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Short communication

High performance liquid chromatographic determination of some co-administered anticancer drugs in pharmaceutical preparations and in spiked human plasma

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Abstract

Two HPLC methods are introduced in this paper for the simultaneous determination of doxorubicin hydrochloride (DOX) and 5-fluorouracil (5-FU), combination I, and of cytarabine (CYT) and etoposide (ETO), combination II, as co-administered drugs. In both combinations, a 250 mm × 4.6 mm C-18 column is used. The mobile phase for combination I consists of a mixture of acetonitrile and 0.05 M disodium hydrogenphosphate (50:50, v/v) containing 0.1% sodium laurylsulfate (SLS) adjusted to pH 3.7 at a flow rate 1 ml/min, with UV detection at 260 nm and ambient temperature. For combination II, the mobile phase consists of a mixture of 0.02 M sodium dihydrogenphosphate aqueous solution adjusted to pH 6.0 (with 0.2 M orthophosphoric acid or sodium hydroxide) and acetonitrile in a ratio of (7:3) at a flow rate 1 ml/min, with UV detection at 254 nm and ambient temperature. The methods also permitted the determination of methyl hydroxybenzoate (MHB) which is used as a preservative in DOX vials, combination I, and of benzyl alcohol (BZA) preservative in ETO vials, combination II. The proposed HPLC methods were successfully applied to the determination of the investigated drugs, of the two combinations, both in injection solutions and spiked human plasma samples with high precision and accuracy. Linearity, validation, accuracy, precision, limits of detection, limits of quantitation, and other aspects of analytical validation are presented in the text.

Keywords: Determination; HPLC; Co-administered anticancer drugs; Doxorubicin; 5-Fluorouracil; Cytarabine; Etoposide; Plasma

1. Introduction

All four drugs: doxorubicin hydrochloride (DOX), 5-fluorouracil (5-FU), cytarabine (CYT), and etoposide (ETO, Fig. 1) are official in both the BP [1] and the USP [2].

Numerous HPLC methods have been applied for the determination of DOX and its metabolites in pharmaceutical dosage forms and biological fluids which are discussed in several excellent review articles [3–5].

Various HPLC methods have been widely used for the quantitative measurement of 5-FU in pharmaceutical preparations [6], biological fluids [7,8] and in environmental samples [9].

Different HPLC methods with UV detection have been applied for the determination of CYT in various dosage forms [10] and in biological fluids [11].

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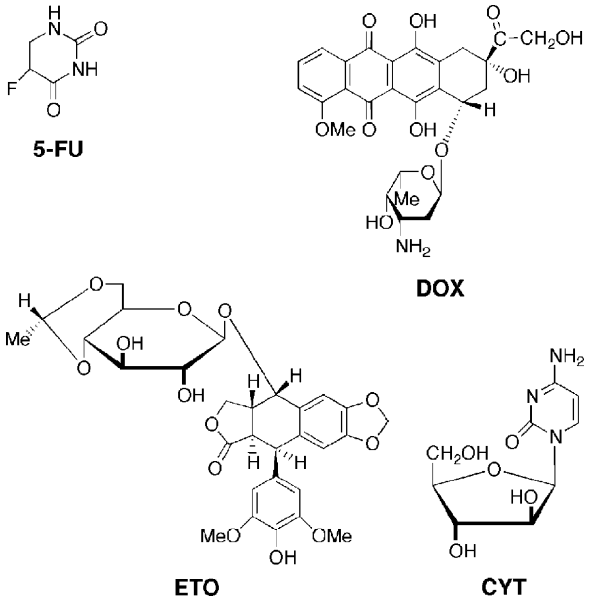

Fig. 1. Chemical structures of the four investigated drugs: 5-FU; DOX; ETO; and CYT.

HPLC has been used for the quantitation of ETO in different matrices. This has been summarized in a recent review article [3].

5-FU may be given in combination with DOX (combination I) for the treatment of advanced gastrointestinal cancer [12], non-small cell lung carcinomas [13], advanced or recurrent salivary gland carcinoma [14] or breast cancer [15,16]. On the other hand, CYT and ETO (combination II) can be co-administered in patients suffering from certain types of leukaemia [17] or non-Hodgkin's lymphoma [18]. So it is important to monitor the drug levels in plasma in order to get the required synergistic action and avoid drug toxicity. In spite of the importance of the simultaneous determination of drugs in both combinations, no such method was reported in the literature.

This work describes two rapid, specific, reliable and sensitive analytical methods based on reversed-phase high performance liquid chromatography with UV detection for the quantitative determination of drugs in both combinations whether in injection solutions or in the plasma of patients receiving any of the two combinations.

DOX injection is available as freeze-dried powder containing lactose as a diluent and methyl hydroxybenzoate (methyl paraben) (MHB) as a preservative. Review of the literature reveals that there is no method reported for the determination of MHB in such a combination. On the other hand, 5-FU is marketed as injectable solution containing only the active drug (5-FU).

CYT is available as multi-dose vials containing only the active drug. On the other hand, ETO is commercially available as multi-dose or single-dose vials containing the active drug in addition to citric acid, benzyl alcohol (BZA), polysorbate 80, polyethylene glycol, and alcohol. There is no method reported for the determination of benzyl alcohol preservative in this preparation.

The developed methods allowed the quantitation of MHB and BZA used as preservatives in combinations I and II, respectively.

Generally, anticancer drugs are highly toxic with a narrow margin of safety. Therefore, patients should be carefully supervised since therapeutic response is unlikely to occur without some evidence of toxicity. Combined anticancer therapy is used to produce a synergistic anticancer effect and to decrease the toxic side effects. So it was necessary to develop a method that permits simultaneous determination of both combinations in plasma of cancerous patients.

2. Experimental

2.1. Instrumentation

The chromatographic system consisted of S 1121 solvent delivery system (Sykam GmbH, Germany), S 3210 variable-wavelength UV-Vis detector (Sykam GmbH, Germany) and S 5111 Rheodyne injector valve bracket fitted with a 20 μl sample loop. HPLC separations were performed on a stainless-steel Thermo-Hypersil C-18 analytical column (250 mm × 4.6 mm) packed with 5 μm diameter particles. Data were processed using EZChrom™ Chromatography Data System, version 6.8 (Scientific Software Inc., CA, USA) on an IBM-compatible PC connected to a printer.

2.2. Materials and reagents

DOX (Adriblastina® vials) was obtained from (Pharmacia and Upjohn S.P.A, Milan, Italy) labeled to contain 10 or 50 mg DOX per vial. 5-FU (fluorouracil

vials, 5 ml) was supplied by (Biosyn Arzneimittel GmbH, Felldach, Germany) and each milliliter is labeled to contain 50 mg 5-FU. CYT (Aracytin™ vials) was supplied from (Pharmacia & Upjohn N.V./S.A., Puurs, Belgium) and each powder for injection vial is labeled to contain 100 mg CYT. ETO (Vepesid™ vials, 5 ml) was obtained from (Bristol-Myers Squibb, Caribbean Company, Princeton, New Jersey) labeled to contain 20 mg/ml ETO. All reagents were of analytical grade, namely: acetonitrile and methanol (Panreac Co., E.U.), disodium hydrogenphosphate, phosphoric acid, sodium hydroxide and sodium laurylsulfate (SLS, BDH, Poole, England). The water for HPLC was double glass distilled.

2.3. Chromatographic conditions

For combination I, the mobile phase consisted of a mixture of 0.05 M disodium hydrogenphosphate in 0.1% (w/v) aqueous sodium laurylsulfate adjusted to pH 3.7 (using 0.2 M orthophosphoric acid or sodium hydroxide) and acetonitrile in a ratio of 1:1. For combination II, it consisted of a mixture of 0.02 M sodium dihydrogenphosphate aqueous solution adjusted to pH 6.0 (with 0.2 M orthophosphoric acid or sodium hydroxide) and acetonitrile in a ratio of 7:3. The mobile phase was degassed and filtered by passing through a 0.45 μm pore size membrane filter (Millipore, Milford, MA, USA) prior to use. The flow rate was 1 ml/min. All determinations were performed at ambient temperature. The detection wavelength was 260 and 254 nm for combinations I and II, respectively.

2.4. Standard solutions and calibration graphs

For combination I, stock solutions were prepared by dissolving DOX, MHB, and 5-FU in water to obtain concentrations of 0.2, 0.02, and 0.1 mg/ml, respectively. For combination II, stock solutions were prepared by dissolving ETO, BZA, and CYT in water to obtain concentrations of 2, 3, and 0.3 mg/ml, respectively. These stock solutions were further diluted with the mobile phase to obtain working standard solutions of suitable concentrations (corresponding to the linearity range stated in Table 3). Triplicate 20-μl injections were made for each concentration and are chromatographed under the above-mentioned conditions. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph for each compound.

2.5. Assay of mixtures containing doxorubicin hydrochloride, methyl hydroxybenzoate and 5-fluorouracil or containing cytarabine, benzyl alcohol and etoposide

Accurate volumes of each of DOX, MHB and 5-FU (combination I) or of CYT, BZA, and ETO (combination II) stock solutions were transferred into 10-ml volumetric flasks and diluted to volume with the mobile phase such that the ratios between drugs are as mentioned in Table 4. Triplicate 20-μl injections were made for each mixture solution and chromatographed under the conditions described above.

2.6. Analysis of pharmaceutical formulations

For combination I, an accurate weight of the powder content of DOX vial was transferred into a 25-ml volumetric flask with few millimeters of water, dissolved and completed to volume with the same solvent such that the concentration of the final solution is 0.2 mg/ml DOX (and to 0.02 mg/ml MHB). Since 5-FU is available as injectable solution for intravenous administration, a stock solution of 0.1 mg/ml was prepared after diluting accurate volume of the injectable solution with water.

For combination (II), an accurate weight of the powder content of CYT vial was transferred into a 25-ml volumetric flask with few millimeters of water and completed to volume with the same solvent such that the concentration of the final solution is 0.3 mg/ml. Since ETO is available as injectable solution together with benzyl alcohol, a stock solution of ETO containing 2 mg/ml (and 3 mg/ml BZA) was prepared by diluting accurate volume of the injectable solution with water. Further dilutions were made to the suitable concentrations (similar to standard solutions) then chromatographed exactly as under the assay of mixtures containing combinations I and II as presented in Table 5.

2.7. Analysis of spiked human plasma samples

The frozen plasma to be used was thawed at room temperature. Separate aliquots of 2.0 or 0.5 ml plasma

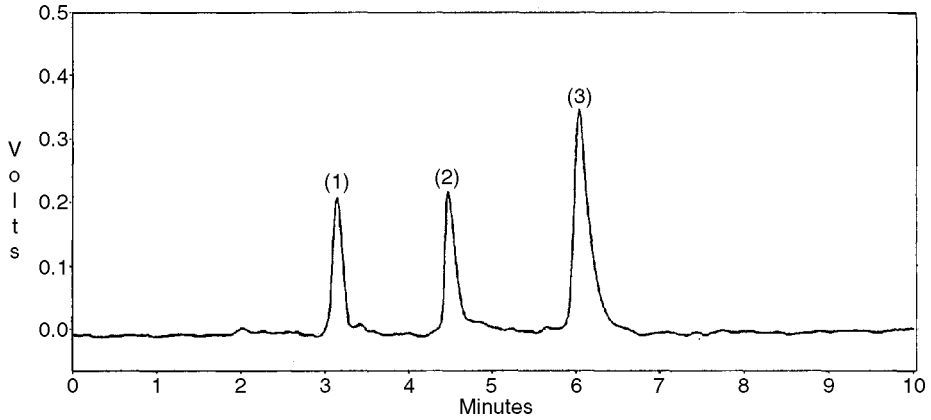

Fig. 2. A chromatogram of a 20-μl injection of a plasma sample spiked with 20 μg/ml of 5-FU (1); 4 μg/ml of MHB (2); and 40 μg/ml of DOX (3).

were transferred into two sets of centrifuge tubes for combinations I and II, respectively. The plasma sample in each tube was spiked with a suitable amount of standard DOX and 5-FU solutions or of standard CYT and ETO solutions as presented in Table 3. For protein precipitation, 8 or 2 ml methanol, for combinations I and II, respectively, was mixed with each sample then centrifuged for 10 min at $450 \times g$. The solutions were evaporated under nitrogen at room temperature and then the residue was re-dissolved in 500 μl water. These final assay solutions were analyzed as described.

3. Results and discussion

The absorption spectrum of DOX in aqueous acid exhibits two maxima in the UV region at 233 and 253 nm while that of 5-FU shows a single maximum at 266 nm followed by a sharp absorbance decrease. Therefore, the wavelength of 260 nm was selected for the simultaneous determination of DOX and 5-FU, which also allowed MHB preservative to be detected with increased sensitivity. These experimental conditions allowed the simultaneous determination of 5-FU and DOX peaks at retention times of 3.0 and 5.9 min, respectively (Fig. 2) together with MHB (preservative) which appeared at 4.2 min. The chromatographic characteristics of 5-FU, DOX, and MHB (combinations I) are summarized in Table 1.

The wavelength of 254 nm was selected for the simultaneous determination of CYT and ETO, which also allowed the determination of BZA preservative in ETO vials with high sensitivity. Fig. 3 shows the typical chromatogram of a plasma sample spiked with the three compounds. The method permitted adequate resolution of the mixture components within reasonable run-time, CYT being eluted at 3.0 min, ETO at 9.2 min, and BZA at 6.1 min. The chromatographic characteristics of combinations II components (CYT, ETO, and BZA) are summarized in Table 2.

3.1. Optimization of chromatographic conditions

To optimize the HPLC assay conditions, the effects of acetonitrile percentage, concentration of sodium laurylsulfate (for combination I) as well as the effect of pH of the mobile phase were studied.

Table 1
Chromatographic characteristics of 5-FU, DOX, and MHB (combination I)

Compound	t_R [a]	N [b]	k [c]	α [d]	R [e]
5-FU	3.0	1600	0.20		
				3.36	4.00
MHB	4.2	2304	0.68		
				2.00	3.50
DOX	5.9	1900	1.37		

[a] Retention time, in minutes.
[b] Number of theoretical plates.
[c] Retention factor.
[d] Selectivity, between each two successive peaks.
[e] Resolution, between each two successive peaks.

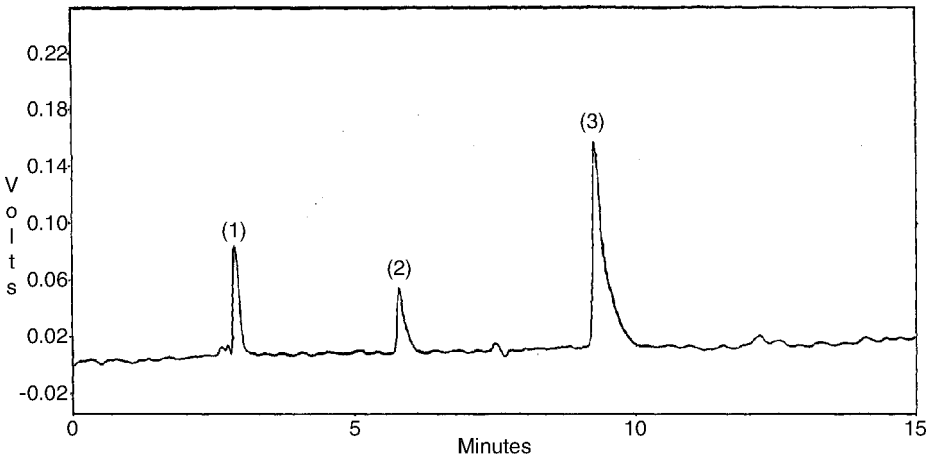

Fig. 3. A chromatogram of a 20-μl injection of a plasma sample spiked with 3 μg/ml of CYT (1); 45 μg/ml of BZA (2); and 30 μg/ml of ETO (3).

3.1.1. Effect of acetonitrile percentage in the mobile phase

The results showed that a satisfactory separation was obtained with a mobile phase consisting of 50% acetonitrile for combination I and 30% for combination II. Figs. 4 and 5 show the retention times obtained for combinations I and II, respectively as a function of acetonitrile percentage in the mobile phase. As can be seen, 50 or 30% acetonitrile for combinations I and II, respectively, provided optimum resolution with the most symmetric and well-defined peaks. At lower acetonitrile concentrations, separation occurred but with excessive tailing and increased retention times. Increasing acetonitrile concentration led to loss of resolution and overlapped peaks of DOX and MHB, or of CYT and BZA for combinations I and II, respectively.

Table 2
Chromatographic characteristics of CYT, ETO, and BZA (combination II)

Compound	t_R [a]	N [b]	k [c]	α [d]	R [e]
CYT	3.0	1089	0.20		
				7.10	7.60
BZA	6.1	2873	1.45		
				1.86	5.80
ETO	9.2	3397	2.69		

[a] Retention time, in minutes.
[b] Number of theoretical plates.
[c] Retention factor.
[d] Selectivity, between each two successive peaks.
[e] Resolution, between each two successive peaks.

3.1.2. Effect of sodium laurylsulfate concentration

SLS was added to improve the sharpness and symmetry of the DOX peak in combination I. It was found that SLS has an effect on MHB and DOX peaks, it will increase the retention times of both drugs and improves the peak symmetry of DOX. A concentration of 0.1% of SLS in the mobile phase was found optimum and produced maximum sharpness and symmetry of the DOX peak.

3.1.3. Effect of pH

The influence of the pH of the aqueous component of the mobile phase was studied by using aqueous phases at various pH values between 3.0 and 7.0 (adjusted using orthophosphoric acid or sodium hydroxide). These solutions with 50 or 30% acetonitrile were used as the mobile phase for combinations I and II, respectively. The pH had only a marked effect on the retention of DOX in combination I, where a pH 3.7 was selected as it provided optimum resolution which was similar to that achieved at higher pH values but with the added advantage of increased speed, the last compound being eluted within 5.9 min. For combination II, increasing the pH had nearly no effect on the retention times of CYT and BZA and only a slight increase in the retention time of ETO was noticed with the increase in pH. The separation was carried out at pH 6.0 since the highest symmetry and peak height was observed for both ETO and CYT at such pH.

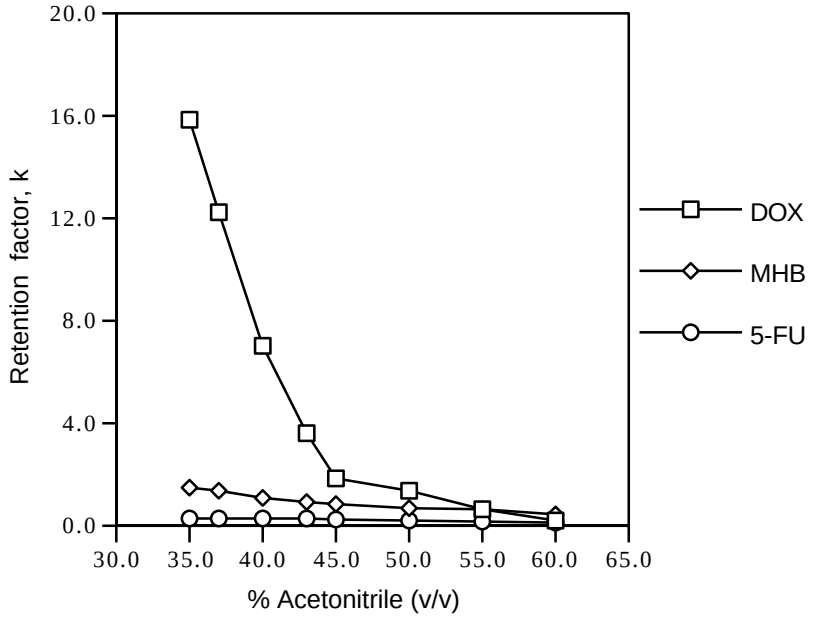

Fig. 4. Variation of the retention times of combination I components as a function of the percentage of acetonitrile in the mobile phase.

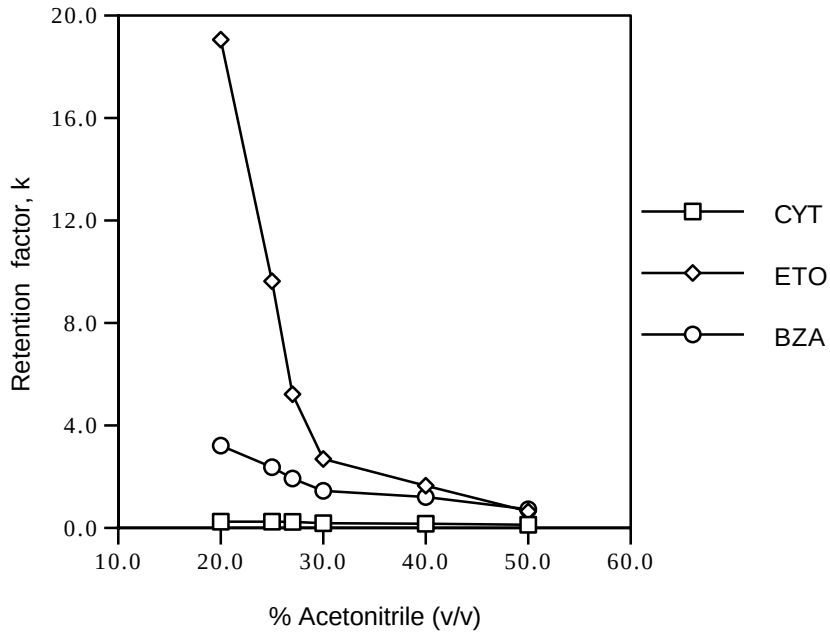

Fig. 5. Variation of the retention times of combination II components as a function of the percentage of acetonitrile in the mobile phase.

Table 3
Regression and statistical parameters for the determination of drug combinations I and II by the proposed HPLC method

Linearity range (μg/ml)		Regression data			$S_{y/x}$[d]	S_a[e]	S_b[f]	LOD[g] (μg/ml)	LOQ[h] (μg/ml)
		a[a]	b[b]	r[c]					
Combination I									
DOX	15–80	−124900	167000	0.9998	104000	112100	2040	1.82	6.07
5-FU	2–40	99150	391900	0.9999	83030	55790	2703	0.49	1.62
MHB	1.5–8	−39290	165100	0.9999	16990	20760	3790	0.30	1.30
Combination II									
CYT	0.5–30	89945	489410	0.9998	125962	71006	5065	0.15	0.45
ETO	1.5–200	−17366	182935	0.9999	1197741	59518	682	0.45	1.23
BZA	2–300	−66821	49026	0.9999	89267	40100	326	0.62	2.08

[a] Intercept.
[b] Slope.
[c] Correlation coefficient.
[d] Standard deviation of residuals.
[e] Standard deviation of intercept.
[f] Standard deviation of slope.
[g] Limit of detection.
[h] Limit of quantitation.

3.2. Statistical analysis of results

3.2.1. Concentration ranges and calibration graphs

Under the above described experimental conditions, linear relationship was observed by plotting drug concentrations against peak area for each compound, the corresponding concentration ranges are listed in Table 3. The slopes, intercepts and correlation coefficients obtained by the linear least squares regression treatment of the results are also given. The high values of the correlation coefficients (r-values >0.999) with negligible intercepts indicate the good linearity of the calibration graphs. Standard deviations of residuals ($S_{y/x}$), of intercept (S_a), and of slope (S_b) are presented for each compound. ($S_{y/x}$) is a measure of the extent of deviation of the found (measured)

Table 4
Evaluation of the precision and accuracy for the determination of drug combinations I and II in synthetic mixtures by the proposed HPLC method

Nominal value in synthetic mixture (μg/ml)			Percentage recovery ± S.D.[a]			R.S.D.[b] (%)			E_r[c] (%)		
DOX	5-FU	MHB	DOX	5-FU	MHB	DOX	5-FU	MHB	DOX	5-FU	MHB
20	40	2	99.6 ± 0.52	99.8 ± 0.34	99.3 ± 0.31	0.52	0.34	0.31	−0.4	−0.2	−0.7
30	30	3	100.4 ± 0.29	99.4 ± 1.21	100.4 ± 1.04	0.29	1.21	1.04	0.4	−0.6	0.4
40	20	4	99.8 ± 0.74	100.1 ± 0.44	100.1 ± 0.80	0.74	0.44	0.80	−0.2	0.1	0.1
60	10	6	99.9 ± 0.19	100.0 ± 0.89	100.0 ± 0.69	0.19	0.89	0.69	−0.1	0.0	0.0
80	2	8	99.4 ± 0.10	100.2 ± 0.98	98.7 ± 0.90	0.10	0.98	0.91	−0.6	0.2	−1.3
CYT	ETO	BZA	CYT	ETO	BZA	CYT	ETO	BZA	CYT	ETO	BZA
1	200	300	100.6 ± 0.54	100.1 ± 0.05	100.1 ± 0.03	0.54	0.05	0.03	0.6	0.1	0.1
5	80	120	100.9 ± 1.12	100.2 ± 1.00	100.1 ± 0.12	1.11	1.00	0.12	0.9	0.2	0.1
10	40	60	101.1 ± 1.00	100.1 ± 0.12	100.0 ± 0.02	0.99	0.12	0.02	1.1	0.1	0.0
20	10	15	100.8 ± 0.56	100.0 ± 0.10	99.9 ± 0.03	0.56	0.10	0.03	0.8	0.0	−0.1
30	2	3	99.9 ± 0.15	100.2 ± 0.25	100.2 ± 0.14	0.15	0.25	0.14	−0.1	0.2	0.2

[a] Mean ± standard deviation of three determinations.
[b] Percentage relative standard deviation.
[c] Percentage relative error.

Table 5
Evaluation of the precision and accuracy for the determination of synthetic mixtures of the pharmaceutical preparations of drug combinations I and II by the proposed HPLC methods

Nominal value in synthetic mixture (μg/ml)			Percentage recovery ± S.D.[a]			R.S.D.[b] (%)			E_r[c] (%)		
DOX	5-FU	MHB	DOX	5-FU	MHB	DOX	5-FU	MHB	DOX	5-FU	MHB
20	40	2	100.6 ± 0.83	99.5 ± 0.54	99.4 ± 0.61	0.83	0.54	0.61	0.6	−0.5	−0.6
30	30	3	100.0 ± 1.17	99.8 ± 0.32	100.0 ± 0.66	1.17	0.32	0.66	0.0	−0.2	0.0
40	20	4	99.6 ± 0.38	100.1 ± 0.40	99.3 ± 0.54	0.38	0.40	0.54	−0.4	0.1	−0.7
60	10	6	100.2 ± 0.39	100.2± 0.52	99.8 ± 0.50	0.39	0.52	0.50	0.2	0.2	−0.2
80	2	8	99.0 ± 0.20	99.9 ± 1.07	100.4 ± 0.89	0.20	0.1.07	0.89	−1.0	−0.1	0.4
CYT	ETO	BZA	CYT	ETO	BZA	CYT	ETO	BZA	CYT	ETO	BZA
1	200	300	100.2 ± 0.67	100.1 ± 0.07	100.0 ± 0.08	0.67	0.07	0.08	0.2	0.1	0.0
5	80	120	100.6 ± 1.53	100.3 ± 0.05	99.9 ± 0.11	1.53	0.05	0.11	0.6	0.3	−0.1
10	40	60	101.0 ± 0.83	100.1 ± 0.17	100.1 ± 0.15	0.82	0.17	0.15	1.0	0.1	0.1
20	10	15	100.0 ± 0.66	100.3 ± 1.10	100.6 ± 0.26	0.66	1.10	0.26	0.0	0.3	0.6
30	2	3	99.9 ± 0.41	99.4 ± 1.60	100.1 ± 0.60	0.41	1.61	0.60	−0.1	−0.6	0.1

[a] Mean ± standard deviation of three determinations.
[b] Percentage relative standard deviation.
[c] Percentage relative error.

y-values from the calculated ones. The $S_{y/x}$ value is also involved in the calculation of S_a and S_b-values.

3.2.2. Detection and quantitation limits

Limit of detection (LOD) is defined in the BP as the concentration which has a signal-to-noise ratio of 3:1. For limit of quantitation (LOQ), the ratio considered was 10:1 with a R.S.D. value less than 10%. LOD and LOQ for each compound were calculated and are presented in Table 3.

3.2.3. Precision and accuracy

In order to assess the precision, as percentage relative standard deviation (R.S.D.%), and the accuracy,

Table 6
Assay results of drug combinations I and II in spiked human plasma by the proposed HPLC method

Spiked concentration (μg/ml)		Final concentration (μg/ml)		Percentage recovery ± S.D.[a]		R.S.D.[b] (%)		E_r[c] (%)	
DOX	5-FU	DOX	5-FU	DOX	5-FU	DOX	5-FU	DOX	5-FU
5.0	10.0	20	40	99.9 ± 0.46	100.0 ± 0.79	0.46	0.79	−0.1	0.0
7.5	7.5	30	30	100.1 ± 0.45	100.2 ± 0.85	0.45	0.85	0.1	0.2
10.0	5.0	40	20	99.7 ± 1.04	99.4 ± 0.54	1.04	0.54	−0.3	−0.6
15.0	2.5	60	10	99.3 ± 0.55	100.1 ± 0.85	0.55	0.85	−0.7	0.1
20.0	0.5	80	2	100.2 ± 0.70	99.4 ± 1.06	0.70	1.06	0.2	−0.6
CYT	ETO	CYT	ETO	CYT	ETO	CYT	ETO	CYT	ETO
1	40	1	40	101.1 ± 0.33	100.1 ± 0.21	0.33	0.21	1.1	0.1
5	20	5	20	100.6 ± 0.69	99.9 ± 0.26	0.69	0.26	0.6	−0.1
10	10	10	10	100.3 ± 0.62	99.9 ± 0.50	0.62	0.50	0.3	−0.1
20	5	20	5	101.3 ± 0.27	100.3 ± 1.31	0.27	1.31	1.3	0.3
30	2	30	2	100.3 ± 0.29	99.4 ± 1.05	0.29	1.06	0.3	−0.6

[a] Mean ± standard deviation of three determinations.
[b] Percentage relative standard deviation.
[c] Percentage relative error.

as percentage relative error (E_r%), of the proposed HPLC method, triplicate determinations were carried out on synthetic mixtures of different proportions, for both combinations. The data shown in Table 4 indicate good accuracy and precision of the proposed procedure.

3.3. Analysis of pharmaceutical formulations

Synthetic mixtures of DOX (Adriblastina®) vials and 5-FU (Fluorouracil) vials or of CYT (Aracytin™) vials and ETO (Vepesid™) vials, for combinations I and II, respectively, were made and analyzed by the proposed HPLC method. Excipients in the preparations did not interfere in the analysis. In addition, the fact that MHB (the preservative in Adriblastina® vials) or BZA (the preservative in Vepesid™ vials) could also be assayed as separate peaks was a plus. The results obtained are listed in Table 5. The accuracy and precision were satisfactory to the label claim.

3.4. Analysis of spiked human plasma samples

The proposed HPLC method was applied for the simultaneous determination of CYT and ETO as well as of DOX and 5-FU in plasma of healthy volunteers spiked with both drugs. The concentrations of these compounds in plasma of cancerous patients vary widely in the literature, depending on the dose used and the drug administration regimen prescribed (it is highly specific case for cancerous patients). However, their usual concentration ranges according to the PDR [19] are: 4.0–8.5, 10–125, 0.5–5.0, and 17–88 μg/ml for DOX, 5-FU, CYT, and ETO, respectively.

Methanol was used for protein precipitation prior to sample preparation. Specificity of the method was assessed after carrying out the chromatographic procedure on blank plasma samples (after protein precipitation) and since no interfering peaks were detected at the retention times of the analytes, it was concluded that no endogenous substances from plasma interfered with the assay. To assess method precision, three determinations for each concentration examined were conducted and the standard deviation was calculated. The results obtained are listed in Table 6. The method is quite fast and effective for the determination of both combinations in plasma samples.

4. Conclusion

The proposed HPLC methods can be readily applied for the simultaneous determination of both 5-FU and DOX or of CYT and ETO in injection solutions and in plasma samples of cancerous patients. The proposed methods are specific and there is no interference from any of the sample components, in addition, they can also be used to determine the MHB preservative in DOX vials (combination I) or the BZA preservative in ETO vials (combination II). The methods are quite selective, sensitive and are suitable for routine blood drug-monitoring of both combinations.

References

[1] British Pharmacopoeia, HMSO, London, 2001.
[2] United States Pharmacopoeia 24, The National Formulary 19, US Parmacopoeial Convention Inc., Rockville, MD, 2000.
[3] C.L. Chen, K.K. Thoen, F.M. Uckun, J. Chromatogr. B: Biomed. Appl. 764 (2001) 81–119.
[4] P.M. Loadman, C.R. Calabrese, J. Chromatogr. B: Biomed. Appl. 764 (2001) 193–206.
[5] G. Zagotto, B. Gatto, S. Moro, C. Sissi, M. Palumbo, J. Chromatogr. B: Biomed. Appl. 764 (2001) 161–171.
[6] Y.S.R. Krishnaiah, R.S. Karthikeyan, V. Satyanarayana, Indian Drugs 39 (2002) 23–26.
[7] C.H. Liu, S.L. Liu, J.F. Bian, Z. Geng, Y. Lu, H.Y. Hu, Yaowu Fenxi Zazhi 21 (2001) 95–97.
[8] J. Escoriaza, A. Aldaz, E. Calvo, J. Giraldez, J. Chromatogr. B: Biomed. Appl. 736 (1999) 97–102.
[9] G. Micoli, R. Turci, M. Arpellini, C. Minoia, J. Chromatogr. B: Biomed. Appl. 750 (2001) 25–32.
[10] L.D. Kissinger, N.L. Stemm, J. Chromatogr. 26 (1986) 353309–353318.
[11] S.V. Galushko, I.P. Shishkina, J. Pharm. Biomed. Anal. 10 (1992) 1093–1095.
[12] S.G. Arbuck, Y. Silk, H.O. Douglass Jr., H. Nava, Y.M. Rustum, S. Milliron, Cancer 65 (1990) 2442–2445.
[13] M. Volm, J. Mattern, Anticancer Res. 12 (1992) 2293–2296.
[14] I.W. Dimery, S.S. Legha, M. Shirinian, W.K. Hong, J. Clin. Oncol. 8 (1990) 1056–1062.
[15] S. Paik, J. Bryant, E. Tan Chiu, G. Yothers, C. Park, D.L. Wickerham, N. Wolmark, J. Natl. Cancer Inst. 92 (2000) 1991–1998.
[16] E.M. Ritzi, S. Pyo, Life Sci. 54 (1994) 887–900.
[17] J.H. Lee, K.H. Lee, S. Kim, M. Seol, S.H. Kim, W.K. Kim, J.S. Lee, Leukemia Res. 25 (2001) 305–312.
[18] C. Gisselbrecht, E. Oksenhendler, U. Tirelli, E. Lepage, J. Gabarre, J.P. Farcet, R. Gastaldi, B. Coiffier, A. Thyss, et al., Am. J. Med. 95 (1993) 188–196.
[19] Physician's Desk Reference Electronic Library, ver. 6.0.0a, 57th ed., Medical Electronics, 2003.