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TEST AND VALIDATION OF AN AUTOMATIC BAGS COMPOUNDING SYSTEM WITH NON-HAZARDOUS INJECTABLE DRUGS

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To my family

I. Acknowledgements

Before proceeding with the discussion of this thesis, I would like to dedicate a few lines to all those who have been close to me in this journey of personal and professional growth.

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1 Abstract

This thesis investigates the development of robotics in hospital pharmacy. In this regard, the company Loccioni, since 2006, has undertaken a development in the market to design automated systems aimed at the compounding of both hazardous and non-hazardous drugs for their centralised preparation in pharmacies. The latter is an integral part of the health structure as it is necessary for the organisation and fulfilment of institutional tasks related to drug management. For example, regarding the use of toxic drugs, different regulations impose their preparation within the pharmacy. The advantages are an increase in the quality and safety of the final preparations in terms of accuracy and sterility, a reduction in preparation time and the workload of medical staff and, finally, comprehensive documentation of each step. In the case of non-hazardous drugs preparation, however, the production is not always centralised, and the alternative is the compounding on the ward by the nursing staff which leads to a high probability of preparation errors, high drug wastage and inadequate traceability. Hence, the importance of robotics for drug preparation becomes evident in order to introduce standard procedures and product quality control.

Since 2016, Loccioni's project has widened the vision also on the automated preparation of non-hazardous drugs to overcome the limitations present today. This has led to the design of a prototype called APOTECAbag, which is an automated system for the preparation of bags batches with a standard dosage of both liquid and powder non-hazardous drugs. The key design specification for the automated system is high productivity up to 100 bags per hour. To ensure this specificity, several solutions were designed.

This thesis focuses on two main critical components: vial clamps and peristaltic pumps. The first are devices composed of a homopolymer and copolymer blend whose function is to fix the vial at the neck. In this way, the benefits of using them are the possibility to define a standard gripping point for the anthropomorphic and cartesian

robots and ensure dedicated vials housings to free the robot gripper which in turn can simultaneously perform other functions to optimise the production cycle time. Three peristaltic pumps inside the APOTECAbag ensure automatic and accurate dosing without the need for additional components to complete the preparation, such as syringes. Pump dosage introduces the need for two different types of peristaltic tubes made of PVC. To date, the automated system is still a prototype and, therefore, requires a testing and validation procedure before its market introduction. The thesis will focus on the testing and validation of vial clamps and peristaltic pumps. The first step is to define testing procedures for the two critical components that will assess their validity. The objective of the testing procedure for the vial clamps is to validate them in terms of mechanical resistance and handling by the anthropomorphic and cartesian robot when applied to vials with different neck diameters. This can be explained since there are two clamp versions: one for vials with a small neck and the other for vials with a large neck. The validation of peristaltic pumps consists in defining two crucial aspects. The first involves the estimation of the calibration coefficient for each pump in order to optimise the final product quality and the system cycle time dedicated to the dosing phase. This is the parameter that correlates the revolutions number of the peristaltic pump with the delivered volume by the tube. The second aspect is to assess the performance of the two types of the peristaltic tube about wear due to use and the time of non-use in order to establish when it should be replaced. The pump testing procedures guide an initial experimental phase that will lead to the acquisition of information relating to the volume dispensed by the pump and its error in reference to the theoretically expected dosage. Subsequently, data processing and analysis is performed using MATLAB software to estimate the calibration factor using regression models. The calibration factors for each peristaltic pump have also been experimentally verified on the APOTECAbag system. Furthermore, analysing the error and delivered volume datasets separately, it was also possible to characterise the peristaltic tube. Once the procedures have been conducted, valid results are obtained. Concerning the clamps, it is possible to define the validity and the necessity to use the two versions, respectively for small-neck and large-neck vials, to ensure both a good

fixing of the vial and good handling by the anthropomorphic and cartesian robot. The results obtained concerning peristaltic pumps are different. The estimated and verified calibration factors are 1.63 for the pump in the reconstitution area, 1.66 and 1.06 for those dispensing drug and solvent in the dosage area respectively. The validation of the peristaltic tube in terms of the time of use led to a conclusion that the one present in the reconstitution area can deliver up to a maximum of 19 l, while the tube branch dispensing drug in the dosing area up to approximatively 7 l and the adjacent branch dispensing solvent up to 19 l. Since the tube in the reconstitution area and the drug-dosing branch in the dosage area have the same internal diameter and are inserted into the pump in the same way, it is concludable that both deliver up to 19 l. Once the tubes dispense such quantities, it is recommended to replace them to ensure the performance of the calibrated peristaltic pumps with the estimated optimal factors. Finally, the validation on the peristaltic tubes in terms of the time of non-use has defined that once their use is finished because of batch-end or reached the maximal deliverable quantity, they cannot be reused for dosing the same active substance since a pause of 1.30 h already results in a decrease in dosage accuracy.

La tesi indaga lo sviluppo della robotica nella farmacia ospedaliera. A tal proposito, dal 2006 l'azienda Loccioni ha intrapreso uno sviluppo nel mercato per progettare sistemi automatizzati finalizzati all'allestimento centralizzato di farmaci sia tossici che non tossici in farmacia. Quest'ultima è parte integrante della struttura sanitaria in quanto necessaria per l'organizzazione e l'adempimento dei compiti istituzionali legati alla gestione del farmaco. Ad esempio, per quanto riguarda l'uso dei farmaci tossici, diverse normative impongono la loro preparazione all'interno della farmacia. I vantaggi sono un aumento della qualità e della sicurezza delle preparazioni finali in termini di precisione e sterilità, una riduzione dei tempi di preparazione e del carico di lavoro del personale medico e, infine, una documentazione completa di ogni fase. Tuttavia, nel caso di preparazioni di farmaci non tossici la produzione non è sempre centralizzata e l'alternativa è l'allestimento in reparto da parte del personale infermieristico comportando un aumento della probabilità di errori di preparazione, un elevato spreco di farmaci e una tracciabilità inadeguata. Quindi, l'importanza della robotica per la preparazione dei farmaci diventa evidente al fine di introdurre procedure standard e il controllo della qualità del prodotto.

Dal 2016 il progetto di Loccioni ha ampliato la visione anche sulla preparazione automatizzata di farmaci non tossici per superare le limitazioni ad oggi presenti. Questo ha portato alla progettazione di un prototipo chiamato APOTECAbag, un sistema automatizzato per la preparazione di lotti di sacche con un dosaggio standard di farmaci non tossici sia liquidi che in polvere. La specifica di progetto che caratterizza il sistema automatizzato è l'elevata produttività fino a preparazioni di 100 sacche per ora. Per garantire questa specifica diverse soluzioni sono state progettate.

L'elaborato di tale tesi si focalizza su due componenti critici principali: clamp per i flaconi e le pompe peristaltiche. I primi sono dispositivi composti da una miscela di omopolimeri e copolimeri la cui funzione è l'ancoraggio del flacone all'altezza del collo. In tal modo è possibile definire una presa standard del robot antropomorfo e cartesiano ma, nello stesso tempo, garantire alloggiamenti dedicati ai flaconi per svincolare la continua presa da parte dei robot che a loro volta possono svolgere altre task per un'ottimizzazione del tempo ciclo di produzione. Tre pompe peristaltiche

all'interno di APOTECAbag assicurano un dosaggio automatico e accurato senza la necessità di componenti aggiuntivi per completare la preparazione, come le siringhe. Il dosaggio mediante la pompa introduce la necessità di utilizzo di due diversi tipi di tubi peristaltici in PVC. Ad oggi, il sistema automatizzato è ancora un prototipo e, dunque, una fase di test e validazione è necessaria prima della sua introduzione nel mercato.

Pertanto, la tesi verterà sul test e validazione delle clamp per i flaconi e delle pompe peristaltiche. Il primo passo è quello di definire le procedure di test per i due componenti critici che ne valuteranno la loro validità. L'obiettivo della procedura di test delle clamp per i flaconi è la validazione in termini di resistenza meccanica e di manipolazione da parte del robot antropomorfo e cartesiano quando applicate a fiale con diversi diametri di collo. Questo può essere spiegato dal fatto che ci sono due versioni di clamp: una per flaconi con un collo piccolo e l'altra per quelli con un collo grande. La validazione delle pompe peristaltiche consiste nel definire due aspetti cruciali. Il primo riguarda la stima del coefficiente di calibrazione per ogni pompa al fine di ottimizzare la qualità del prodotto finale e il tempo di ciclo del sistema dedicato alla fase di dosaggio. Quest'ultimo è il parametro che correla il numero di giri della pompa peristaltica con il volume erogato dal tubo. Il secondo aspetto consiste nell'andare a valutare le prestazioni dei due tipi di tubo peristaltico in merito all'usura dovuta all'utilizzo e al tempo di non utilizzo al fine di stabilire un criterio di sostituzione. Le procedure di test della pompa guidano ad una prima fase sperimentale che porterà all'acquisizione di informazioni relative al volume erogato dalla pompa e al suo errore in riferimento al dosaggio teoricamente atteso. Successivamente, l'elaborazione e l'analisi dei dati viene condotta tramite il software MATLAB per stimare il fattore di calibrazione utilizzando modelli di regressione. In seguito, i fattori di calibrazione stimati per ciascuna pompa peristaltica sono stati verificati sperimentalmente mediante il sistema APOTECAbag. Inoltre, analizzando i dataset relativi all'errore e al volume erogato è stato possibile caratterizzare anche il tubo peristaltico. Una volta che le procedure sono state condotte, si ottengono risultati validi. Per quanto riguarda le clamp, è possibile definire la validità del dispositivo

mediante un utilizzo differenziato in accordo alla dimensione del collo del flacone e della sua capacità dal momento che influisce sul peso da sostenere, il tutto per garantire sia un buon fissaggio del flacone che una buona manipolazione da parte del robot antropomorfo e cartesiano. I risultati ottenuti in riferimento alle pompe peristaltiche sono diversi. I fattori di calibrazione stimati e verificati sono 1,63 per la pompa nell'area di ricostituzione, 1,66 e 1,06 per quelle che erogano rispettivamente farmaco e solvente nell'area di dosaggio. La validazione del tubo peristaltico in termini di tempo di utilizzo conduce a una conclusione che quello presente nella zona di ricostituzione può erogare fino a un massimo di 19 l, mentre il ramo di tubo che eroga il farmaco nella zona di dosaggio fino a circa 7 l e il ramo adiacente che eroga il solvente fino a 19 l. Dal momento che il tubo nella zona di ricostituzione e il ramo che eroga il farmaco nella zona di dosaggio hanno lo stesso diametro interno e sono inseriti nella pompa nella stessa maniera, si può concludere che entrambi erogano fino a 19 l. Una volta che i tubi erogano tali quantità, si raccomanda di sostituirli per garantire le prestazioni delle pompe peristaltiche calibrate con i fattori ottimali stimati. Infine, la validazione sui tubi peristaltici in termini di tempo di non utilizzo ha definito che una volta terminato il loro utilizzo per motivazioni di fine lotto o raggiunta quantità massima erogabile, non possono essere riutilizzati per dosare lo stesso principio attivo poiché un'interruzione di un'ora e mezza comporta già una diminuzione della precisione di dosaggio.

2 Introduction

This chapter will present the automated APOTECAbag system, from its origins to its development. It is a solution that allows to centralise the pharmaceutical production in hospitals of non-toxic drugs. In addition, the general aspects of the Loccioni company and its APOTECA market project, centred on robotics in the pharmaceutical field, will also be described.

2.1 Loccioni enterprise

2.1.1 History, values, missions

Loccioni is an Italian family business founded in 1968 by Enrico Loccioni and his wife Graziella Rebichini, located in a small town in the hinterland of the Marche region. It is an enterprise specialized in the design of measurement and control systems for the improvement of quality, safety and sustainability of processes and products in different sectors. The enterprise initially started as a micro-enterprise in the field of electrical systems, but as the years went by it embraced businesses in different market areas: mobility, energy, health and environment. A typical map describing the enterprise's business is called Polaris (Figure 2.1), where at its centre is highlighted how all projects have as their mission the wellness of people and the planet. Today the enterprise has 500 employees in 45 countries with six offices abroad, in addition to the reference office in Italy: United States, Germany, China, Japan, India and South Korea. In a knowledge-based enterprise everyone is an entrepreneur and uses his/her talent to develop business from within. The enterprise model adopts a network organization, with a non-hierarchical but horizontal structure, characterized by strong business culture and sharing of values that eliminates the need for formal controls. With these characteristics, Loccioni enterprise is a "technological tailoring", which focuses on projects and solutions integrated to the customer's needs, rather than

on standard products or solutions. There are three fundamental points on which the enterprise's activity has been based in all these years:

- Solving costly and annoying problems: a customized solution for a client can be replicated for other clients in the same industry;
- Working with the world's biggest: being in close contact with industry leaders allows them to follow future development trajectories in advance;
- Technological barrier: solving problems that require overcoming a technological barrier. Knowledge in these cases will be limited.

The strength of this enterprise is the measurement, the mission is to transform data into value. As founder Enrico Loccioni said, "*measuring means transforming data into value and transforming data into value is the commitment that moves us to improve the future*". This implies a willingness to exceed oneself, to improve, to go beyond and to take up new challenges. Thus, the data that are managed, created, measured, transferred, are not only used to improve products and processes, but through intelligence, knowledge, the network of the open enterprise they turn into values to improve the quality of life on this planet. The main goal is to leave the world a little better than we found it.

Up to now it has been discussed about Loccioni as an enterprise and not as a company, because the mission is to create on the territory an entrepreneurial model that develops work and knowledge by integrating ideas, people and technologies. Loccioni's corporate values can be summarized as follows:

- Imagination: having the ability to dream, to see the invisible, to ask oneself useful questions to reach the realization of the imagined dream. It is the desire to participate in the construction of the future;
- Energy: putting enthusiasm and passion, courage and motivation into the things the do. It is the highest expression of doing;
- Responsibility: it is taking charge of the future, aware that every action and every project has consequences. It is the response to the trust offered (by customers,

suppliers, collaborators). It is the ethical dimension of being a person in a community;

- Tradinnovation: indicates the link with tradition, listening and using experience to project into the future, to innovate.

Values are the true identity of the group because they provide a common language, give strength to the enterprise and guide it in its market performance. In general, without values, there is no development because they give strength and consistency to actions.

Figure 2.1 Polaris: representation of all Loccioni's projects.

2.1.2 APOTECA project

Going back to Polaris, in the business related to wellness it is possible to identify the APOTECA market project that integrates the competence of measurement with people's health. This project has as a pillar the Lab@AOR, a research and development laboratory born in 2006 from the collaboration between public and private of the

Azienda Ospedaliera Universitaria Ospedali Riuniti of Ancona and Loccioni. The main objective is to promote the quality of care through technology, in a project space in which clinicians, referents of the academic world and technology experts continuously dialogue to put together their respective competencies to develop the hospital of the future: safe, efficient and economically sustainable. The collaboration has allowed the development of the wellness market project shown in the Polaris (Figure 2.1) which enclose a highly innovative technological solutions (APOTECA platform) and the re-engineering of clinical processes to improve the quality and safety of patient care pathways. The laboratory is also the site of scientific validation of APOTECA platform solutions in the clinical environment and the results have been made available to the international scientific community through publications.

Along this sector, Loccioni supplies different solutions for APOTECA platform that can be both hardware and software. Hardware solutions include APOTECAchemo, APOTECAunit, APOTECAps, APOTECAped, while software solutions are APOTECAmanager and APOTECAm@a. All hardware solutions correspond to automated systems intended for safe and efficient preparation of injectable drugs that may be toxic and non-toxic. APOTECAchemo [1][2] is the first technology within the healthcare sector introduced to the market at the beginning of 2007. The technology is a robotic system intended for the preparation of both liquid and powder toxic drugs. The system can prepare doses customized to the patient's chemotherapy treatment in syringe or bag format. The technology has been developed to minimize the risk of error for the patient due to the therapeutic relationship of the drugs, as well as to decrease the possible negative effects on the operators performing compounding. In addition, the robotic system reduces the variability of compounding procedures and standardizes the workflow regarding human factors and, in turn, the occurrence of therapeutic errors. Hence, the system was designed to isolate the surrounding area from toxic substances through a negative pressure environment with laminar flow.

Subsequently, in 2016, APOTECAunit [3], a robotic system capable of automating the compounding of both liquid and powder non-toxic drugs, was developed. The technology is designed for both standard and patient-specific batch productions of

therapies, although such drugs are rarely used for custom dosages due to their nature. The system is capable of preparing bags and syringes with an automated closure using a tamper-proof cap in a microbiologically controlled environment under positive pressure to ensure product sterility.

Contrary to APOTECAchemo and APOTECAunit, APOTECAps [4] is a semi-automatic system that guides the operator during manual preparation of both toxic and non-toxic drugs that are found to be unmanageable by the previously disclosed systems.

Finally, in 2018 APOTECAped [5][6] was developed, a robotic system for the automatic compounding of non-toxic paediatric therapies within a controlled atmosphere environment to ensure product sterility. The system was designed because doses for young patients require different precautions than preparations for adults. The robotic system can produce therapies for both standard and patient-specific batches, in syringe and bag formats with low and accurate dosages through the use of drugs in both liquid and powder formats. Complementing the hardware solutions there are the software solutions previously listed. The first is APOTECAmanager, management software for the entire production of the hospital pharmacy, used to:

- Manage operations: from validation to preparation delivery;
- Track materials, information, people and procedures;
- Communicate with computerized prescribing software used on the wards;
- Control multiple robotic systems from a single location.

The second is APOTECAm@a, which is a statistical tool for data processing that improves the decision-making process of hospital pharmacists and directors.

Since hospitals are the reference target of the APOTECA project, the physical place where the technologies are installed is the internal pharmacy of a hospital. The latter is an integral part of the health structure as it is necessary for the organisation and fulfilment of institutional tasks related to drug management. In Italy, pharmacy is mandatory in general or specialized hospitals. From an organisational point of view,

when the drug compounding takes place within the hospital pharmacy, we talk about centralised production. However, the production is not always centralized and the alternative is the compounding on the ward by the nursing staff.

Regarding toxic drugs, normally the production is performed in a centralized way, following the directive of the Ministry of Health [7] . All this leads to a reduction of risks for the operator and the patient. The main risk on the operator's side is the occupational risk due to handling and exposure to highly toxic substances, while for the patient is the adverse therapeutic events, due to non-standard compounding procedures [2]. The same reasoning cannot be made for pharmaceutical production related to non-toxic drugs, since they are often prepared on the ward by nurses. This all follows:

- High probability of error;
- Poor level of oversight by the hospital pharmacist;
- High wastage of medication since once a vial of medication is opened and partially used, issues related to sterility arise;
- Inadequate traceability and non-exhaustive documentation.

In particular, to overcome the problem of medication errors, it is necessary to introduce a series of measures to control the clinical risk related to the use of drugs. A therapy error is any preventable event that can cause or lead to inappropriate use of the drug or a danger for the patient. Thus, the error in the preparation of therapy is mainly due to incorrect formulation, for example: incorrect dilutions and reconstructions, physically or chemically incompatible drug combinations or inappropriate packaging of drugs [8][9]. Hence, it is clear the importance and the need for centralization in hospital pharmacy which is able to bring qualitative and quantitative benefits [2][10].

Qualitative benefits:

- Increased quality and safety of final preparations, in terms of accuracy and sterility;

- Improvement of patient safety, due to the very low variability caused by standardized compounding procedures, and of the operator, who only performs loading and unloading activities;
- Exhaustive documentation, related to every single step: from the request of the drug to its delivery to the ward;
- Increased control over drug stock on the ward and drug handling.

Quantitative benefits:

- Reduction in the incidence of administration errors;
- Reducing drug waste;
- Reduction in preparation time and workload of medical and nursing staff, who can be redirected to activities with greater added value;
- Reduction of drug stocks on the ward.

However, the centralization of preparations in the pharmacy has critical points to resolve. It is necessary to implement an effective communication system between the hospital staff and the pharmacy, both to respond promptly to urgent requests and to program and plan production in advance.

What's more, with planned production, drug compounding takes place in advance. Thus, there will be a time lag between preparation and administration that could increase the risk of contamination and use beyond the expiration date. Hence, standard procedures and proper facilities for process and product quality control and assurance are required. The development of centralisation, therefore, requires a restructuring of structures, processes and procedures which often involves substantial financial outlays. This means that for small hospitals, investments may not be possible or convenient.

2.1.3 Apoteca community

The positive experience of public-private collaboration found with Lab@AOR has highlighted how the conversation with its users and the exchange of technical knowledge is a winning weapon to ensure a high standard of quality and to address new needs. Based on this experience, the APOTECAcommunity project was born. It

aims to share experience, knowledge and best practices associated with the optimal use of technologies to anticipate the future role of robotics in hospital pharmacies [11]. APOTECAcommunity is the international scientific network of APOTECA users, which brings together people with different skills, but with the same passion and desire to implement robotics in pharmacies [11]. Through this project, the customer becomes an integral part of the innovation and development flow of the enterprise, which implies a very close collaboration between the APOTECA technical-scientific team and the hospital pharmacy staff. Therefore, through the community, the customer does not become the endpoint of the production process but is involved in a co-creation process of the service offered by ensuring continuous engineering and new solutions. This is important because the hospital environment is always in continuous evolution since there are changes on new drugs, new required functionalities, new services but, above all, the need to manage useful information in real-time. For Loccioni enterprise, this project represents an important opportunity to develop new businesses and to improve the existing ones. Indeed, the APOTECAcommunity project is characterized by events in which all users' contributions are collected to codify possible process upgrades transferable from one context to another. On this approach resides the ability of the enterprise to sense the practices development trajectory in use to identify opportunities for improvement and standardization towards new practices that are presented to a diffusion of the whole sector.

Therefore, the importance of APOTECAcommunity for the enterprise lies also in the fact that the development of new solutions starts with the ideas coming from the Community, which are then developed by the Research and Development (R&D) and tested at first within the enterprise and finally at Azienda Ospedaliera Universitaria Ospedali Riuniti of Ancona. Once validated, the solution is ready to be put on the market (Figure 2.2).

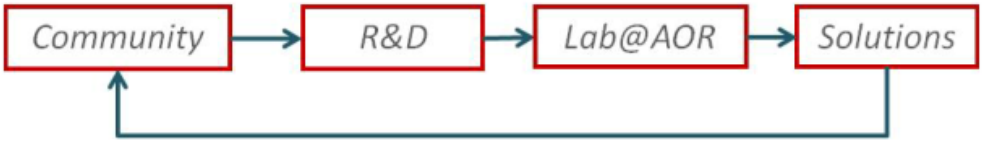


Figure 2.2. Workflow within APOTECA projects.

The Community is not only a virtual group. It takes shape with periodic meetings between users and developers to contribute to the process of improving the system, promote scientific research, build a strong network spirit and encourage users to open up, sharing problems and needs that will later be resolved.

The Community is continuously growing with users in over 25 countries. Users are spread worldwide with a presence in Asia, Europe, America, Middle East, South America Japan and South Korea. For this reason, in 2012 the International Community was born, where once every two years the main worldwide users meet at Loccioni headquarters to discuss international standards for pharmacy automation based on clinical experiences, technological know-how and scientific facts. In addition to the biennial meeting, a series of annual meetings are organized for different countries including Italy, Germany, Japan, USA, Denmark and Spain. The national meeting brings together users from each country to present the annual update of APOTECA solutions in order to hear users' thoughts on the development of the system.

2.2 APOTECAbag: robotic system for bags preparation

2.2.1 APOTECAbag system

The project of this thesis will be based on a new prototype of a robotic system belonging to the APOTECA platform. The technology in question is APOTECAbag (Figure 2.3), a robotic system capable of producing large quantities of sterile bags with standard dosages starting from both liquid and powder drugs. The idea of the APOTECAbag project was born in 2018 during the International Community. Given the importance of this event, and especially of its function and validity, the need arose to achieve a large production of bags using non-toxic drugs [12].

To consolidate this idea, through a careful workflows analysis of different hospitals spread in the world, it was noticed that many pharmacies use APOTECAunit to produce bags and syringes with standard dosages. Of course, the demand needed to meet the usage requirements was lower since APOTECAunit is able to produce about

20 bags per hour because of the slow reconstitution procedure. The remaining quantity required was obtained in outsourcing from external compounding centres with consequent risks of sudden drug shortages, inefficiency in the management of stocks and an increase in the capital used. The need to develop the APOTECAbag system was identified mainly for the USA market after a careful analysis of the production workflow regarding APOTECAunit, but also through the international community.

Figure 2.3. APOTEGAbag system.

Differently, in Europe (Italy, Germany, France, Spain, Scandinavian countries) many of these therapies are still prepared on the ward and the need for a robotic system in the pharmacy is limited to a few drug classes. This aspect highlights the concept of the lack of product centralisation in the pharmacy for non-toxic drugs. Although they are considered less critical and dangerous than hazardous drugs, these parenteral products must comply with sterility requirements and must be prepared in appropriately classified premises in accordance with the relevant national (Official Pharmacopoeia X Ed. [13] and relevant Good Manufacturing Practices[14]) and international (USP <797>[15], PIC/S PE 010-04 [16]) regulations.

Having ascertained the need to develop a robotic system capable of setting up a high quantity of standard-dose of non-hazardous drugs in infusion bags, the acquisition of project requirements began in 2019 by interacting with Community USA hospitals: Wake Forest Baptist Health (WFBH), Johns Hopkins (JHH), Cleveland Clinic Foundation (CCF), Cone Health. Subsequently, other requirements were acquired employing meeting with different clients both in the Loccioni enterprise and within the corresponding hospitals and in the end, to have refinements and improvements, a further fillable questionnaire was drawn mainly concerning the most used dosages and the final bag size. Once the requirements were collected it became possible to divide them into:

- General Requirements, are the requirements linked to general characteristics and features expected from the information system and/or to system hardware and software components;
- Process Requirements, are the requirements directly linked to the processes managed by the system;
- Equipment Requirements, are the requirements to be satisfied by the equipment controlled by the system in terms of performances and mechanical requirements;
- Interface Requirements, are the requirements to be satisfied by the system in terms of interface with other systems, equipments or software;
- Automation Requirements, are the requirements to be satisfied by the control system;
- Regulatory Requirements, are the requirements linked to relevant and applicable regulations;

All the collected requirements are reported in Table 7.4 in Appendix.

Given the requirements, the next step involve the definition of project technical specifications which can be then grouped into technical characteristics and functionality.

Regarding the technical characteristics, the APOTECAbag robotic system includes:

- Loading and unloading area;

- Double carousel capable of holding up to 42 bags;
- Magazine capable of holding up to 24 vials;
- Anthropomorphic robotic arm for handling vials and bags;
- Reconstitution form for powdered medicines;
- Dedicated area for transferring the drug into bags;
- Internal areas classified by Good Manufacturing Practise (GMP) as Grade A (ISO 5) and characterized by laminar flow;
- Positive pressure gradient with respect to the external environment (+10 Pa);
- High precision peristaltic pumps;
- Clamp for vial support;
- Closed system.

As for as the functionality, the APOTECAbag robotic system is designed to:

- Manage liquid drugs, powdered drugs and solvents from vials with prickly septum;
- Prepare bags of various sizes (50-250ml) at standard dosage ready for use;
- Prepare sterile empty bags in 500 and 1000ml format;
- Up to 100 preparations per hour;
- Faster powder reconstitution process.

Today, the robotic system APOTECAbag is a prototype, but the development planning of this project provides a series of executive steps that will end with the installation of the system by the end of 2021, at Azienda Ospedaliera Universitaria Ospedali Riuniti of Ancona for a clinical validation.

2.2.2 Description of the APOTECAbag system

The APOTECAbag system is very complete and well organized in order to guarantee a bag compounding procedure in compliance with many regulations. It is possible to define seven different functional areas: ventilation area, loading area, dosage area, reconstitution area, printing area, final product area, waste area. The different functional areas will be discussed individually below.

Ventilation system.

APOTECAbag consists of nine HEPA filters with a dedicated fan for each in the upper part. The main purpose of the ventilation system is to ensure sterile environment of ISO 5 class according to UNI EN ISO14644-04 [17]. This aspect is of fundamental importance to ensure the sterility of the final product. The ventilation system can guarantee a sterile environment through a laminar flow and a positive pressure environment. The laminar flow is ensured by several recovery grids through which air passes, which will then be drawn in by fans in the lower part of the robotic system and then expelled. Regarding the positive pressure gradient, it is of fundamental importance to avoid microbiological contamination by the external environment.

Loading area.

This is the sector dedicated to loading the material necessary for the compounding inside the robotic system (Figure 2.4): bags, vials with drug both liquid and powder. Before loading the different products, it is important to scan each one through a barcode reader to avoid an error in product insertion by the operator. The sector is developed on two levels divided by a grid designed to ensure a laminar flow.

Each level is accessible through two sliding doors, useful to avoid contamination from the external environment, allowing access to the carousel. In particular, the sector consists of two concentric carousels, one for each level, able to contain up to a total of 42 bags and 24 vials (Figure 2.5). It is important to underline that for both bags and vials there is a very precise mechanism that allows the hook.

As far as the bag is concerned, there are specific clamps that adhere perfectly to the upper profile of the bag allowing the hooking on the carousel. For the vials the logic is always the same, in fact is used a clamp made of polymeric material which adheres to the neck of the vial and then hooks it on the carousel.

Figure 2.4. APOTECAbag: loading area.

Figure 2.5. Internal view of APOTECAbag loading area.

Dosage area.

The sector (Figure 2.6) is characterised internally by the presence of a 6-axis anthropomorphic robot capable of managing the movement of both bags and vials.

The robot can take the product from the carousel since the loading area is communicating with the latter. Also, internally (Figure 2.7) there is a gravimetric control which is important to ensure the correct dosage of both solvent and drug in the final bag. On the floor of this area is present an automatic lifter on which the final product will be placed and, also, a trap door with an automatic opening for unloading an empty vial. The part of the area described is accessible through a large door. Moreover, in the dosage area, there is a small loading sector which is accessible by the operator to load the solvent bag, used to dilute the drug in the final bag, but also to change the peristaltic tube. This last component is extremely important since the dosing of the drug and solvent is performed automatically by two peristaltic pumps.

In particular, the peristaltic tube is made of two branches to dose both the drug and the solvent flow into the final bag. Importantly, both the drug vial and the solvent bag are positioned in dedicated housings during the dosing procedure. In addition to the explained roles of this loading area, the function of the double door was designed to avoid contamination from the external environment but also to build a security system during the robotic system activity while the operator loads the products.

Figure 2.6. APOTECAbag: dosage area.

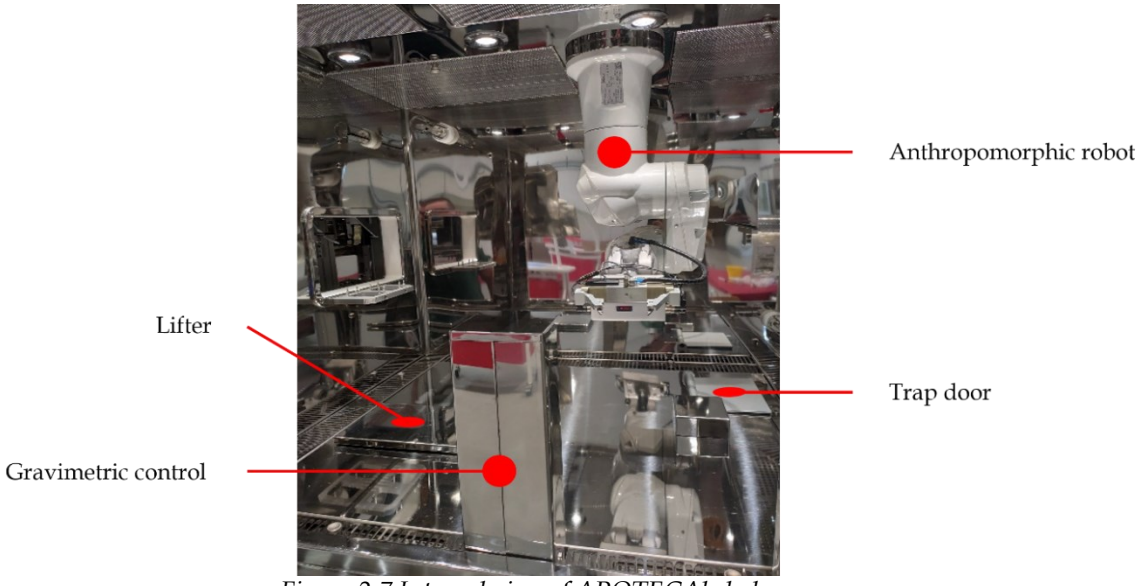


Figure 2.7.Internal view of APOTECAbab dosage area.

Reconstitution area.

This area is used only when it is decided to make preparations using powdered drugs. As well as in the previous area, the actual one is accessible through two doors (Figure 2.8). The large one allows access to the internal part of the system where there are two shaking systems useful to perform the reconstitution procedure. In particular, the two stations can house three vials each and through vibrations at a given frequency they allow the reconstitution phase to be performed. There is also a gravimetric control inside the area which is useful for defining the correct solvent dosage in the vial containing the powdered drug. In addition, the dosage area communicates with the latter using an opening on which up to two vials can be housed. The movement of the vials inside this area is managed by a cartesian robot. Even here, the small door allows loading the solvent bag and the peristaltic tube. Indeed, in this sector the dosage of the solvent useful to dilute the powdered drug is conducted automatically utilizing a peristaltic pump. Unlike the peristaltic tube used in the dosage area, the latter consists of a single branch since it has to dose only the solvent from the solvent bag to the vial.

The function of the double door function has been designed also for this area in order to avoid contamination from the external environment but also for a security system during the robotic system activity.

Figure 2.8. APOTECAbag: reconstitution area.

Printing area.

This area is below the reconstitution area and contains a printer useful for labelling the final product that comes from the dosage area via the automatic lifter (Figure 2.9). On the label there will be all the information regarding the final product, such as the type and quantity of drug and solvent used.

Figure 2.9. APOTECAbag printer.

Final product area.

The area is below the dosage area and consists of a container in which the final product will be automatically deposited once labelled (Figure 2.10).

Figure 2.10. Final products area.

Waste area.

This area is also below the dosage area and inside it there is a waste bin in which the empty vial will be deposited. The waste product passes from the dosage area to the latter through a trap door with an automatic opening. Once the bin is full it is automatically sealed with a lid.

Figure 2.11. Waste area with waste bin presence.

2.3 Thesis aim: testing and validation of critical components

2.3.1 The importance of the testing and validation phase

The APOTECAbag system is classified by the Food and Drug Administration (FDA) as a class two medical device since in March 2001 the pharmacy compounding systems are classified in this way [18].

The main risks to health associated with these kinds of systems according to the FDA are:

- Incorrect use or faulty device design or software that could lead to inaccurate concentrations or volumes, non-sterility, or incompatibilities resulting in therapeutic failures, overdoses, fluid or electrolyte imbalances, sepsis, or other adverse events;
- Incorrect use or faulty device design or software that could cause or contribute to the cross-contamination or adulteration of drug products
- Faulty electrical design or shielding that could lead to unsafe current leakage and/or electromagnetic interference, resulting in operational failure, electric shock, burns, or death.

From this it is clear the importance of the validation step in the project development process of a medical devices. In fact, the regulatory bodies of the various countries ask that after the development, the various solutions are validated before starting the commercialization. In many countries is used as a reference the FDA, in particular the standard 21 CFR Part 820 (Quality System Regulation) section 820.30(g) Design Validation [19].

As reported by the regulation, the validation means to confirm through examinations and provision of evidence objectives that the particular requirements for a specific intended use can be consistently met.

In detail, it is possible to define:

- A validation process, in which it is established through objective evidence that a process consistently produces a result or that a product meets predetermined specifications;
- A validation design, which means establishing through objective evidence that the device specifications meet the user's requirements and intended use.

The previously mentioned standard defines the main characteristics of design validation:

- Each manufacturer shall establish and maintain procedures for validating the device design;
- Design validation shall be performed under defined operating conditions on initial production units, lots, or batches, or their equivalents;
- Design validation shall ensure that devices conform to defined user needs and intended uses and shall include testing of production units under actual or simulated use conditions;
- Design validation shall include software validation and risk analysis, where appropriate;
- The results of the design validation, including identification of the design, method, date, and individual performing the validation, shall be documented in the design history file.

For what has been said so far, design validation is important to ensure that the design will conform with the customer's needs and intended use. Several aspects are important to ensure proper validation. First, a validation plan should be defined at the beginning of the design process. The performance characteristics that are to be assessed should be identified and validation methods, together with acceptance criteria, should be established. During the product development process, the validation plan should be reviewed for appropriateness, completeness, and to ensure that user needs and intended uses are addressed. The devices require a clinical evaluation and should be tested in the actual or simulated use environment as a part

of validation. Additionally, the validation should include simulation of the expected environmental conditions, such as temperature, humidity, shock and vibration, corrosive atmospheres. Validation is a compilation of the results of all validation activities. For a complex design, the detailed results may be contained in a variety of separate documents and summarized in a validation report. Supporting information should be explicitly referenced in the validation report and available in the design history file.

There is also another standard that refers to the validation of medical devices, the standard ISO 13485:2016 [20]. In detail, in the normative there is a paragraph in section 7.3 dedicated to Design and Development validation.

Concerning the APOTECAbag system, in parallel with the start of the design phase (2019), a plan was formulated for the validation activities necessary for placing the product on the market. In particular, the document reports that all validation tests must be performed on the prototype, which is identical in components, assembly and functionality to the product that will be placed on the market. After passing all the verification and validation tests, the checks foreseen by the product quality plan must be performed on each device produced before it is placed on the market. If hardware or software changes are made to the design, these must be subjected to verification and validation before being transferred to production or products already on the market.

The verification and validation protocols and related evidence must be kept in the technical file of the product in question.

2.3.2 Testing and validation of vial clamps and peristaltic pumps

In the previous paragraph, the concept of testing and validation was introduced to better understand the central topic of this thesis, since it will focus on testing and validation of some critical components of the APOTECAbag system. It is important to remark that the system in question is still a prototype that needs to undergo validation of each component.

The thesis is focused only on two characteristic components: peristaltic pumps and vial clamps. These are two innovative components in the APOTECA platform since to date no robotic system already designed contain such innovations. Essential to highlight that for each component has been defined a test e validation procedure. The testing and validation part related to the clamps aims in a first phase in testing the clamp mechanical properties in order to verify a possible breakage of the device during its application and the correct fixing of the vial. The second phase of testing is aimed at assessing the manipulation of the clamp with vial by the anthropomorphic and cartesian robots during the automatic system cycle.

Regarding the testing and validation of peristaltic pumps, the procedure is a little more articulated. The purpose of the test is to define the calibration factor for each peristaltic pump through an accurate elaboration and analysis of the acquired experimental data. In this way it is possible to correlate the number of revolutions made by the pump with the quantity of drug or solvent dosed. For completeness, of course, the calibration factors for each pump will then be experimentally verified on the cyclic of the robotic system by changing those parameters in the software system and then defining the optimal one. At the same time, the analysis of the experimental data also leads to a characterization of the peristaltic tube. It is possible to define a criterion such that after a certain time of use, quantified in terms of quantity of solvent or drug dispensed, it is recommended to replace the peristaltic tube in order to ensure accurate dosing. This is because one of the initial specifications of the project was to ensure a great amount of bags with standard dosage, about 100 bags per hour. As soon as a production batch with a high quantity of bags is run, the peristaltic tube will start to wear and therefore lose its properties. The wear linked to the time of use is due to the numerous revolutions made by the cylinders inside the pump, which press the tube guaranteeing the peristaltic phenomenon. In addition to the characterisation of the peristaltic tube by the time of use, assessments were also made regarding the replacement of the peristaltic tube after a certain time of non-use since the material properties of the tube may change.

3 Material and Methods

This section presents the innovative components for the APOTECA platform that will be analysed: vial clamps and peristaltic pumps. For each of these components, their characteristics will be described as well as the useful testing procedures to define their validation.

3.1 Vial clamp

3.1.1 Component description

Vial clamps are innovative components for APOTECA platform solutions since they are not present in the already robotic systems designed. The need to design a new component is related to the fact that the vials handling, within the robotic systems designed so far, represents one of the main operations performed. Therefore, focusing on this phase was of crucial importance for optimising the robotic system operation.

In fact, through the analysis of vial handling during the cyclic of APOTECA platform systems, it is possible to find some limitations. In the already designed robotic systems, the vial is grab by an electric gripper directly on the neck and brought to the various designated positions to ensure the automated compounding of the final product. However, the electrical gripping system could introduce some limitations. In fact, by gripping the glass vial with the metal components of the electric gripper, a vial breakage and a consequent machine stopping for cleaning procedure may occur. In addition, it should be considered that the geometrical dimensions of the different vials vary according to the pharmaceutical company. Indeed, by examining the different diameters of the vial necks used by the customers, it is possible to underline a variation from a minimum of 9.5 mm to a maximum of 30 mm. This means that the robotic arm has to be programmed according to the neck size of the vial used. The remarks made so far, emphasize in the previous APOTECA platform solution some restrictions from

both mechanical and a software point of view. Therefore, taking into account both these disadvantages and the APOTECAbag design specifications, the idea of designing an adaptor device that would allow always the same vials grip diameter was reached.

The main clamp characteristics to be respected during the design phase were the following:

1. No device contact with the vial membrane in order to guarantee the sterility of the product;
2. The device must be disposable, thus avoiding cleaning and sterilisation after use;
3. Design as few versions as possible to fit different vial necks sizes;
4. The gripping geometry of the device must conform to the robot gripper profile;
5. The device must be equipped with suitable interfaces in order to interact with the different system areas;
6. Breaking strength at the time of its application.

The design phase of the vial clamp started in late 2019 and only recently a suitable prototype has been defined and needs to be validated. The device is produced by injection moulding using a well-defined mould. The moulding is performed in a cleanroom to guarantee the sterility of the final product. In detail, the manufacturing method is performed in a cold chamber, which means that once the material has been injected through ducts at specific points, a cooling time is required to allow the device to separate from the mould. The mould is a two-cavity mould able to produce two clamp versions by applying insertions with specific shapes. The reason for this is that two clamp versions have been designed with the same function (Figure 3.1).

The first version is used to properly adhere and fix vials characterized by a large neck, while the second version is for those with a small neck. From a geometric point of view, the clamps have the same dimensions, the main difference is the presence of support in the second version in order to reinforce the adhesion between the clamp flexible part and the vial neck. In detail, the flexible part of the device, which allows the fixing of the vial, is located in the central part. This means that the vial will be fixed centrally in a perpendicular position with the clamp.

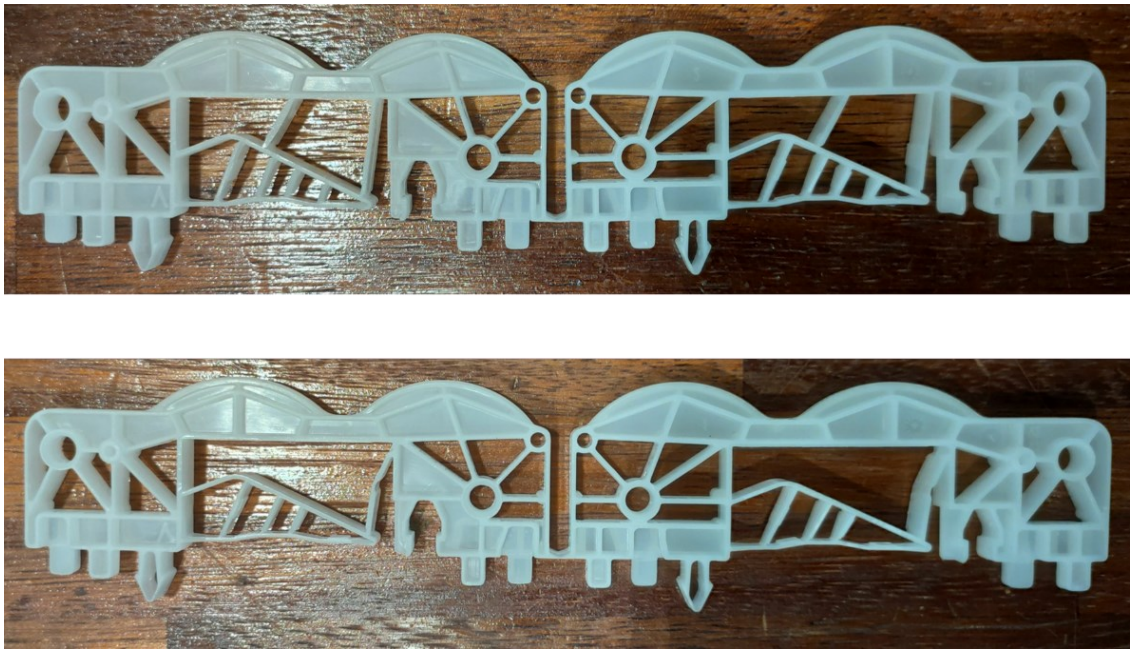


Figure 3.1.The two clamp versions. In the upper part the version two, in the lower one the version one.

The application of the clamp to the vial is not handled automatically but is done by the operator. When personnel load the vials, they are already equipped with the clamp because they need to position them on the carousel where there are holes that match perfectly to the protrusions of the device. (Figure 3.2).

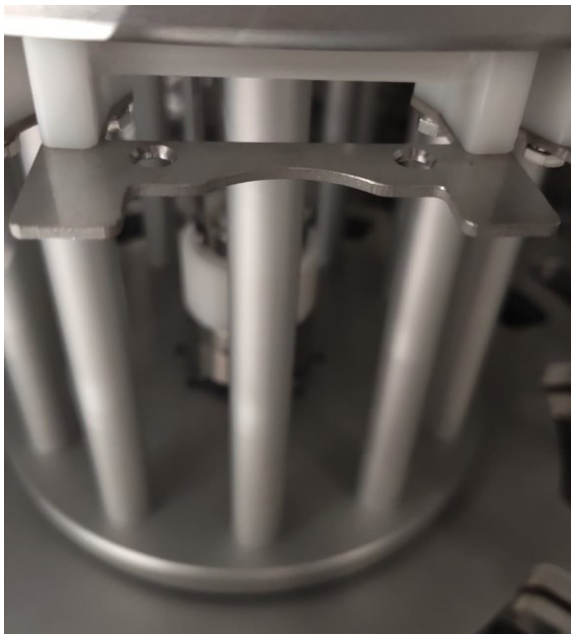


Figure 3.2. Representation of vial clamp housing in the carousel.

The material used to produce the clamp is a blend characterized by less homopolymer and more copolymer. This mixture is suitable for providing both rigidity and flexibility to the device in order to ensure the adhesion and fixing of the vial. In detail, the homopolymer used is a type of polypropylene homopolymer that is produced with a phthalate-free catalyst, characterized by high flowability and a narrow to medium molecular weight distribution. As for the copolymer, the heterophasic copolymer is used. This product is characterized by an optimum combination of very high stiffness and high impact strength. These materials have been chosen because are easy to process with standard injection moulding machines and, also, for their physical properties.

3.1.2 Impact in the automated system

There are many advantages introduced by the use of the clamp in the automated system. The first is related to the fact that the gripper of anthropomorphic and cartesian robot is not in direct contact with the vial neck, thereby preventing it from breaking. This is an advantage compared to previous APOTECA solutions in which before using a drug vial, it is necessary to ensure that its geometric characteristics are available in the database. In this way the algorithm that managed the movement and closure of the robot's gripper was able to ensure the correct handling of the glass vial and, more importantly, avoid its breakage. The inclusion in the database of the geometric characteristics of the drug vial is a limiting aspect that is evident daily in previous APOTECA solutions since in several countries there are pharmaceutical companies that produce the same drug vials with different geometries.

With the clamp introduction, this limitation is overcome because the use of that device standardises the handling and, therefore, the gripping point of the vials independently from its geometrical characteristics. This means that is no longer necessary to use an electrical robot gripper whose opening can be easily controlled by software once the necessary vials geometrical data have been provided. Indeed, unlike the previous APOTECA solutions which us an electric robot gripper with high

precision and sensitivity, the APOTECAbag system introduces a pneumatic one which is also more economical.

Another relevant aspect introduced by the use of the clamps is the reduction of the system cycle time which is the time taken by the machine to produce the final product. Considering the dosing phase in the other available APOTECA solutions, the robot remains involved in the entire phase affecting the compounding time. With the standardization of the vials size, alternative gripping points have been introduced allowing the vial to be positioned in various housing and taken once the dosage phase is complete. This frees the robotic arm from the continuous vial handling, which can in the meantime perform other tasks such as gripping other vials or bags.

There are several alternative gripping points in APOTECAbag. In the dosage area, there is the dosage unit which corresponds to the machine station where the drug is sucked from the vial. The dosage is achieved through a mini-spike moved by a pneumatic actuator in order to penetrates the vial membrane positioned upside down by the robot (Figure 3.3).

Figure 3.3. Representation of vial clamp housing in the dosage area.

Another vial housing is located in the reconstitution area, specifically in the scale unit. In this area, the scale has a dual role: to perform an accurate measurement and to support the vial during dosing. The balance has been designed from a mechanical point of view to perfectly guarantee the housing of the vial with clamp and, simultaneously, fix it to support the vertical effort caused by the insertion of the mini-spike for the solvent dispensing.

The shacking group, made up of two shakers (Figure 3.4), is also considered as an alternative gripping point for housing the vials since they free the cartesian robot's grip during the reconstitution phase which, in turn, is rapid because it can mix up to six vials simultaneously.

Figure 3.4. Representation of vial clamp housing on the shakers.

The last housing station in the system is the one between the dosage and reconstitution area. This station can accommodate up to two vials by interlocking the holes in the clamp vertices with the station protrusions (Figure 3.5). This is the place where the anthropomorphic and cartesian robots interact to exchange vials.

Figure 3.5. Representation of vial clamp housing on station communicating both with anthropomorphic and cartesian robots.

3.1.3 Design evolution

The design of the vial clamp started in July 2019 with the definition of the first CAD model. After that, 3D printing was made to analyse the feasibility of the first prototype. The conducted analysis proved to be valid, therefore the thinking began on what material would be suitable for the device realisation. Initially, it was decided to use a homopolymer thanks to its rigid properties useful to properly fix the vial. Once the possible material was chosen, the product engineering phase in terms of moulding began. As already mentioned in paragraph 3.1.1, injection moulding was chosen because it is quick and economical. This last aspect is important since the device is a disposable and, thus, is useful to obtain an economic product for the customers. After produced several quantities of clamps, their validity was tested by applying to the vials. It was noted that clamp version two was able to correctly handle the small-neck vials, while version one was not suitable because when applied to the large-neck vial it broken. For this reason, it was decided to replace the homopolymer with the copolymer to provide more elastic properties.

Once the new device was produced and subsequently tested, the result was the opposite of the previous one. Indeed, version one proved to correctly handle large neck vials. On the other hand, version two did not manage the small-neck vials since the central part of the clamp did not adequately fix the vial and, when it was used during

the machine cycling, the vial was leaking due to the force generated by the spike insertion into the vial membrane. In addition, it also happened that the robot, both the anthropomorphic and cartesian ones, could not handle in terms of grip and release the full vial. Regarding the grip, the vial tilted the position of the clamp and, therefore, it resulted in the robot inability to grab the vial since the theoretical gripping position was different from the actual one. Concerning the release phase, it happened that while the robot was handling the clamp, the vials started to oscillate and, thus, failed the insertion in the various housing stations.

These results have led to the idea of mixing the homopolymer with the copolymer in order to ensure simultaneous rigid and flexible performance. A geometric modification of the flexible anchorage system was made before testing various blends on version one. Then, the first blend consisted of more homopolymer and less copolymer. When the device was produced and tested later, it was found that the previous problems had improved since version one did not break during application to large neck vials and version two fix small neck vials better. The decision to use a blend as a moulding material was promising, although not definitive since the vials were not yet perfectly fixed and, additionally, episodes of vial leakage during the dosing phase occurred. The latest idea, exactly two years after the design of the first device prototype (July 2021), has been to create a blend exactly opposite to the previous one: less homopolymer and more copolymer. The latter devices still need to be tested and validated, which is why the development of this thesis will also focus on this aspect.

3.1.4 Testing procedure

To test and validate the vial clamp, several materials were required. In detail, has been used a calliper, several samples of two clamp versions and the 2ml, 20ml, 80ml and 100ml drug vials (Figure 3.6). In addition, Table 3.1 shows the vials neck diameter used during the test and validation phase since they are useful information to write the subsequent procedures.

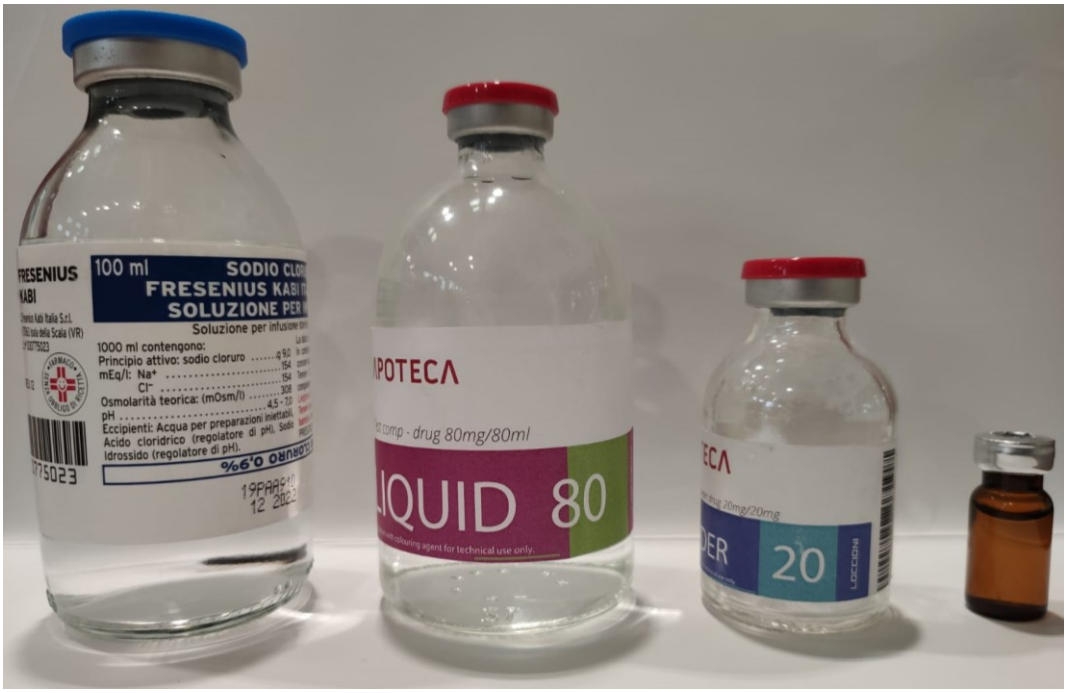


Figure 3.6. Vials used during the clamp testing procedures. Going from left to right there are the following vial formats: 100ml, 80ml, 20ml, 2ml.

Two procedures have been written: one test the clamp mechanical properties of the clamp to verify its possible breakage and the correct fixing of the vial, the second to evaluate the manipulation of the vial with clamp by the anthropomorphic and cartesian robots.

Table 3.1. Vials neck diameter used during the test and validation phase.

VIALS NECK DIAMETER			
Vial 100ml	Vial 80ml	Vial 20ml	Vial 2ml
25 mm	16 mm	17 mm	10 mm

In detail, the first testing procedure is the following:

1. Take four clamps of version one;
2. Check the geometry using the callipers to ensure that the device conforms to the CAD model;

3. Check the moulding quality and any imperfections caused by burrs or the air presence inside the material;
4. Check the eventual breakage of the device;
5. Check that the vial is properly fixed by applying manual pressure to the membrane;
6. Set the APOTECAbag system to manual configuration;
7. Open the dosage area door;
8. Insert 20ml drug vial with clamp into the dedicated housing of the dosing area;
9. Close the dosage area door;
10. Through the use of the software activate the pneumatic actuator allowing the mini-spike insertion of the tube "B Double Filling Line" in the vial membrane;
11. Evaluate the fixing of the vial;
12. Through the use of the software activate the pneumatic actuator allowing the mini-spike of the "B Double Filling Line" tube to exit from the vial membrane;
13. Evaluate the fixing of the vial;
14. Repeat from steps 10 to 13 for 15 times;
15. Open the dosage area door;
16. Remove the vial with a clamp from the dedicated housing;
17. Close the dosage area door;
18. Open the reconstitution area door;
19. Insert the vial with clamp into the dedicated housing of the balance in the reconstitution area;
20. Close the reconstitution area door;
21. Through the use of the software operate the pneumatic actuator allowing the mini-spike insertion of the tube "B Recon Line" in the vial membrane;
22. Evaluate the fixing of the vial;
23. Through the use of the software operate the pneumatic actuator allowing the mini-spike of the "B Double filling line" tube to exit from the vial membrane;
24. Evaluate the fixing of the vial;
25. Repeat from steps 20 to 24 for 15 times;

26. Open the reconstitution area door;
27. Remove the vial with a clamp from the dedicated housing of the balance;
28. Close the reconstitution area door;
29. Repeat from steps 7 to 28 two more times for the 80ml and 100ml vial;
30. Repeat from steps 1 to 29 using the clamp version two;
31. Repeat from steps 1 to 28 using clamp version two for small neck vials and version one for large neck vials;
32. Repeat from steps 1 to 31 five times using new clamps.

It should be underlined that the drugs vial were filled manually by using a syringe with 0.9% NaCl concentration. In addition, small-neck vials were considered to be the 2ml, 20ml, 80ml and large-neck vial the 100ml size. The reasons are related to the geometric dimensions of the neck visible from Table 3.1. As can be seen from the procedure, the 2ml vial is only used to manually assess the correct fixing of the vial. It is not tested within the APOTECAbag system because its membrane is smaller than the size of the mini-spike and, thus, it is not handled.

The second procedure involve:

1. Using the APOTECAmanager software production batches defined in Table 3.2;
2. Set the APOTECAbag system to automatic configuration;
3. Select production lot number one;
4. Application clamp version one;
5. Bag clamp application;
6. Load the required material using the loading wizard;
7. Once the required material has been loaded, assess the machine cyclic by carefully analysing the anthropomorphic and cartesian robot's handling of the vial with clamp and its insertion into the various dedicated housings;
8. Repeat from steps 2 to 7 for batch one e two;
9. Repeat from steps 2 to 8 for two more times. The first using clamp version two for all defined batches respectively, the second differentiating the use of clamp version two for batches 1,2 and version one for batch 3.

Table 3.2. Batches used during the testing phase generated by using APOTECAmanager.

Batch	Reconstitution	Final bag	Drug	Vial format	Number of vials	Drug dosage	Number of bags
1	Yes	100ml NaCl	Powder	20ml	3	6ml	9
2	Yes	100ml NaCl	Powder	80ml	3	20ml	9
3	Yes	100ml NaCl	Powder	100ml	3	35ml	9

It is important to underline that, for more safety of the operator, the vials containing powdered drugs have been simulated by using empty vials. The choice of the differentiated use of the clamp concerning the batch is strictly due to the geometric characteristics of the vial necks used.

3.2 Peristaltic pump

3.2.1 Peristaltic pump description

The APOTECAbag system has three peristaltic pumps: two in the dosage area and one in the reconstitution area. In detail, the two peristaltic pumps in the dosage area have the function of dosing the drug and the solvent in the final bag. As for the pump in the reconstitution area, its function is to dilute the powder drug. The use of the peristaltic pump introduces the usefulness of automatic dosing.

The pumps under analysis are "Watson - Marlow Pump" series 114. The dimensions of the device are 64mm x 64mm x 42mm for a total weight of 0.1kg [21]. The pump consists of a front cover at the top which can be raised and lowered. When it is raised, it is possible to notice the presence of four rollers that guarantee the peristaltic phenomenon. The rollers can rotate clockwise and anticlockwise depending on the direction of the required dosage flow.

The use of the peristaltic pump introduces the need to use tubes capable of ensuring the peristaltic phenomenon. The pump allows the tube to be positioned with adequate pressure inside the device preventing operator intervention for the adjustment of the relative position important to ensure high accuracy and repeatability of the dosage (Figure 3.7).

Figure 3.7. Peristaltic pump together with the tube.

By analysing the pump datasheet, the following specifications can be defined:

1. Recommended for continuous duty;
2. Four-roller pumphead;
3. Max speed: 400 rpm continuous and 600 rpm intermittent;
4. Up to 340ml/min continuous flow and up to 510ml/min intermittent flow;
5. Two tube-holder positions to accept tube in bore sizes from 0.5mm to 4.8mm;
6. Models with occlusion settings for standard or high-pressure operation;
7. Universal drive connection for shafts from 6mm diameter to 10mm diameter;
8. Operating temperature: -10° to 45°C.

Peristaltic pumps are self-priming and self-sealing against backflow. No valves are required in inlet or discharge lines. In detail, the peristaltic pump can avoid backflow due to the presence of sliders in the side parts of the device that allow the tube to be accommodated in the inlet and outlet part of the pump. In addition, the two sides are also used to ensure the correct fixing of the tube in order to avoid slipping during the peristaltic phenomenon. These two functions are ensured when the pump front cover is lowered until it completely touches the remaining part, inducing compression of the

tube by the sliders previously defined. It should be noted that tubes with different internal diameters can be used according to the required flow rate. The pump in question is capable of accommodating tubes with an internal diameter in the range of 0.5 mm to 4.8 mm. To ensure the functions of the pump side part, there are two sliders on both sides, as previously mentioned, whose height can be set on two different levels according to the diameter of the tube used. It is possible to define the "inner position" for small diameter tubes and the "outer position" for large diameter ones.

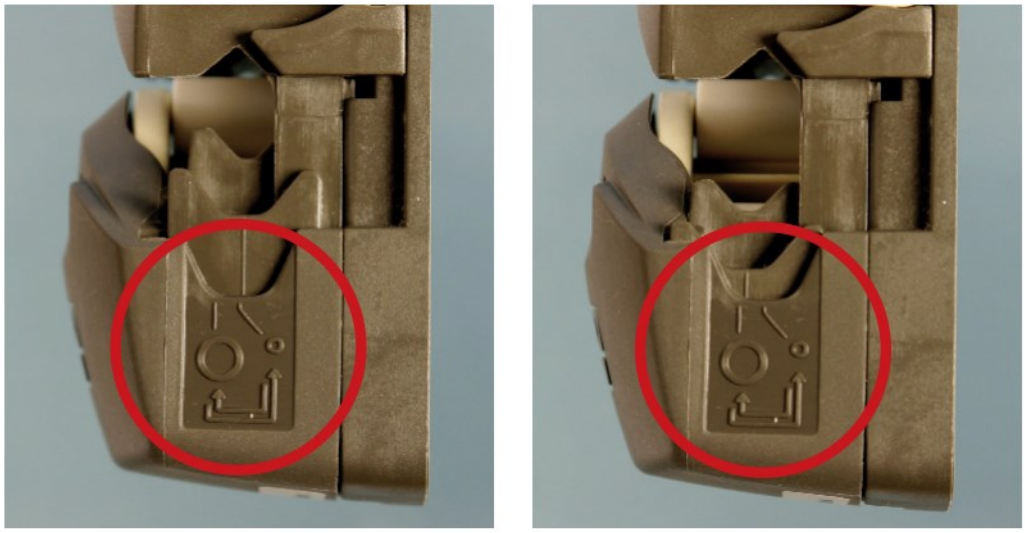


Figure 3.8. Representation of lateral sliders. On the left the 'inner position', on the right the 'outer position.

With the smaller bore tubes of 0.5mm, 0.8mm and 1.6mm the inner position must be used to prevent the risk of the tube slipping through the sliders and wandering across the rollers causing premature tube rupture. With the larger bore tubes of 4.0mm and 4.8mm the outer position must be used to prevent the flow rate from being excessively reduced. For tubing bores of 2.4mm and 3.2mm either setting may be used, as appropriate for the application. The inner setting will clamp the tube harder, reducing tube slip but has the potential to marginally reduce flow rate. The outer setting will optimise flow rate but the risk of tube slip is increased.

The main difference in using tubes with various internal diameters is the desired flow rate, as shown in Table 3.3, for precise and repeatable pump performance.

Table 3.3. Changes in flow rates according to the pump speed and tube bore size.

Flow rates,ml/min							
Tube bore size	0,5mm	0,8mm	1,6mm	2,4mm	3,2mm	4,0mm	4,8mm
ml/rev	0,02	0,04	0,14	0,29	0,47	0,67	0,85
30 rpm	0,7	1,3	4,2	8,7	14	20	25,5
60 rpm	1,4	2,6	8,4	17,5	28,5	40,5	51
100 rpm	2,2	4,3	14	29	47,5	67	85
190 rpm	4,3	8,2	26,5	55	90,5	128	160
200 rpm	1,6	8,6	28	58	95	135	170
350 rpm	8	15	49	100	165	235	300
400 rpm	9,1	17	56	115	190	270	340
600 rpm	13,5	26	84	175	285	405	510

As can be seen from the table, the flow rate, not only depends on the internal diameter of the tube used, but is also related to the speed of the pump. Indeed, the device can work with different levels of the rollers rotational speed: 30 rpm, 60 rpm, 100 rpm, 190 rpm, 200 rpm, 350 rpm, 400 rpm, 600 rpm.

It is important to mention how to insert the peristaltic tube. First is to be set the height level of the side sliders according to the diameter of the tube used and, thus, between inner position and outer position. Then open the upper front cover and place the tubing inside the pump: over the rollers and on the two side sliders. Once the tube is in place, lowering the front cover ensures that it is locked in the right position by compressing the inlet and outlet sides of the pump by the sliders. It is important to emphasise that to maintain a high degree of accuracy and repeatability of the dosage, the kinking of tube must be avoid because could cause a slight obstruction.

3.2.2 Peristaltic tube description

The use of the peristaltic pump introduces the need for tubes. In particular, the tubes required must comply with the position of the various components within the APOTECAbag system, but also depend on the location of the initial and final

container. The tubes used by the APOTECAbag system are called "B Dispensing Line". They consist of single-use tubes to be inserted in peristaltic pumps for transferring solvent and drug with the APOTECAbag automated compounding systems from an initial container to a final one. The initial and final container changes according to the variant considered: vial or bag. In detail, the liquids of the initial container are sucked and then enter into the tubing system. The liquids inside the device are forced to pass through the tubing system from the inlet to the outlet under the action of a peristaltic pump. Finally, the automated system provides a gravimetric control after the injection of the liquids in the final container, in order to verify the accuracy of the dosage. B Dispensing Line is intended to work only with non-toxic drugs. In addition to its role within APOTECAbag, it can be used in combination with other medical devices, for the treatment or alleviation of disease. For this reason, according to the definition provided in Directive 93/42/EEC [23] and Medical Device Regulation (MDR) [24], B Dispensing Line is qualified as a medical device. Furthermore, the device is considered a non-invasive and non-active medical device and it is classified as class I in compliance with rule 2. Moreover, class I medical devices can be further divided into different categories:

- Is, for class I device delivered sterile;
- Im, for class I device with measurement function;
- I, for all the other class I devices.

B Dispensing Line, according to the classification rules of Annex IX of Directive 93/42/EEC and the class I categories reported above, is a class Is medical device.

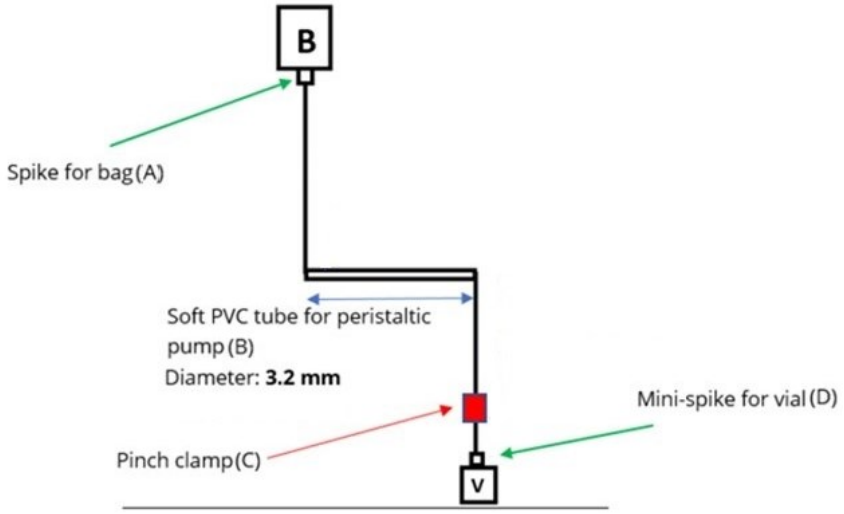
For this reason, several tests have been conducted to assess the product biocompatibility to avoid any risk related to its use. To avoid unnecessary tests and increasing awareness about the possible risks correlated to the medical device, has been defined a biological evaluation plan according to the Standard EN ISO 10993-1 [25] and the FDA Guidance "Use of International Standard ISO 10993-1", in order to plan biocompatibility testing within a risk management process. In addition, the tube is manufactured with biocompatible materials, DEHP PVC, and it is sterilized by

ethylene oxide (EO) sterilization method and then packed. B Dispensing line packaging consists of a blister with a plastic film on one side and medical paper on the other side, which is EO sterilization compatible. Subsequently, have been done also tests to evaluate transparency, tensile strength and leakage, which reported positive results.

The tubes used inside the robotic system are of two different versions: "B Recon Line" and "B Double Filling Line".

B Recon Line (Figure 3.9) is intended to transfer solvent from a bag to a vial in an automated way by means of a peristaltic pump. Therefore, the tube is located within the reconstitution area since the solvent is sucked from the bag and dispensed into the vial containing the powdered drug. This tube consists of a single channel and its main components are:

A. Spike for the connection of the device to the medication port of the solvent IV bag;
B. Section of the tube to be inserted in the peristaltic pump;
C. Pinch clamp.
D. Mini-spike for the connection of the device to a vial;



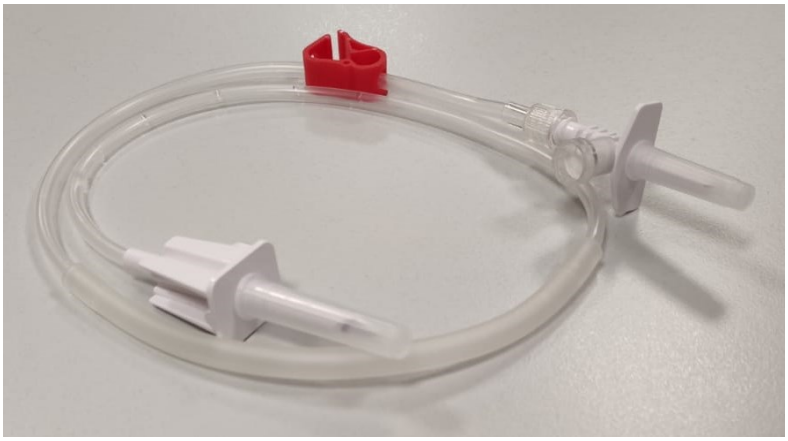


Figure 3.9. B Recon Line. In the upper panel a conceptual design, in the lower one a real representation.

As shown in the figure, the internal diameter is 3.2 mm, hence the side sliders of the peristaltic pump have been set to the inner position. A small internal diameter has been chosen since a high flow rate is not required to fill a vial. In addition, a spike and a mini-spike are present in the initial and terminal parts respectively. The functions of the latter tube components are important to suck and dispense solvent.

As for regard, the B Double Filling Line (Figure 3.10) is intended to transfer solvent from a bag and drug from a vial to the final bag in an automated way employing a peristaltic pump. This means that this tube is present in the dosage area. It has a Y-shape conformation. Indeed, in the upper part, drug and solvent flow separately in two different channels confluence in the same lower channel. Its main components are:

A. Spike for the connection of the device to the medication port of the solvent bag;
B. Section of the tube to be inserted in the peristaltic pump;
C. Mini-spike for the connection of the device to a vial;
D. Section of tube to be inserted in the peristaltic pump;
E. Two Pinch clamps, one for each branch;
F. Y-connection with the two branches;
G. Needle for the connection of the device to the bag.

As shown in the figure, the internal diameter of the tube is 3.2 mm for the branch that doses the drug and 4.8 mm for the one that doses the solvent.

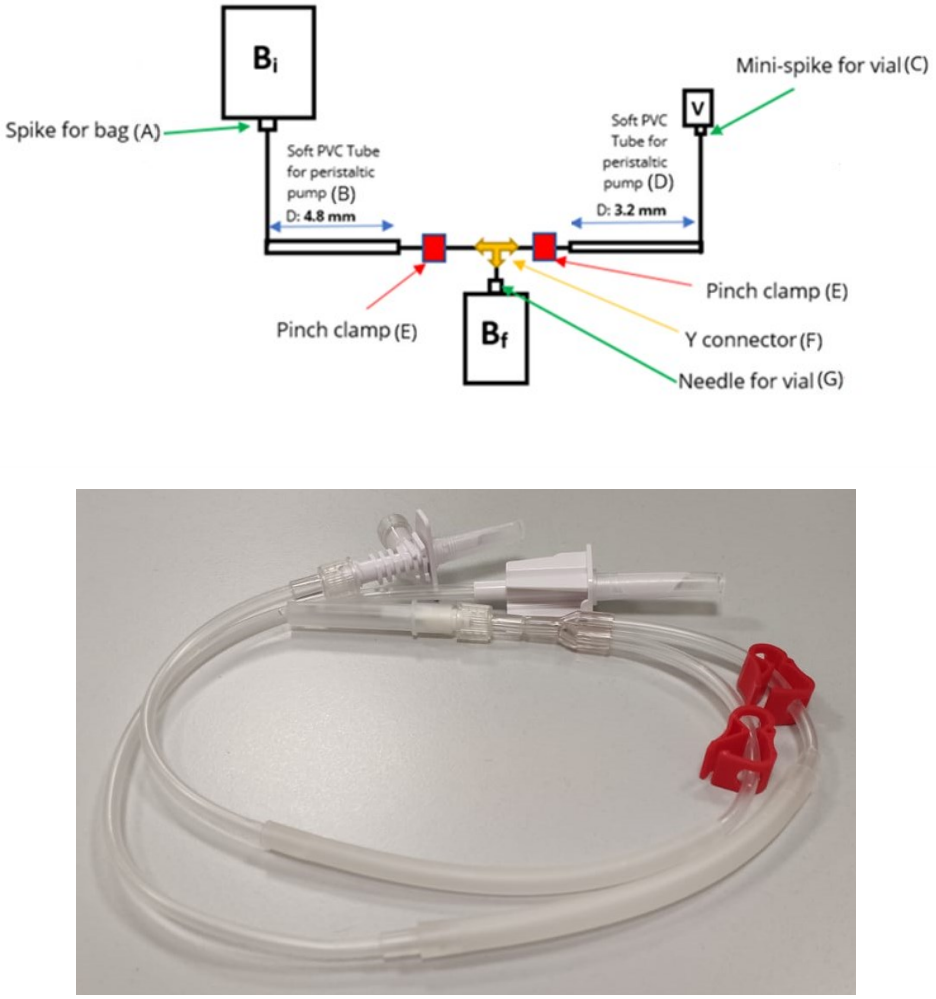


Figure 3.10. B Double Filling Line. In the upper panel a conceptual design, in the lower one a real representation.

This choice was made because the tube with a smaller diameter must dose the drug and, therefore, a high flow rate is not required, while for the other branch a large internal diameter was chosen because a high flow rate is required to eventually fill the entire final bag with solvent. Due to the different diameters, the side sliders of the peristaltic pump have been set to inner position and outer position for the pump that doses the drug and the one that doses the solvent, respectively. In addition, a mini-spike and a spike are present at both beginning of the two branches, for the vial and the solvent bag respectively. The functions of the latter tube components are important to suck and dispense solvent and drug through a needle in the final bag.

It is important to note that for both described models used in the APOTECAbag system, the portion of the tube inserted inside the pump will be softer and larger than the portion that will be outside.

The loading operation of the tubes inside the peristaltic pumps is performed by the operator. For this reason, it is necessary to define some precautions:

1. Before the use, check that the sterile package is unbroken and the validity of the product;
2. Open the package containing the single product in a sterile environment;
3. Make sure that the device is not damaged in any way;
4. Remove the protective caps and check that the pinch clamp is open, insert the spike into the solvent bag medication port and then insert the connector into the appropriate housing in the reconstitution area or the two connectors in the appropriate ones in the dosage area. Open the peristaltic pump, pulling the mobile section of the device upwards, and insert the peristaltic section of the device on the rotors. Then close the pump. After the positioning inside the system of the containers to which it is connected, the device is ready for use by APOTECAbag;
5. Be sure that the air filter of the spike is open, if it is closed there may be a blockage in the transfer of Liquid;
6. A priming cycle is not necessary before the use of the device because it is automatically performed by a robotic system;
7. Once the work cycle of the system is completed, close the pinch clamp, open the peristaltic pump and remove the peristaltic section of the tube. Open the housing where the connector or connectors is/are placed and remove the device connected to the empty bag.

It is important to underline that each tube is intended to be used only with one active principle. This means that when is started a batch with a different drug than the one used in the previous batch, the APOTECAbag system software will ask the user to change the tube in order to avoid cross microbiological contamination.

3.2.3 Impact in the automated system

The use of peristaltic pumps within the APOTECAbag system is an innovation compared to the previous APOTECA platform solutions. In fact, in APOTECAchemo, APOTECAunit and APOTECAped the dosage is performed by using a syringe which, once housed on an automatic mobile system, is able to suck the drug from the vial kept in the correct position by the anthropomorphic robot. The process described involves the robot during the entire dosing phase and, therefore, there is an increase in the compounding cycle time. An analysis of the design specifications showed that an automatic system capable of producing 100 bags per hour has to be designed. For this reason, the dosing mechanism of the previous solutions are not suitable.

After these considerations, it is possible to highlight the impact of the use of peristaltic pumps within the automated system. In fact, its use allows automated dosing to be performed more rapidly for different reasons. The first dealing with the rotational speed of the rollers inside the pump. Indeed, it is possible to set different speeds in order to change the flow rate. The second reason is that there is no need to use a syringe to perform the dosing task, thus reducing the machine cycle time addressed to the loading phase. The corresponding function of the syringe is performed by the peristaltic tubes that are equipped with spikes capable of suck and deliver the fluid from an initial container to a final one through the peristaltic phenomenon guaranteed by the presence of the pump. Another reason can be associated with the use of the vial clamp because it frees the anthropomorphic and cartesian robot from the constant grip of the vial for the entire dosing phase since the vial with clamp can be placed in special housings that interact with the dosing mechanism made up of pump and peristaltic tubes. All this results in a reduction of the machine cycle time since during the dosing phase the robots can simultaneously execute other tasks. In addition, it should be emphasised that the dosing time is significantly reduced, especially for the dosage area, because the housed vial can be used for dosing several final products. Everything is faster because the mini-spike, positioned in a dedicated housing, is upward moved by the use and the activation of

a pneumatic actuator. This means that once the spike penetrates the vial membrane, it is possible to dose for different bags by an intermittent rotating action of the rollers inside the peristaltic pump without activating its downward motion.

In the APOTECAbag system, before starting the preparation of a bag batch, the software interface asks to replace the peristaltic tube when the scheduled preparation employ a different active ingredient from the previous batch. All this is programmed to avoid microbiological cross-contamination that prevents a sterile final product. This implies a frequent interaction of the operator with the automated system due to tube changes through the double door present both in the dosing and reconstitution area. One might think that such interaction causes risks on the final product sterility but, in reality, there is no contamination risk thanks to the function of both the double loading door and the inside positive pressure environment compared to outside. In addition, when the preparation batch involves the same active ingredient as the previous batches, no tube replacement is required and theoretically could be used over and over again. Thus, the pump validation will also focus on the characterization of the tube replacement in function of time of use and non-use.

3.2.4 Testing procedure

The validation of the peristaltic pump aims to define the calibration factor for each pump in the APOTECAbag system. The parameter describes the relationship between the number of revolutions made by the pump and the corresponding volume dosed. In addition, the pump validation will also focus on the characterization of the peristaltic tubes. By testing the peristaltic pump, it will be possible to analyse the behaviour of the peristaltic tube in accordance with the time of use and/or non-use in order to define after how long it should be replaced to ensure accurate dosing.

To validate both the pump and the peristaltic tubes it is necessary as a first step to test them during their use in order to acquire experimental data that will be subsequently processed and analysed. For this reason, detailed testing procedures have been defined.

To define the testing procedure, as a first step the requirements of three hospitals have been analysed: Johns Hopkins, Cleveland Clinic and Ospedale Riuniti di Ancona. In detail, the production of their bag with standard dosage through the use of non-toxic drugs has been analysed. The aspects that have been mainly considered are the batches average production of standard dosage bags, the typical drug dosage values, the solvent dosage quantity for dilution and, finally, the dosage of solvent in the final bag. The analysis led to an average bag production at a standard dosage of 200 units in the three hospitals defined above. Instead, the dosages typically used for each step of bags compounding which were subsequently used in testing procedures are reported in Table 3.4.

Table 3.4. Dosages defined at the end of the hospital requirements analysis.

Reconstitution Area	**Dosage Area**	
Pump 1	Pump 2	Pump 3
10ml	3ml	30ml
30ml	10ml	50ml
50ml	15ml	70ml
70ml	20ml	100ml
100ml	35ml	/

Where:

- Pump 1 is the one present in the reconstitution area and it is associated with the solvent dosing for the dilution of the powdered drug;
- Pump 2 is the one present in the dosage area and it is associated with the drug dosing in the final bag;
- Pump 3 is the one present in the dosage area and it is associated with the solvent dosing in the final bag.

Having available this information, the definition of the testing procedure has been accomplished. Several materials have been used to perform the different tests: 10 vial clamps version one, 10 vials of 80ml, 10 bags of 250ml, bag clamps, syringe, solvent.

Before starting the test procedures, the calibration of the scales in the dosing and reconstitution area respectively has been verified by placing a calibration weight equal to 200 g on the scale by means of a plate and then its measured weight was checked (Figure 3.11).

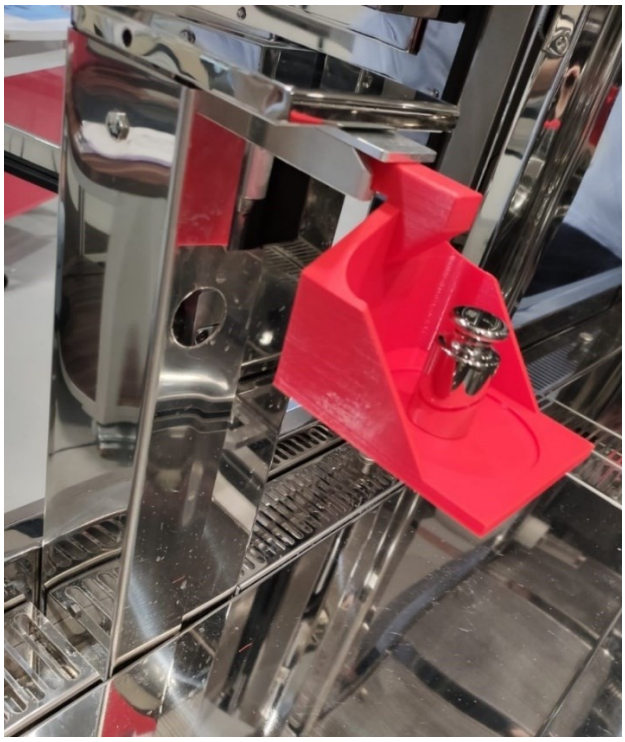


Figure 3.11. Balance calibration check.

Once verified the correct calibration of each balance, three testing procedures for each pump have been defined. It is important to underline that for each procedure 0.9% NaCl solvent was used as the fluid.

A preliminary testing procedure has been also defined for each pump in order to find the initial calibration factor that will be used later in the testing procedure associated with each pump.

The preliminary testing procedure for pump 1 is as follows:

1. Set the APOTECAbag to manual operation;
2. Activate the ventilation system to exactly reproduce the working environment of the automated system;

3. Make sure that the rotational speed of the peristaltic pump is set to 200 rpm, otherwise change it;
4. Open the package containing the B Recon Line peristaltic tube and insert it correctly into pump 1 via the opening of the small loading door in the reconstitution area. If a tube is already present substitute it making sure to close the pinch clamp to prevent solvent spillage;
5. Remove the protective caps from both the spike and mini-spike;
6. Insert correctly the spike in the solvent bag and the mini-spike in the specific dedicated housing able to move vertically by the activation of pneumatic actuator;
7. Make sure that the pinch clamp of the peristaltic tube is open;
8. Be sure to use 10 x 80ml vials with intact membrane, otherwise change the membrane using suitable forceps;
9. Apply version two of the clamp to all vials;
10. Ensure that the solvent bag is appropriately filled, otherwise fill it manually using the syringe by opening the small door of the reconstitution area. Then close it;
11. Ensure that the 80ml vial to be used is empty, otherwise empty it using the syringe;
12. Open the reconstitution area door to properly position the vial with clamp in the housing in the scale;
13. Close the reconstitution area door;
14. Using the software interface on the APOTECAbag system monitor, set the initial calibration factor for the pump;
15. Lower the scale via the software interface;
16. Reset the weight measured by the scale to zero;
17. Set 5ml as the amount of solvent to be dispensed and then activate dispensing;
18. Once dispensing is complete, operate the pneumatic actuator in order to raise the mini-spike;
19. Lower the scale to weigh;

20. Store the measured weight information in grams (g) on an Excel spreadsheet and simultaneously calculate the percentage error to define how much the actual measurement deviates from the ideal measurement;
21. If the vial is empty to the point of containing more of the required amount of dosage leave the vial on the balance, otherwise replace it with an empty vial;
22. Make sure that the bag is filled with solvent, otherwise refill it manually using the syringe;
23. Repeat from steps 12 to 22 three times;
24. Repeat steps 12 to 23 three times set for each repeat in step 17 a dispensing volume of 10ml, 15ml and 20ml;
25. Repeat from step 12 to 24 several times changing the calibration factor in step 14 until seems to achieve a possible factor associated with a minimum error.

The testing procedure for pump 1 is as follows:

1. Set the APOTECAbag to manual operation;
2. Activate the ventilation system to exactly reproduce the working environment of the automated system;
3. Change the calibration factor of pump 1 to the optimal one found in the preliminary testing procedure;
4. Make sure that the rotational speed of the peristaltic pump is set to 200 rpm, otherwise change it;
5. Open the package containing the new B Recon Line peristaltic tubing and insert it correctly into pump 1 via the opening of the small loading door in the reconstitution area. If a tube is already present substitute it making sure to close the pinch clamp to prevent solvent spillage;
6. Remove the protective caps from both the spike and mini-spike;
7. Insert correctly the spike in the solvent bag and the mini-spike in the specific dedicated housing able to move vertically by the activation of pneumatic actuator;
8. Make sure that the pinch clamp of the peristaltic tube is open;

9. Be sure to use 10 x 80ml vials with intact membrane, otherwise change the membrane using suitable forceps;
10. Apply version two of the clamp to all vials;
11. Ensure that the solvent bag is appropriately filled, otherwise fill it manually using the syringe;
12. 11.Ensure that the 80ml vial to be used is empty, otherwise empty it using the syringe;
13. 12.Open the reconstitution area door to properly position the vial with clamp in the housing in the scale;
14. 13.Close the reconstitution area door;
15. 14.Using the software interface on the APOTECAbag system monitor, lower the scale;
16. 15.Reset the weight measured by the scale to zero;
17. 16.Lift the scale via the software interface;
18. 17.Through the software interface activate the pneumatic actuator to lower the mini-spike in order to perforate the vial membrane;
19. 18.Using the software interface, set 10ml as the amount of solvent to be dispensed then activate dispensing;
20. 19.Once dispensing is complete, activate the pneumatic actuator in order to raise the mini-spike;
21. 20.Lower the scale to weigh;
22. 21.Store on an Excel sheet the information of the weight measured in grams (g);
23. 22.If the vial is empty to the point of containing more of the required amount of dosage leave the vial on the balance, otherwise replace it with an empty vial following steps 13-14;
24. .Make sure that the bag is filled with solvent, otherwise refill it manually using the syringe;
25. 24.Repeat from steps 16 to 24 for 200 times;

26. 25.Repeat from steps 4 to 25 for four more times setting 30ml, 50ml, 70ml and 100ml respectively as the desired dispensing volume in step 19 for each time. In addition, use new clamps for each test.

Moving on to the dosing area, it is possible to define two more procedures, with the associated preliminary testing procedure for choosing the initial calibration factor for pumps 2 and 3 respectively.

The preliminary testing procedure for pump 2 is:

1. Set the APOTECAbag to manual operation;
2. Activate the ventilation system to exactly reproduce the working environment of the automated system;
3. Make sure that the rotational speed of the peristaltic pump is set to 200 rpm, otherwise change it;
4. Open the package containing the peristaltic tubing B Double Filling Line and insert it correctly into pump 1 and pump 2 via the opening of the small door in the dosage area for loading. If a tube is already present remove it making sure to close the pinch clamps on the two branches to prevent solvent leakage;
5. Remove the protective caps from the spike, the mini-spike and the needle;
6. Insert correctly the spike in the solvent bag, the mini-spike and the needle in the specific dedicated housings respectively in a position proximal to the vial and to the scale on which the final bag will be placed. These housing stations can move by the activation of a pneumatic actuator;
7. Make sure that the pinch clamps on both branches of the peristaltic tube are open;
8. Be sure to use 10 x 80ml vials with intact membrane, otherwise change the membrane using suitable forceps;
9. Apply version two of the clamp to all vials;
10. Ensure that the 80ml vial to be used is empty, otherwise empty it using the syringe;
11. Make sure to use a 250ml capacity bag as the final product and check that it is empty, otherwise empty it manually using the syringe;

12. Apply the bag clamp;
13. Open the door of the dosage area to properly position the vial with clamp in the dedicated housing and the bag with clamp in the location in the scale;
14. Close the dosage area door;
15. Using the software interface on the APOTECAbag system monitor, set an initial calibration factor for pump 2;
16. Lower the scale via the software interface;
17. Reset the weight measured by the scale to zero;
18. Lift the scale via the software interface;
19. Using the software interface, activate the pneumatic actuator for lifting the mini-spike in order to perforate the vial membrane and the other for lowering the needle that will perforate the spongy part of the bag intended for needle insertion;
20. Using the software interface, set 5ml as the amount of drug to be dispensed then activate dispensing;
21. When dispensing is complete, operate the pneumatic actuator to raise the needle;
22. Lower the scale to weigh;
23. Store the measured weight information in grams (g) on an Excel spreadsheet and simultaneously calculate the percentage error that defines how much the actual measurement deviates from the ideal measurement;
24. If the vial is full to the point of dispensing another quantity of the required dosage, leave the vial on the appropriate housing, otherwise activate the pneumatic actuator to lower the mini-spike and then replace it with a full one by opening the door of the dosage area. Then close the door and activate the pneumatic actuator to raise the mini-spike;
25. If the bag is empty to the point of containing more of the required amount of dosing, leave the bag on the scale, otherwise replace it with an empty one by opening the door of the dosage area. Then close the door;
26. Repeat from steps 12 to 24 three times;

27. Repeat steps 12 to 25 three times, setting a dispensing volume of 10ml, 15ml and 20ml for each repeat in step 17;
28. Repeat from step 12 to 26 several times changing the calibration factor in step 15 until seems to achieve a possible factor associated with a minimum error.

Once performed and stored the information coming from the above procedure, it is possible to proceed with the following testing procedure for pump 2:

1. Set the APOTECAbag to manual operation;
2. Activate the ventilation system to exactly reproduce the working environment of the automated system;
3. Change the calibration factor for pump 2 to the optimum one found in the preliminary testing procedure;
4. Make sure that the rotational speed of the peristaltic pump is set to 200 rpm, otherwise change it;
5. Open the packet containing the new B Double Filling Line peristaltic tubing and insert it correctly into pump 2 and pump 3 through the opening of the small door in the dosage area dedicated to loading. If a tube is already present remove it making sure to close the pinch clamps on the two branches to prevent solvent leakage;
6. Remove the protective caps from the spike, the mini-spike and the needle;
7. Insert correctly the spike in the solvent bag, the mini-spike and the needle in the specific dedicated housings respectively in a position proximal to the vial and to the scale on which the final bag will be placed. These housing stations can move by the activation of a pneumatic actuator;
8. Make sure that the pinch clamps on both branches of the peristaltic tube are open;
9. Be sure to use 10 x 10ml vials with intact membrane, otherwise change the membrane using suitable forceps;
10. Apply version due of the clamp to all vials;
11. Ensure that the 80ml vial to be used is empty, otherwise empty it using the syringe;

12. Make sure to use a 250ml capacity bag as the final product and check that it is empty, otherwise empty it manually using the syringe;
13. Apply the bag clamp;
14. Open the door of the dosage area to properly place the vial with clamp in the dedicated slot and the bag with clamp in the location on the scale;
15. Close the dosage area door;
16. Using the software interface on the APOTECAbag system monitor, lower the scale;
17. Reset the weight measured by the scale to zero;
18. Lift the scale via the software interface;
19. Using the software interface, activate the pneumatic actuator for lifting the mini-spike in order to perforate the vial membrane and the other for lowering the needle that will perforate the spongy part of the bag intended for needle insertion;
20. Using the software interface, define 3ml as the desired drug delivery quantity for pump 2 and then activate the delivery;
21. Once dispensing is complete, operate the pneumatic actuator to lift the needle from the final bag;
22. Lower the scale to weigh;
23. Store the information of the measured weight in grams (g);
24. If the vial is full to the point of dispensing another quantity of the required dosage leave the vial on the appropriate housing, otherwise activate the pneumatic actuator to lower the spike and then replace it with a full one by opening the door of the dosage area. Then close the door and activate the pneumatic actuator to raise the mini-spike;
25. If the final bag is empty to the point of containing more of the required amount of dosing, leave the bag on the scale, otherwise replace it with an empty bag with a clamp by opening the door of the dosage area. Then close the door;
26. Repeat from steps 16 to 25 for 200 times;

27. Repeat from steps 4 to 26 four more times setting 10ml, 15ml, 20ml and 35ml as the desired dispensing volume in step 20 respectively. In addition, use new clamps for each test.

The following steps can be defined for the preliminary procedure of pump 3:

1. Set the APOTECAbag to manual operation;
2. Activate the ventilation system to exactly reproduce the working environment of the automated system;
3. Make sure that the rotational speed of the peristaltic pump is set to 200 rpm, otherwise change it;
4. Open the package containing the peristaltic tubing B Double Filling Line and insert it correctly into pump 1 and pump 2 via the opening of the small door in the dosage area for loading. If a tube is already present remove it making sure to close the pinch clamps on the two branches to prevent solvent leakage;
5. Remove the protective caps from the spike, the mini-spike and the needle;
6. Insert correctly the spike in the solvent bag, the mini-spike and the needle in the specific dedicated housings respectively in a position proximal to the vial and to the scale on which the final bag will be placed. These housing stations can move by the activation of a pneumatic actuator;
7. Make sure that the pinch clamps on both branches of the peristaltic tube are open;
8. Be sure to use 10 x 80ml vials with intact membrane, otherwise change the membrane using suitable forceps;
9. Apply version two of the clamp to all vials;
10. Ensure that the 80ml vial to be used is empty, otherwise empty it using the syringe;
11. Make sure to use a 250ml capacity bag as the final product and check that it is empty, otherwise empty it manually using the syringe;
12. Apply the bag clamp;
13. Open the door of the dosage area to properly place the vial with clamp in the dedicated housing and the bag with clamp in the location in the scale;

14. Close the dosing area door;
15. Using the software interface on the APOTECAbag system monitor, choose an initial calibration factor for pump 3;
16. Lower the scale via the software interface;
17. Reset the weight measured by the scale to zero;
18. Lift the scale via the software interface;
19. Using the software interface, activate the pneumatic actuator for lifting the mini-spike in order to perforate the vial membrane and the other for lowering the needle that will perforate the spongy part of the bag intended for needle insertion;
20. Set for pump 3 as the amount of drug to be dispensed 5ml and then operate the dispensing;
21. When dispensing is complete, operate the pneumatic actuator to raise the needle;
22. Lower the scale to weigh;
23. Store the measured weight information in grams (g) on an Excel spreadsheet and simultaneously calculate the percentage error that defines how much the actual measurement deviates from the ideal measurement;
24. Ensure the solvent is present in the solvent bag, otherwise refill it manually using the syringe by opening the small door of the dosage area. Then close the door;
25. If the final bag turns out to be empty to the point of containing more of the required amount of dosing leave the bag on the scale, otherwise replace it with an empty one by opening the door of the dosage area. Then close the door;
26. Repeat from steps 12 to 24 three times;
27. Repeat from step 12 to 25 three times, setting a dispensing volume of 10ml, 15ml and 20ml for each repeat in step 17;
28. Repeat from steps 12 to 26 several times changing the calibration factor in step 15 until seems to achieve a possible factor associated with a minimum error.

Once you have performed the preliminary procedure for pump 3, you can move on to the following testing procedure:

1. Set the APOTECAbag to manual operation;
2. Activate the ventilation system to exactly reproduce the working environment of the automated system;
3. Change the calibration factor of pump 3 to the optimal one found in the preliminary testing procedure;
4. Make sure that the rotational speed of the peristaltic pump is set to 200 rpm, otherwise change it;
5. Open the package containing the new B Double Filling Line peristaltic tubing and insert it correctly into pump 2 and pump 3 through the opening of the small door in the dosage area dedicated to loading. If a tube is already present remove it making sure to close the pinch clamps on the two branches to prevent solvent leakage;
6. Remove the protective caps from the spike, the mini-spike and the needle;
7. Insert correctly the spike in the solvent bag, the mini-spike and the needle in the specific dedicated housings respectively in a position proximal to the vial and to the scale on which the final bag will be placed. These housing stations can move by the activation of a pneumatic actuator;
8. Make sure that the pinch clamps on both branches of the peristaltic tubing are open;
9. Be sure to use 10 x 80ml vials with intact membrane, otherwise change the membrane using suitable forceps;
10. Apply version two of the clamp to all vials
11. Ensure that the 80ml vial to be used is empty, otherwise empty it using the syringe;
12. Make sure to use a 250ml capacity bag as the final product and check that it is empty, otherwise empty it manually using the syringe;
13. Apply the bag clamp;
14. Open the door of the dosage area to properly place the vial with clamp in the dedicated housing and the bag with clamp in the location in the scale;
15. Close the dosage area door;

16. Using the software interface on the APOTECAbag system monitor, lower the scale;
17. Reset the weight measured by the scale to zero;
18. Lift the scale via the software interface;
19. By means of the software interface, activate the pneumatic actuator for lifting the mini-spike in order to perforate the vial membrane and the other for lowering the needle that will perforate the spongy part of the bag intended for needle insertion;
20. Using the software interface, define 3ml as the desired drug delivery quantity for pump 3 and then activate the delivery;
21. Once dispensing is complete, operate the pneumatic actuator to lift the needle from the final bag;
22. Lower the scale to weigh;
23. Store the information of the measured weight in grams (g);
24. Ensure the solvent is present in the solvent bag, otherwise refill it manually using the syringe by opening the small door of the dispensing area. Then close the door;
25. If the final bag is empty to the point of containing more of the required amount of dosing leave the bag on the scale, otherwise replace it with an empty bag with a clamp by opening the door of the dosing area. Then close the door;
26. Repeat from steps 16 to 25 for 200 times;
27. Repeat from steps 4 to 26 three more times setting 30ml, 50ml, 70ml and 100ml as the desired dispensing volume in step 20 respectively. In addition, use new clamps for each test.

So far, testing procedures have been defined to acquire experimental data which will be subsequently processed and analysed in order to define the calibration factor for each pump. After that, the estimated factors will be verified experimentally with APOTECAbag through other procedures.

The verification procedure for pump 1 is the following:

1. Set the APOTECAbag to manual operation;

2. Activate the ventilation system to exactly reproduce the working environment of the automated system;
3. Change the calibration factor for pump 1 to the optimum factor found at the end of the data analysis;
4. Make sure that the rotational speed of the peristaltic pump is set to 200 rpm, otherwise change it;
5. Open the package containing the new B Recon Line peristaltic tubing and insert it correctly into pump 1 via the opening of the small loading door in the reconstitution area. If a tube is already present remove the tube making sure to close the pinch clamp to prevent solvent spillage;
6. Remove the protective caps from both the spike and mini-spike;
7. Insert correctly the spike in the solvent bag and the mini-spike in the specific dedicated housing capable of moving vertically through the activation of a pneumatic actuator;
8. Make sure that the pinch clamp of the peristaltic tube is open;
9. Be sure to use 10 x 80ml vials with intact membrane, otherwise change the membrane using suitable forceps;
10. Apply version two of the clamp to all vials, if already present remove it and replace it with a new one;
11. Ensure that the solvent bag is appropriately filled, if necessary, fill it manually using the syringe;
12. Ensure that the 80ml vial is empty, otherwise empty it using the syringe;
13. Open the reconstitution area door to properly position the vial with clamp in the housing in the scale;
14. Close the reconstitution area door;
15. Using the software interface on the APOTECAbag system monitor, lower the scale;
16. Reset the weight measured by the scale to zero;
17. Lift the scale via the software interface;

18. Through the software interface activate the pneumatic actuator to lower the mini-spike in order to perforate the vial membrane;
19. Using the software interface, define 10ml as the desired solvent delivery quantity for pump 1 and then switch on the delivery;
20. Once dispensing is complete, operate the pneumatic actuator in order to raise the mini-spike;
21. Lower the scale to weigh;
22. Store on an Excel sheet the information of the weight measured in grams (g);
23. If the vial is empty to the point of containing more of the required amount of dosage leave the vial on the balance, otherwise replace it with an empty vial following steps 13-14;
24. Make sure that the bag is filled with solvent, otherwise refill it manually using the syringe;
25. Repeat from step 16 to 24 10 times;
26. Repeat from step 4 to 25 for four more times setting 30ml, 50ml, 70ml and 100ml respectively as the desired dispensing volume in step 19 for each time;
27. Repeat from step 3:26 for a number of times equivalent to the number of calibration factors found by analysis of the experimental data. For each repetition, set the estimated factor in step 3 as the factor and for each time the procedure is repeated, use a new B Recon Line tube.

The verification procedure for pump 2 is:

1. Set the APOTECAbag to manual operation;
2. Activate the ventilation system to exactly reproduce the working environment of the automated system;
3. Change the calibration factor for pump 2 to the optimum factor found at the end of the data analysis;
4. Make sure that the rotational speed of the peristaltic pump is set to 200 rpm, otherwise change it;

5. Open the package containing the new B Double Filling Line peristaltic tubing and insert it correctly into pump 2 and pump 3 through the opening of the small door in the dosage area dedicated to loading. If a tube is already present remove it making sure to close the pinch clamps on the two branches to prevent solvent leakage;
6. Remove the protective caps from both the spike, mini-spike and the needle;
7. Insert correctly the spike in the solvent bag, the mini-spike and the needle in the specific dedicated housings respectively in a position proximal to the vial and to the scale on which the final bag will be placed. These housing stations can move by the activation of pneumatic actuator;
8. Make sure that the pinch clamps on both branches of the peristaltic tube are open;
9. Be sure to use 10 x 80ml vials with intact membrane, otherwise change the membrane using suitable forceps;
10. Apply version two of the clamp to all vials, if already present remove it and replace it with the new one;
11. Make sure that the 80ml vial is full, otherwise fill it using the syringe;
12. Be sure to use a 250ml capacity bag as the final product bag and check that it is empty, otherwise empty it manually using the syringe;
13. Apply the bag clamp;
14. Open the door of the dosage area to properly place the vial with clamp in the dedicated housing and the bag with clamp in the location in the scale;
15. Close the dosage area door;
16. Using the software interface on the APOTECAbag system monitor, lower the scale;
17. Reset the weight measured by the scale to zero;
18. Lift the scale via the software interface;
19. By means of the software interface, activate the pneumatic actuator for lifting the mini-spike in order to perforate the vial membrane and the other for lowering the needle that will perforate the spongy part of the bag intended for needle insertion;

20. Using the software interface, define 3ml as the desired drug delivery quantity for pump 2 and then activate the delivery;
21. Once dispensing is complete, operate the pneumatic actuator to lift the needle from the final bag;
22. Lower the scale to weigh;
23. Store the information of the measured weight in grams (g);
24. If the vial is full to the point of containing another quantity of the required dosage leave the vial on the appropriate housing, otherwise activate the pneumatic actuator to lower the spike and then replace it with a full one by opening the door of the dosage area. Then close the door and activate the pneumatic actuator to raise the mini-spike;
25. If the final bag is empty to the point of holding more of the required amount of dosing, leave the bag on the scale, otherwise replace it with an empty bag with a clamp by opening the door of the dosage area. Then close the door;
26. Repeat from step 16 to 25 10 times;
27. Repeat from step 4 to 26 four more times setting 10ml, 15ml, 20ml and 35ml as the desired dispensing volume in step 20 respectively;
28. Repeat from step 3:27 for a number of times equivalent to the number of calibration factors found by analysis of the experimental data. For each repetition, set the estimated factor in step 3 as the factor and use a new Double Filling Line B-tube each time the procedure is repeated.

The last verification procedure is for pump 3:

1. Set the APOTECAbag to manual operation;
2. Activate the ventilation system to exactly reproduce the working environment of the automated system;
3. Change the calibration factor for pump 3 to the optimum factor found at the end of the data analysis;
4. Make sure that the rotational speed of the peristaltic pump is set to 200 rpm, otherwise change it;

5. Open the package containing the new B Double Filling Line peristaltic tubing and insert it correctly into pump 2 and pump 3 through the opening of the small door in the dosage area dedicated to loading. If a tube is already present remove the tube making sure to close the pinch clamps on the two branches to prevent solvent leakage;
6. Remove the protective caps from both the spike, mini-spike and the needle;
7. Insert correctly the spike in the solvent bag, the mini-spike and the needle in the specific dedicated housings respectively in a position proximal to the vial and to the scale on which the final bag will be placed. These housing stations can move by the activation of a pneumatic actuator;
8. Make sure that the pinch clamps on both branches of the peristaltic tube are open;
9. Be sure to use 10 x 80ml vials with intact membrane, otherwise change the membrane using suitable forceps;
10. Apply version two of the clamp to all vials, if already present remove it and replace it with the new one;
11. Make sure that the 80ml vial is full, otherwise fill it using the syringe;
12. Be sure to use a 250ml capacity bag as the final product bag and check that it is empty, otherwise empty it manually using the syringe;
13. Apply the bag clamp;
14. Open the door of the dosage area to properly place the vial with clamp in the dedicated housing and the bag with clamp in the location in the scale;
15. Close the dosage area door;
16. Using the software interface on the APOTECAbag system monitor, lower the scale;
17. Reset the weight measured by the scale to zero;
18. Lift the scale via the software interface;
19. By means of the software interface, activate the pneumatic actuator for lifting the mini-spike in order to perforate the vial membrane and the other for lowering the needle that will perforate the spongy part of the bag intended for needle insertion;

20. Using the software interface, define 3ml as the desired drug delivery quantity for pump 3 and then activate the delivery;
21. Once dispensing is complete, operate the pneumatic actuator to lift the needle from the final bag;
22. Lower the scale to weigh;
23. Store the information of the measured weight in grams (g);
24. Ensure the solvent is present in the solvent bag, otherwise refill it manually using the syringe by opening the small door of the dosage area. Then close the door;
25. If the final bag is empty to the point of containing more of the required amount of dosing leave the bag on the scale, otherwise replace it with an empty bag with a clamp by opening the door of the dosage area. Then close the door;
26. Repeat from step 16 to 25 10 times;
27. Repeat from step 4 to 26 three more times setting 30ml, 50ml, 70ml and 100ml as the desired dispensing volume in step 20 respectively;
28. Repeat from step 3:27 for a number of times equivalent to the number of calibration factors found by analysis of the experimental data. For each repetition, set the estimated factor in step 3 as the factor and use a new Double Filling Line B-tube each time the procedure is repeated.

The scale present in both dosage and reconstitution areas is the Mettler Toledo WMS weigh module [22]. The WMS weigh modules guarantee high accuracy with a readability up to 10 mg for any environment thanks to the individual selection of filter characteristics and stability criteria. The extremely robust stainless-steel design and the integrated overload protection guarantee high reliability, long service life and extreme handling. With three programmable digital outputs, the WMS weighing module can control check weighing and filling/dosing applications quickly and responsively. In addition, the three digital inputs allow high-speed software commands to be sent to the module. Important to highlight that the customer-specific adapters can easily be fixed to the square-shaped weighing platform which is rigidly connected through a patented locking device to the weigh module. Indeed, thanks to

this property it has been possible to customize the weighing platform according to the bag clamp and vial clamp for dosage and reconstitution area, respectively.

3.2.5 Data analysis technique

Before proceeding with the analysis of the acquired experimental data, it is important to define the methodology with which the initial calibration factor was defined for each peristaltic pump. This last parameter is important since it will be the main variable at the base of the testing procedures.

Once the preliminary testing procedure has been performed for each peristaltic pump, the percentage error associated with dosing according to a given calibration factor set a-priori in the APOTECAbag system was evaluated. In particular, by averaging the absolute values of the percentage errors, the calibration factor associated with the lowest average error was chosen. The various acquisitions were made by changing the calibration factor using the APOTECAbag software until relatively low percentage errors were obtained. The information acquired has been used within the testing procedures defined in paragraph 3.2.4.

Once a large number of experimental data has been acquired through the procedure, a data processing phase was conducted to assure their in-depth analysis. The experimental data were collected on an Excel datasheet. In detail, for each defined dosage relative to each pump, has been acquired information regarding the measured volume expressed in grams. Having acquired this information, the first step was to define for each measurement the percentage error relative to the measured volume (Eq. 1), the dosed volume expressed in millilitres and the corrective calibration factor (Eq. 2). The conversion of the dosed volume from grams to millilitres was possible since 0.9% NaCl was used as a fluid in the acquisition phase, thus its density is known (1.0052 g/ml).

Eq. 1 $$e\%_{,j} = \frac{V_{m,j} - V_{t,j}}{V_{t,j}} \times 100$$

Eq. 2 $$K_{c,j} = K_{i,j} \times \frac{V_{t,j}}{V_{m,j}}$$

In Eq. 1, $V_{m,j}$ corresponds to the measured volume dosed by the pump after conversion to millilitres, $e\%_{,j}$ represents the percentage error, while $V_{t,j}$ is referred to the theoretical dosing volume that is set within the APOTECAbag software. In Eq. 2, $K_{i,j}$ corresponds to the initial calibration factor defined during the preliminary testing phase associated to each pump, V_t the theoretical volume, V_m the measured volume and $K_{c,j}$ is the corrective calibration factor that defines which factor should be assigned to the pump in order to obtain a zero-percentage error. The index j corresponds to the number of acquisitions for a given dosage and, therefore, varies from 1 to 200. For this reason, the variables that appear in Eq. 1 and Eq. 2 are relative to the j -th acquisition.

The information obtained until now have been calculated through an Excel spreadsheet. After that, the data processing and analysis has been performed entirely through MATLAB software. It is important to note that the entire data processing and analysis has been conducted separately for each peristaltic pump considering as a dataset the experimental data associated with the dosage defined in the testing procedure (Table 3.4). Nevertheless, the approach for processing the experimental data is the same for all pumps. As a first step, the Excel data concerning the measured volume expressed in millilitres, the percentage error and the correct calibration factor K_c for each dosage have been imported into MATLAB software. Following, the not a number (NaN) related to those measurements that failed during the acquisition phase was removed. The next step was to analyse the trends of each variable imported into the software to see the presence of outliers. The latter are data points that differ significantly from other observations. After viewing the trends, has been performed the identification and removal of outliers using two different algorithms depending on the pump under analysis. In detail, for pumps 1 and 2, which dose solvent and drug in the area of reconstitution and dosage respectively, to perform the identification and removal has been applied a moving method called 'Moving Median' in which the outliers are defined as elements more than three local scaled median absolute deviations (MAD) from the local median over a window length equal to 20 samples. As far as pump 3 is concerned, the one that doses solvent in the dosing area, to identify and remove outlier has been chosen the method based on quartiles, in which the

outliers are defined as elements more than 1.5 interquartile ranges above the upper quartile (75 %) or below the lower quartile (25 %). For precision the 1.5 of interquartile has been replaced with a threshold of 2.25. Following the definition of a new function (Eq. 3) in which the corrective calibration factor depends on the desired absolute error has been defined. It is important to highlight that the latter corrective calibration factor corresponds exactly to the pump calibration factor being investigated.

Eq. 3 $$K_j = K_{i,j} \times \frac{V_{t,j} + E_{t,j}}{V_{m,j}}$$

In Eq. 3, K_j corresponds to the calibration factor of the pump under analysis, $E_{t,j}$ represents the desired theoretical absolute error, V_t the theoretical volume, V_m the measured volume and K_i the initial calibration factor obtained from the preliminary testing procedure. Also, for this last equation, the index j corresponds to the acquisitions number for a given dosage and, therefore, varies from 1 to 200. For this reason, the variables that appear in Eq. 3 are relative to the j -th acquisition.

Subsequently, it has been represented the trend of $e\%$ and eventual pauses, relative to interruptions during the acquisition phase, in function of the variable K per dosage of each pump. After viewing the trends and calculating the Pearson correlation coefficient (ρ), a static model based on a polynomial regression was created to characterize the relationship between $e\%$ e K. In fact, within the model it has been defined as a predictive variable K and as response one $e\%$. For each dataset, related to the defined dosages of each pump, two linear models have been defined and analysed. Firstly, a model characterized by a first-order regression polynomial, thus a simple linear regression, and then a second one described by a second-order regression polynomial, therefore a quadratic regression. The algorithm used to estimate the regression coefficients for both models is the least squares algorithm. Once the models were defined, the goodness of fit has evaluated through a quantitative statistical approach and a qualitative one by analysing the graphs concerning the residuals. To evaluate the goodness of the fitting from a statistical point of view, different parameters were calculated: determination coefficient (R-square), error sum of square

(SSE) and root mean square error (RMSE). To assess the fitting quality by means of residuals, the assumptions imposed a priori to develop a regression model has been verified: linearity, independence, normality and equal variances about the error [26]. To analyse the linearity and the error variance, the residual scatter plot is defined, while to verify the independence of the errors the plot of the residuals is evaluated as a function of the j index. Finally, to verify the presence of a normal distribution of the error, the Q-Q plot is analysed. Taken note of the fitting goodness, the predictive model is evaluated through the resolution of the inverse problem. In fact, by means of a precise criterion, the absolute error in which to evaluate the regression polynomial is defined to predict what the corresponding calibration factor should be. The criterion used to choose the absolute error is based on the splitting of the error dataset, associated with the acquisitions that occurred before any interruption, into two sub-datasets depending on whether it is positive or negative and then calculate a weighted average. In detail, to define the weight of the average two histograms are defined, one for each dataset associate to the positive and negative error, to represent the occurrence frequency of a given error. The two histograms are subsequently fit by a normal distribution to define the mean value and its probability of occurrence. Thus, the weighted mean of the two datasets will correspond to the mean of the two normal distributions weighted by their probability of occurrence.

Repeating this method of data processing and analysis on each dataset corresponding to the dosages defined for each pump in the testing procedures (Table 3.4) can be achieved different calibration factors using the predictive model and setting the desired absolute error (E_t) in the Eq. 3:

- 5 calibration factors for pump 1 concerning dosages 10ml, 30ml, 50ml, 70ml, 100ml;
- 5 calibration factors for pump 2 concerning dosages 3ml, 10ml, 15ml, 20ml, 35ml;
- 4 calibration factors for pump 3 concerning dosages 30ml, 50ml, 70ml, 100ml.

Among all estimated factors for each pump, they were averaged to define one for each pump.

The described approach was repeated four times by setting 1%, 0%, -0.5% and -1% as the desired absolute error value (E_t) in the Eq. 3. This means that at the end of the entire data processing there will be four calibration factors for each pump.

The different values obtained will then be experimentally verified with APOTECAbag to assess their feasibility according to the verification procedure defined in paragraph 3.2.4. From the new acquisitions relative to each estimated calibration factor associated with a given pump, it will be possible to calculate the average of the measurement errors in order to choose the calibration factor corresponding to the smallest average error. This verification criterion shall be repeated for each peristaltic pump to define their optimal calibration factor.

The main steps of the data analysis technique followed to choose the calibration factor for each pump are summarized in Figure 3.12.

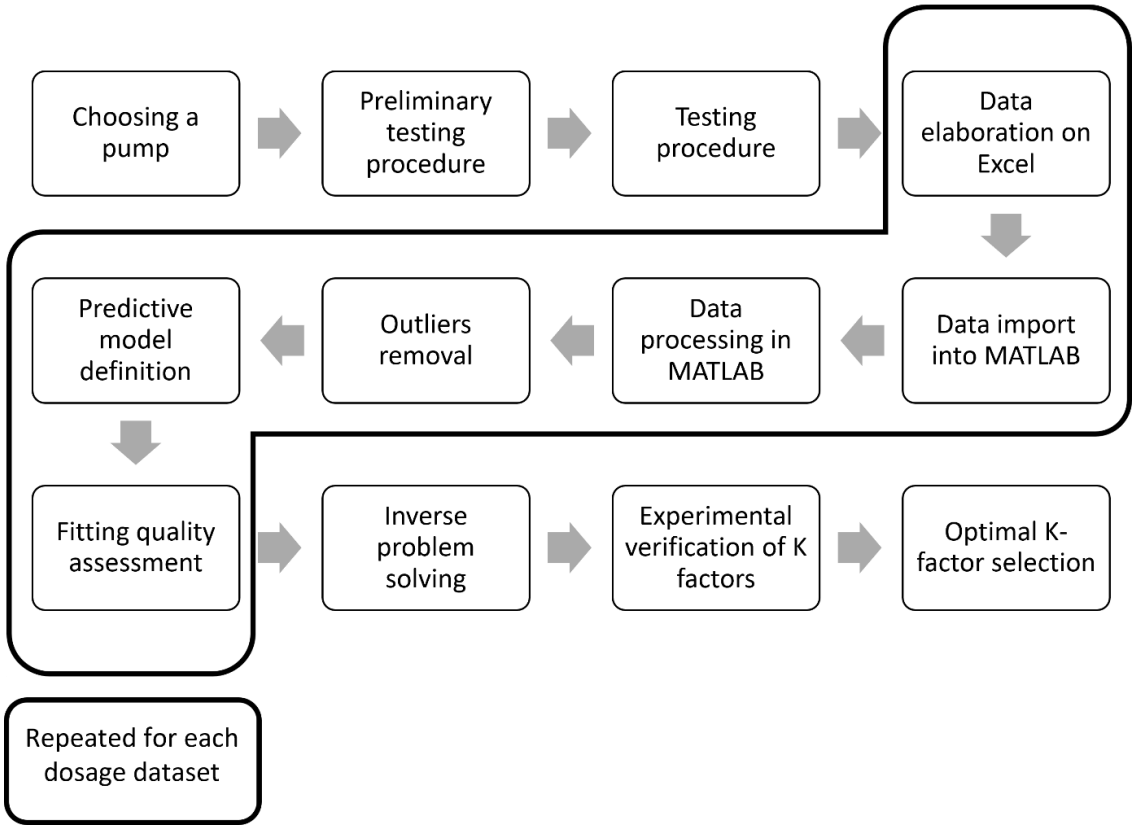


Figure 3.12. The main steps followed in the data analysis technique.

The method of data processing and analysis described so far relates to estimating the optimal calibration factor for each peristaltic pump. For the analysis concerning

the behaviour of the peristaltic tube, the data of the absolute error and the volume delivered, related to each dosage of each pump, after the removal of NaN and outliers have been used. To facilitate the analysis of the peristaltic tubes characterisation in terms of replacement after a period of use and non-use, a smoothing procedure on errors data has been done using the Gaussian method. This consists of a Gaussian-weighted moving average over each window along the vector dimension. The window size is determined heuristically and can be scaled according to the defined threshold, which is set equal to 0.45. Subsequently, the smoothed error trend was analysed in function of the delivered volume increment, with the superimposition of the interruption information that occurred during the acquisition phase.

4 Results and Discussion

In this chapter will be presented and discussed firstly the results of the different tests performed on the two vial clamp versions following the steps defined in the procedure given in the section 3.1.4. Subsequently, the results and discussions concerning the peristaltic pumps and tubes will be reported according to the procedures defined in section 3.2.4.

4.1 Test vial clamp

The validation of the vial clamps has been conducted through the testing procedure defined in section 3.1.4. In detail, two procedures have been executed. The first one focuses on the vials validation concerning the mechanical properties to verify both the breaking strength at the moment of its application and the quality of the vial fixing. The second procedure is aimed at validating the manipulation by the anthropomorphic and cartesian robots of the vial with clamp during the automated cycle of the APOTECAbag system.

Placing the focus on the first testing procedure, several results were obtained. One of the first steps in the procedure involved checking the geometrical characteristics of the two clamp versions using a caliper. The analysis proved to be positive for both versions one and two since the measured geometries matched those of the CAD model. Subsequently, the moulding qualities of the two versions have been assessed. In version two, manufacturing defects in the vial flexible fixing section were found as a result of air bubbles during the moulding process (Figure 4.1). In addition, a careful visual analysis for both versions have been performed in order to verify the integrity of the device in terms of no initial damage.

At this point, the testing procedure proceeded to the analysis of the two clamp versions application to 2ml, 20ml, 80ml and 100ml drug vials whose neck diameters

are shown in Table 3.1. The different results achieved by applying the two versions of the clamps are reported separately.

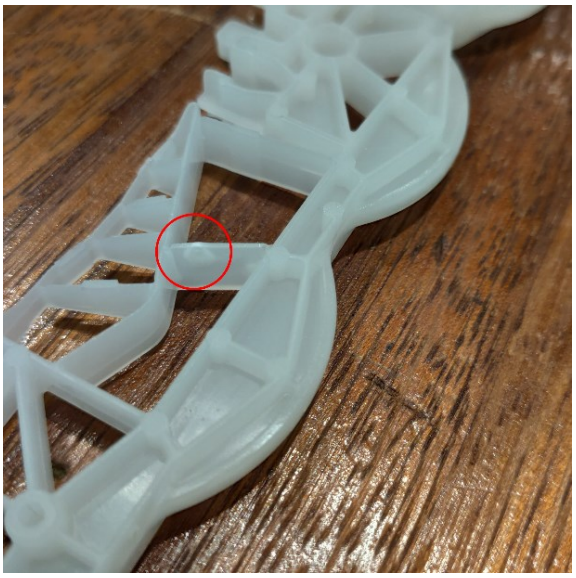


Figure 4.1. Manufacturing defect in clamp version two.

Starting with version two, after an application on the 2ml vial is found to be unbreakable due to the relatively small neck diameter (Figure 4.2.a). In addition, the vial appears to be well fixed and when applying manual vertical force to the membrane there is no leakage.

Turning to the analysis concerning the application of version two to the 20ml vial, no breaks are noted and the fixation appears to be resistant despite the force applied perpendicular to the membrane (Figure 4.2.b).

The application of version two to the 80ml vial shows no breakage of the device and, furthermore, a good vial fixation is noticed even during a manual pressure on the membrane (Figure 4.2.c).

Finally, the 100ml vial application does not induce any breakage and the fixing of the vial is very good during the manual pressure even if there is slight forcing to close the clamp. Moreover, the vial is decentralised in the clamp's flexible fixing section (Figure 4.2.d). These limitations result from fitting the version two of the clamp to a vial with a large neck.

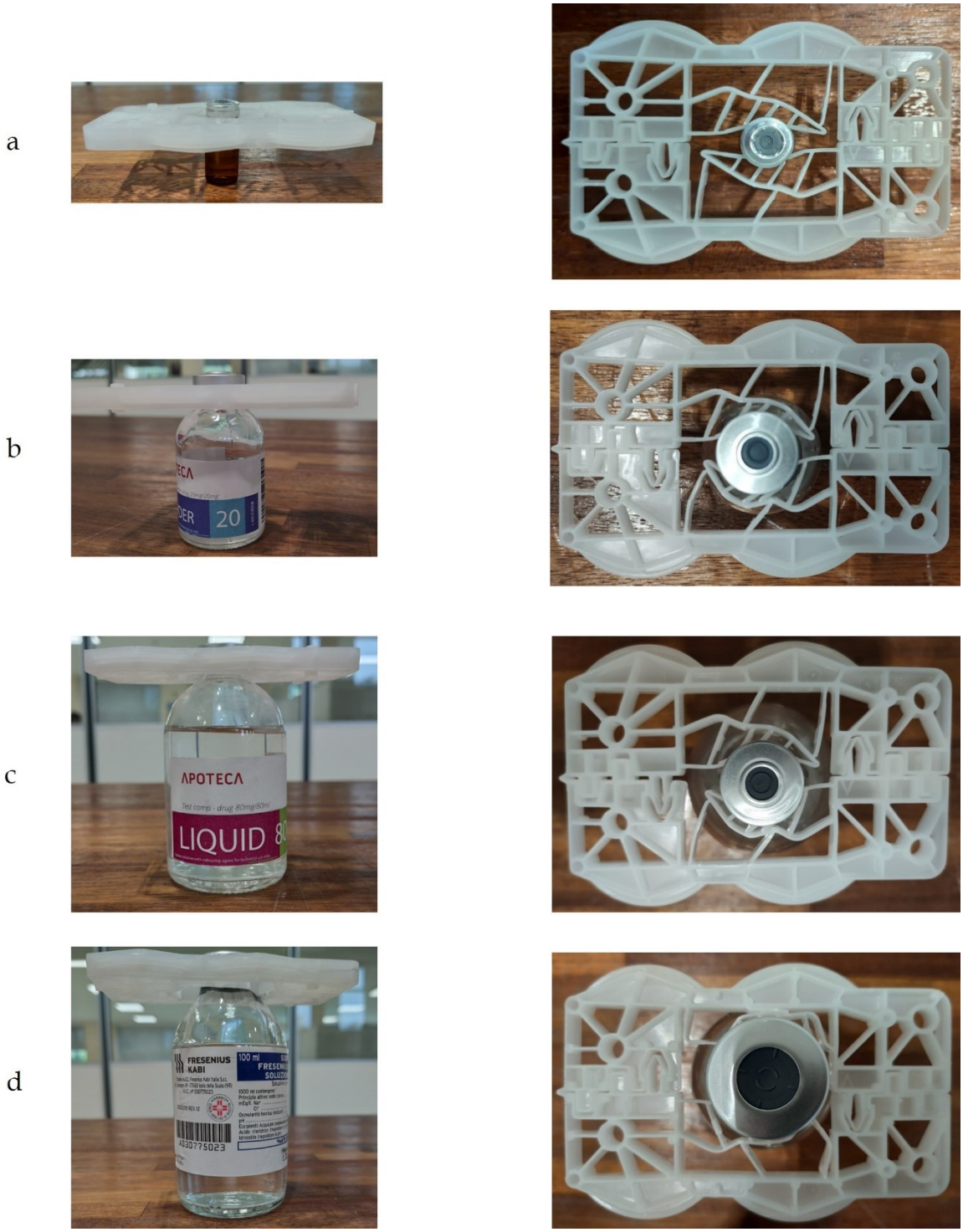


Figure 4.2. Application clamp version two to vial: (a) 2ml; (b) 20ml; (c) 80ml; (d) 100ml. In the left side a frontal view, on the right a top one.

Moving on to the analysis of clamp version one, its application on the 2ml vial does not present any breakage since the flexible fixing part is not subjected to great stress by the small neck (Figure 4.3.a). In addition, no oscillations occur since it has a good

fixation despite the application of the version dedicated to vials with a large neck. When manual force is applied to the vial, it remains fixed by the clamp except for an intense effort that leads to its exit.

No breakage occurs when applying version one of the device to the 20ml vial. Furthermore, the vial does not seem to be very fixed and, thus, it oscillates so as to assume a tilted position with respect to the clamp (Figure 4.3.b). The application of manual pressure does not result in any leakage, although the possibility cannot be totally excluded.

As regards the application of the device to the 80ml vial there were no episodes of breakage and its fixation is weak. Indeed, at the moment of the manual pressure application the vial escaped. The weak anchorage of the vial can also be evidenced by the inclination of the clamp with respect to the vial (Figure 4.3.c).

Finally, the use of the clamp to the 100ml vial revealed no breakage and a good fixing when applied a manual pression. Unlike the version 2 clamp, the version one allows the vial to be positioned centrally in the flexible fixing area (Figure 4.3.d).

Of course, the assessment of how the vial fixed by the clamp behaves when a manual force is applied is limiting for an accurate analysis. For that reason, the first testing procedure reported in section 3.1.4 allows also to evaluate the fixation during the automated cycle of the APOTECAbag system. This let an evaluation of the force applied by the mini-spike on the vial membrane at both inlet and outlet.

Using the defined procedure, it is possible to obtain different results for the 20ml, 80ml, and 100ml vials. The analysis on the 2ml vial was not conducted because the APOTECAbag system does not handle these sizes since the spike is larger than its membrane.

Looking first at version two, it was possible to observe how the 20ml vial is able to withstand the pressure while ensuring an insertion of the mini-spike central to the membrane.

A similar behaviour was observed for the 80ml vial fixed by the clamp.

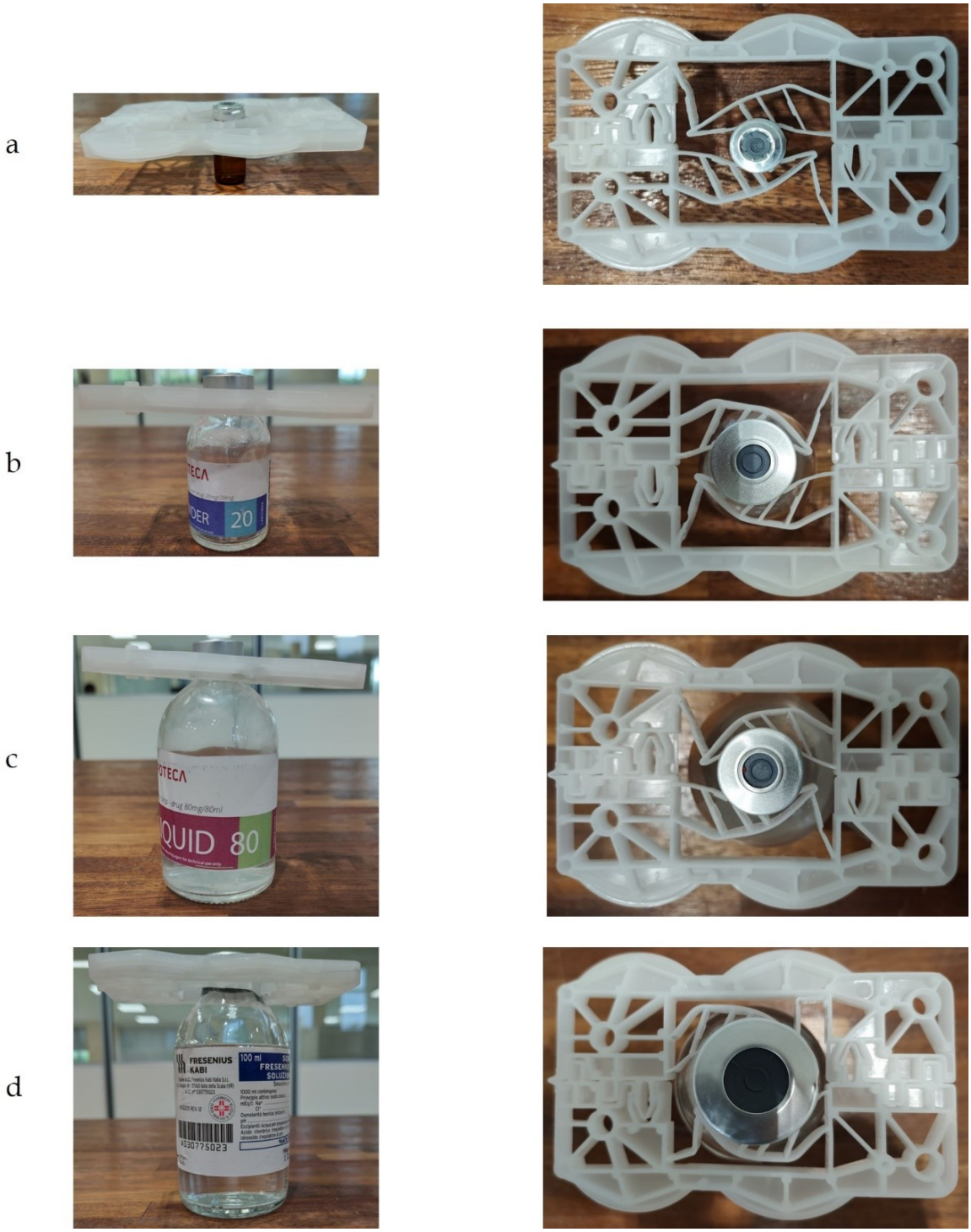


Figure 4.3. Application clamp version one to vial: (a) 2ml; (b) 20ml; (c) 80ml; (d) 100ml. In the left side a frontal view, on the right a top one.

When analysing the 100ml vial, problems arise because the vial's position is not central to the flexibly anchored section of the clamp. This results in an off-centre insertion of the mini-spike on the circumferential part of the membrane, which is

stiffer. Therefore, the spike will exert a greater effort to cross the membrane causing a visible slope of the vial with respect to the clamp (Figure 4.4).

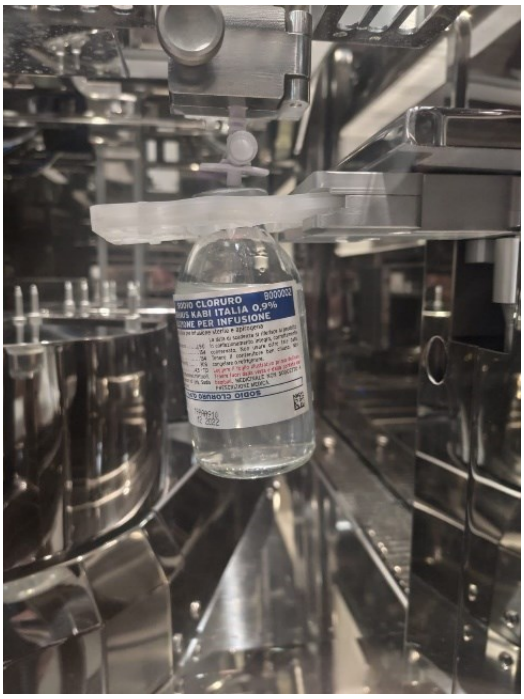


Figure 4.4. Forced insertion of the mini-spike into the 100ml vial. A tilt is visible.

Going on to the results obtained by using the clamp version one, it is possible to state that 20ml vial is able to withstand the pressure generated by the spike inlet and outlet without any episodes of vial escape from the clamp. Spike insertion is not always centred in the membrane because the lack of strong fixation causes the vial to be inserted in different positions along the longitudinal axis of the clamp. In addition, a slight oscillation of the vial was noted during the mini-spike.

When testing the 80ml vial fixed by clamp version two, a central insertion of the spike into the vial membrane was noted as well as accentuated oscillations resulting from the light fixation of the vial.

Finally, the tests conducted on the 100ml vial led to positive results regarding both the central insertion of the spike and the good fixing of the clamp.

The behaviours described for versions one and two relate to the positioning of the vial with clam in the housing in both the dosing and reconstitution area.

Once these results were obtained, the second testing procedure was conducted by creating batches defined in Table 3.2 with the use of the two clamp versions. Starting from the results obtained from batch 1 by using version two, it has been possible to observe a correct vial handling by the cartesian and anthropomorphic robots both before the reconstitution phase, so when the vial is empty, and afterwards when it is fulfilled. In fact, the robots are able to hook and position the clamp correctly. Comparable results were obtained by running the same production batch using version one. The only difference present is a slight oscillation of the vial during manipulations without the occurrence of falling vials.

With regard to production batch 2 involving the 80ml vial, correct handling by the two robots was noted during the use of version two both before and after the reconstitution procedure. On the other hand, when the same batch was run using version one, the two robots were able to handle the 80ml vial correctly before reconstitution, but after this procedure the filled vial fluctuated considerably during handling by the anthropomorphic robot until the vial escaped from the clamp.

Lastly, when analysing the batch 3 involving the 100ml vials with clamp version two, solvent spillage was observed during the dilution phase. This was caused by the decentralisation of the vial in the flexible fixing section of the clamp, which prevent a proper insertion of the mini-spike into the membrane. Despite this, handling by the anthropomorphic and cartesian robots was appropriate before and after the reconstitution phase. Running the same batch using version one, no improper spike insertion occurred during the dilution phase and, moreover, a correct handling by both robots was found.

4.2 Test peristaltic pump

The initial calibration factor for each peristaltic pump was found according to the preliminary testing procedure defined in section 3.2.4.

The a-priori calibration factors used to conduct the preliminary testing procedure are as follows:

- For pump 1: 1.68, 1.69, 1.7;
- For pump 2: 1.66, 1.67, 1.68;
- For pump 3: 1.08, 1.1, 1.21.

The percentage error was simultaneously calculated using the Excel spreadsheet during the acquisition of the measured volume, which was previously converted from grams to millilitre since during the preliminary procedure the use of the drug was simulated with the solvent 0.9% NaCl and, therefore, a known density of 1.0052 g/ml. The results of the different acquisitions for pump 1 are shown in Table 7.1, for pump 2 in Table 7.2 and, finally, those for pump 3 in Table 7.3. All of these tables are reported in Appendix.

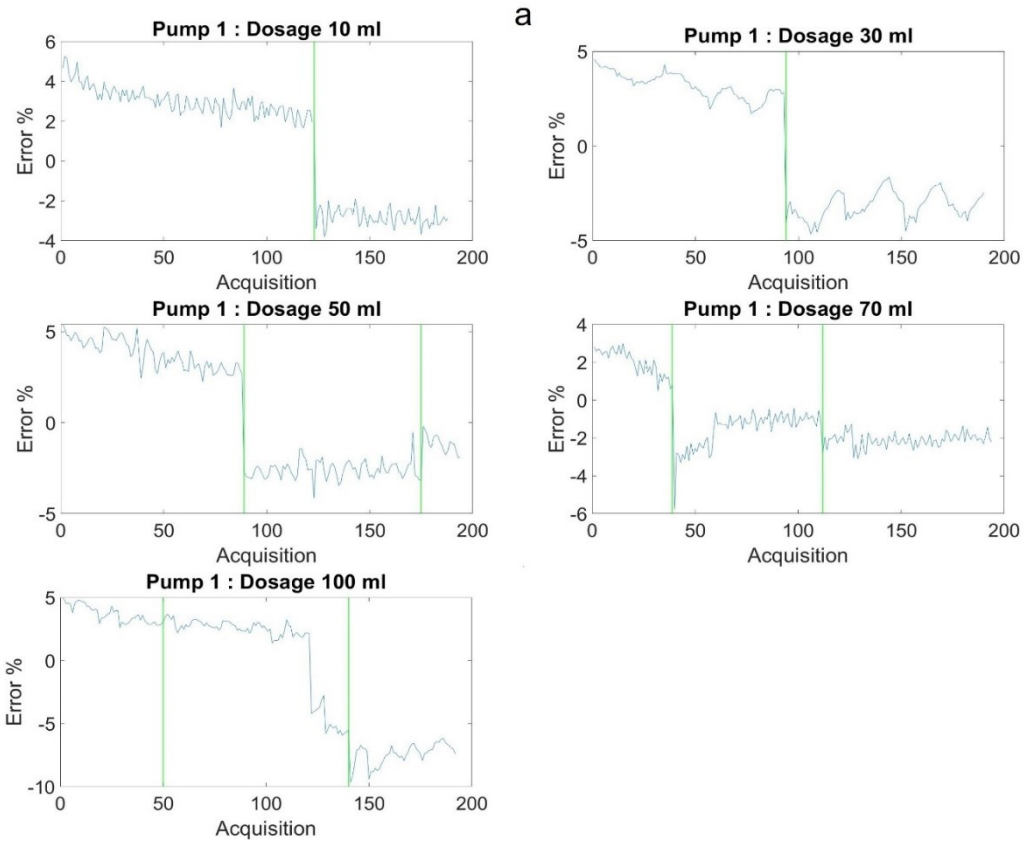
Analysing the error in the tables, it is noticeable that positive and negative values are associated with a well-defined meaning. In fact, a positive value represents an overdose condition, while a negative one reflects an underdose condition. Therefore, in the evaluation of the error for the choice of initial calibration factors, the absolute value was considered and its average was calculated for each dosage of a given factor. On the basis of this data processing, the lowest mean value obtained is the one associated with a 1.69 calibration factor and, thereby, the latter was chosen for pump 1. With the same evaluations for Table 7.2 and Table 7.3, the initial calibration factor for pump 2 can be defined as 1.67 and for pump 3 as 1.1.

The initial calibration factors obtained are the ones used for the testing procedure. Then, experimental measurements on the measured volume have been acquired. Given this information, the delivered volume expressed in millilitres, the corresponding percentage error and the corrective calibration factor (K_c) were calculated. This last parameter corresponds to the factor that should be set in the APOTECAbag software in order to obtain a measurement error equal to zero. When the data was imported into MATLAB, the NaN were removed from each data variable. The percentage error data vector was then converted to absolute error. With each

pump data available, the measurement error ($e\%$) trends for each dose and the corrective calibration factors K_c were evaluated in function of the number of acquisitions. The trends described for pump 1 (Figure 4.5), pump 2 (Figure 4.6) and pump 3 (Figure 4.7) are shown below. It should be noted that the error has been represented in percent for easier visual analysis, although the absolute error has been used for the entire data processing and analysis technique.

A common aspect for Figure 4.5, Figure 4.6, Figure 4.7 is that for each pump the absolute error has opposite trend with respect that of the corrective calibration factor for the same dose. This can be explained by the meaning of K_c because when the error is positive, that means an overdose condition, it must decrease in order to reduce the delivery. Conversely, when the error is negative, a condition of under-dosage, the K_c must increase to assure more dispensing with the assumption of a theoretical zero error.

a



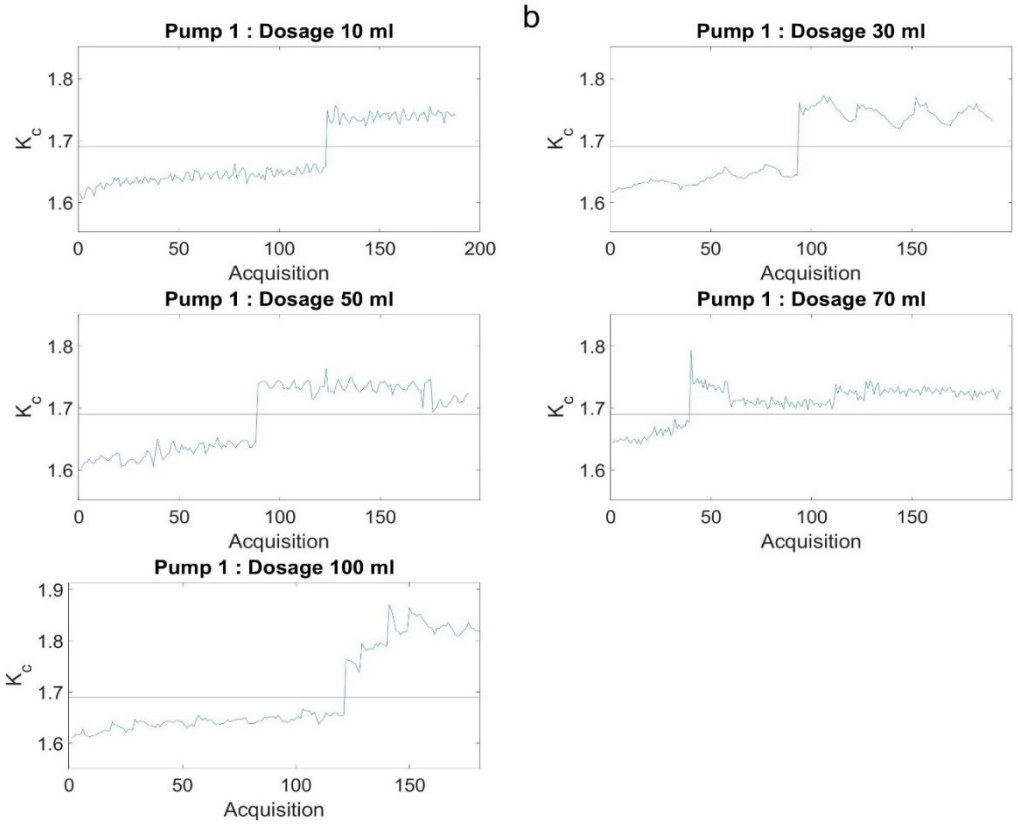
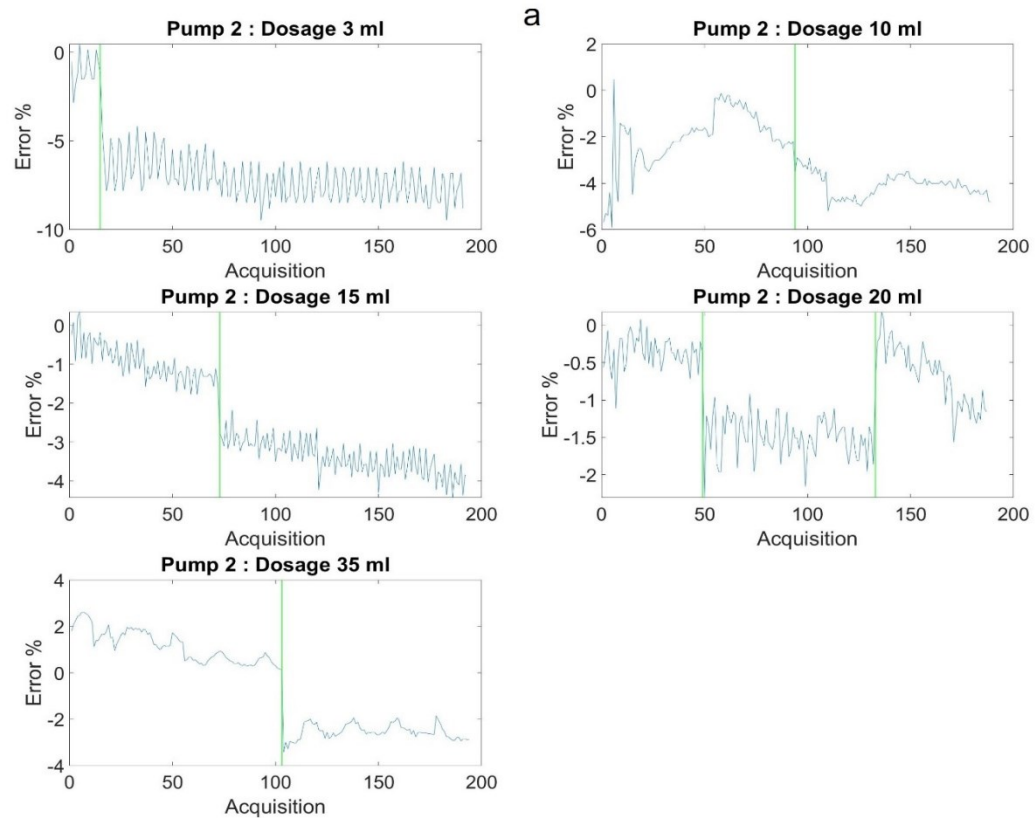


Figure 4.5. Pump 1: (a) percentage error (e%); (b) corrective calibration factor (K_c).



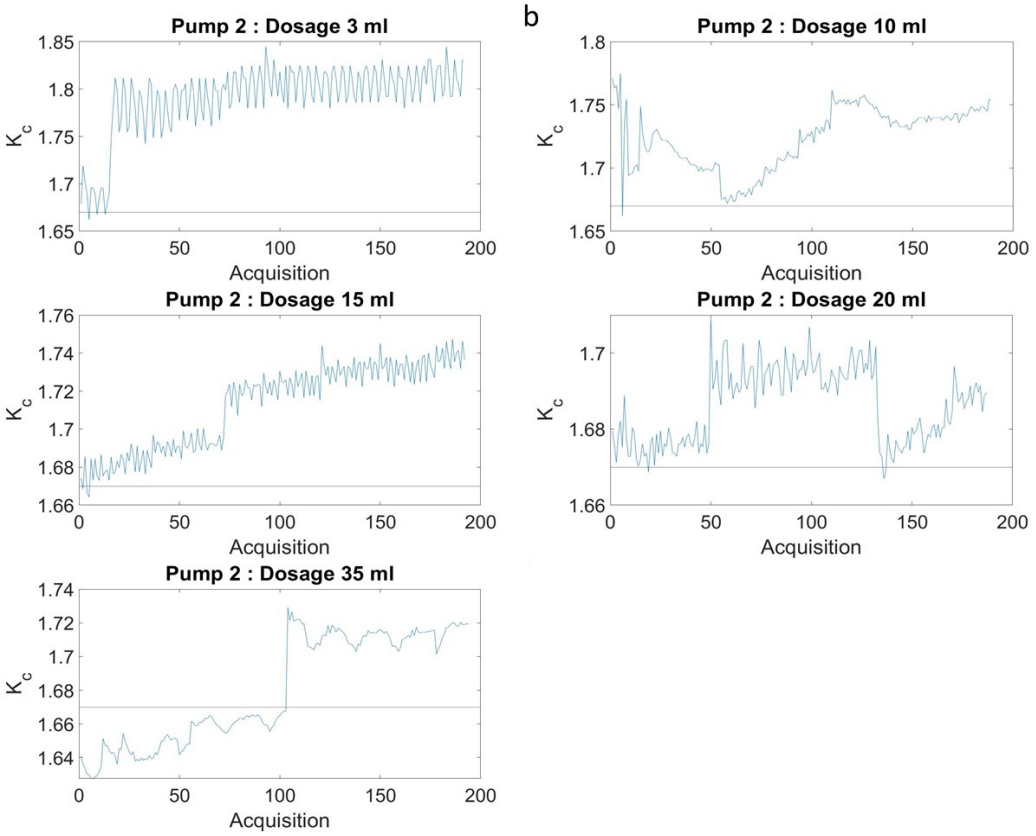
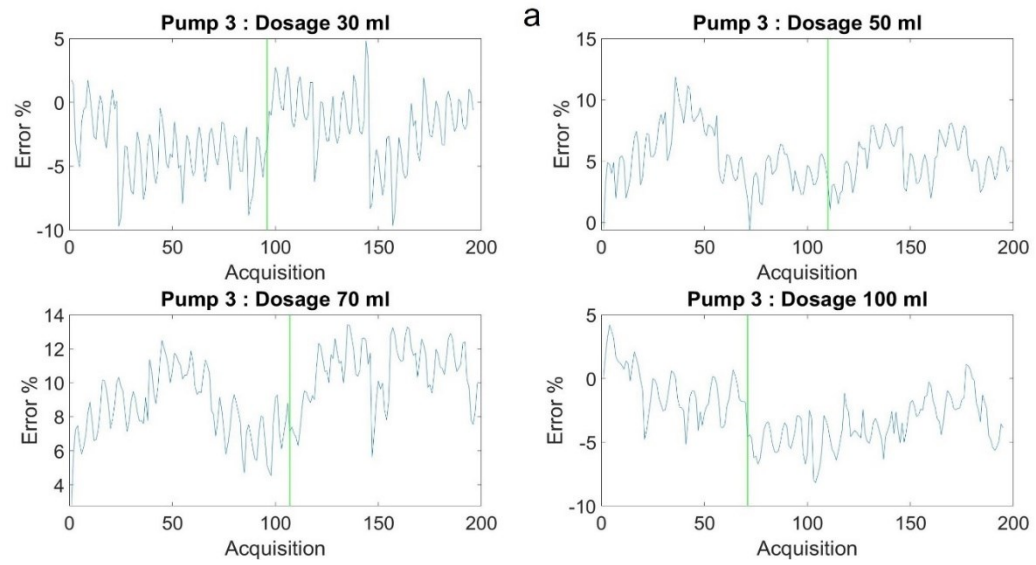


Figure 4.6. Pump 2: (a) percentage error (e%); (b) corrective calibration factor (K_c).



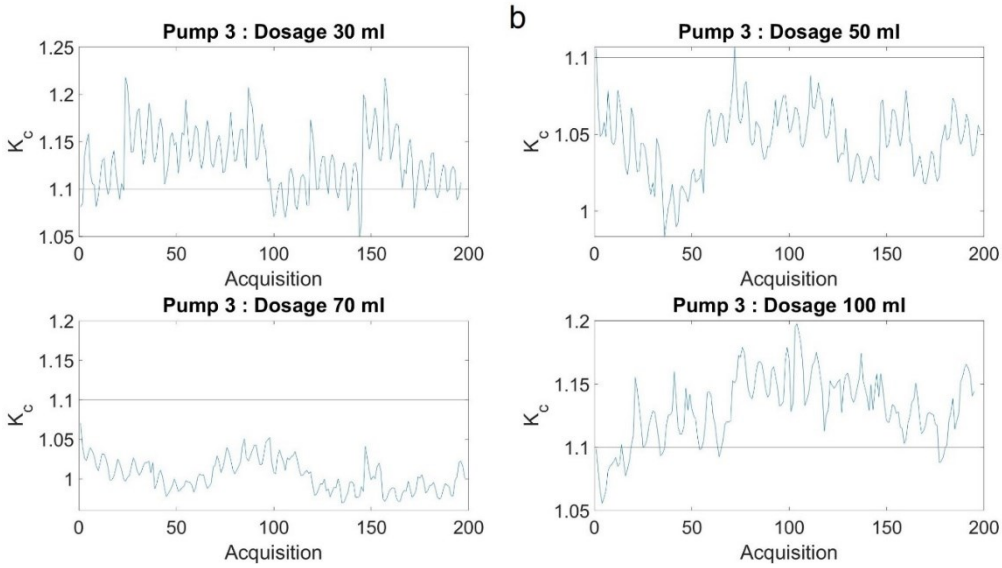


Figure 4.7. Pump 3: (a) percentage error (e%); (b) corrective calibration factor (K_c).

The variation of the calibration factor is correlated with the amount of dosing by a direct proportionality relationship. In fact, the meaning of the calibration factor within the APOTECAbag system corresponds to that parameter which correlates the number of pump revolutions with the quantity of fluid delivered. Thus, increasing the calibration factor will increase the number of revolutions performed by the peristaltic pump and, consequently, the quantity of fluid delivered.

In Figure 4.5.b, Figure 4.6.b, Figure 4.7.b, superimposed on the K_c trend, the initial calibration factor calculated by means of the preliminary testing procedure for each peristaltic pump is shown in black. This illustrates how K_c varies with respect to K_i.

Another aspect that can be seen, especially in figures Figure 4.5.a, Figure 4.6.a is the rapid variation of the percentage error in correspondence with the pause marked in the figures with the green colour. The pause represents the interruption of variable time during the data acquisition phase.

In Figure 4.5 is noticeable how the percentage error variation maintains a similar range for the dosages 10ml, 30ml, 50ml, 70ml (-5%/5%) and increases for the high dosage of 100ml (-10%/5%).

Looking at Figure 4.6, the range of variation of the error in percentage is very high due to the presence of outliers visible from the deviation from the trend of

neighbouring samples. In addition, for the dosages 3ml, 10ml, 15ml, 20ml there is always a condition of negative percentage error and, thus, a condition of underdosing except for the presence of outliers.

Analysing pump 3 (Figure 4.7), it is possible to notice the presence of outliers, present above all for the dosages 50ml and 70ml, and in addition there is always a positive percentage error corresponding to an overdosage condition.

This initial analysis allows us to understand how the percentage error and the calibration factor vary according to different types of dosage relative to each pump.

In accordance with the analysis method described in section 3.2.5, the next step was the identification and removal of outliers. This is important since their presence can negatively influence the accuracy and analysis of data with incorrect information.

To prevent this problem, a moving method called 'Moving Median' was applied to pump 1 and 2 data, while a quartile-based method was applied to pump 3. The decision to use different methods came after the trend analysis of Figure 4.5, Figure 4.6, Figure 4.7. In the moving median the outliers are defined as elements more than three local scaled median absolute deviations (MAD) from the local median over a window length equal to 20 samples. The quartile method is based on the identification, and subsequent removal, of those values that are more than threshold to interquartile ranges above the upper quartile (75 percent) or below the lower quartile (25 percent). The set threshold is 2.25, this means that has been decreased the sensitivity range for outliers' decision rules. Indeed, a bigger scale would make the outliers to be considered as data points, while a smaller one would make some of the data points to be perceived as outliers.

The choice of using a different method for pump 3 lies in the fact that the quartile method is more effective when the data within a vector are not normally distributed. In fact, analysing the trend in Figure 4.7.a, it is possible to notice the high variability of the data with respect to the trends of the percentage error present in Figure 4.5.a and Figure 4.6.a.

The identification and removal of outliers conducted on the absolute error data vectors is shown for pump 1 in Figure 4.8, for pump 2 in Figure 4.9 and for pump 3 in Figure 4.10.

The figures show the absolute error trend before the identification of outliers, which are highlighted with a red cross, and superimposed the signal after removal. In addition, the threshold beyond which a data point is considered as an outlier and therefore removed is shown in black.

In Figure 4.8 several outliers were identified: 4 for the 10ml and 30ml dosages, 1 for the 50ml dosage, 3 and 2 for the 70ml and 100ml dosages respectively. The outliers identified and eliminated for pump 2 (Figure 4.9) are: 1 for the 3ml dosage, 6 for the 10ml and 35ml dosages, 5 for the 15ml and 20ml dosages.

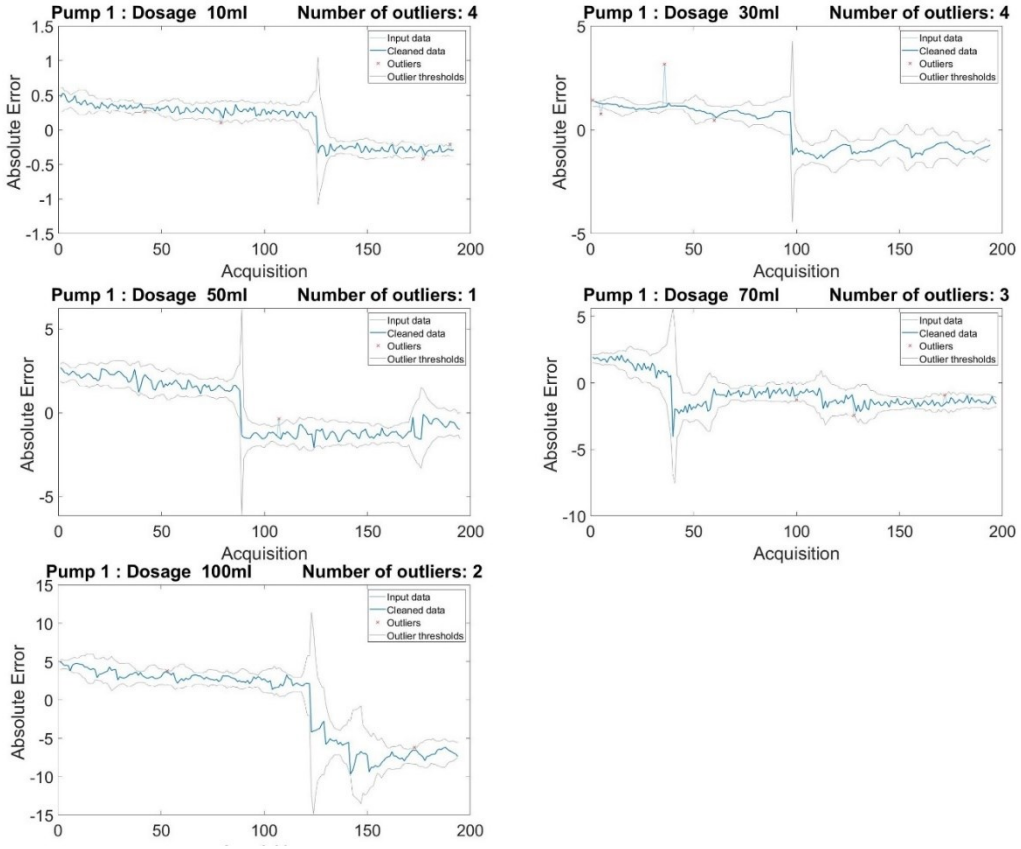


Figure 4.8. Pump1: outliers removal form error dataset.

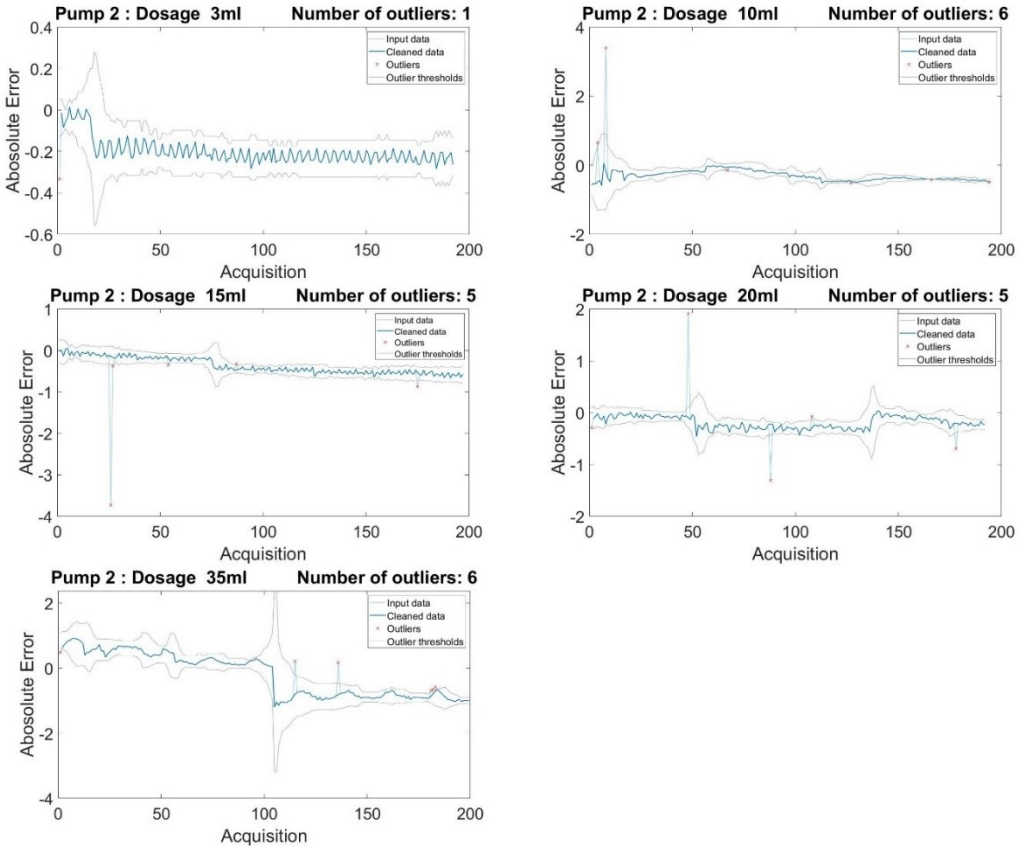


Figure 4.9. Pump 2: outliers removal from error datasets.

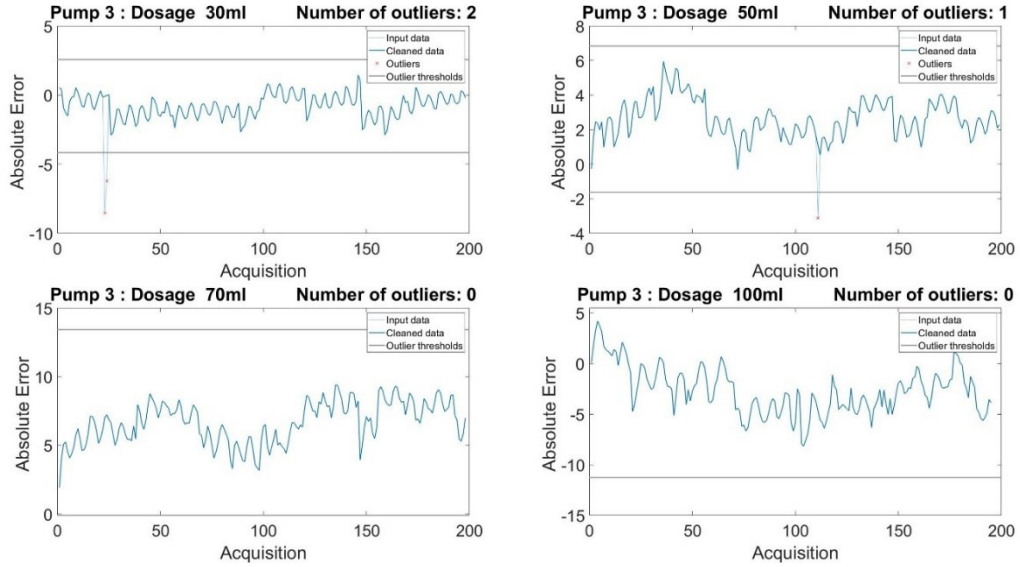


Figure 4.10. Pump 3: outliers removal from error datasets.

For pump 3 (Figure 4.10), the number of outliers identified and removed is lower than for pumps 1 and 2 for the reason related to the use of a high threshold. In fact, the outliers removed are 2 and 1 for the 30ml and 50ml doses respectively.

The presence of outliers is mainly associated with an underdosing condition due to air intake caused by membrane wear. All because the repetitive insertion of the mini-spike inside the vial causes enlargement of the inlet hole and, consequently, the dosage not only contains fluid but also air. In addition, other outliers are also related to a measurement error by the balance caused as a result of the ventilation system activity. In fact, during the acquisitions performed in accordance with the testing procedure defined in paragraph 3.2.4, it was always necessary to reset the balance before proceeding with a new acquisition and at the first attempt it was not possible to perfectly zero the weight because of the laminar flow generated by the ventilation system.

Subsequently, a new corrective calibration factor has been calculated that differs from the one defined in Eq. 2 since instead to reflect what should be the parameter needed to dose with a theoretical zero percent error, it is defined to achieve a slight underdose condition with an error equal to that set a-priori in Eq. 3. The equation purpose is not to theoretically obtain a measurement with a zero percent error since the real measurement most often corresponds to an overdose condition. The intention is to define a slightly negative measurement error to achieve under-dosages that subsequently the system can correct by the injecting the missing quantity. As such, it is recommended to ensure dosages with slightly negative errors, with a possible subsequent correction, and not positive errors where corrections cannot be made. It should be emphasized that when assuring an underdosing condition, one must take into account that a large negative error corresponds to multiple dosage corrections and, in turn, an increase in machine cycle time. It follows that the objective is to ensure optimal underdosing to affirm both the possibility of an eventual correction and a decrease in the machine cycle time related to dosing.

With this consideration, the meaning of the latter corrective calibration factor (K) can be defined as corresponding to the parameter being sought.

Setting the theoretical dosing error E_t of -1%, Eq. 3 has been used to calculate the K parameter for all acquisitions relative to each dose defined in the test procedures of each pump. This means that all the consecutive results refer to an E_t set to -1%. The defined K -factors have a vector size equal to that of the error because in Eq. 3 is needed the information relative to the measured volume on which also the outliers removal has been performed for the peristaltic tube characterization. Consequently, the evolution of the error $e\%$ as a function of K can be represented for pump 1 (Figure 4.11), for pump 2 (Figure 4.12) and for pump 3 (Figure 4.13).

The trends in Figure 4.11, Figure 4.12, Figure 4.13 suggest a linear relationship between the error $e\%$ and the K factor. In detail, it is evident that increasing the K factor decreases the error.

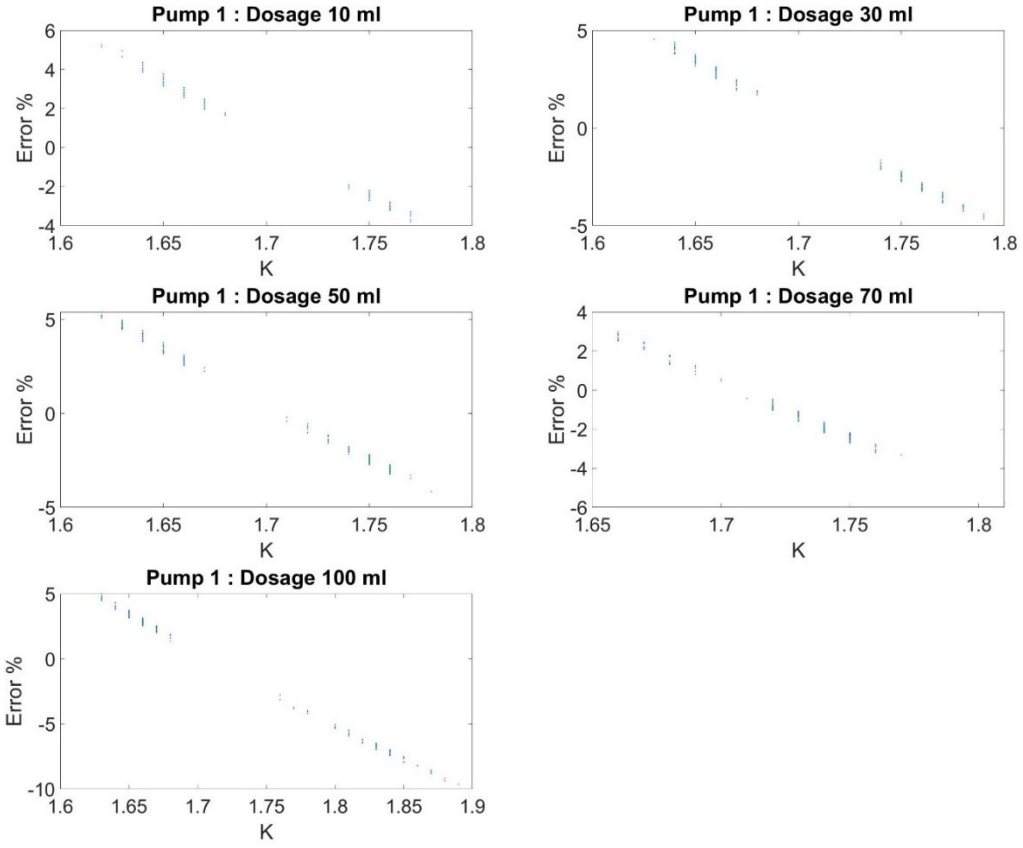


Figure 4.11. Pump 1: trend of percentage error in function of corrective calibration factor K.

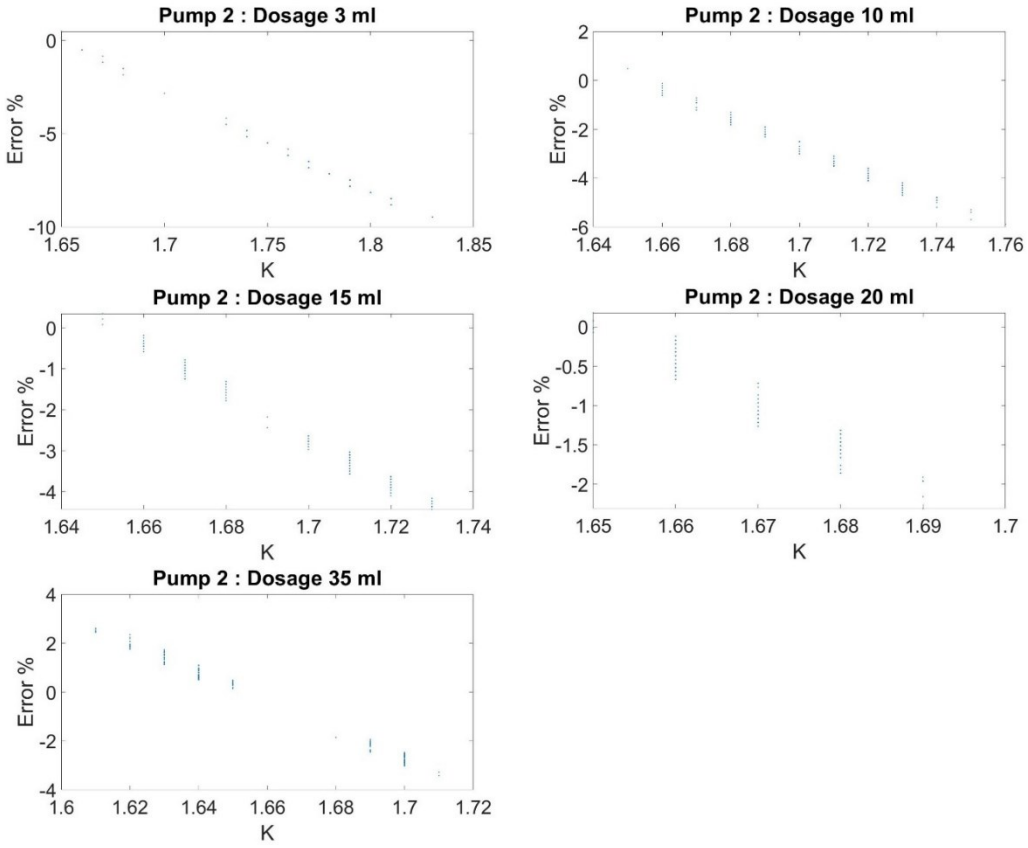


Figure 4.12. Pump 2: trend of percentage error in function of corrective calibration factor K.

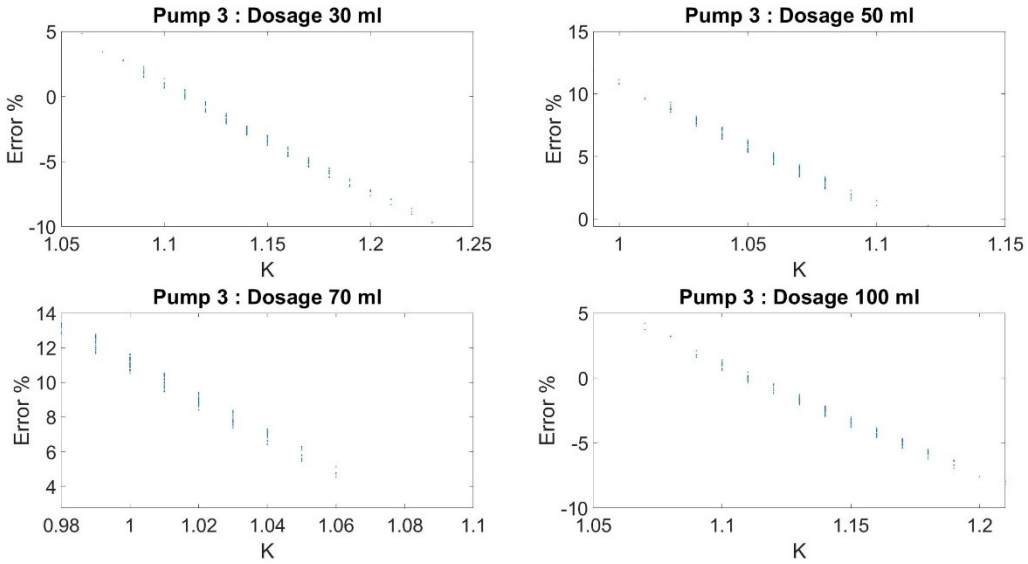


Figure 4.13. Pump 3: trend of percentage error in function of corrective calibration factor K.

This reflects the correction meaning associated with *K*. In fact, when the error is high positively, thus an overdose condition, a correction with a lower calibration fact

is required and vice versa when there is a negative error an increase in the k-factor is required. The variation of the K -factor is aimed to guarantee a dosage with a theoretical error predetermined in Eq. 3, and therefore -1% of the analysed dosage.

Another parameter that strengthens the suggestion of a linear relationship is the Pearson correlation coefficient (ρ) between the two variables shown in Table 4.1.

The reported Pearson correlation coefficients suggest a strong inverse linear relationship between the two variables. Taking into account the linear trend and the strong inverse linear correlation of the error e% with the K factor, several simple linear regression models were initially developed. The K -factor was considered as the predictor variable and the error as the response.

Table 4.1. Pearson correlation coefficient associated for each dosages per pump.

PEARSON CORRELATION COEFFICIENT				
Pump 1: Dosage				
10ml	30ml	50ml	70ml	100ml
-0,998	-0,998	-0,998	-0,994	-0,999
Pump 2: Dosage				
3ml	10ml	15ml	20ml	35ml
-0,996	-0,992	-0,992	-0,957	-0,996
Pump 3: Dosage				
30ml	50ml	70ml	100ml	/
-0,996	-0,991	-0,9892	-0,993	/

Of course, 4 assumptions were made for the development of the simple linear regression model [26]:

- The mean of the response, at each predictor value, is a linear function of the predictor;
- The errors are independent;
- The errors at each value of the predictor are normally distributed;
- The errors, at each value of the predictor, have equal variances.

Based on these assumptions and by application of the least squares method, regression coefficients were defined for each model associated with each pump dose. The simple regression lines calculated for pump 1 are shown in Figure 4.14, for pump 2 in Figure 4.15 and for pump 3 in Figure 4.16. The figures show the estimated simple regression line in red and the corresponding observed points in blue.

Once the different models have been created, it is necessary to examine their validity. As defined in section 3.2.5, the analysis of the fitting quality, and thus the validity of the model, is performed using a quantitative statistical approach and a qualitative one by analysing the residuals plots.

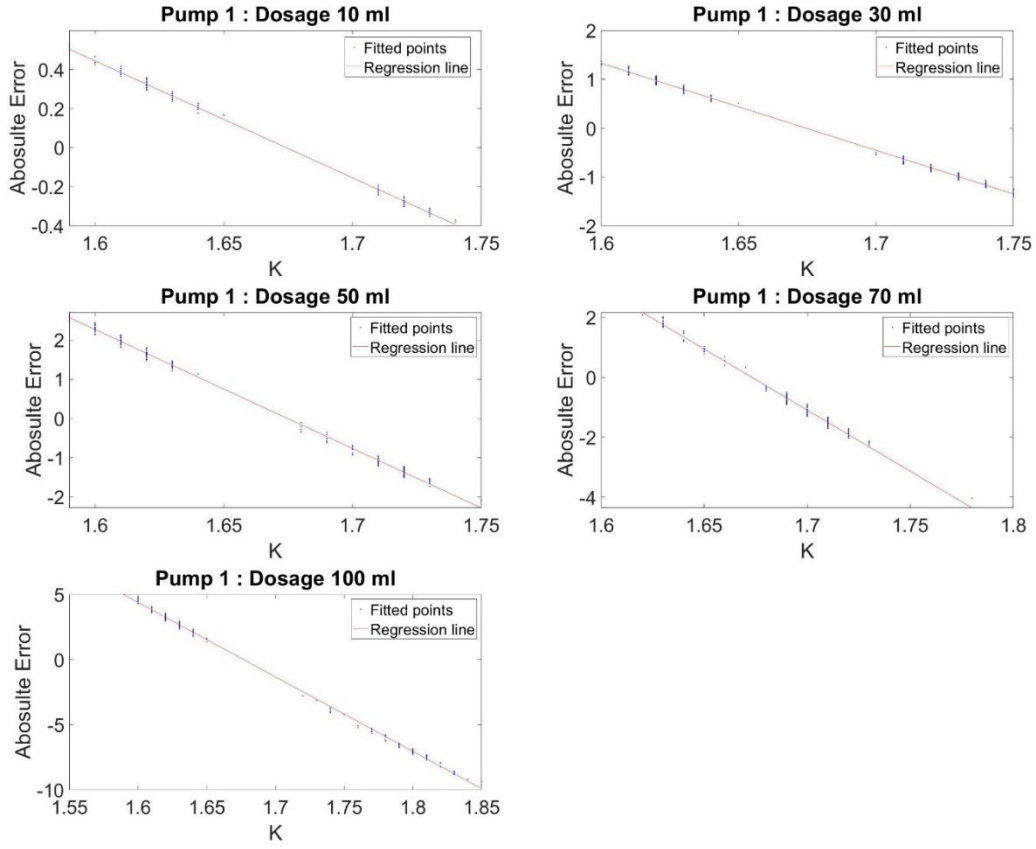


Figure 4.14. Pump 1: simple linear regression model.

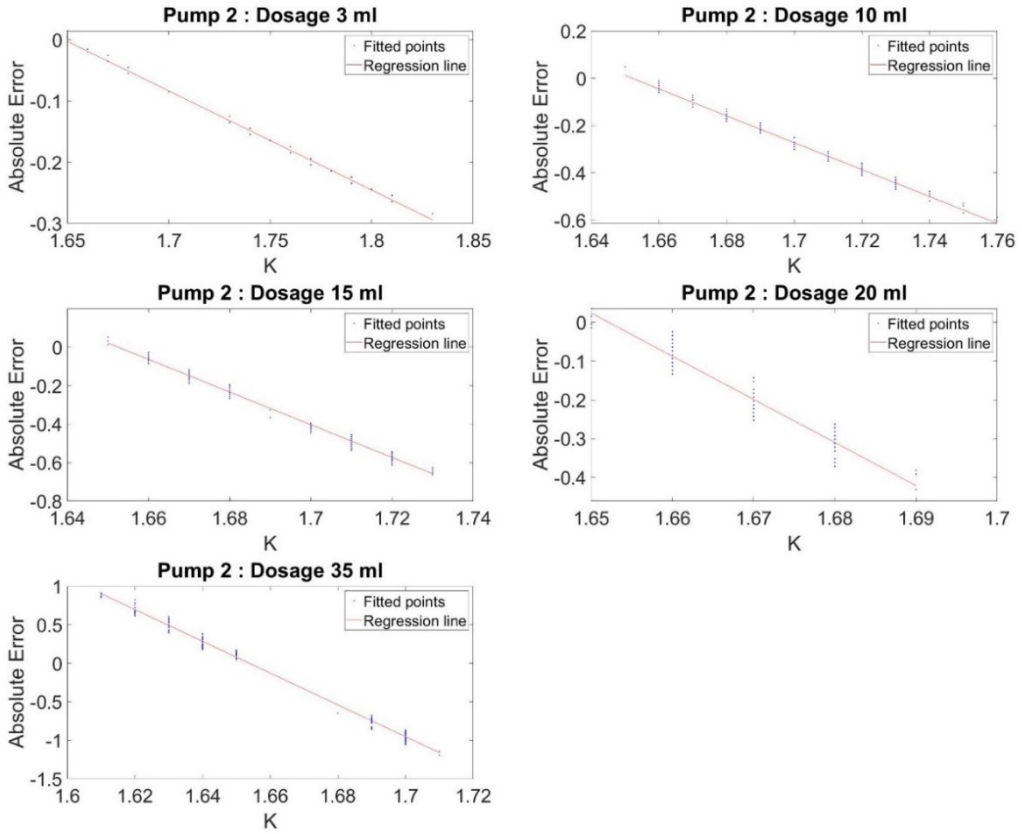


Figure 4.15. Pump 2: simple linear regression model.

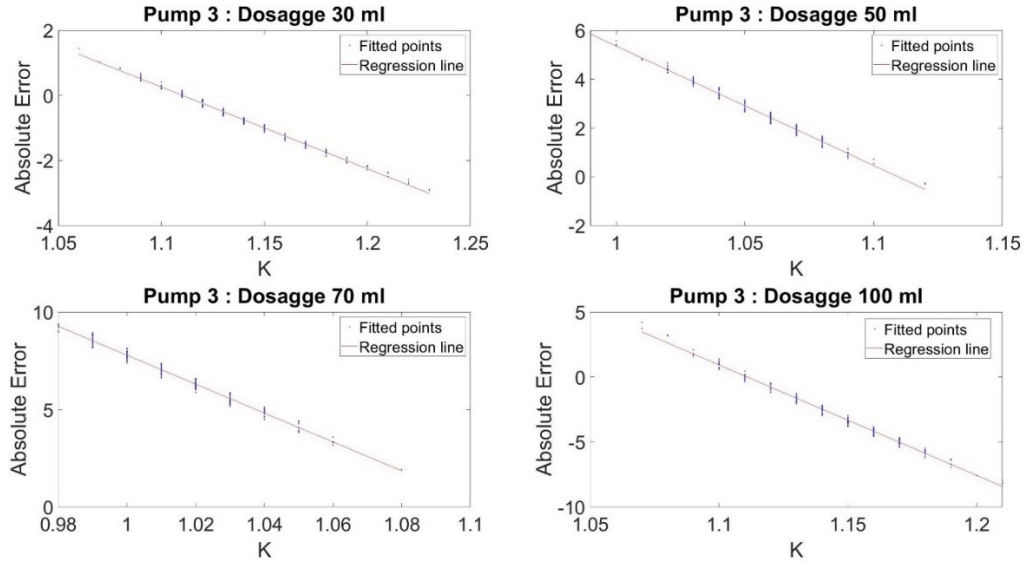


Figure 4.16. Pump 3: simple linear regression model.

Beginning with the quantitative approach, R-square, SSE and RMSE were calculated. The values of these parameters for each model relative to the dosages of each pump are shown in Table 4.2.

Table 4.2. Representation of quantitative statistical variable for the dosages related to each pump by using linear regression model.

	Dosage (ml)	R-square	SSE	RMSE
Pump 1	10	0,996	0,061	0,018
	30	0,997	0,575	0,055
	50	0,997	1,643	0,092
	70	0,990	2,857	0,122
	100	0,999	6,571	0,186
Pump 2	3	0,993	0,005	0,005
	10	0,986	0,052	0,017
	15	0,984	0,111	0,024
	20	0,918	0,208	0,034
	35	0,992	0,699	0,060
Pump 3	30	0,991	1,218	0,079
	50	0,982	4,237	0,147
	70	0,979	9,418	0,219
	100	0,989	11,405	0,243

The R-square values are typically high. The latter parameter assesses the dispersion of the data points around the estimated regression line and, usually, its high value means that there is little difference between the observed data and the model values. However, it is not possible to use R-squared to determine whether the coefficient estimates and predictions are biased, which is why a high value of it is not sufficient to assess the correctness of the model, but further qualitative analysis of the residuals is required.

The SSE parameter, which measures the variability of the measure of the response variable with respect to the value given by the regression model, is found to be high and therefore not negligible for pump 1 and 3.

As for regard RMS which is the standard deviation of the residuals the values are low and, thus, define that data are concentrated around the line of best fit.

Once seen these values, the next step was to perform the analysis of the residuals in order to verify the correctness of the assumptions made a-priori to the model development. In order to verify the linearity and equal variance of the errors, scatter plots have been defined for each model relative to each dosage of each peristaltic

pump. These plots are shown in Figure 4.17 for pump 1, in Figure 4.18 for pump 2 and in Figure 4.19 for pump 3.

There are mainly two distinctive aspects to be considered for a visual analysis of the scatter plot. The first is that the residues are randomly disposed around the zero line. This suggests that the assumption of linearity is respected. The second is that the residuals form a horizontal band around the zero line such that the variances of the error terms are equal.

In the scatter plots of pumps 1 and 3, the residuals are not randomly distributed around the zero line, but have a parabolic profile. This shows that the linearity imposed as initial assumption for the model development is not verified. In addition, the trend of the residuals also reflects a high SSE reported in Table 4.2.

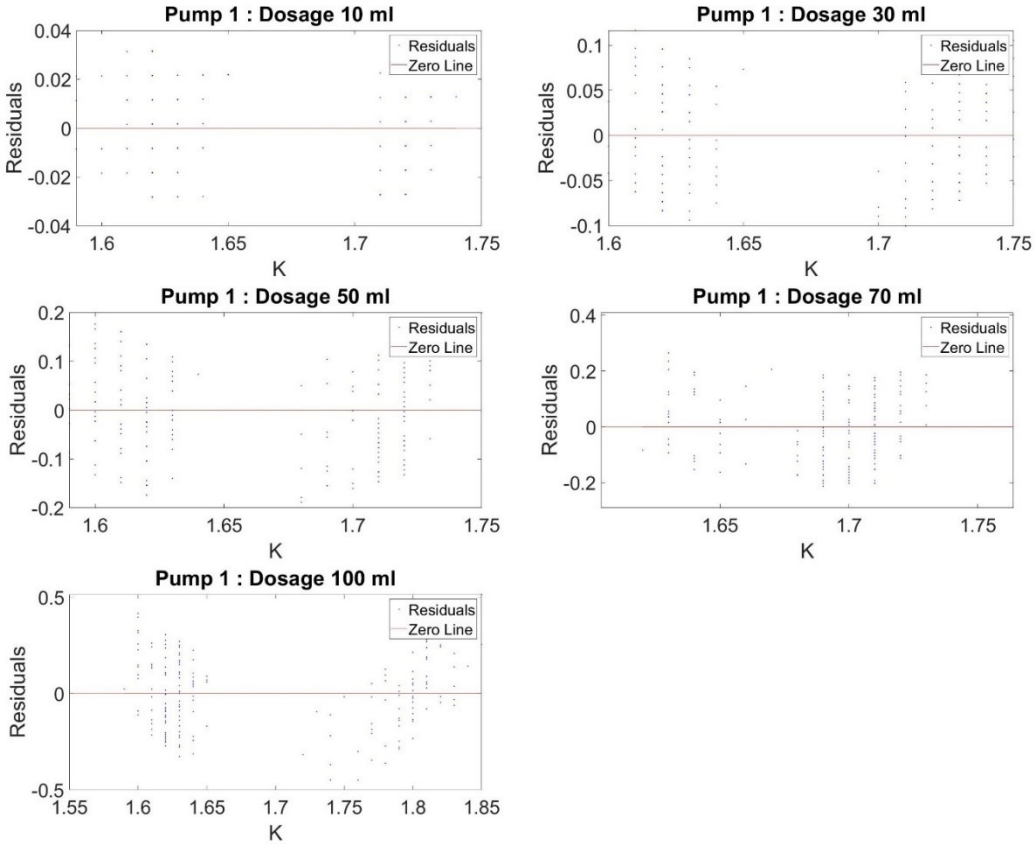


Figure 4.17. Pump 1: residuals scatter plot related simple linear regression model.

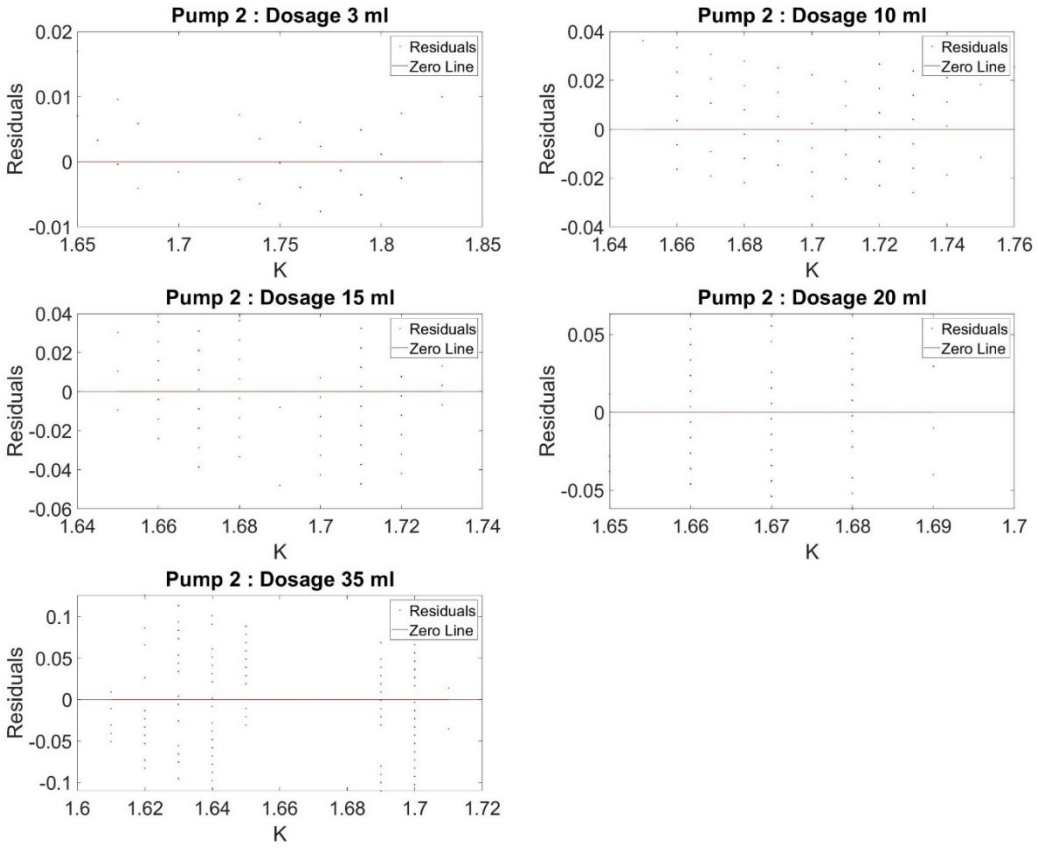


Figure 4.18. Pump 2: residuals scatter plot related simple linear regression model.

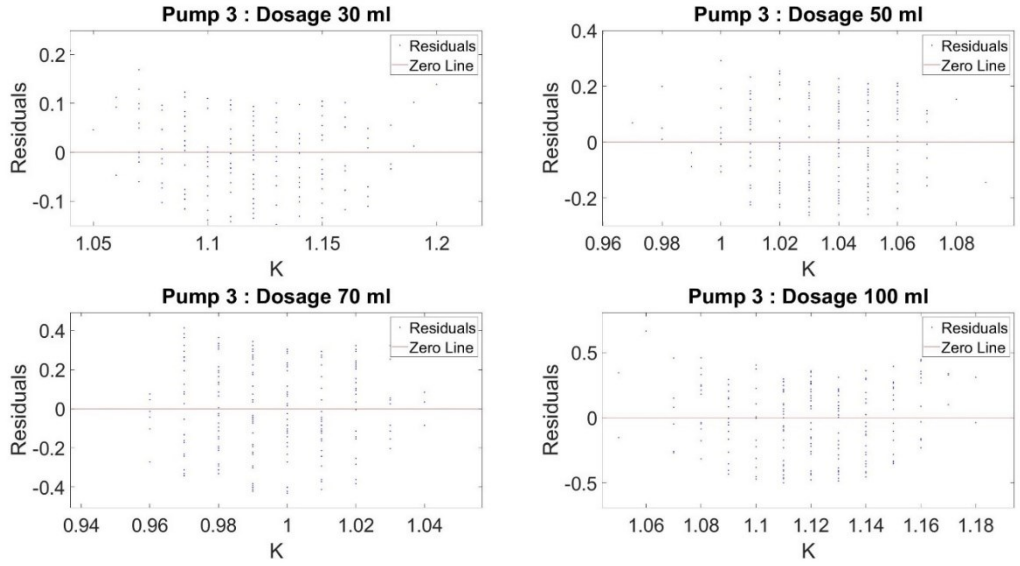


Figure 4.19. Pump 3: residuals scatter plot related simple linear regression model.

The lack of linearity is an aspect that might be in contrast to the high R-square values but, in reality, it highlights how a high R-square is not always an indicator of whether

the regression model fits the data adequately. Indeed, a higher value may also reflect other limitations found in the scatter plot, such as a lack of linearity. This occurs when the linear model is underspecified. In other words, it is missing significant independent variables, polynomial terms and interaction terms. For this reason, it was decided to replace the use of a simple linear regression model with a quadratic order polynomial regression model. Although this type of model allows for a non-linear relationship between the predictor variable and the response, polynomial regression is still considered a linear regression model since it is linear in its regression coefficients. Also by employing the least squares method the regression coefficients were estimated and the regression lines obtained from the polynomial regression model are shown for pump 1 in Figure 4.20, for pump 2 in Figure 4.21 and for pump 3 in Figure 4.22 for each corresponding dosage.

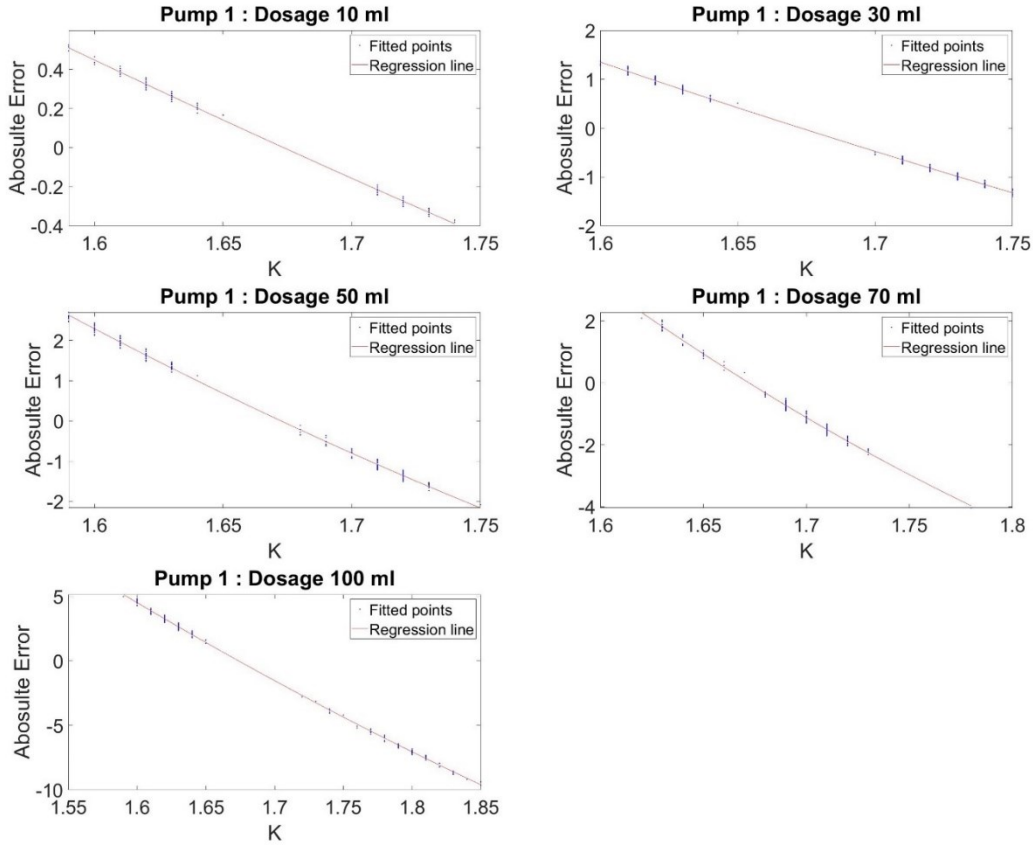


Figure 4.20. Pump 1: regression lines obtained from the polynomial regression model.

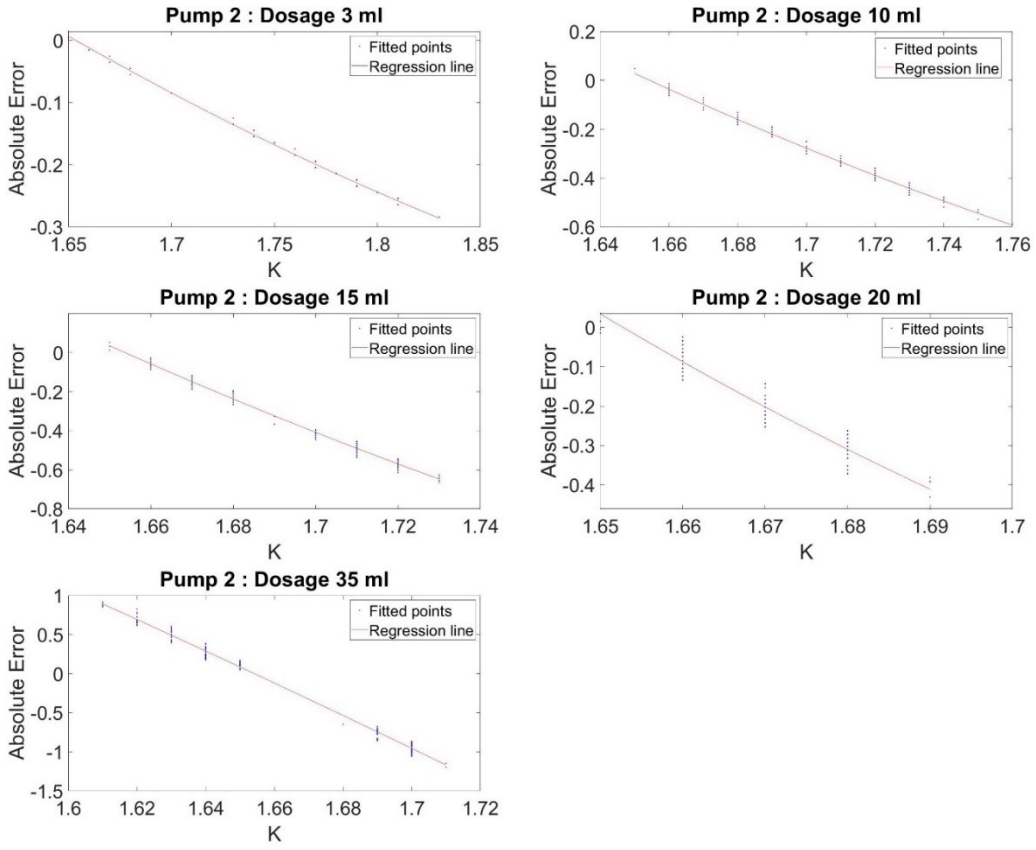


Figure 4.21. Pump 2: regression lines obtained from the polynomial regression model.

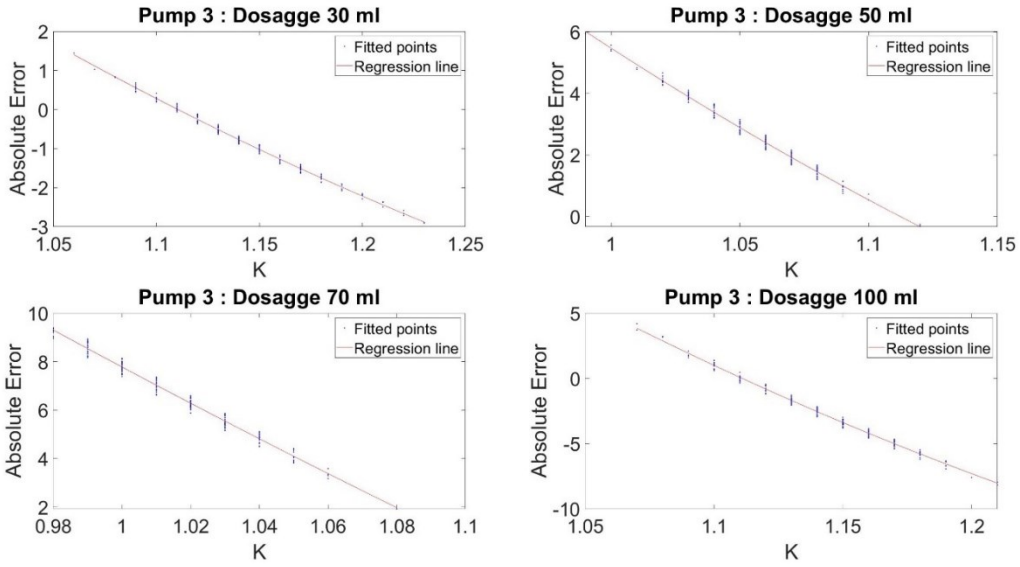


Figure 4.22. Pump 3: regression lines obtained from the polynomial regression model.

The next step is the validation of the model, always following a quantitative approach by calculating statistical variables and a qualitative one through the analysis of residue graphs. Starting from the evaluation of the quantitative approach, the R-

square, RMSE and SSE values obtained for the models of each pump dosage are shown in Table 4.3.

Table 4.3. Representation of quantitative statistical variable for the dosages related to each pump by using polynomial regression model.

	Dosage (ml)	R-square	SSE	RMSE
Pump 1	10	0,996	0,060	0,018
	30	0,997	0,554	0,054
	50	0,997	1,449	0,087
	70	0,991	2,506	0,115
	100	0,999	5,200	0,166
Pump 2	3	0,995	0,003	0,004
	10	0,987	0,048	0,016
	15	0,985	0,109	0,024
	20	0,918	0,206	0,033
	35	0,992	0,695	0,060
Pump 3	30	0,991	1,195	0,078
	50	0,982	4,255	0,147
	70	0,979	9,354	0,219
	100	0,978	9,772	0,223

As can be seen in the table, the R-square values are slightly lower with respect those of simple linear regression, but globally high enough to define a minimal difference between the observed data and the model values. The RMSE values are relatively low, thus indicating a fit that is useful for prediction, whereas the SSE values for pump 1 and 3 are slightly higher.

Subsequently the analysis on the model validity by means of residual analysis has been performed. Seeing the scatter plots of pump 1 (Figure 4.23) and pump 2 (Figure 4.24) the parabolic pattern of the residuals, which was found when using the simple linear regression model, is no longer visible. The concentration of the residuals around the zero line with a random arrangement confirms the hypothesis of linearity and an error with equal variance.

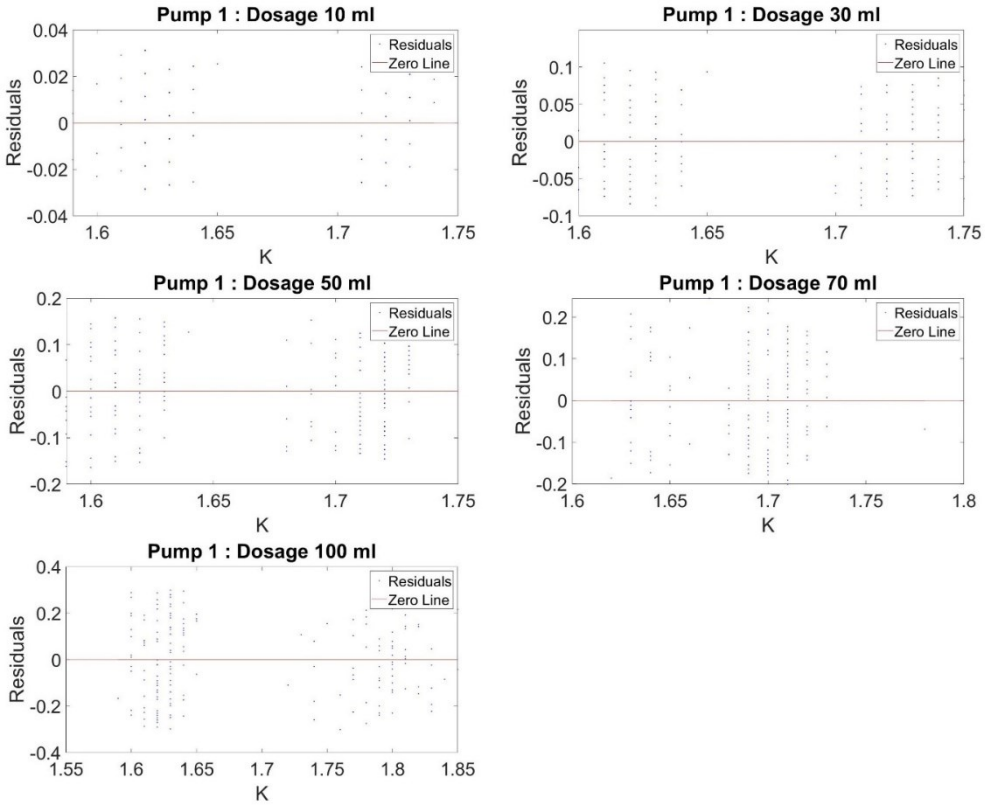


Figure 4.23. Pump 1: residuals scatter plot related to polynomial regression model.

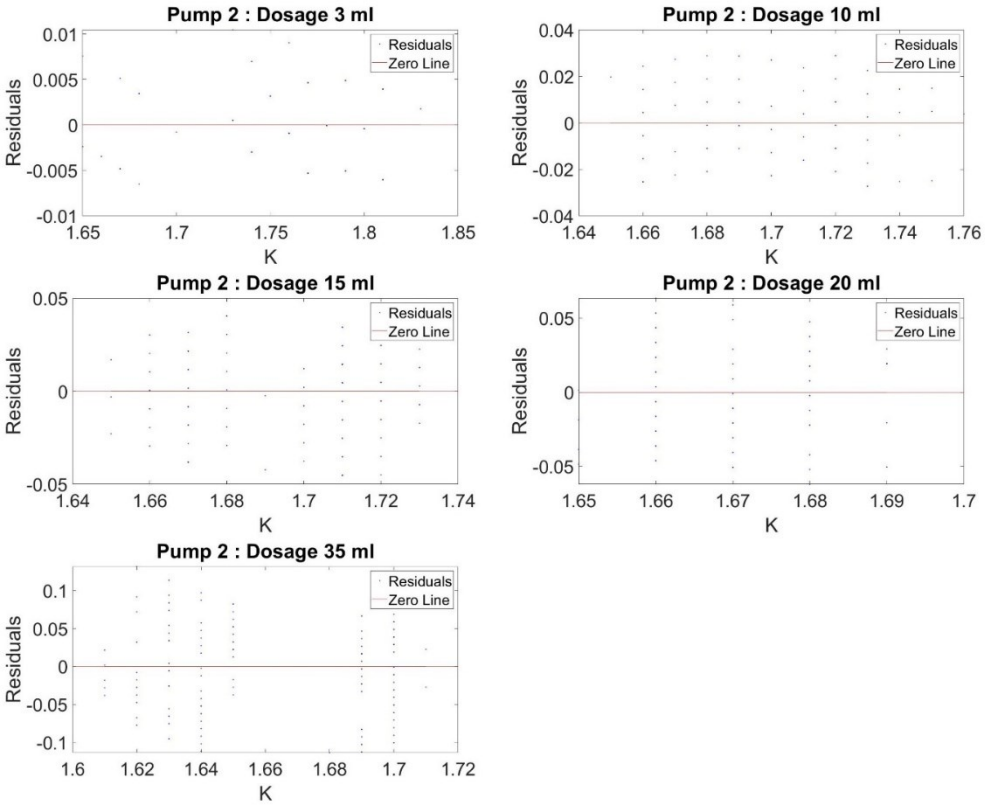


Figure 4.24. Pump 2: residuals scatter plot related to polynomial regression model.

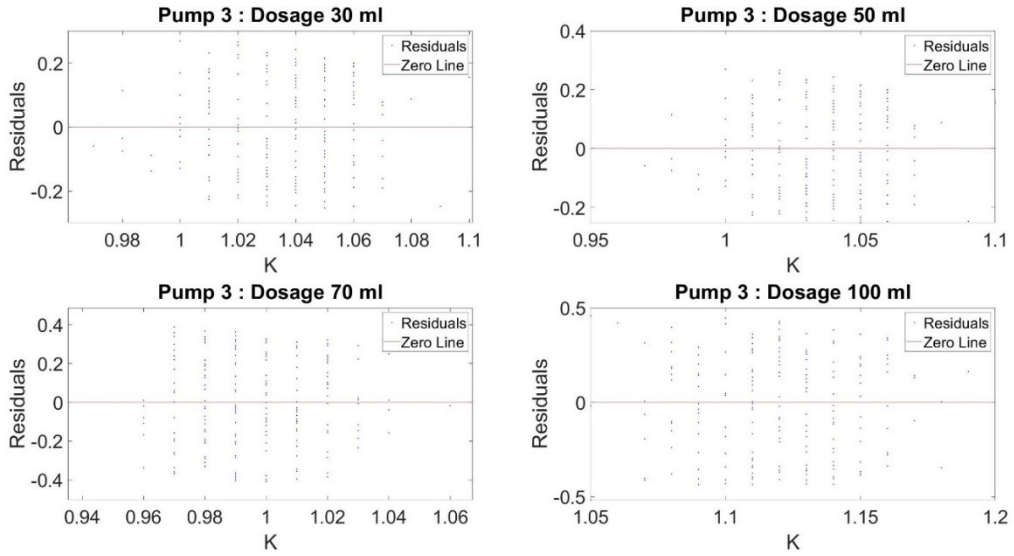


Figure 4.25. Pump 3: residuals scatter plot related to polynomial regression model.

To test the hypothesis concerning the independence of the errors, the plot of the residuals was evaluated as a function of the observation order for each dose relative to each pump. The latter trend is depicted in Figure 4.26 for pump 1, in Figure 4.27 for pump 2 and in Figure 4.28 for pump 3.

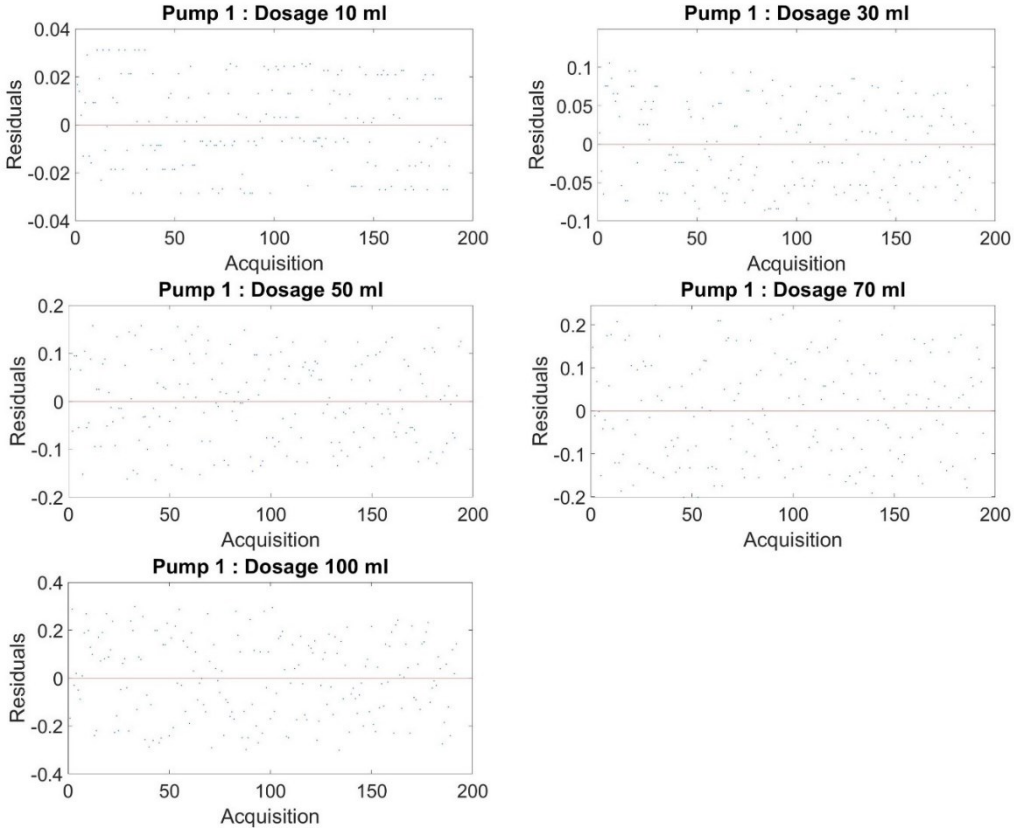


Figure 4.26. Pump 1: residuals trend in function of acquisitions number.

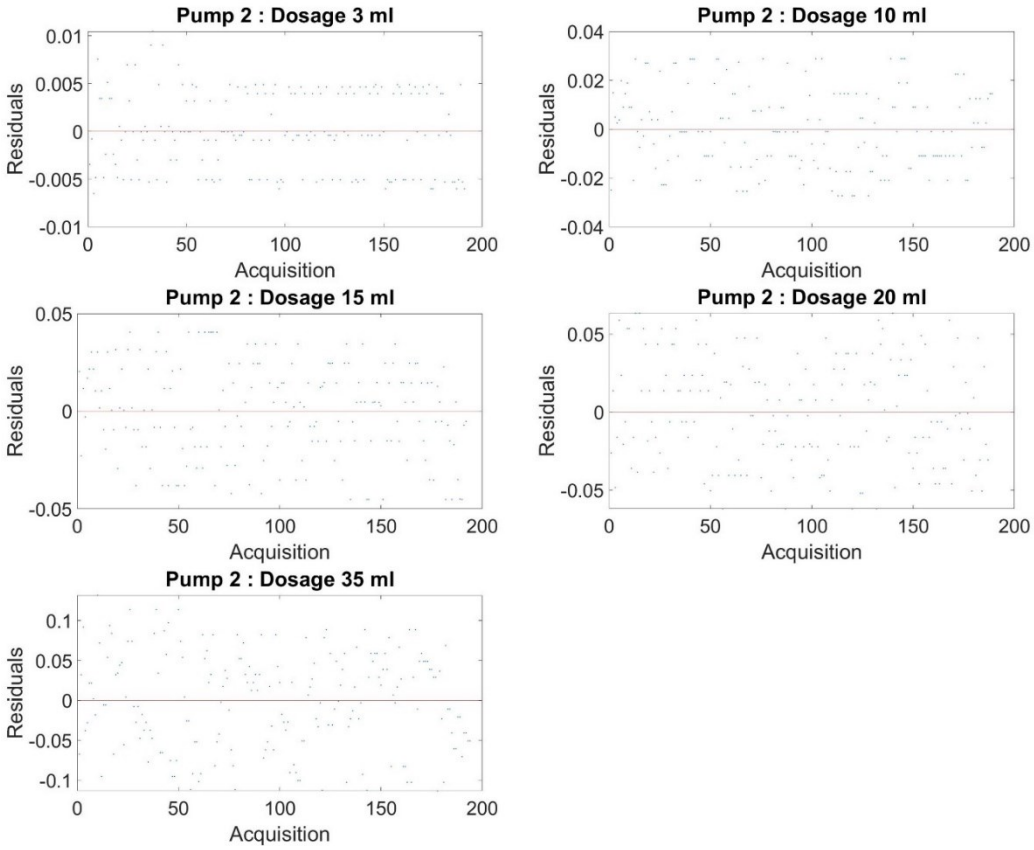


Figure 4.27. Pump 2: residuals trend in function of acquisitions number.

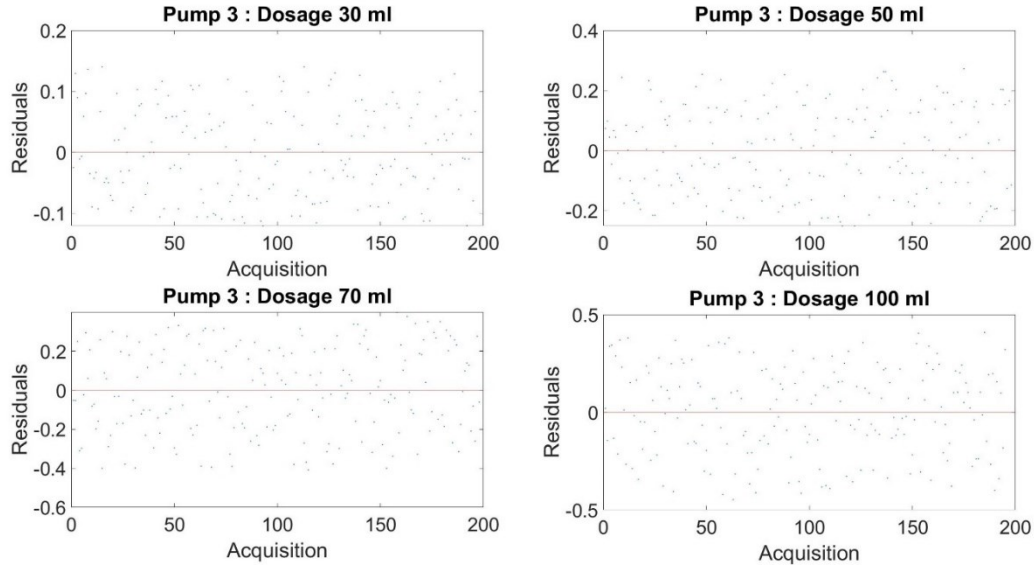


Figure 4.28. Pump 3: residuals trend in function of acquisitions number.

As shown in these figures, the residuals randomly bounce around the zero-line highlighted in red. This implies that the residuals exhibit normal random noise around the zero residual line suggesting that there is no serial correlation.

The verification of the last assumption concerning the normal distribution of the error has been tested by the Q-Q plot. It is a representation of residuals quantiles versus the theoretical quantile values from a normal distribution. If the distribution of residual is normal, then the data plot appears linear. The Q-Q plots for the residuals of each model are depicted in Figure 4.29 for pump 1, Figure 4.30 for pump 2 and Figure 4.31 for pump 3.

In these figures is evident that the relationship between the theoretical percentiles and the sample percentiles is approximately linear for all the samples with a little deviation along the tails. Thus, the normal probability plot of the residuals suggests that the residuals are normally distributed.

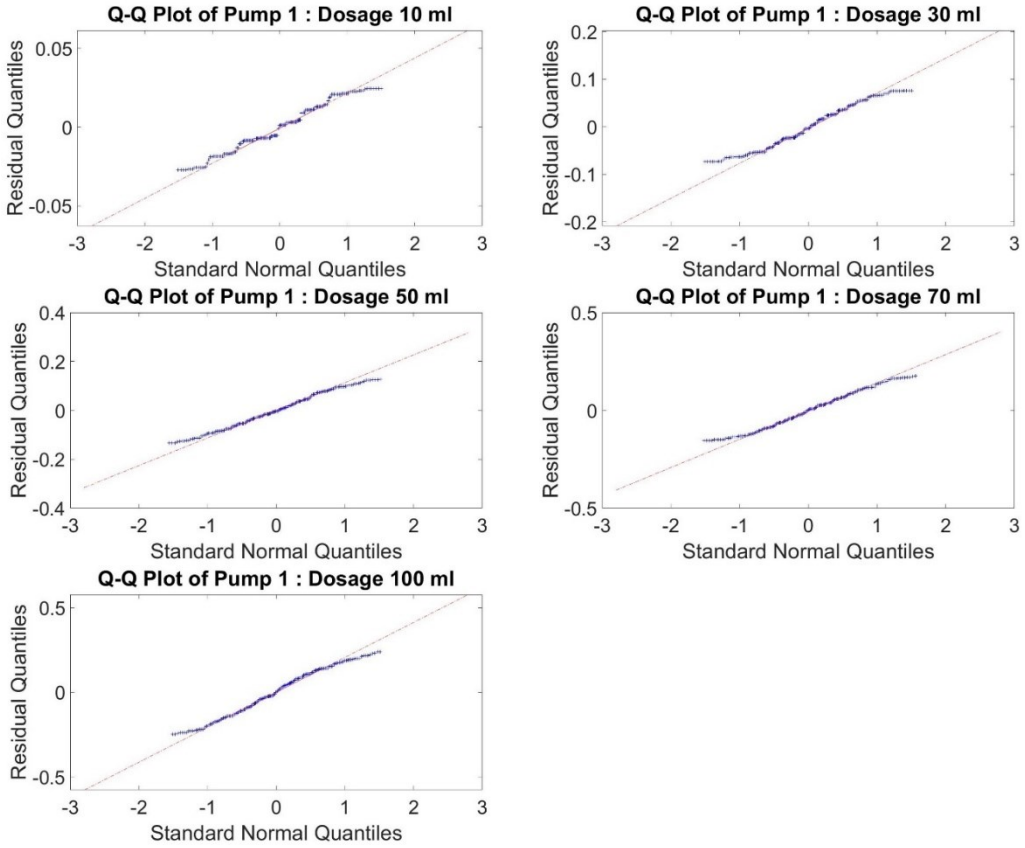


Figure 4.29. Pump 1: residuals Q-Q plot for polynomial regression model.

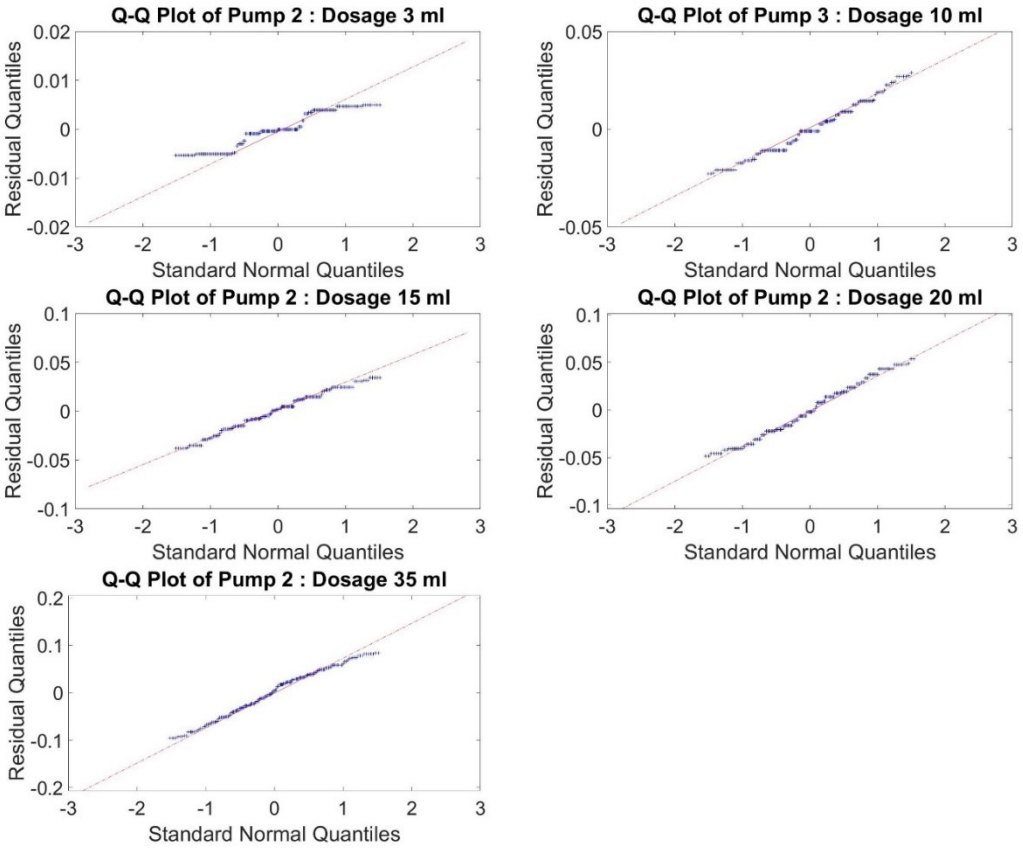


Figure 4.30. Pump 2: residuals Q-Q plot for polynomial regression model.

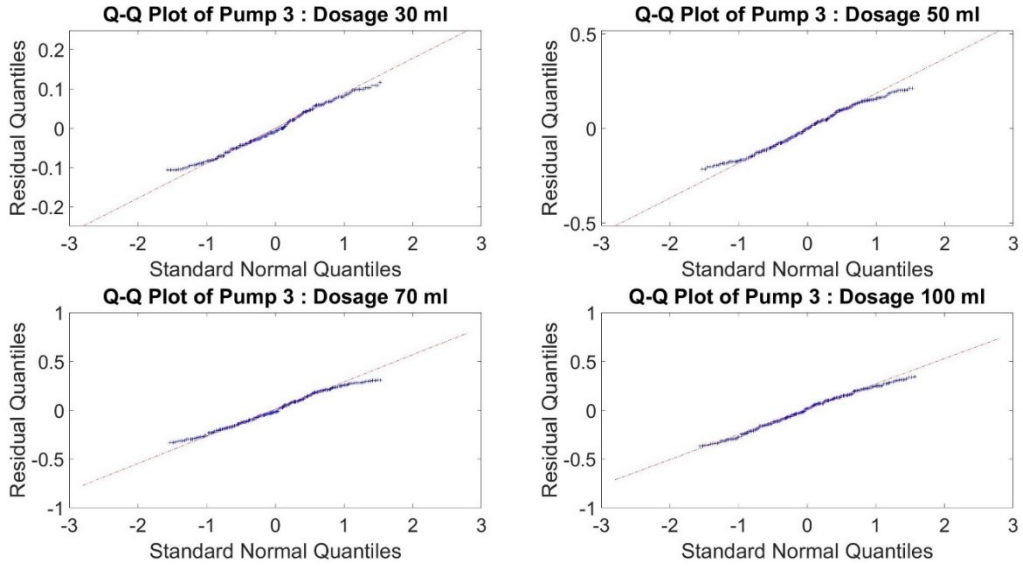


Figure 4.31. Pump 3: residuals Q-Q plot for polynomial regression model.

Importantly to remark that the reported results are for a K -factor whose theoretical measurement error (E_t) set a priori is -1% of the theoretical dosage.

Once the validity of the different models, and thus the quality of the fitting, has been established, the step forward is the evaluation of the model in order to predict the K variable.

The evaluation of the model was performed by inverse problem solving since the predictor variable is K and the response is the absolute error. Therefore, considering that the objective is to define the correction calibration factor, which corresponds to parameter being sought, an absolute error was defined in which to evaluate the model. The error choice to estimate the optimal calibration factor is based on the calculation of a weighted average according to the repetitions number of a given error.

Specifically, the absolute errors relative to the acquisitions performed before the presence of pauses were considered and then divided into two vectors containing positive and negative values respectively. The choice of considering only the errors before a break was made in order to increase the validity of the calibration factor estimate without introducing the variability related to the break. The two new vectors were analysed via a histogram fitted by a normal distribution to define the mean value of the error and the corresponding number of occurrences. Knowing this latter information, the weighted mean of the two datasets has been performed between the mean of the two normal distributions weighted by their probability of occurrence.

The value obtained will correspond to the absolute error in which the model will be evaluated. The absolute errors calculated by weighted averaging are given in Table 4.4 for each dosage per pump. In addition, the calibration factors estimated from the corresponding model are also reported.

Table 4.4. Representation of the weighted error used as input of the polynomial regression model and the factor K as the prediction of the model.

	Dosage (ml)	Weighted Error	K
Pump 1	10	0,303	1,66
	30	0,9605	1,65
	50	1,9016	1,64
	70	1,4043	1,67
	100	1,4987	1,68

Pump 2	3	-0,0301	1,67
	10	-0,2051	1,69
	15	-0,208	1,67
	20	-0,0732	1,66
	35	0,4065	1,63
Pump 3	30	-0,9725	1,15
	50	2,7602	1,05
	70	5,9688	1,02
	100	-0,6529	1,12

Considering the calibration facts estimated by means of the polynomial regression models shown in Table 4.4, it is possible to define a single factor per pump by averaging those relative to each dosage.

The data processing and analysis that led to the definition of an optimal calibration factor for each peristaltic pump by using a polynomial regression model was repeated three more times by setting the following values as the theoretical dosing error (E_t) imposed a priori in Eq. 3 for the calculation of K: -0.5%. 0% and 1%. The different calibration factor for each pump given the a-priori imposed measurement error are shown in Table 4.5.

Table 4.5. Estimated calibration factor for each pump according to the theoretical absolute error E_t.

Calibration Factor K			
e %	**Pump 1**	**Pump 2**	**Pump 3**
1	1,66	1,70	1,09
0	1,65	1,68	1,08
-0,5	1,64	1,67	1,07
-1	1,63	1,66	1,06

The following step is to experimentally verify the values obtained through the verification procedure illustrated in section 3.2.4. Analysing the values of the dosage error per acquisition, the coefficient relative to the smallest average of the absolute errors was chosen as the optimal calibration factor K for the pump given a present a-priori error.

The results for the mean value of the percentage errors module for each imposed error in Eq. 3 are shown in Table 4.6.

Table 4.6. Mean value of the percentage errors module for each imposed theoretical error.

	Pump 1			
MEAN OF THE PERCENTAGE ERROR MODULE	K = 1,66	K = 1,65	K = 1,64	K = 1,63
	6,14	3,81	1,92	1.32
	Pump 2			
	K = 1,7	K = 1,68	K = 1,67	K = 1,66
	5,84	4,62	2,33	1,7
	Pump 3			
	K = 1,09	K = 1,08	K = 1,07	K = 1,06
	3,62	2,55	2,07	2

As can be seen in Table 4.6, the smallest average errors for each pump are associated with the following optimal calibration factors:

- K = 1.63 for pump 1;
- K = 1.66 for pump 2;
- K = 1.06 for pump 3.

The calculated actual mean error does not coincide with the corresponding theoretical error (E_t) present in Eq. 3. This is due to a non-negligible value of the SSE variable (Table 4.3) which reflects a certain degree of model error in the prediction of the variable. In addition, the verified estimated calibration factor value corresponds to an average of the factors for the individual dosages relative to a pump. This means that the final calibration factor considered for each pump will be slightly different from that corresponding to the individual doses and will therefore induce an overdosing and/or underdosing effect depending on whether the difference is positive or negative.

4.3 Test peristaltic tube

The testing procedure defined in paragraph 3.2.4 is also useful for the characterisation of peristaltic tubes in order to define when its replacement is recommended for the assurance of good dosing accuracy. The processing and analysis conducted concerns the measured volume data in millilitres and the corresponding error. The error data are the same as those used in the 4.2 paragraph, thus after removal both NaN and outliers. The vector containing the delivered volume measurements was processed in the same way as the error. Indeed, firstly a removal of the Nan which will result to be the same samples removed from the vector containing the errors. Secondly, for pump 1 and 2 a subsequent identification and removal of the outliers by the median method applied in a 20 samples window movable along the entire vector. Differently, for a pump 3 a quartile-based method has been applied by setting the threshold equal to 2.25. The identification and removal of outliers for dispensed volumes for each test are shown in Figure 4.32 for pump 1,Figure 4.33 for pump 2, and Figure 4.34 for pump 3.

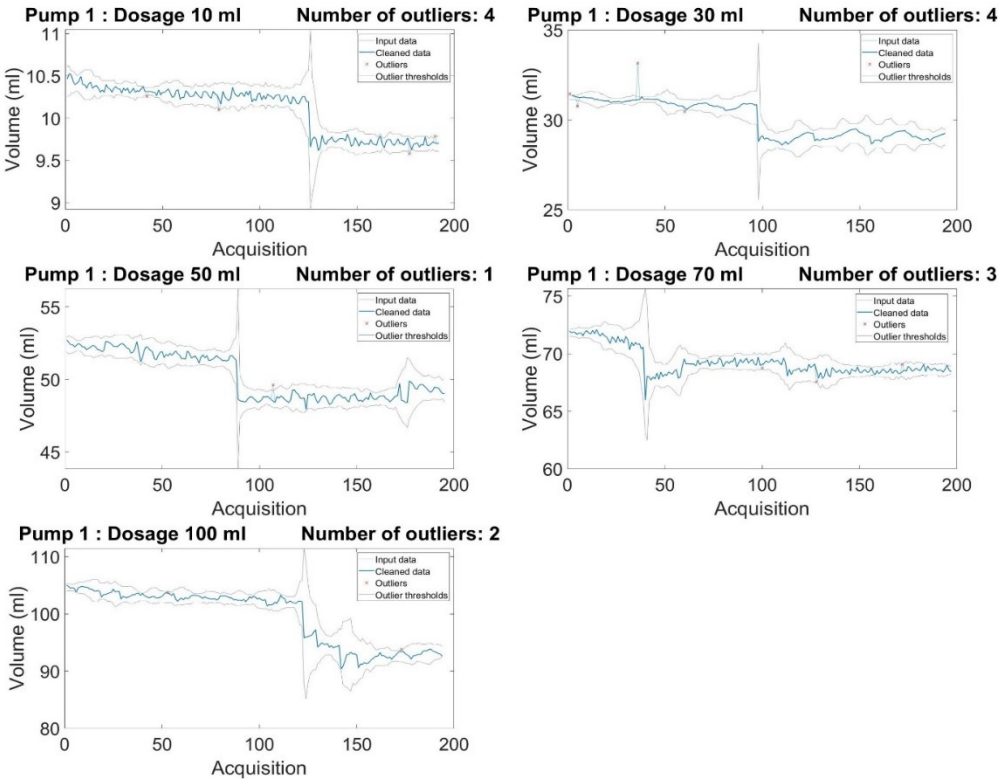


Figure 4.32. Pump 1: outliers removal from delivered volume datasets.

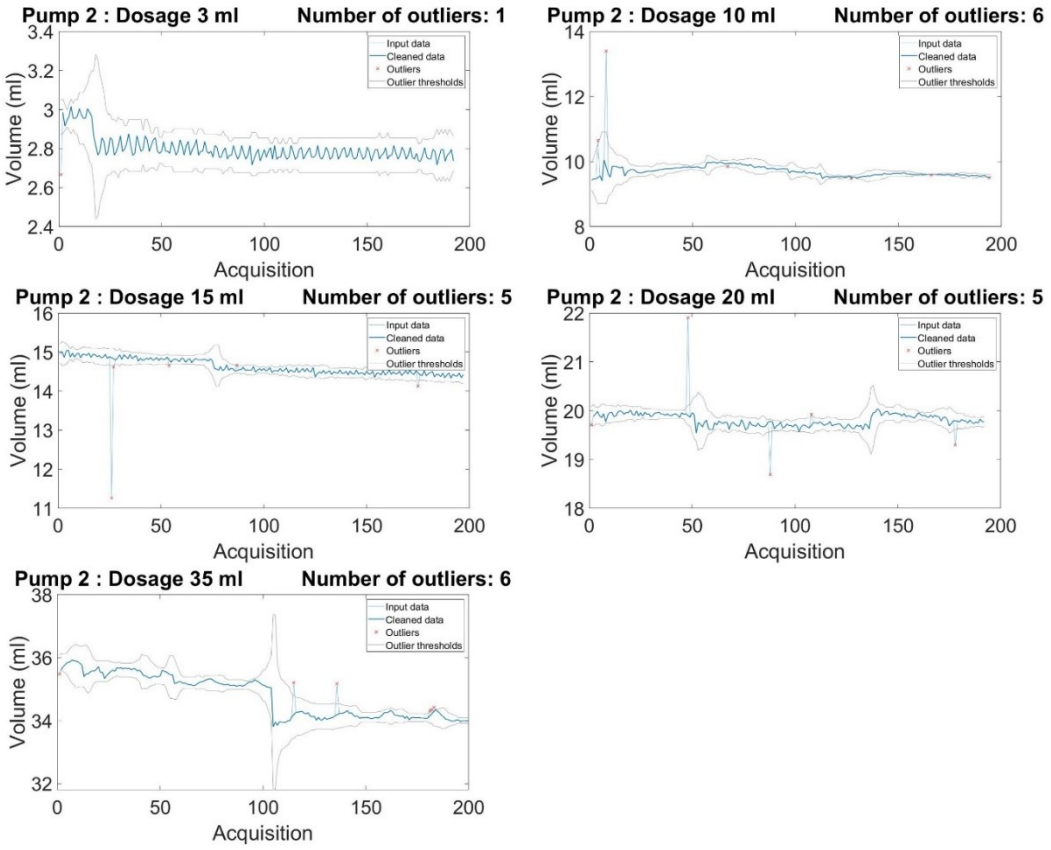


Figure 4.33. Pump 2: outliers removal from delivered volume datasets.

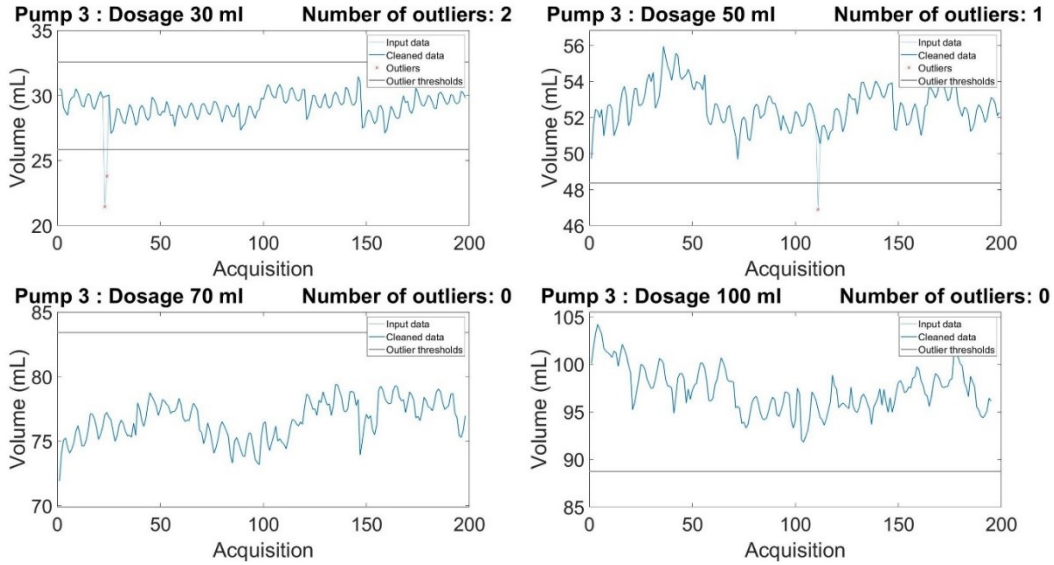


Figure 4.34. Pump 3: outliers removal from delivered volume datasets.

The Figure 4.32 shows the identification and removal of several outliers: 4 for the 10ml and 30ml dosage, 1 for the 50ml dosage and, finally, 3 and 2 for the 70ml and 100ml dosage. For pump 2 (Figure 4.33), 1 data sample was removed for the 3ml dosage, 6 for the 10ml and 35ml dosages and 5 for the 15ml and 20ml dosages. In contrast, the outliers removed for pump 3 (Figure 4.34) are 2 for the 30ml dosage, 1 for the 50ml dosage and zero for the 70ml and 100ml dosages. Note that in the latter two dosages no data sample was removed, as a fairly high threshold was chosen for the quartile method. This means that has been decreased the sensitivity range for outliers decision rules. Indeed, a bigger scale would make the outliers to be considered as data points, while a smaller one would make some of the data points to be perceived as outliers.

For a better evaluation of the error variation a smoothing procedure of the error vectors associated with each dose of pumps was performed. It is important to note that the data processing is based on the absolute error, while for a better visual analysis the corresponding percentage error is represented. The smoothing method applied, as defined in paragraph 3.2.5, is a Gaussian-weighted moving average over each window along the vector dimension. The window size is determined heuristically and can be scaled according to the defined threshold. Values near zero produce smaller moving window lengths, resulting in less smoothing, while values near to one produce larger moving window lengths, producing more smoothing. The threshold used to smooth the data is equal to 0.45.

The evolution of the error after the smoothing procedure was analysed as a function of the increase in volume delivered by the peristaltic tube. These trends are shown for each dosages of pump 1 in Figure 4.35, for pump 2 in Figure 4.36 and finally in Figure 4.37 for pump 3. In the figures, a green delimiter (or delimiters) representing a pause during the acquisition phase is highlighted. In particular, the pause may correspond to a duration of 1.30h, 14 h or even several days. The pauses differ from each other depending on the situation in which the tube is left during the breaks. Indeed, for some breaks the tube is left inside the peristaltic pump, while for others it is removed and placed outside the APOTECAbag system.

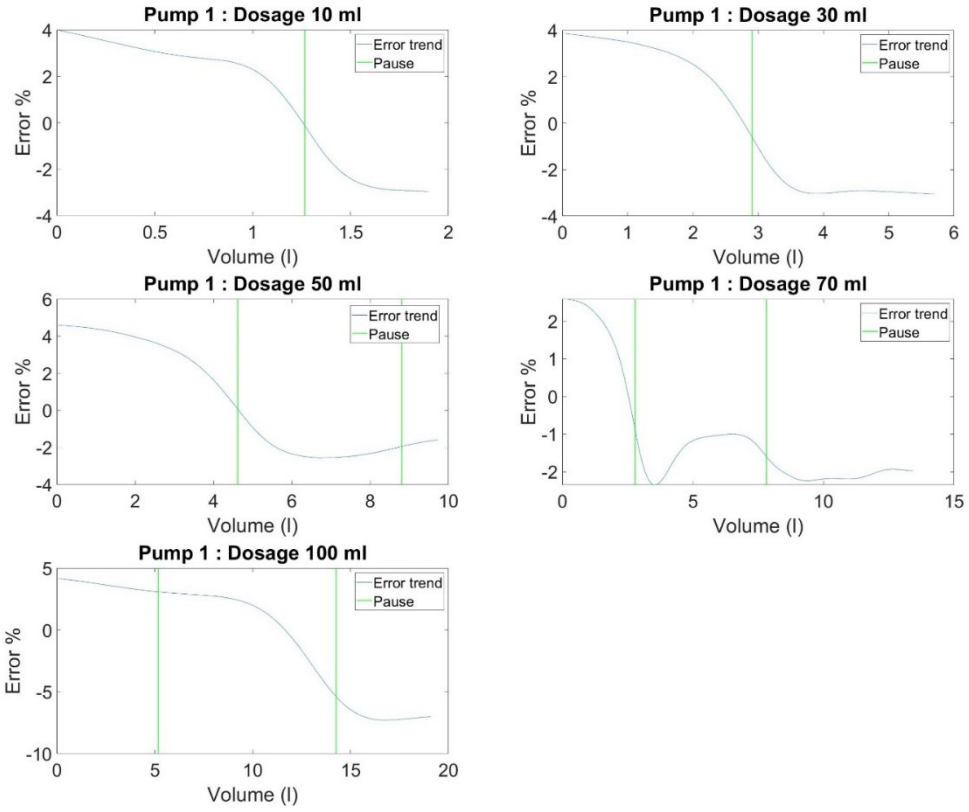


Figure 4.35. Pump 1: smoothed error trend in function of measured volume.

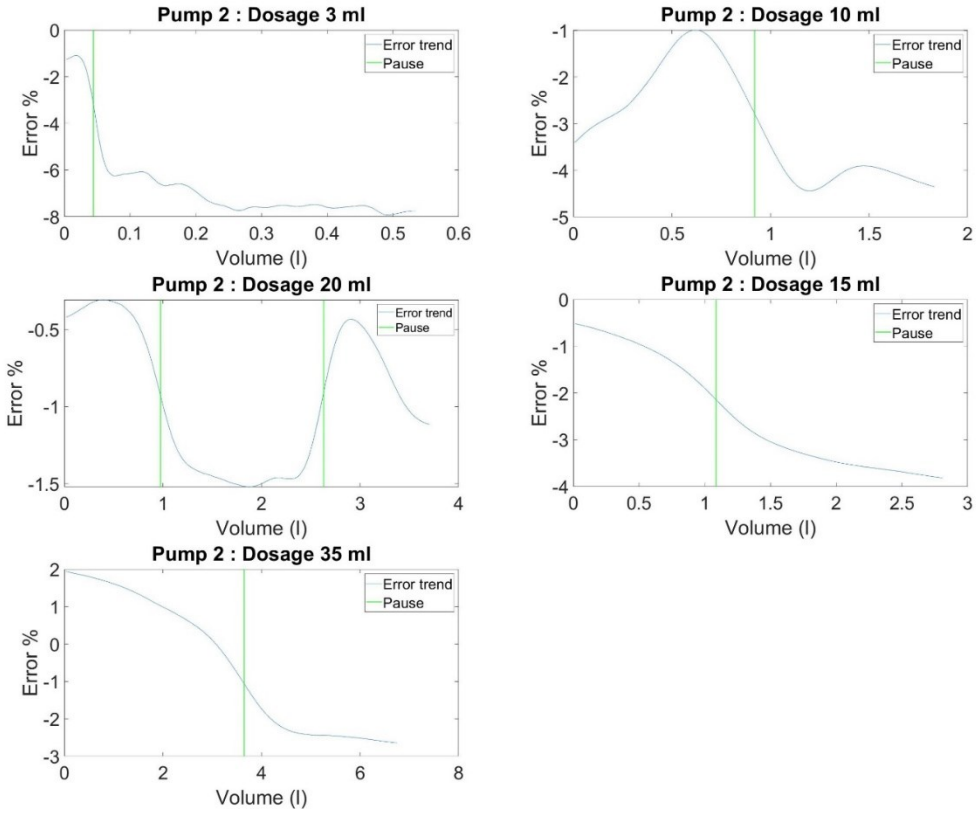


Figure 4.36. Pump 2: smoothed error trend in function of measured volume.

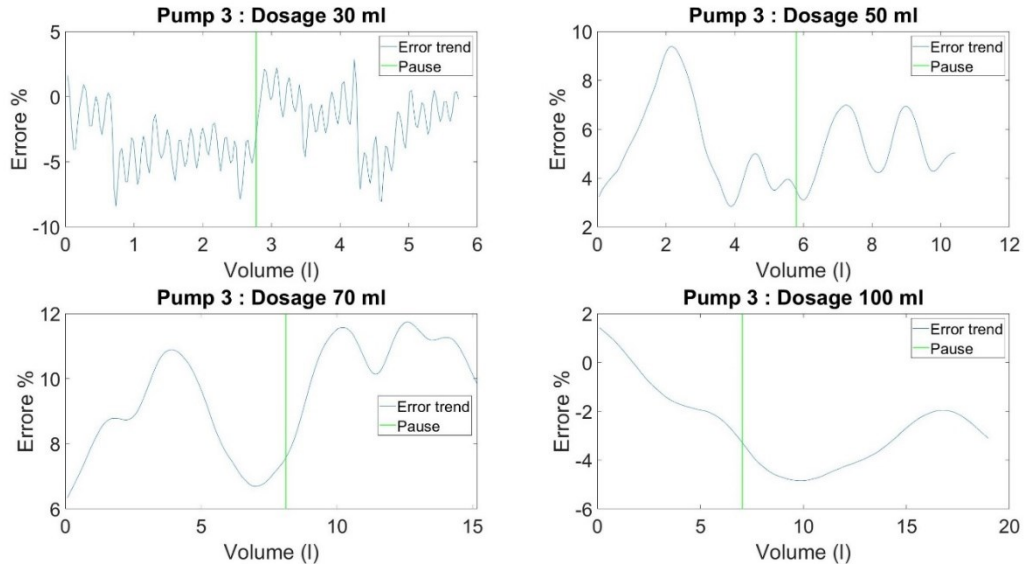


Figure 4.37. Pump 3: smoothed error trend in function of measured volume.

Analysing the effect of the pause in the use of the peristaltic tube on the error of the dosages relative to pump 1 (Figure 4.35), the following changes in the percentage error can be noted:

- Dosage 10ml: from 2% to -3.5% after a 1 h pause in which the tube was removed from the pump;
- Dosage 30ml: from 2.8% to -4% after a pause of 14 h in which the tube was left inside the pump;
- Dosage 50ml: from 2.7% to -3% after a first pause of 1 h and another step from -3% to -0.2% after a 14 h pause. For both pauses the tube was left inside the pump;
- Dosage 70ml: 0.8% to -5.7% after a 14 h pause and another step from -1.1% to -2.8% after a 1 h pause. For both pauses the tube was left inside the pump;
- Dosage 100ml dosage: after a first pause of 1 h there is no noticeable change, while after a pause of 14 h there is a jump from-5.6% to -9.6%. For both pauses the tube was left inside the pump.

The effects of breaks in use relative to pump 2 (Figure 4.36) on the percentage error are as follows:

- Dosage 3ml: -1% to -7.8% after a 14 h break in which the tube was removed from the pump;

- Dosage 10ml: -2.2% to -3.5% after a break of 6 days in which the tube was removed from the pump;
- Dosage 15ml: from -1.5% to -2.7% after a break of 1 h in which the tube was left inside the pump;
- Dosage 20ml: from -1.8 % to -0.2 % after a pause of 1 h in which the tube was removed from the pump
- Dosage 35ml: from 0.1% to -3.4% after a pause of 1 h in which the tube was left inside the pump.

Finally, the effects of pump 3 interruption (Figure 4.37) on the change in error in percentage are:

- Dosage 30ml: from -5.5% to -0.7% as a result of a 3 day break in which the tube was removed from the pump;
- Dosage 50ml: from 4.4% to 1% as a result of a 1 h pause in which the tube was removed from the pump;
- Dosage 70ml: from 8.8% to 6% as a result of a pause of 1 h in which the tube was removed from the pump;
- Dosage 100ml: from -1.8% to -4.4% after as a result of a pause of 1 h in which the tube was removed from the pump.

In addition, with a close look at sample number 120 of the 100ml dose for pump 1 and the sample 50 of the 20ml dose for pump 2, there is a further sharp variation in the percentage error despite the absence of a pause. These variations are due to rapid removal and subsequent insertion of the tube. The reason why the variation occurs may be related to the different pump pinching point on the peristaltic tube and, therefore, the fluid will be affected by the slight obstruction due to the tube deformation associated with the previous pinching point.

To obtain a clear view of the effect of interruption use on the accuracy of the peristaltic tube, Table 4.7 shows the error variation of the sample before the break compared to the one after. All this is categorised according to the pause duration and whether the tube is left inside or outside the pump.

Analysing the variation of the error when the pause occurs, it can be seen that for all doses there is a decrease in error both when the tube is left inside the pump and when it is removed. In particular, these changes induced by the effect of the utilization pause cause a decrease in error and therefore a lower dosage. The negative effect of breaks on dosing accuracy is found for all doses other than 20ml for pump 2 and 30ml for pump 3 where there is a positive change in percentage error. Using these results, it is possible to evaluate the effect of non-use time of the peristaltic tube on dosing accuracy.

Table 4.7. Representation of the errors variation when occurs a use interruption of use. e%↓ means a reduction; e%↑ means an increment; = no variation.

		1 h pause		1h or more pause	
	Dosage	**Tube out pump**	**Tube in pump**	**Tube out pump**	**Tube in pump**
Pump 1	**10ml**	e%↓			
	30ml				e%↓
	50ml		e%↓		e%↓
	70ml		e%↓		e%↓
	100ml		=		e%↓
Pump 2	**3ml**			e%↓	
	10ml			e%↓	
	15ml		e%↓		
	20ml	e%↑			
	35ml		e%↓		
Pump 3	**30ml**			e%↑	
	50ml	e%↓			
	70ml	e%↓			
	100ml	e%↓			

In accordance with the aim of the thesis, it is also important to characterise the wear of the peristaltic tube according to time of use by quantifying the maximum volume that can be delivered with appropriate accuracy. The wear linked to the time of use is due to the numerous revolutions made by the cylinders inside the pump, which press the tube guaranteeing the peristaltic phenomenon. To verify this last aspect, the error trends relative to the maximum dosages for each peristaltic pump were considered.

Taking into consideration the 100ml dosage relative to pump 1 (Figure 4.35) it is possible to verify how the B Recon Line tube is able to ensure an accuracy of -7% up to a total delivery of 19 l. For the B Double Filling Line, considering the 35ml dosage for the branch relative to pump 2 (Figure 4.36) the tube manages to dispense up to approximately 7 l with an accuracy of -2.5%, while for the 100ml dosage associated with pump 3 (Figure 4.37) the tube is able to dispense up to 19 l with an error of -3%. It should be remarked that the different peristaltic tubes are able to ensure a good accuracy for a given maximum delivery volume despite pauses occurring.

5 Conclusion

The main focus of this thesis has been the testing and validation of two critical components in the automated APOTECAbag system: the peristaltic pump and the vial clamp. The work performed was of fundamental importance for the validation of a prototype that will soon be on the market. The tests conducted on the two versions of the vial clamp were very useful in confirming the usefulness of this device. Indeed, its use reflects numerous benefits both in terms of production time, by introducing alternative gripping points, and economically thanks to the use of a pneumatic rather than an electric robot gripper.

An analysis of the results obtained from the two testing procedures shows the need to use the two clamp versions according to the neck diameter. In fact, it can be seen that version two is able to properly fix the 2ml, 20ml and 80ml vials, and for these last two formats there is good handling by the anthropomorphic and cartesian robots in both full and empty vial conditions. It should be emphasised that a low fixing and a quite good handling by the robots is also visible with the use of version one, mainly in the condition in which the vials are empty. In fact, during the analysis of the 80ml vial, good handling of the robots was observed when the vial was empty but, after the reconstitution phase, the fulfilled vial escaped from the clamp during the different movements. With regard to the 20ml vial, good manipulation was appreciated both in the full and empty vial condition. For the 100ml vial, it is strictly necessary to use version one to avoid problems connected with both the decentralised fixation of the vial with respect to the device and inadequate handling by the robot.

From the present analysis, it is evident how the different use of the two clamp versions is not only strictly linked to the vial diameter but also to its capacity, and therefore its weight. In fact, the 20ml vial, which has a slightly larger neck diameter than the 80ml vial, can be handled by both versions because it is not heavy when full. On the other hand, the 80ml vial, despite the small difference in the neck diameter, its use is restricted to version two since when full it is necessary to ensure a strong fixation

to support the high weight. The conclusion of the testing is summarised in Table 5.1, which shows the different use of the versions according to the vials.

Table 5.1. Clamp validation conclusions. The cross marks the proper version choice according to the vial. V2 refers to clamp version two; V1 refers to clamp version one.

Vial	Neck Diameter	V2 Clamp	V1 Clamp
20ml empty	17 mm	X	X
20ml filled	17 mm	X	X
80ml empty	16.5 mm	X	X
80ml filled	16.5 mm		X
100ml empty	25 mm		X
100ml filled	25 mm		X

Thus, it can be concluded that the testing conducted leads to a clamp validation that differentiates the use of its two versions according to diameter and weight relative to the maximum capacity.

Moving on to the peristaltic pump, the tests conducted aim to validate the device itself but also the tubing used. The estimated factor for the pump within the reconstitution area is 1.63 ensuring an average error of 1.32%. The estimated calibration factor for the pump located in the dosing area that doses the drug is 1.66 with an average accuracy of 1.7% while for the pump that doses solvent it is 1.06 with an average error of 2%.

Although the error percentages obtained with the estimated calibration factors do not perfectly coincide with the theoretical error defined in Eq. 3, they are still valid and adequate to obtain a good quality of the final product according to regulations that impose a maximum dosing error of 10%[13].

The conclusion is that the validation of peristaltic pumps conducted in this study was extremely important for the definition of the optimal calibration factor. Moreover, the latter is relevant to ensure a good quality of the final preparation but, above all, to reduce the compounding time of the APOTECAbag system in order to comply with the project specifications, which envisage an average productivity of about 100 bags per hour.

Focusing on the peristaltic tubes, it is important to remark that within the APOTECAbag system, before starting the preparation of a bag batch, the software interface asks to replace the peristaltic tube when the scheduled preparation employ a different active ingredient from the previous batch. When the preparation batch involves the same active ingredient as the previous batches, no tube replacement is required and theoretically could be used over and over again. Thus, one of the main questions concerning the tube that this thesis answered was its validation to characterize the replacement both in terms of time of use and non-use. The results obtained from the testing procedure reflect how the tubes are able to dose up to a well-defined volume ensuring a given accuracy within the 10% dosing error, after which the tube wear becomes significant leading to a high error.

Based on the results obtained from the analysis, it was possible to validate the B Recon Filling Line tube defining a replacement after the delivery of 19 l.

The estimated value is also valid for the branch that doses the drug in the B Double Filling line tube since comparing it with the previous one has the same internal diameter, the same material and inserted in the pump with the same inner position. The validation of the branch that doses the solvent lead to define the replacement after a dose of 19 l. Thus, it is possible to conclude that B Double Filling line tube replacement will occurs after delivery of 19 l.

From the results concerning the tube replacement after a time of non-use, the performances decrease after 1.30 h of inactivity both in the condition in which the tube is left inside the pump and outside. To sum up, the work also validated the characterisation of the peristaltic tubes that is relevant for ensuring the performance of the calibrated peristaltic pumps with the estimated optimal factors.

It should be considered that the obtained tests and validations have some limitations to be evaluated. Regarding the use of the clamp, the tests were conducted on a limited number of laboratory vials in comparison with the large different vial geometries present in the market.

Therefore, future studies will be necessary to validate the clamps on a larger number of vials but, above all, to define dimensional criteria within the drug market for which a vial should be considered as one with a large or small neck.

For the validation of the peristaltic pump, one limiting aspect is the testing performed using the solvent NaCl at 0.9% concentration to simulate the drug. This introduces a future development of the thesis, which will focus on analysing and verifying whether the densities and/or viscosities of the different drugs can influence the accuracies obtained by the estimated optimal calibration factors.

The future validation developments defined can be conducted in the Ospedali Riuniti of Ancona thanks to the research and development laboratory, Lab@AOR.

6 Bibliography

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7 Appendix

7.1 Definition of initial calibration factor

In this section the results obtained from the preliminary testing procedure to define the initial calibration factors are reported. These parameters will be used in the testing procedures for each peristaltic pump defined in section 3.2.4. Table 7.1 for pump 1, Table 7.2 for pump 2 and Table 7.3 for pump 3 show the experimental values obtained by changing different calibration factors set a priori in the APOTECAbag system. The lowest average value of the error modulus has been evaluated to choose the corresponding initial factors.

Table 7.1. Experimental data obtained from the pump 1 preliminary testing procedure.

Pump 1												
Calibration Factor	**1,68**				**1,69**				**1,7**			
$V_{teorico}$ (ml)	**$V_{misurato}$ (g)**	**$V_{misurato}$ (ml)**	**e %**	**$\lvert e_{assoluto} \rvert$**	**$V_{misurato}$ (g)**	**$V_{misurato}$ (ml)**	**e %**	**$\lvert e_{assoluto} \rvert$**	**$V_{misurato}$ (g)**	**$V_{misurato}$ (ml)**	**e %**	**$\lvert e_{assoluto} \rvert$**
5	4,87	4,84	-3,10	0,16	4,97	4,94	-1,11	0,06	4,94	4,91	-1,71	0,09
5	4,85	4,82	-3,50	0,18	4,96	4,93	-1,31	0,07	4,99	4,96	-0,72	0,04
5	4,87	4,84	-3,10	0,16	4,96	4,93	-1,31	0,07	4,99	4,96	-0,72	0,04
10	9,89	9,84	-1,61	0,16	10,03	9,98	-0,22	0,02	10,1	10,05	0,48	0,48
10	9,88	9,83	-1,71	0,17	9,88	9,83	-1,71	0,17	10	9,95	-0,52	0,05
10	9,96	9,91	-0,92	0,09	9,93	9,88	-1,21	0,12	10,03	9,98	-0,22	0,02
15	14,91	14,83	-1,11	0,17	15	14,92	-0,52	0,08	15,08	15,00	0,01	0,00
15	14,91	14,83	-1,11	0,17	15	14,92	-0,52	0,08	15,24	15,16	1,07	0,16
15	14,91	14,83	-1,11	0,17	14,85	14,77	-1,51	0,23	15,24	15,16	1,07	0,16
20	19,88	19,78	-1,11	0,22	20,12	20,02	0,08	0,02	20,14	20,04	0,18	0,04
20	19,93	19,83	-0,87	0,17	20,02	19,92	-0,42	0,08	20,14	20,04	0,18	0,04
20	19,94	19,84	-0,82	0,16	20,1	20,00	-0,02	0,00	20,27	20,17	0,83	0,17
			Mean =	**0,16**			**Mean =**	**0,08**			**Mean =**	**0,11**

Table 7.2. Experimental data obtained from the pump 2 preliminary testing procedure.

Pump 2												
Calibration Factor	**1,66**				**1,67**				**1,68**			
$V_{teorico}$ (ml)	**$V_{misurato}$ (g)**	**$V_{misurato}$ (ml)**	**e %**	**$\|e_{assoluto}\|$**	**$V_{misurato}$ (g)**	**$V_{misurato}$ (ml)**	**e %**	**$\|e_{assoluto}\|$**	**$V_{misurato}$ (g)**	**$V_{misurato}$ (ml)**	**e %**	**$\|e_{assoluto}\|$**
5	4,87	4,84	-3,10	0,16	4,91	4,88	-2,31	0,12	4,98	4,95	-0,92	0,05
5	4,91	4,88	-2,31	0,12	4,89	4,86	-2,71	0,14	5,11	5,08	1,67	0,08
5	4,9	4,87	-2,51	0,13	4,85	4,82	-3,50	0,18	5,02	4,99	-0,12	0,01
10	9,77	9,72	-2,81	0,28	9,94	9,89	-1,11	0,11	10,29	10,24	2,37	0,24
10	9,86	9,81	-1,91	0,19	9,95	9,90	-1,01	0,10	10,17	10,12	1,17	0,12
10	9,87	9,82	-1,81	0,18	9,94	9,89	-1,11	0,11	10,28	10,23	2,27	0,23
15	14,77	14,69	-2,04	0,31	14,88	14,80	-1,31	0,20	15,49	15,41	2,73	0,41
15	14,74	14,66	-2,24	0,34	15	14,92	-0,52	0,08	15,41	15,33	2,20	0,33
15	14,69	14,61	-2,57	0,39	14,9	14,82	-1,18	0,18	15,45	15,37	2,47	0,37
20	19,84	19,74	-1,31	0,26	20,03	19,93	-0,37	0,07	20,68	20,57	2,87	0,57
20	19,93	19,83	-0,87	0,17	19,96	19,86	-0,72	0,14	20,68	20,57	2,87	0,57
20	19,94	19,84	-0,82	0,16	20,03	19,93	-0,37	0,07	20,72	20,61	3,06	0,61
			Mean =	**0,22**			**Mean =**	**0,12**			**Mean =**	**0,30**

Table 7.3. Experimental data obtained from the pump 3 preliminary testing procedure.

Pump 3												
Calibration Factor	**1,08**				**1,1**				**1,21**			
$V_{teorico}$ (ml)	**$V_{misurato}$ (g)**	**$V_{misurato}$ (ml)**	**e %**	**$\|e_{assoluto}\|$**	**$V_{misurato}$ (g)**	**$V_{misurato}$ (ml)**	**e %**	**$\|e_{assoluto}\|$**	**$V_{misurato}$ (g)**	**$V_{misurato}$ (ml)**	**e %**	**$\|e_{assoluto}\|$**
5	4,59	4,57	-8,67	0,43	4,64	4,62	-7,68	0,38	5,76	5,73	14,60	0,73
5	4,73	4,71	-5,89	0,29	4,86	4,83	-3,30	0,17	5,42	5,39	7,84	0,39
5	4,89	4,86	-2,71	0,14	4,93	4,90	-1,91	0,10	5,51	5,48	9,63	0,48
10	9,59	9,54	-4,60	0,46	9,71	9,66	-3,40	0,34	11,75	11,69	16,89	1,69
10	9,65	9,60	-4,00	0,40	10,08	10,03	0,28	0,03	11,35	11,29	12,91	1,29
10	9,83	9,78	-2,21	0,22	10,27	10,22	2,17	0,22	11,73	11,67	16,69	1,67
15	14,98	14,90	-0,65	0,10	15,48	15,40	2,67	0,40	16,98	16,89	12,61	1,89
15	15	14,92	-0,52	0,08	14,82	14,74	-1,71	0,26	17,33	17,24	14,94	2,24
15	15,04	14,96	-0,25	0,04	14,99	14,91	-0,58	0,09	17,21	17,12	14,14	2,12
20	20,15	20,05	0,23	0,05	20,63	20,52	2,62	0,52	23,18	23,06	15,30	3,06
20	19,81	19,71	-1,46	0,29	20,19	20,09	0,43	0,09	23,3	23,18	15,90	3,18
20	19,74	19,64	-1,81	0,36	19,87	19,77	-1,16	0,23	23,41	23,29	16,44	3,29
			Mean =	**0,24**			**Mean =**	**0,23**			**Mean =**	**1,84**

7.2 Definition of APOTECAbag requirements

The Table 7.4 shows the requirements for the APOTECAbag system: general requirements, process requirements, equipment requirements, interface requirements, automation requirements, regulatory requirements.

Table 7.4.APOTECAbag requirements.

General Requirements	Requirement Description
Language	Interface language and Manual language must be English
Installation	Correct installation of the System must be executed and documented in a proper way.
Qualification Document	The system must have the following document: • Factory and Site Acceptance Test Protocol
Maintenance	The system has to guarantee the possibility of and accessible maintenance by appointed and properly trained personnel. The system shall be supported by an adequate Maintenance Manual
Cleaning	The manufacturer shall provide the cleaning instructions and a list of cleaning material.
Maintenance Schedule	The manufacturer shall provide schedule that identifies maintenance items and duration intervals.
Training	The system has to be provided together with an adequate training program at the various user's levels configured
Data Backup	The system has to guarantee the capability to reconstruct all relevant documentation from the backup copies (availability of the stored data).
Safety	The system has to satisfy the safety requirements related to the environment characteristics of the facility area in which the system will be installed and taking into account the local regulations.
Review and Change Control	Change Control procedures must be in place
Ease of Use	The system has to be easily configurable and user friendly
User Manual	The system has to be provided together with a User Manual
Upgrade	The System has to assure the possibility to upgrade and expand the configuration; in the case these changes are required for a better management of data

General Requirements	Requirement Description
Spare Parts	A spare parts listing shall be provided that includes: • Normal wear parts • Parts that are easily broken • Parts that can wear out, and are long lead time availability. The Manufacturer shall have a stock of frequently required spare parts
Quality Plan	The system shall have a Quality Plan defined by the Manufacturer
Quality System	The Manufacturer shall have a quality system in place.
Documentation	Following document shall be available (paper or pdf): • Technical Specifications • Factory Acceptance Test • Site Acceptance Test • Software Integration Test • User Manual • Maintenance Manual • Process and Instrumentation Diagram • Instrument Listing • Electrical Diagram • Assembly Drawings • Mechanical Drawings • Assembly Drawings • Design verification and validation • Spare Parts List • Risk Analysis
Process Requirements	Requirements Description
Work Instructions	The system shall be able to fill IV bags with non-hazardous sterile drugs starting from drugs in glass vials both in liquid form and powder form

General Requirements	Requirement Description
	The system shall be able to perform bath production.
	The system shall be able to receive from a preparation management software all the data for a batch production: 1. Drug (name, batch, quantity) 2. Solvents (name, batch, quantity) 3. Bags 4. Preparation date 5. Use by date 6. Storage condition 7. Final dose 8. Final total volume
	The batch must contain only one type of final container
	Batch number must be unique
	For each batch the following information must be defined and traced: 1. Batch number 2. Preparation date 3. Use by date 4. Number of preparations 5. Identification barcode 6. Drug (name, batch, quantity) 7. Solvents (name, batch, quantity) 8. Storage conditions 9. Final dose 10. Final total volume
	The system shall allow to pharmacist the selection of: • Bag size • Drug

General Requirements	Requirement Description
	• Powder reconstitution • Drug dilution • Use by date • Storage condition of the preparation
Materials	The system shall be able to compound drugs for the administration to the patient both in NaCl/dextrose bags and in empty IV bags.
	The system shall be able to fill with drugs intermediate IV bags, to be used for: • subsequent dosing in syringes out of the system • direct administration to the patient
	The system shall be able to compound drugs both in liquid and powder forms.
	The system shall be able to handle and to use for drug compounding following I.V. injection bag brands: 1. Baxter Viaflo 2. Baxter Viaflex 3. Baxter Intravia
	The system shall be able to handle and to use empty I.V. bags as final container for preparation
	The system shall be able to handle and to use for powder reconstitution, for dilution activities, as final container and as intermediate bags following bags: 1. 0.9% Sodium Chloride Baxter Viaflo 2. 5% Glucose Baxter Viaflo 3. Water for Injection Baxter Viaflo 4. 0.9% Sodium Chloride Baxter Viaflex 5. 5% Glucose Baxter Viaflex 6. Water for Injection Baxter Viaflex
	The system shall be able to use as final container IV bags of following volumes: 1. 50ml 2. 100ml

<table>
<tr><th>General Requirements</th><th>Requirement Description</th></tr>
<tr><td></td><td>
3. 250ml
4. 500ml
</td></tr>
<tr><td></td><td>The system shall be able to use as intermediate container, empty IV bags with a volume of 1000ml.</td></tr>
<tr><td></td><td>The system shall be able to use only for drug dilution or powder reconstitution, at least one bag of following volumes:
1. 1000ml
2. 2000ml
3. 3000ml
4. 4000ml
5. 5000ml</td></tr>
<tr><td></td><td>The system shall be able to handle and to use at least following drug vials:
<table>
<tr><th>ID</th><th>Generic Name</th><th>Vial Dimension</th><th>Form</th><th>Concentration</th><th>Average dosage (mg)</th><th>Final volume (ml) of bag</th></tr>
<tr><td>1.</td><td>Vancomycin</td><td>10g</td><td>Powder</td><td>10g in 95ml WFI</td><td>7.5ml
10ml
12.5ml
15ml
17ml
20ml</td><td>250ml
500ml</td></tr>
<tr><td>2.</td><td>Cefazolin</td><td>10g</td><td>Powder</td><td>10g</td><td>2g</td><td>50ml</td></tr>
<tr><td>3.</td><td>Cefoxitin</td><td>10g</td><td>Powder</td><td>10g</td><td>2g/7ml</td><td>50ml</td></tr>
<tr><td>4.</td><td>Ampicillin</td><td>10g</td><td>Powder</td><td>10g</td><td>2g/6.8ml</td><td>100ml</td></tr>
<tr><td>5.</td><td>Methohexital</td><td></td><td>Powder</td><td></td><td>4 vials</td><td>1000ml</td></tr>
<tr><td>6.</td><td>Phenylephrine</td><td>10ml</td><td>Liquid</td><td>10mg/ml</td><td>1ml
8ml</td><td>250ml</td></tr>
</table>
</td></tr>
</table>

General Requirements	Requirement Description						
	7.	Norepinephrine	4ml	Liquid	1mg/ml	4ml	250ml
	8.	Heparin	30ml	Liquid	1000units/ml	4ml 5ml	1000ml 500ml
	9.	Epinephrine	30ml	Liquid	1mg/ml	4ml	250ml
	10.	Calcium Chloride	10ml	Liquid	10mg/ml	10ml	100ml
	11.	Dexamethasone	1ml	Liquid	4mg/ml	2ml	50ml
	12.	Lidocaine	50ml	Liquid	10mg/mL		1000ml
	13.	Phenylephrine	10ml	Liquid	100mcg/mL	10.8	1000ml
	14.	Epinephrine	1 - 10 - 30ml	Liquid	10mcg/mL	10.8	1000ml
	15.	Neostigmine	10ml	Liquid	1mg/mL		1000ml
	16.	Nitroglycerin	10ml	Liquid	5mg/mL	21.5ml	1000ml
	17.	Norepinephrine	4ml	Liquid	1mg/mL	4.4ml	500ml
	18.	Ephedrine		Liquid	50mg/mL	117ml	1000ml
	19.	Epinephrine	1 - 10 - 30ml	Liquid	1mg/mL	0.5ml 5.5ml	500ml
	20.	Phenylephrine	10ml	Liquid	10mg/mL	0.55	500ml
	21.	Potassium Chrloide	20ml - 30ml	Liquid	2 mEq/mL	10ml	100ml
	22.	Hydralazine	1ml	Liquid	20 mg/mL	0.5ml	50ml
	23.	Potassium Phosphate	50ml	Liquid	3 mmol/mL	10mmol	100ml
	24.	Sodium Phosphate	5ml - 15ml	Liquid	3 mmol/mL	3.3ml	100ml
	25.	Bupivacaine	30ml	Liquid	7.5 mg/mL	20.8ml	250ml
	26.	Midazolam	10ml	Liquid	5 mg/mL	20ml	100ml
	27.	Magnesium Sulfate	50ml	Liquid	500 mg/mL	6g/12ml	50ml

<table>
<tr><th>General Requirements</th><th>Requirement Description</th></tr>
<tr><td rowspan="6"></td><td>
<table>
<tr><td>28.</td><td>Calcium Gluconate</td><td>100ml</td><td>Liquid</td><td>100 mg/mL</td><td>10ml
20ml
30ml</td><td>50ml
100ml</td></tr>
<tr><td>29.</td><td>Fentanyl Citrate</td><td>5ml-50ml</td><td>Liquid</td><td>50 mcg/mL</td><td>50ml</td><td>250ml</td></tr>
<tr><td>30.</td><td>Norepinephrine Bitartrate</td><td>4ml</td><td>Liquid</td><td>1 mg/mL</td><td>8ml</td><td>250ml</td></tr>
</table>
</td></tr>
<tr><td>The system shall be able to store all materials required by the work order in an internal warehouse</td></tr>
<tr><td>The system shall be able to handle automatically all the materials needed to perform the filling activities.</td></tr>
<tr><td>The transfer of drug from bags to vials for reconstitution and from vials to bags must be performed automatically by the system through disposable items.</td></tr>
<tr><td>At the end of a batch production, the system shall ask to operator to throw and replace the disposable items used.</td></tr>
<tr><td>If the disposables items include needles or spike with sharp tip, the operator must load the items with the protective cap and the system shall automatically remove the cap for use and put it back before the manual unloading of used disposables.</td></tr>
<tr><td rowspan="4">Loading</td><td>The system shall allow the loading of following materials used in preparations in aseptic conditions:
Bags
Drug vials
Tubes, connectors, spikes</td></tr>
<tr><td>Following operations must be performed in a dedicated zone of Grade A of the machine in aseptic conditions and under a laminar flow:
Removal of the IV bags from their primary packaging
Removal of tubes/connectors/spikes from their primary packaging
Removal of caps from vials</td></tr>
<tr><td>The system shall allow the loading of more than one component at a time.</td></tr>
<tr><td>During the loading of vials, the system shall allow the verification of the proper drug vial loading through a barcode reader or a vision system.</td></tr>
</table>

General Requirements	Requirement Description
	During the loading of bags, the system shall allow the verification of the proper bag loading through a barcode reader or a vision system.
Powder reconstitution	The system shall allow the reconstitution of powder drug with the right solvent.
	The system shall allow to reconstitute more than one vial at a time
	The system shall be able to verify the accuracy of solvent dosage for drug reconstitution
Drug dilution	The system shall allow the dilution of drug in IV bags with the right sterile solvent.
	The system shall allow the aspiration of solvent from bags.
	The system shall allow the injection of solvent in empty bags.
Drug dosage	The system shall be able to fill IV bags with the correct drug, dose and concentration.
	The system shall be able to verify the accuracy of dose in each IV bags
	The limits of dose accuracy for which a bag is accepted or automatically rejected should be configurable by the system administrator.
Labeling	If the accuracy is less than the acceptable limit, the bag must be defined as FAILED and identified with an appropriate label.
	If the accuracy is equal or better than the acceptable limit, the bag must be defined as successfully filled.
	In case of final bags for the patients the system shall allow the printing of a label with following information: 1. Batch Number 2. Number compared to the total 3. Preparation date 4. Use by date 5. Identification barcode 6. Storage condition 7. Drug (name, batch, quantity) 8. Final dose 9. Final total volume

General Requirements	Requirement Description
	If the intermediate bag is successfully filled with drug and the work instructions provide that it must be unloaded, the system shall allow the printing of a label with following information: 1.Drug 2.Concentration 3.Volume 4.Identification barcode 5.Use by date 6.Preparation date
	The system shall be able to print and to affix the label automatically on the final bags for the patients
	The system shall be able to print and to affix the label automatically on the intermediate bag if it must be unloaded.
	The system shall be able to store the filled bags in an internal warehouse
	The system shall be able to use RFID labels
	The RFID labels affixed in the IV bags, shall be programmed by the equipment before the unloading of IV bags.
Unloading	The system shall be equipped with a storage warehouse for the compounded bags in order to allow the operator to unload a defined number of bags at once.
	The area for the unloading of IV bags must be separated from the loading area
Waste management	The system shall be able to throw away in the internal waste container the empty vials after the compounding activities
	The system shall allow the replacement of a full waste container.