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HPLC-UV and LC-MS/MS analysis to study formulation and long-term stability of some anticancer drugs in elastomeric pumps

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Abstract

Drug stability evaluations in elastomeric pump represent the first step to certify the safety of a therapeutic treatment in oncology. Since the stability of several anticancer molecules is due to their reactivity and stability in elastomeric pumps, made up of different materials, several experimental conditions, such as temperature, pH, concentration and possible chemical interactions among drugs in a single formulation, should be always investigated. Galenic preparation of anticancer drugs is an important prerogative of Anticancer Units within hospital pharmacies and, considering the burden of COVID-19 pandemic event, specific guidelines for therapeutic administration in elastomeric pumps reducing hospitalization both for post-surgical treatment and for therapeutic treatment have been worldwide elaborated. In the present study, the stability of Doxorubicin and Vincristine as a single formulation at different experimental conditions has been investigated. Moreover, we report a systematic study of 5-FU, which is known to be largely used in these medical devices, although its criticisms in terms of solubility, pH effect, storage time and conditions. Our results demonstrate that doxorubicin and vincristine can be mixed safely as a single formulation and 5-FU is stable for 32 days at different temperatures and concentrations in elastomeric pumps.

Keywords: Elastomeric pump; Continuous infusion; Chemotherapy; HPLC and LC/MS analyses; Chemical stability in solution

Introduction

Pharmaceutical research cannot stop after the commercial availability of a drug. To better exploit their efficacy, researchers and hospital pharmacists have to deepen the study of drugs in solution at the real handling and administration conditions, particularly in terms of chemical and physical stability at different concentrations, within all clinical schedules of treatment, independently and in parallel to drug producers' data [1]. This concept is even more substantial for intravenous antitumor drugs, whose handling takes place in strictly controlled conditions of temperature, humidity, microbiological and particulate contamination, within a biological class II cabin, due to their intrinsic toxicity by inhalation or contact with skin and mucous membranes (mouth, conjunctiva) [2-4]. Even if several antitumor drugs have known for decades and don't represent recent discoveries in clinics, from a pharmaceutical point of view each single galenic product (in pump devices, bags, syringes) is unique in its final formulation, also because most drugs can be administrated at the same dosage as bolus or some-hours-to-some-days infusion intervals. This is the reason why producers often report chemical, physical and microbiological stability in the technical sheet as a range of possible concentrations of use, specifying that, outside aseptic technique of handling, the clinical use of the drug is upon exclusive responsibility of the pharmacist. Hospital pharmacists know that aseptic technique is the result of different concomitant conditions: among these, the absence or very low particulate contamination within the cabin, operators' expertise, the correct use of closed system and transfer devices (CSTD). The variability of these conditions in worldwide hospital pharmacies justifies the need of an independent research, which clarifies and extends producers' data and gives to the pharmacists the certainty of the chemical and physical stability of each preparation, also considering the precarious clinical conditions of oncologic patients that often cause delay in administration. In this paper, we report our data on Doxorubicin and Vincristine mixture and 5-FU in terms of stability in solution in pump devices by means of HPLC and LC-MS analyses.

Pump devices grant continuous intravenous infusion of many antitumor drugs and consent to patients the administration of their long-lasting therapy at home, without hospitalization, which often reduces their compliance to treatment. The elaboration of a database of all experimental data on physical and chemical stability for drugs at different concentrations should be a goal of all hospital pharmacies, to rationalize the clinical use of drugs beyond the producers' prescriptions [5].

The aims of these studies is to verify the compatibility of Doxorubicin and Vincristine in the same final container and final formulation and the limit of stability for 5-FU considering experimental variables, such as storage time and temperature. The mixture Doxorubicin and Vincristine is world-wide well-known as part of the therapeutic schedule of different refractory hematologic malignancies [6], but in some nations, included Italy, technical sheets of both drugs prescribe no mixture with any molecule.

Material and Methods

All compounds as standards have been studied in HPLC and LC-MS at established concentrations.

Doxorubicin 330 µg/mL and Vincristine 11.5 µg/mL in 100 ml final volume filled in disposable pumps.

5-FU has been studied in the same medical device at 50 mg/mL for 28 days at 25° C and at 32° C observed for additional 7 days.

Instruments and analytical conditions

HPLC-UV Apparatus: HPLC Analyses were performed on a Shimadzu Prominence Modular HPLC equipped with System Controller CBM-20A, Photo-diode Array detector SPD-M20A and controlled by Lab Solutions WS-Single PDA (Vers. 5) WS Software. The detector was employed at 254 nm. For 5-FU, the mobile phase consisted in Acetonitrile and water (10:90), at 1 mL/min flow rate. The injection volume was 20 µl.

Table 1: Eluent A: Water / 0.1% formic acid; Eluent B: Acetonitrile / 0.1% formic acid

Time (min)	Eluent A	Eluent B
2	97	3
2.50	97	3
10	76	24
13.0	76	24
13.50	66	34
15.50	44	56
16.00	97	3
21.00	97	3

LC-MS/MS Apparatus: Shimadzu LC-MS 8040 TripQuad MS equipped with System Controller CBM-20A, Trip Quad Mass Spectrometer detector and controlled by Lab Solutions WS Software. A Luna® Omega 5 µm PS C18 100 Å column with 150x4.6 mm dimensions was used (Phenomenex QC MIX 870). For Doxorubicin and Vincristine, the mobile phase flow rate was 0.2 ml/min using the gradient elution program described in Table 1, using water/formic acid and acetonitrile/formic acid mixtures. The thermostatic auto-sampler was maintained at 15° C and the

injection volume was 25 µL. The column was set at 30°C. Positive ESI conditions were capillary temperature set at 250° C, spray voltage 4kV and stealth and auxiliary gas (nitrogen) flow rate at 45 and 2 psi, respectively. MS/MS was acquired in multiple reaction monitoring (MRM) mode Q1 and Q3.

The collection of the samples was carried out directly by the terminal part of the plastic infusion line in a tube before transferring them into vials for LC-MS/MS analysis. The samples were used after dilution in LC-MS/MS analyses.

Stability Criterion

For all the selected drugs, the qualitative and quantitative results have been compared with the data reported in literature about substance identification, drug shelf life, safety dose and degradation products [7].

Experimental section

a) Doxorubicin and Vincristine mixture

Doxorubicin (330 µg/ml) and Vincristine (11.5 µg/ml) as a single formulation has been stored and handled as reported in the following Table 2 (h = hours and d = days).

Table 2: Quantitative and qualitative conditions of analyses for Doxorubicin/Vincristine mixture

Doxorubicin Chlorohydrate	**330 µg/ml**	
Vincristine sulfate	11.5 µg/ml	
Diluent	Normal Saline	
Storage condition	25° C	
Determinations	0 h	48 h

In Table 3, 5-FU analytical conditions are reported (h= hours, d = days).

Table 3: Quantitative and qualitative conditions of analyses for 5-FU.

Concentration	4.86 mg/mL		55.36 mg/mL		57.86 mg/mL		
Diluent	Normal Saline		Normal Saline		Normal Saline		
Storage condition	25° C		25° C		25° C	32° C	
Determinations	0 h	21 d	0 h	21 d	0 h	28 d	35 d

Curve calibration performed in LC-MS

In Figure 1A-B the calibration curves of Doxorubicin and Vincristine and in Figure 2A-B the corresponding chromatograms are reported, respectively.

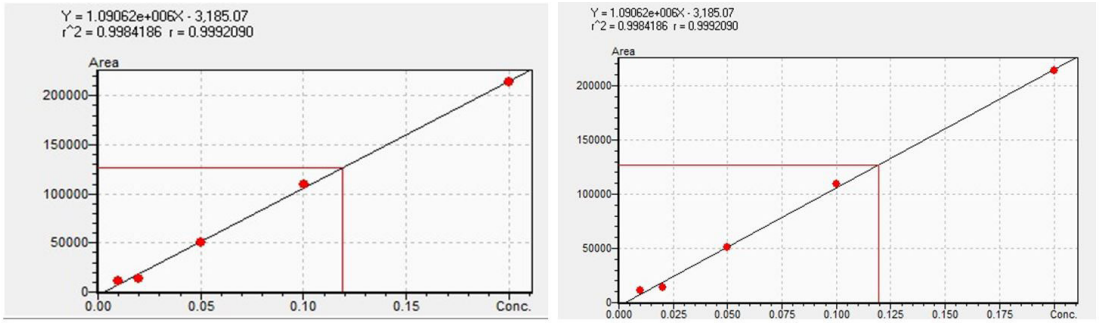
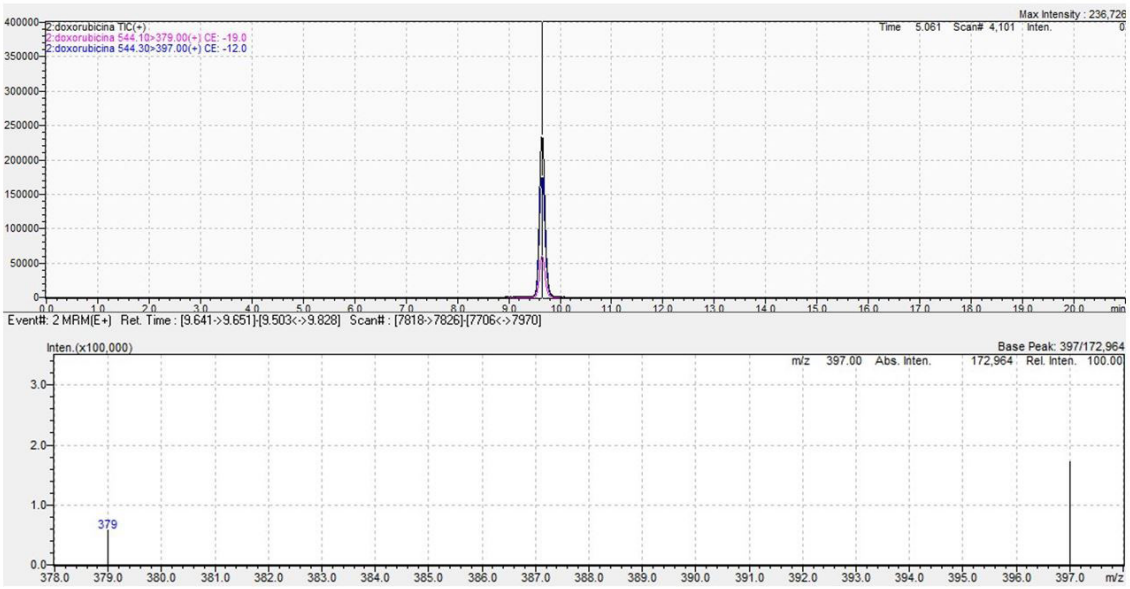
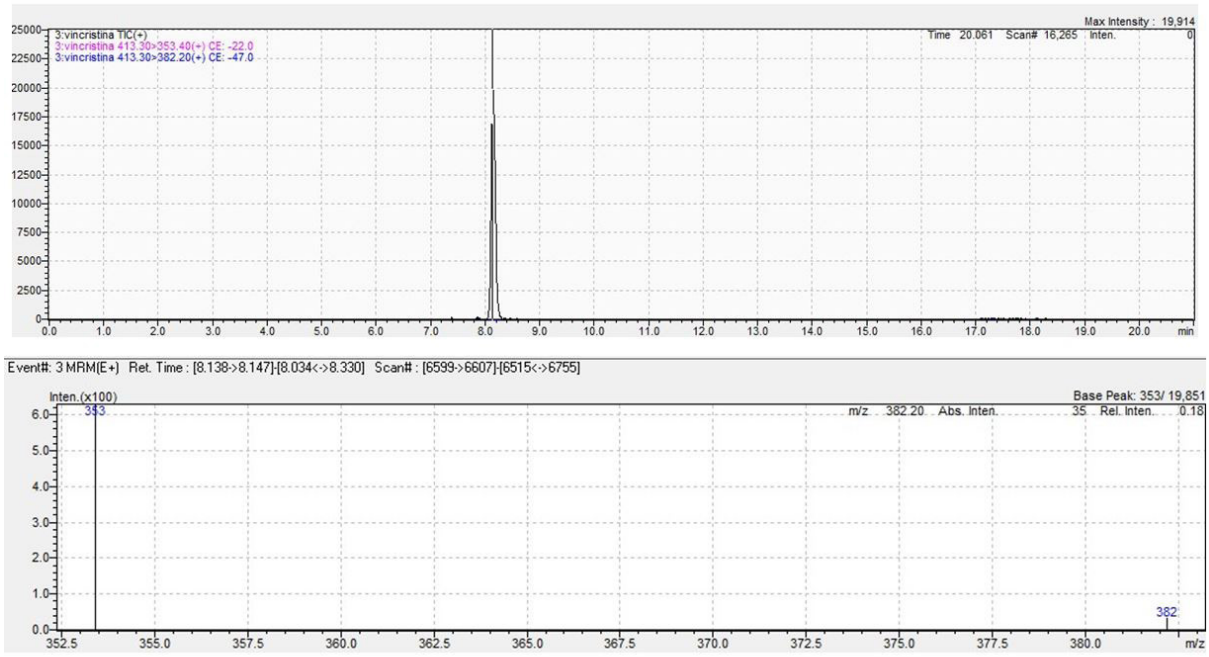


Figure 1: Calibration curves for Doxorubicin (A) and Vincristine (B)



2A



2B

Figure 2A-B: HPLC and LC/MS chromatograms for Doxorubicin (A) and Vincristine (B)
b) 5-Fluorouracil (5-FU)

Curve calibration in UV-HPLC

The calibration curve of 5-FU in UV-HPLC is reported in Figure 3.

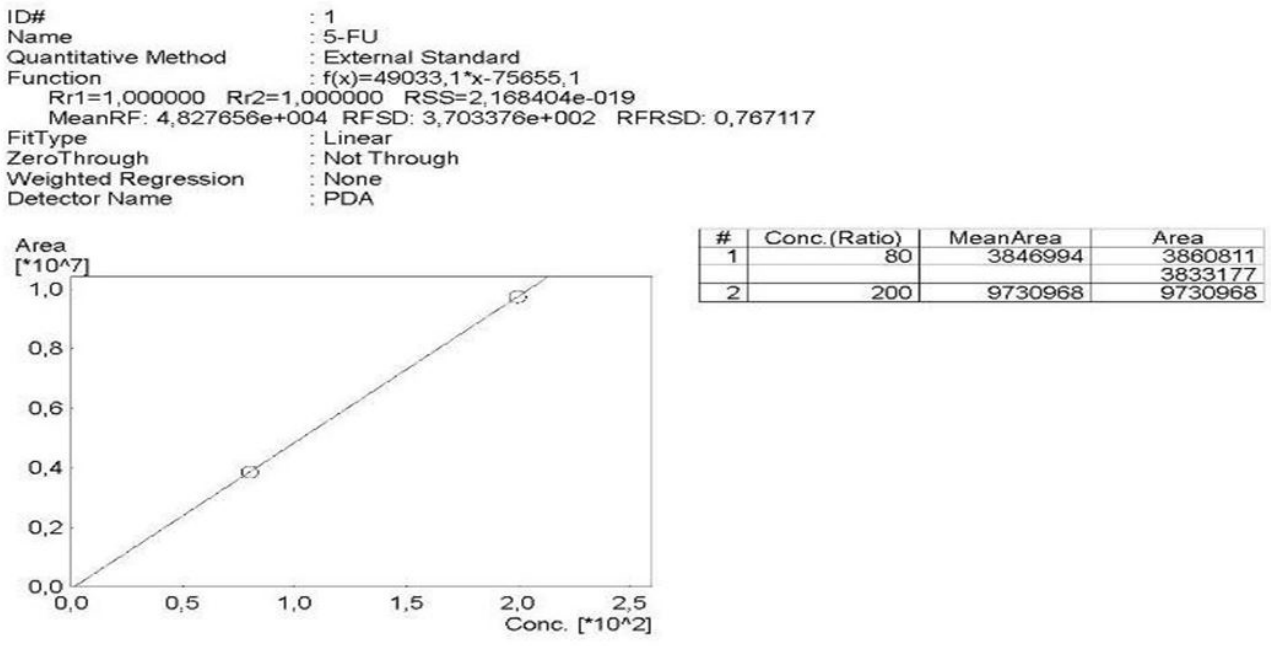


Figure 3: Calibration curve for 5-FU

Results and Discussion

The mixture of Doxorubicin and Vincristine has been studied under the experimental conditions reported in Table 2. The results demonstrated that both compounds are stable in elastomeric pumps and quantitative results are reported in Table 4. After 48 h from the start of analyses, the change (%) in concentration of each drug filled into elastomers is less than 5%. Other peaks till the end of the analyses are not detected.

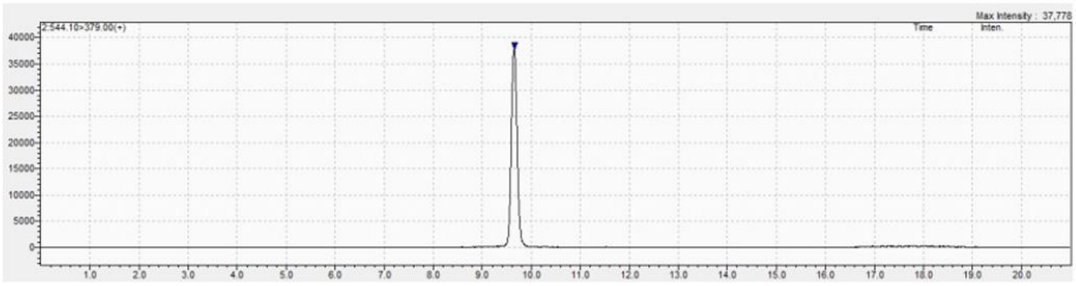
Table 4: LC-MS stability of Doxorubicin and Vincristine solution at Time = 48 h

Temperature	Time	Average concentration (µg/mL)	Average Concentration Change (%)
25° C	0 h	**330±0.07** (Doxorubicin H.) **11.5±0.07** (Vincristine S.)	-
25° C	48 h	**345±0.07** (Doxorubicin H.) **11.8±0.07** (Vincristine S.)	**+ 4.55** (Doxorubicin H.) **+ 2.61** (Vincristine S.)

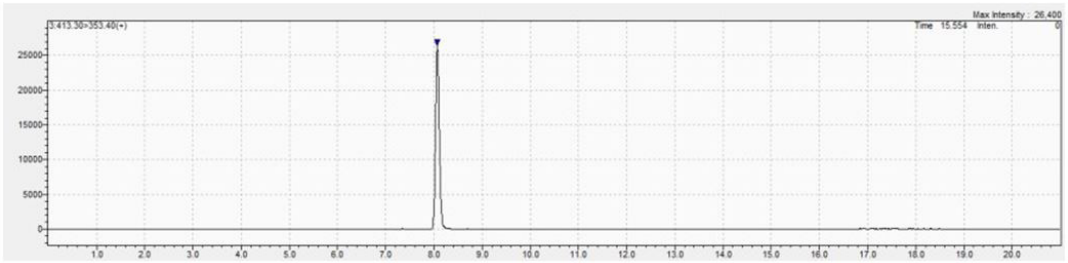
The chromatograms of the mixture containing Doxorubicin and Vincristine are reported in Figure 4A-D.

Recently, many papers reported controversial data on 5-FU solutions filled in elastomeric pumps in relation substantially to the materials of medical devices, that could determine adsorption of drug and, consequently, flocculation and precipitation [8-12]. In addition, the solubility and the pH of 5-FU solutions are other parameters that should be monitored to obtain acceptable drug stability. Obviously, temperature and storage conditions are variables that largely contribute to the drug stability in medical devices [13].

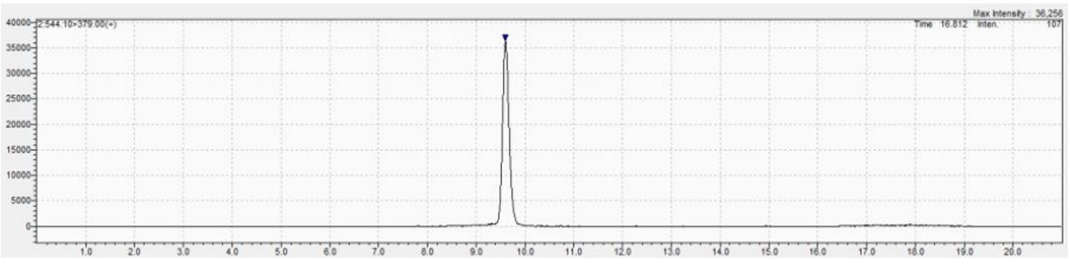
A. Time = **0 h**; 330 µg/ml Doxorubicin Chlorohydrate



B. Time = **0 h**; 11.5 µg /ml Vincristine Sulfate



A'. Time = **48 h**; 330 µg/ml Doxorubicin Chlorohydrate



B'. Time = **48 h**; 11.5 µg/ml Vincristine Sulfate

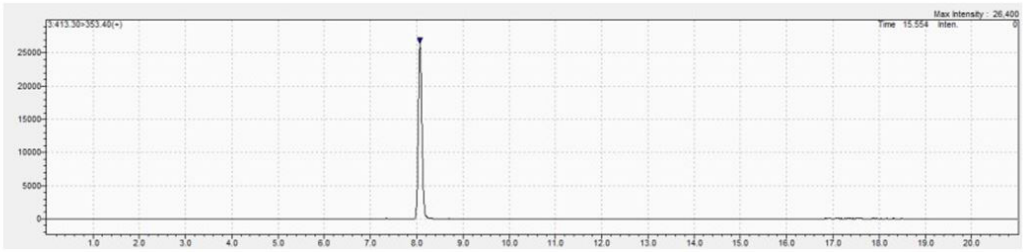
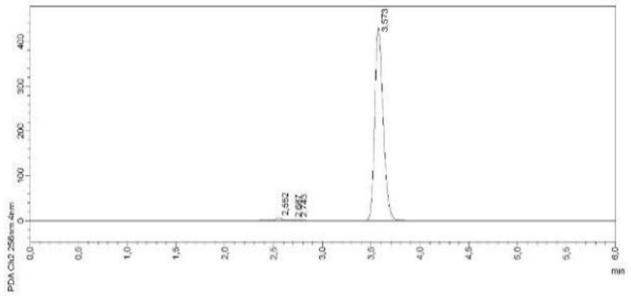


Figure 4A-D: Chromatograms of Doxorubicin and Vincristine mixture at T =0 and T = 48 h at 25° C

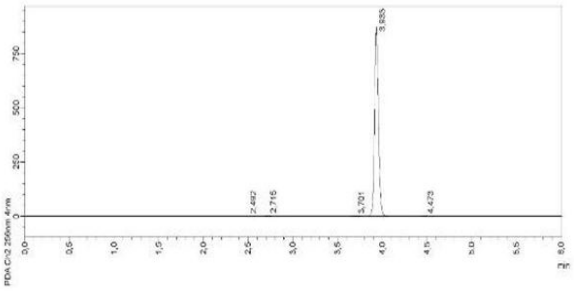
In the present paper, the stability of 5-FU at different experimental conditions is reported. Both at low and high concentration (4.86 mg/mL and 55.36 mg/mL), the stability results after storage at 25° C for 21 days are excellent. The quantitative analysis is reported in Table 5 and the corresponding chromatograms are reported in Figure 5A-D for each 5-FU concentration.

Table 5: Quantitative studies of 5-FU solution at two different concentrations at T = 0 and T = 21 days

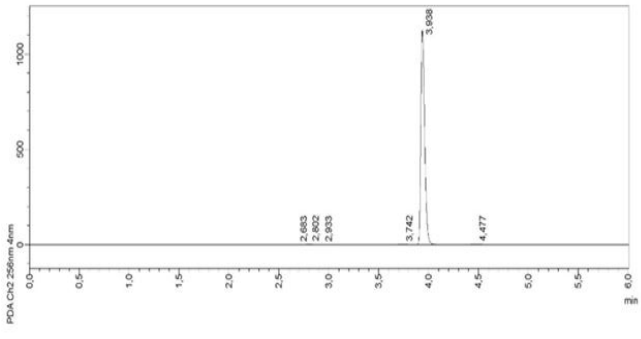
Temperature	Time	Concentration (mg/mL)	Average concentration change (%)
25° C	0	4.86 ± 0.16	0
	21 days	4.88 ± 0.15	+ 0.01
25° C	0	55.36 ± 0.37	0
	21 days	57.24 ± 0.38	+ 3.40



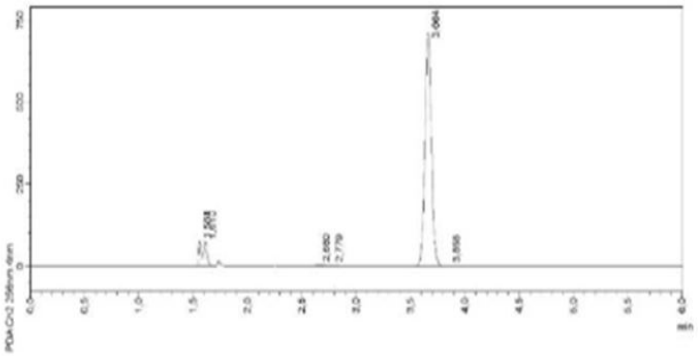
A. 5-FU **4.86 mg/mL** at T = 0



B. 5-FU **4.86 mg/mL** at T = 21 days



C. 5-FU 55.36 mg/mL at T = 0

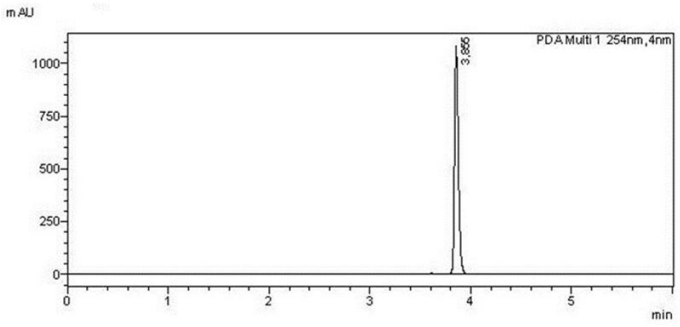


D. 5-FU 55.36 mg/mL at T = 21 days

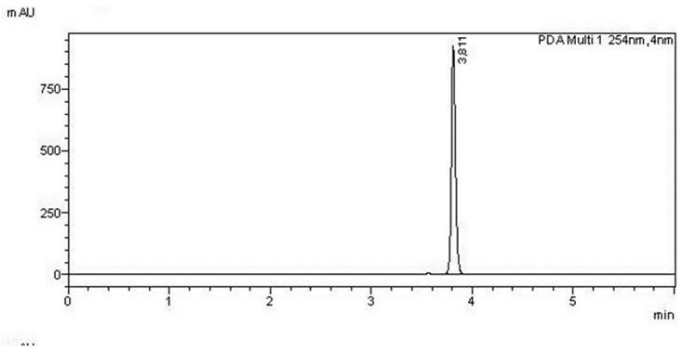
Figure 5A-D: Representative chromatograms of two different 5-FU concentrations at T=0 and T= 21 days

For deepening in this matter, another investigation has been carried out for a even higher concentration (57.86 mg/mL), verifying the stability after 28 days and for additional 7 days after increasing temperature at 32° C (Table 3).

Another calibration curve was performed and the results are reported in Table 6. It is noteworthy that for each lot of filled pumps, the strength of the mother solution was determined and, for this reason, there are low differences between starting solutions. The results, reported in Table 6, demonstrated that in these extreme conditions, 5-FU stability in elastomeric pumps is really acceptable, being the variation in concentration with time less than 5% with respect to the corresponding solution at T= 0 (57.86 mg/mL). The corresponding chromatograms are depicted in Figure 6A-C.



A: 5-FU solution at T= 0



B: 5-FU solution at T= 28 days

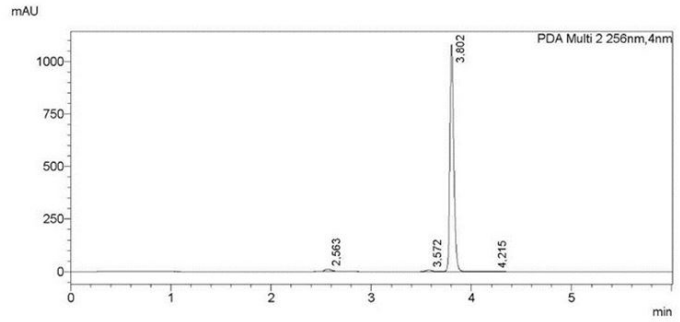


Figure 6A: Representative chromatograms of 5-FU 57.86 mg/mL solution at 28 days and 35 days

Table 6: 5-FU at 57.86 mg/ml solution evaluated at 28 days and 35 days with changing temperature

Temperature	Time	Average concentration	Average concentration change (%)
	0 h	57.86 ± 0.16	0
25° C	28 days	57.13 ± 0.15	+1.26
32° C	+ 7 days	55.56 ± 0.15	+3.98

Discussion

The present work aims at demonstrating that elastomeric pumps are an efficient solution for administrating anticancer drugs in safety. Practise has demonstrated that several factors can influence the quality of the final products: i) the materials of pumps; ii) the expertise in handling anticancer drugs; iii) the analytical methods and the appropriate instrument for carrying out significant results in terms of chemical stability data.

In this study, we tested Doxorubicin and Vincristine in a single formulation by LC-MS and different solutions of 5-FU in UV-HPLC apparatus. The results are very interesting because all requirements have been met. This pharmaceutical preclinical study discloses interesting perspectives in terms of proof of concept and meantime suggests several approaches to investigate the quality of the galenic products prepared in a hospital pharmacy for hematologic and oncologic patients who claim safe therapies to face their personal battle against tumour diseases.

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