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Performance Qualification of Automatic System for Antineoplastic Preparation

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Abstract: The preparation of antiblastic and parental drugs should be carried out by ensuring an aseptic condition and minimizing exposure to toxic drugs. The aim of this study is to evaluate the quality and qualification of these features of an automated dispensing system, called PHARMODUCT®, built by Bioduct s.r.l, part of the Dedalus group. Three antiblastic drugs (cyclophosphamide (powder), 5-fluorouracil and paclitaxel) were used and three preparation and dispensing sessions were carried out for each drug, using PHARMODUCT®. Some of the infusion bags, prepared for each type of antiblastic, were sent to an external laboratory to perform the quantitative dosage analysis and compare it with the quantitative concentration, set on the automatic dispensing equipment, which was found to meet the acceptance criteria of 10%. In addition, to assess the safety of the process for operator exposure to toxic drugs, the differential pressure value between the main chamber and the clean room was measured to be <0 Pa, with an hourly leakage rate of $<2.5 \times 10^{-3}$ h^{-1}. Media fill tests showed no microbiological growth after a 14-day incubation period. The PHARMODUCT® system meets the requirements of safety and repeatability for the dispensation of parenteral antiblastic drugs.

Keywords: antineoplastic drugs; automated compounding; aseptic processing; robotics; dispensing module

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1. Introduction

As the number of new cancer cases increases, advances in medical and public health technology have enabled increasingly early diagnosis of these diseases and their subsequent treatment with chemotherapy. In fact, the demand for the preparation of anticancer drugs is increasing and as a result, automated sterile compounding systems have appeared on the market in recent years as an alternative to manual intravenous drug compounding (IVD) [1].

Although several options for nontoxic drug compounding have since become available, the main focus has remained on robots for cytostatic drug compounding [2]. This is because the proper dosage of parenteral drugs, along with microbiological stability and appropriate and effective cleaning of the environment, is a critical aspect to consider when setting up a galenic pharmacy, especially for oncology patients, who are particularly susceptible to infection and thus serious health damage [3].

The targets of hazardous drugs (HDs), as antineoplastic drugs, are all cells in the body, not only the cancer cells and the metastasis. HDs also affect normal cells inducing side effects such as hair loss, infertility, teratogenicity and immunosuppression. Therefore, it is important to limit exposure to HDs for healthy individuals who do not have cancer

diseases to avoid some adverse health effects. Dangerous effects of HDs can be caused through direct skin contact, inhalation, ingestion and accidental injection [4–6]. Numerous studies over the last decades have indicated a widespread HD contamination in hospital pharmacies and nurse stations, as well as the danger of HD exposure among pharmacy workers and nurses who handle these pharmaceuticals on a daily basis [7–13].

The benefits of robotic compounding over manual compounding, including increased safety for the patient and healthcare worker, improved workflow efficiency and total accountability of the process, have most recently led to the introduction of robotic compounding systems in pharmacies [14].

Automated systems are also important in hospital pharmacy to avoid medication errors, which are one of the major causes of adverse events that may cause serious harm to patients and lead to death. An automated system could support the safety of the patient medication process.

A critical phase of the medication process is the dispensing of specific drugs. This phase used to be performed manually by nurses and pharmacists, but recently, the use of an automated system has improved efficiency, and dispensing errors in the medication process have decreased [15,16].

Currently, there are several automated drug dispensing systems on the market, including some intelligent robotic systems (such as APOTECAchemo, ARCT, Cytocare) [17].

In order to minimize operator exposure [18] and manual handling of toxic drugs [19], which are recognized as potent dangerous drugs due to their intrinsic carcinogenic, mutagenic and nephrotoxic properties [20], while ensuring aseptic production conditions, another important automated system called PHARMODUCT® (Figure 1), built by Bioduct s.r.l. Firenze 50141, Italy part of the Dedalus group, was developed for the compounding and preparation of personalized antineoplastic therapies performed in the hospital [21].

Figure 1. The outside of the PHARMODUCT® automatic system built by Bioduct s.r.l. Firenze 50141, Italy, that is used for antineoplastic preparation.

This innovative system, designed and engineered to maintain a level of tightness appropriate for the proper classification of the laboratory in which the equipment will be installed, is set up with materials capable of separating a differential pressure zone (positive or negative) and acting as a physical separation barrier between the indoor work surface and the outdoor laboratory area.

This is made possible by the application of unidirectional laminar flow and pressure deltas designed to ensure that air never passes from the lower grade environment to the higher grade environment. Moreover, this system is proposed in an entirely niche market, expanding and ensuring constant availability in the face of ever-increasing demands from hospital pharmacies.

To date, in most cases, antiblastic drugs undergo manual compounding under a laminar flow hood in a class B clean room, and there are many risks associated with this activity, such as possible biological and particle contamination of the preparation, dosage and labelling mistakes, errors in prescription and transcription of therapy [22], as well as poor control of drug costs, waste and operator exposure to dangerous drugs. As a result, safety standards often fall short of pharmaceutical industry requirements.

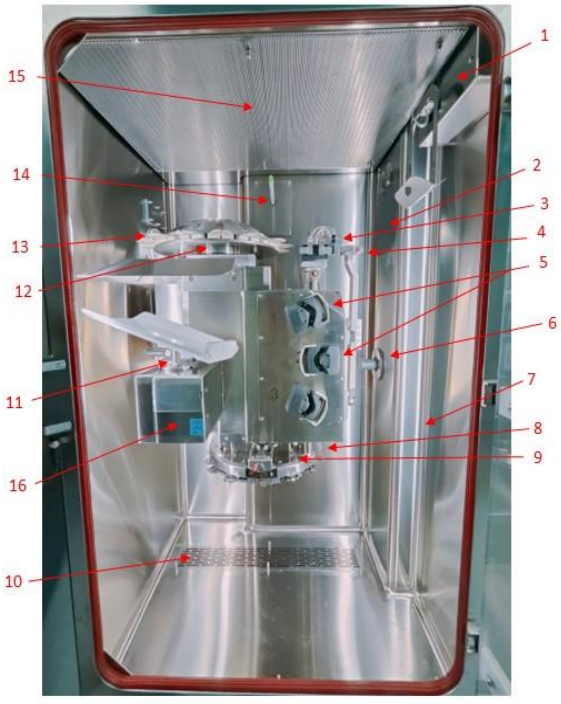
Therefore, the purpose of this study is to evaluate the performance of this robotic preparation system. Specifically, the system allows the automated production of multidose bags and final preparations of antiblastic drugs in a safe mode for the operator and for the preparation itself.

2. Materials and Methods

2.1. Compounding and Formulation System

The automated system is composed of a negative pressure laminar air flow chamber, with ULPA-U15 filters, classified as ISO Class 5, according to EN ISO 14644-1 [23] orresponding to class A-GMP [24].

It is equipped with precision scales, which control the weight accuracy of a set-up product, considering the specific weight of the drug and reconstitution liquid, peristaltic pumps for liquid transfer, a dissolution station for powders, barcode and RFID identification systems for consumables (kits) and a label reader for bottles. The internal view of the machine chamber is shown in Figure 2.



Reference	Description
1	Diluent solution bag scale
2	Automatic internal door
3	Rotor-translating clamp
4	Perforating head
5	Peristaltic pumps
6	Detection sensor tor ozone trend monitoring (maximum level)
7	LED lamps
8	Temperature and humidity monitoring probe
9	Lower rotating plate unit
10	Air extraction grid
11	RFID label reader
12	Upper rotating plate unit
13	Internal camera
14	Airflow speed monitoring probe
15	Air flow diffusor
16	Multidose bag balance

Figure 2. Internal view with technical details of the PHARMODUCT® automated system.

There is also an ozone decontamination tool to ensure proper cleaning of the working chamber and waste areas are isolated with thermally sealed bags.

The automated system thus helps the pharmacist to prepare drugs, according to NBP standards (Italian Pharmacopeia); in particular, it ensures proven rather than assumed dosing accuracy (with an accuracy of ±10%), without risk of microbiological contamination, significantly reducing, as our study showed, operator exposure, as well as waste production and dispensing and administration time also.

To evaluate the performance of the aseptic process of this automated system, three antiblastic drugs, the most representative preparations in clinical practice, were used in clinical practice: cyclophosphamide, available in powder form, 5-fluorouracil and paclitaxel, both available in solution for infusion.

The correct mixing of powders and the correct execution of the various steps in the multidose bag preparation cycle, the filling cycle of the final containers and the operator safety were verified. For each drug included in this study, three preparation and dispensing sessions were performed using the PHARMODUCT® machine built by Bioduct s.r.l. Firenze 50141, Italy.

After disinfection and subsequent bio-decontamination of the instrument, the dispensing module was prepared for the execution of a processing cycle by inserting the final kits, accessories and containers inside the chamber, which were necessary to perform proper mixing of the powders and final dispensing. Subsequently, the antiblastic drug powder container was introduced.

For each work section, concerning each antiblastic, five infusion bags with different concentrations have been prepared, as specified in Tables 1–3. Two of the five infusion bags for each run per type of antiblastic were sampled and sent to a laboratory to perform the quantitative assay analysis.

Table 1. Recipe for preparation of infusion bags at different concentrations for the fluorouracil drug.

Fluorouracil							
	DRUG			Diluent (NaCl 0.9%)	Total (Drug + Diluent)	Bag Name	Theoretical Concentration
RUN° 1 (High dosage)	Fluorouracil 1	6000 mg	120 mL	180 mL	300 mL	Run1_1	20 mg/mL
	Fluorouracil 2	6000 mg	120 mL	180 mL	300 mL	Run1_2	20 mg/mL
	Fluorouracil 3	6000 mg	120 mL	180 mL	300 mL	Run1_3	20 mg/mL
	Fluorouracil 4	6000 mg	120 mL	180 mL	300 mL	Run1_4	20 mg/mL
	Fluorouracil 5	6000 mg	120 mL	180 mL	300 mL	Run1_5	20 mg/mL
RUN° 2 (Low dosage)	Fluorouracil 1	600 mg	12 mL	138 mL	150 mL	Run2_1	4 mg/mL
	Fluorouracil 2	600 mg	12 mL	138 mL	150 mL	Run2_2	4 mg/mL
	Fluorouracil 3	600 mg	12 mL	138 mL	150 mL	Run2_3	4 mg/mL
	Fluorouracil 4	600 mg	12 mL	138 mL	150 mL	Run2_4	4 mg/mL
	Fluorouracil 5	600 mg	12 mL	138 mL	150 mL	Run2_5	4 mg/mL
RUN° 3 (Random dosage)	Fluorouracil 1	600 mg	12 mL	138 mL	150 mL	Run3_1	4 mg/mL
	Fluorouracil 2	1800 mg	36 mL	264 mL	300 mL	Run3_2	6 mg/mL
	Fluorouracil 3	2400 mg	48 mL	252 mL	300 mL	Run3_3	8 mg/mL
	Fluorouracil 4	3800 mg	76 mL	224 mL	300 mL	Run3_4	12.7 mg/mL
	Fluorouracil 5	4200 mg	84 mL	216 mL	300 mL	Run3_5	14 mg/mL

Table 2. Recipe for preparation of infusion bags at different concentrations for the paclitaxel drug.

Paclitaxel							
	DRUG			Diluent (NaCl 0.9%)	Total (Drug + Diluent)	Bag Name	Theoretical Concentration
RUN° 1 (High dosage)	Paclitaxel 1	350 mg	58.33 mL	441.67 mL	500 mL	Run1_1	0.7 mg/mL
	Paclitaxel 2	350 mg	58.33 mL	441.67 mL	500 mL	Run1_2	0.7 mg/mL
	Paclitaxel 3	350 mg	58.33 mL	441.67 mL	500 mL	Run1_3	0.7 mg/mL
	Paclitaxel 4	350 mg	58.33 mL	441.67 mL	500 mL	Run1_4	0.7 mg/mL
	Paclitaxel 5	350 mg	58.33 mL	441.67 mL	500 mL	Run1_5	0.7 mg/mL
RUN° 2 (Low dosage)	Paclitaxel 1	75 mg	12.5 mL	237.5 mL	250 mL	Run2_1	0.3 mg/mL
	Paclitaxel 2	75 mg	12.5 mL	237.5 mL	250 mL	Run2_2	0.3 mg/mL
	Paclitaxel 3	75 mg	12.5 mL	237.5 mL	250 mL	Run2_3	0.3 mg/mL
	Paclitaxel 4	75 mg	12.5 mL	237.5 mL	250 mL	Run2_4	0.3 mg/mL
	Paclitaxel 5	75 mg	12.5 mL	237.5 mL	250 mL	Run2_5	0.3 mg/mL
RUN° 3 (Random dosage)	Paclitaxel 1	75 mg	12.5 mL	237.5 mL	250 mL	Run3_1	0.3 mg/mL
	Paclitaxel 2	100 mg	16.66 mL	233.34 mL	250 mL	Run3_2	0.4 mg/mL
	Paclitaxel 3	150 mg	25 mL	475 mL	500 mL	Run3_3	0.3 mg/mL
	Paclitaxel 4	200 mg	33.33 mL	466.67 mL	500 mL	Run3_4	0.4 mg/mL
	Paclitaxel 5	270 mg	45 mL	455 mL	500 mL	Run3_5	0.54 mg/mL

Table 3. Recipe for preparation of infusion bags at different concentrations for the cyclophosphamide drug.

Cyclophosphamide							
	DRUG			Diluent (NaCl 0.9%)	Total (Drug + Diluent)	Bag Name	Theoretical Concentration
RUN° 1 (High dosage)	Cyclophosphamide 1	1500 mg	75 mL	175 mL	250 mL	Run1_1	6 mg/mL
	Cyclophosphamide 2	1500 mg	75 mL	175 mL	250 mL	Run1_2	6 mg/mL
	Cyclophosphamide 3	1500 mg	75 mL	175 mL	250 mL	Run1_3	6 mg/mL
	Cyclophosphamide 4	1500 mg	75 mL	175 mL	250 mL	Run1_4	6 mg/mL
	Cyclophosphamide 5	1500 mg	75 mL	175 mL	250 mL	Run1_5	6 mg/mL
RUN° 2 (Low dosage)	Cyclophosphamide 1	600 mg	30 mL	220 mL	250 mL	Run2_1	2.4 mg/mL
	Cyclophosphamide 2	600 mg	30 mL	220 mL	250 mL	Run2_2	2.4 mg/mL
	Cyclophosphamide 3	600 mg	30 mL	220 mL	250 mL	Run2_3	2.4 mg/mL
	Cyclophosphamide 4	600 mg	30 mL	220 mL	250 mL	Run2_4	2.4 mg/mL
	Cyclophosphamide 5	600 mg	30 mL	220 mL	250 mL	Run2_5	2.4 mg/mL
RUN° 3 (Random dosage)	Cyclophosphamide 1	600 mg	30 mL	220 mL	250 mL	Run3_1	2.4 mg/mL
	Cyclophosphamide 2	700 mg	35 mL	215 mL	250 mL	Run3_2	2.8 mg/mL
	Cyclophosphamide 3	800 mg	40 mL	210 mL	250 mL	Run3_3	3.2 mg/mL
	Cyclophosphamide 4	900 mg	45 mL	205 mL	250 mL	Run3_4	3.6 mg/mL
	Cyclophosphamide 5	1000 mg	50 mL	200 mL	250 mL	Run3_5	4 mg/mL

In addition, for cyclophosphamide only, sterility and endotoxin tests were performed on the last dispensed bag of each cycle, according to European Pharmacopoeia methods [25,26].

Cyclophosphamide was chosen as the worst case for sterility and endotoxin testing because it is the most complex drug to prepare, in addition to being a contaminant drug, as reported by many studies, often found on the surfaces of pharmaceutical compounding areas [20]. Furthermore, the last dispensed bag of each cycle was chosen as it represents the most stressed unit, i.e., the one most susceptible to possible contamination.

The PHARMODUCT® dispensing module had to guarantee the dispensing of bags with a drug concentration, detected in the analyzed containers in each run, with a maximum error of ±10% (% discrepancy between the set and prescribed amount of drug).

In addition, each container analyzed for sterility had to be sterile for 14 days of incubation (as required by the European Pharmacopoeia) and the bacterial endotoxin content had to be less than 0.625 EU/mL.

The following tables summarized the type of preparations carried out to verify the performance of PHARMODUCT®.

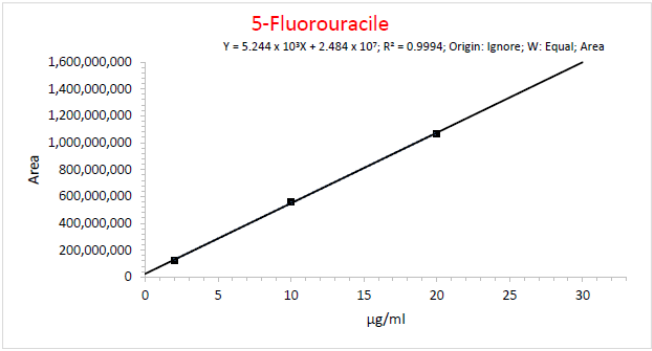
2.2. Quality Control Procedure

To perform quantitative assay analysis, reference standards of fluorouracil, paclitaxel and cyclophosphamide were obtained from Sigma Aldrich Merck (Darmstad, Germany). High-purity water was prepared in-house, using a gradient water purification system. LC-MS grade methanol was purchased from Honeywell–Riedel de Haen.

Formic acid (LC-MS grade) was bought from Carlo Erba. MS-grade ammonium formate was purchased from Sigma Aldrich.

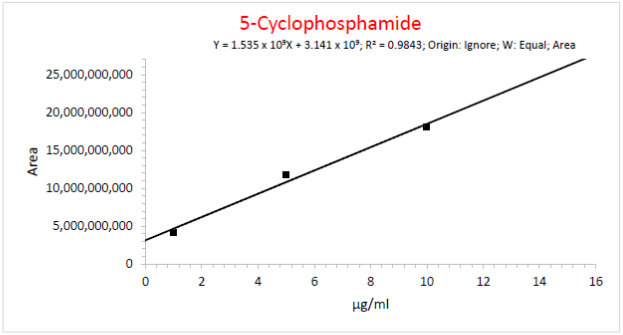
Stock solutions of fluorouracil, paclitaxel and cyclophosphamide were prepared in methanol at a concentration of 1000 µg/mL. Primary dilutions and working standard solutions were prepared from the stock solutions by dilution with water/methanol (90:10, *v*/*v*).

These working standard solutions were used to prepare the quality control samples and calibration curve, which are detailed below for each analyte, as shown in Figures 3–5.



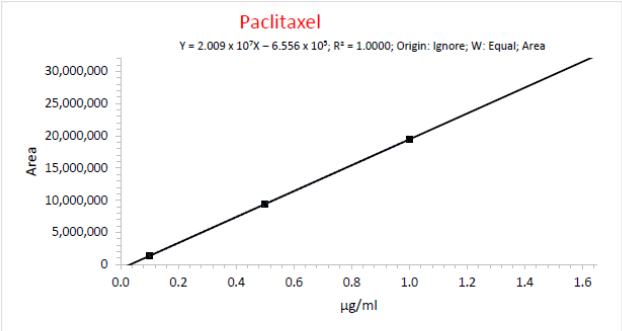
Level	Std Amount	Std Area	Resp factor/ ratio	Calc Amount	Units
L1	2	122259665	61129832.68	1,858	µg/ml
L2	10	562706500	56270649.97	10,256	µg/ml
L3	20	1067727624	53386381.21	19,886	µg/ml

Figure 3. Calibration report of the analyte 5-fluorouracil obtained using a UHPLC system with a high-resolution mass spectrometer.



Level	Std Amount	Std Area	Resp factor/ ratio	Calc Amount	Units
L1	1	4116395761	4116395761	0.636	µg/ml
L2	5	11823138938	2364627788	5.656	µg/ml
L3	10	18044205850	1804420585	9.708	µg/ml

Figure 4. Calibration report of the analyte cyclophosphamide obtained using a UHPLC system with a high-resolution mass spectrometer.



Level	Std Amount	Std Area	Resp factor/ ratio	Calc Amount	Units
L1	0.1	1346990	13469901.62	0.1	µg/ml
L2	0.5	9402642	18805284.48	0.501	µg/ml
L3	1	19431531	19431530.55	1	µg/ml

Figure 5. Calibration report of the analyte paclitaxel obtained using a UHPLC system with a high-resolution mass spectrometer.

Calibration samples were prepared at concentrations of 0.1, 0.5 and 1 µg/mL for paclitaxel, at concentrations of 1, 5 and 10 µg/mL for cyclophosphamide and at concentration of 2, 10 and 20 µg/mL for fluorouracil.

Samples were prepared by dilution in water/methanol (90:10 v/v). A total of 2 µL of the sample was injected into the UHPLC–HRMS system through the autosampler.

Chromatographic separation was performed on a Thermo-Dionex ULTIMATE 3000 UHPLC system using a Thermo Scientific Accucore aQ C18 column built by Thermo Fisher Scientific, Waltham, Massachusetts, MAUSA 02451, (100 × 2.1 mm i.d., 2.6 µm) under multistep gradient conditions. The mobile phase consisted of ammonium formate 5 mM in 0.1% formic acid in water (solvent A) and ammonium formate 5 mM in 0.1% formic acid in methanol (solvent B). The gradient elution program was as follows: 0–2 min 30% B; 2–7 min, 30–70% B; 7–9 min, 70–95% B; 9–12 min, 95% B; 12–12.1 min, 95%- 30% B; 12.1–20 min, 30% B. The column was maintained at 10 °C, the autosampler was set at 10 °C and the injection volume was 2 µL. The HPLC eluent was directly introduced into the heated electrospray ionization source and the total run time for analysis of each sample was 20 min.

The concentration of each analyte was quantified using a Thermo Scientific Orbitrap Q-Exactive focus equipped with a heated ion spray interface. The ion spray voltage was 4500 V with an auxiliary gas temperature of 250 °C and a capillary temperature of 320 °C. Operating conditions were optimized by injecting a mixture of all analytes and were as follows: sheath gas flow 35 arbitrary units (au); auxiliary gas flow 10 au. Quantification was performed in full-scan mode with an operative range of 100 to 900 m/z in polarity switching. The extracted ions, with 5 ppm tolerance, are shown in Table 4.

Table 4. Operational methods adopted for quantification of extracted ions.

Mass (m/z)	Formula	Species	Polarity	Analyte
129.01058	$C_4H_3FN_2O_2$	−H	Negative	Fluorouracil
261.03210	$C_7H_{15}Cl_2N_2O_2P$	+H	Positive	Cyclophosphamide
286.10709	$C_{47}H_{51}NO_{14}$	+H-$C_{31}H_{36}O_{10}$	Positive	Paclitaxel

The orbitrap was set to a resolution of 70,000. Automatic data acquisition and analysis were performed with Trace Finder software (version 3.3). For quantification, the peak area of target ions was compared with least-squares calibration curves in which the peak area of calibration standards was plotted against their concentrations.

2.3. Media Fill Test

A simulation of the automated aseptic robot process, also known as a media fill test, was performed as required by Annex 1 "Manufacture of Sterile Medicines"—Volume 4 of the EU Good Manufacturing Practices, Paragraph "Simulation of the Aseptic Process (APS) (also known as media fill)". The routine aseptic manufacturing process was followed as closely as possible and included all critical steps, such as drug formulation and preparation of final containers and multidose bags [24].

In detail, the test consisted of an exact simulation of the aseptic production process of chemotherapeutic preparations using culture media instead of diluents and antineoplastic products, faithfully reproducing every step of the usual production process.

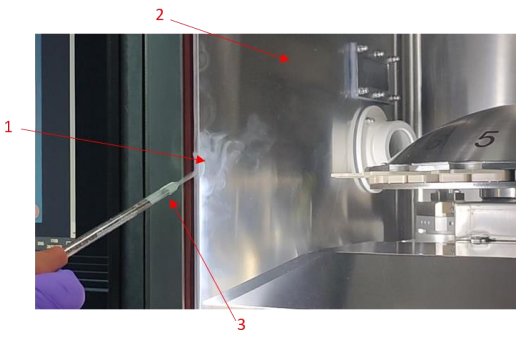
The test consisted of filling units (compounding vials, multidose bags and syringes) with a tryptic soy broth (TSB) which is able to highlight the microbiological contamination after an appropriate incubation time, the fertility of which was previously verified according to the specifications of the European Pharmacopoeia [25].

The units filled during the tests are submitted to a double-temperature incubation for a period of 14 days (7 days at 22.5 °C and 7 days at 32.5 °C); if after this period the media contained in all samples has retained its clearness characteristics, the media fill test will be compliant. Any contamination that occurred during the manufacturing stage can be evidenced by such a test. The test was successful if no turbidity was observed during the incubation time, which would not reveal any microbiological growth [27].

2.4. Differential Pressure of Main Chamber

The differential pressure between the system and the clean room was measured, using a suitable calibrated instrument multifunction model, 9565-P VELOCICALC, built by TSI Shoreview, Minnesota, MN, USA, 55126, and with accuracy equal to 2% of reading. The difference should be understood as positive if the pressure in the main chamber of the system is greater than that of the clean room and negative if the pressure in the main chamber of the system is less than that in the clean room.

Then, a visual test was also carried out to show the differential pressure. A clearly visible white smoke was generated in front of the door of the compartments of the device, using Draeger Tubes™, built by Draeger Italia S.p.A., Corsico 20094 Milano, Italy, containing H_2SO_4 (Figure 6). When air is pumped into the tube by means of the rubber bulb, SO_3 will be released. Per each pump stroke, approximately 3–4 mg of SO_3 is produced and this corresponds to the reaction of air humidity with 4–5 mg of H_2SO_4. By using the tube in a room of 10 m^3, this corresponds to a concentration of 0.5 mg/m^3 H_2SO_4. As shown in Figure 6, the white smoke, generated by the Dragaer Tube™ in the clean room is aspirated into the main chamber of the PHARMODUCT® automated system due to the negative pressure recorded inside.



Reference	Description
1	White smoke, generated by Dragaer Tube™
2	PHARMODUCT® Main Chamber
3	Dragaer Tube™

Figure 6. Visual test of the pressure difference observed at the closed door of the PHARMODUCT® automated system using the Draeger Tubes™.

The air flow, generated by the part of the clean room, must be considered outgoing if it goes from inside the main chamber to the clean room; vice versa, it must be considered incoming.

2.5. Hourly Leak Rate (Tf)

To test the hourly leak rate, air supply/outlet valves and the doors with inflated seals should be closed. Then, the inner box of the system was brought to an initial negative pressure of −250 Pa. The pressure and temperature value in the main chamber of the system was recorded at regular intervals of 15 min for a total time of 60 min using a calibrated datalogger Tracksense ProPressure Combi model built by Ellab.

The hourly leak rate (h^{-1}) was calculated according to the following formula Equation (1):

$$T_f = {}^{60}/_{t} \times \left({}^{P_n T_1}/_{P_1 T_n} - 1\right), \quad (1)$$

where:

- P is the pressure (initial = 1 and final = n) in pascals;
- T the temperature (initial = 1 and final = n) in kelvins;
- t is the duration of the test expressed in minutes.

The system must guarantee an "hourly leak rate" equal to or better than that provided for class 2 insulators according to ISO 10648-2 [28]: $T_f \leq 2.5 \times 10^{-3}$

3. Results and Discussion

The data obtained by the analytical laboratory using the UHPLC–HRMS system were compared with the theoretical dosages. All the samples have met the acceptance criteria of ±10% (Table 5).

Table 5. Comparison of theoretical and found concentrations with relative percentages of deviation.

DRUG	Theoretical Concentration	Found Concentration	Error
Run1_1 5-Fluorouracil	20 mg/mL	19.45 mg/mL	2.75%
Run1_2 5-Fluorouracil	20 mg/mL	20.75 mg/mL	3.75%
Run2_3 5-Fluorouracil	4 mg/mL	3.71 mg/mL	7.25%
Run2_4 5-Fluorouracil	4 mg/mL	3.70 mg/mL	7.50%
Run3_1 5-Fluorouracil	4 mg/mL	3.83 mg/mL	4.25%
Run3_3 5-Fluorouracil	8 mg/mL	7.67 mg/mL	4.13%
Run1_1 Cyclophosphamide	6 mg/mL	6.36 mg/mL	6%
Run1_2 Cyclophosphamide	6 mg/mL	6.26 mg/mL	4.33%
Run2_3 Cyclophosphamide	2.40 mg/mL	2.43 mg/mL	1.25%
Run2_4 Cyclophosphamide	2.40 mg/mL	2.24 mg/mL	6.67%
Run3_1 Cyclophosphamide	2.40 mg/mL	2.25 mg/mL	6.25%
Run3_3 Cyclophosphamide	3.2 mg/mL	3.44 mg/mL	7.50%
Run1_1 Paclitaxel	0.70 mg/mL	0.68 mg/mL	2.86%
Run1_2 Paclitaxel	0.70 mg/mL	0.68 mg/mL	2.86%
Run2_3 Paclitaxel	0.30 mg/mL	0.32 mg/mL	6.67%
Run2_4 Paclitaxel	0.30 mg/mL	0.29 mg/mL	3.33%
Run3_1 Paclitaxel	0.30 mg/mL	0.32 mg/mL	6.67%
Run3_3 Paclitaxel	0.30 mg/mL	0.31 mg/mL	3.33%

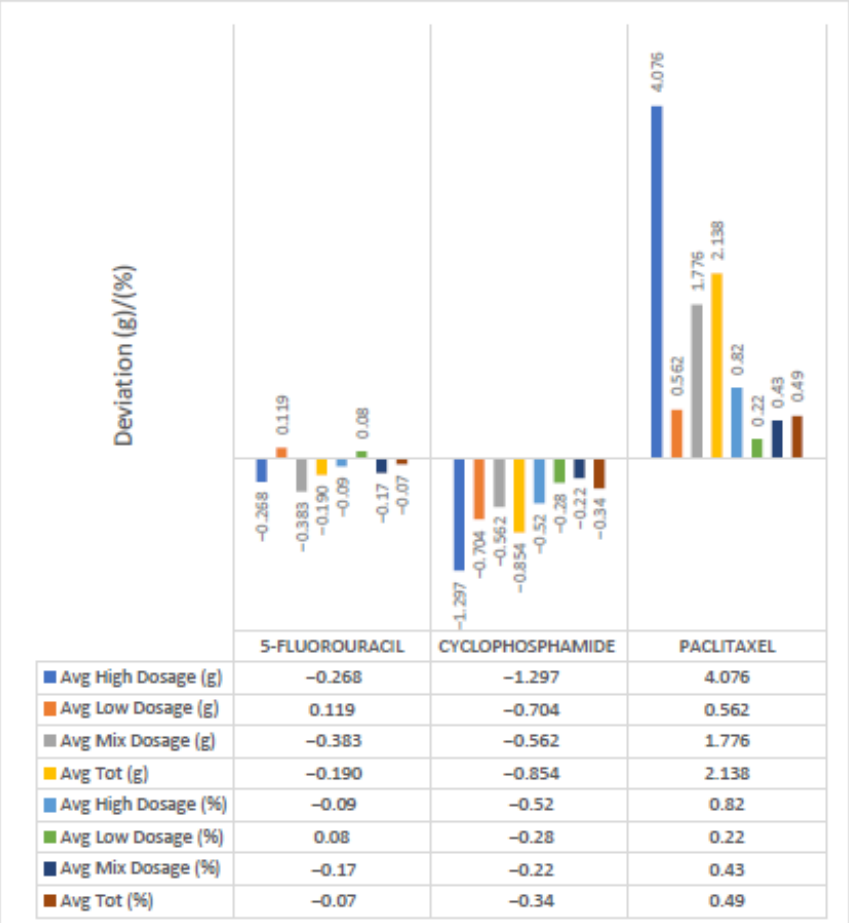
Therefore, the PHARMODUCT® automated system has demonstrated high reliability in the filling accuracy of multidose bags and especially final containers. The data collected showed that when filling the final containers with the three drugs under study, considered representative by formulation (liquid/powder) and by different densities, the maximum total mean deviation was 2.138 g, equal to 0.49% of the expected values, for the drug paclitaxel. The maximum mean deviations obtained during dispensing of the other drugs

studied were −0.854 g or −0.34% of expected values for cyclophosphamide and −0.190 g or −0.07% of expected values for 5-fluorouracil.

Preparations made with these drugs were compared with filling tests using water in-stead of the drug. The maximum average percentage obtained was 0.47%, a value comparable to that obtained in the trials with the drug.

All final preparations were weighed using a reference scale (manufacturer Sartorius, model MCE1202S-2S00-S0, accuracy of 0.01 g) with a valid calibration certificate.

Figure 7 shows the processed data on the conducted trials. For each drug, the deviations measured from the required quantities are expressed by quantity (g) and percentage (%).



	5-FLUOROURACIL	CYCLOPHOSPHAMIDE	PACLITAXEL
Avg High Dosage (g)	−0.268	−1.297	4.076
Avg Low Dosage (g)	0.119	−0.704	0.562
Avg Mix Dosage (g)	−0.383	−0.562	1.776
Avg Tot (g)	−0.190	−0.854	2.138
Avg High Dosage (%)	−0.09	−0.52	0.82
Avg Low Dosage (%)	0.08	−0.28	0.22
Avg Mix Dosage (%)	−0.17	−0.22	0.43
Avg Tot (%)	−0.07	−0.34	0.49

Figure 7. Statistical data related to total mean deviation.

In addition, with regard to cyclophosphamide, the last bags dispensed for each run at high, low and random dosages, chosen as the worst case for sterility testing and bacterial endotoxin determination, were found to comply with the defined acceptance criteria, as well as being sterile and with an endotoxin content of less than 0.625 EU/mL.

As for the media fill test, no signs of microbiological growth were observed after the 14-day incubation period (7 days at 22.5 °C and 7 days at 32.5 °C).

Furthermore, by studying the pressure difference between the system and the clean room, a value of −97.56 Pa was recorded. The negative pressure of the main chamber of the automatic system was also demonstrated by the Draeger tube visual test. In fact, visible white smoke, generated from the clean chamber part, entered the chamber.

At last, the hourly leak rate (h^{-1}) showed a value of 0.002033 h^{-1} (according to acceptance criteria of ISO-10648-2 [28]). The pressure and temperature values at 15-min intervals are shown in Table 6.

Table 6. Data shown by hourly leak rate test.

T (min)	Relative Pressure (Pa)	Absolute Pressure (Pa)	Temperature (°K)	T_f (h^{-1})
0	−255.5	98,809.87	297.43	/
15	−240.3	98,799.16	297.40	/
30	−223.0	98,925.85	297.28	/
45	−203.8	98,919.22	297.20	/
60	−186.3	98,914.23	297.14	0.002033

With these last two tests, we can demonstrate the effectiveness of the instrument to avoid the leakage of cytotoxic drugs during dispensing, improving operator safety [18].

4. Conclusions

This study demonstrates that robotic compounding can play a crucial role in the preparation of chemotherapeutic agents, offering patient safety advantages over conventional parenteral preparation production procedures, due to the high standardization introduced.

In addition, the PHARMODUCT® automated system enables the preparation of antiblastic drugs, both liquid and powdered, with maximum accuracy and precision, minimizing operator exposure to toxic drugs.

At the same time, it prevents the occurrence of chemotherapeutic errors (e.g., due to miscalculation of concentrations, inaccurate preparations and the use of incorrect diluents) [27,29] throughout the entire process, reducing drug waste to zero and cutting costs. In addition, it fulfils sterility requirements, as established in ISO14644-1 [23] and EU GMP standards for hospital pharmacy and the pharmaceutical industry in general [30].

So, this article could provide an important reference point for the development of a qualification protocol, as required by EU GMP Volume 4—Annex 15 [31], for such complex equipment to be used for the preparation of sterile drugs in the hospital setting [30].

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