

Liquid-liquid extraction combined with high performance liquid chromatography-diode array-ultra-violet for simultaneous determination of antineoplastic drugs in plasma

Ananda Lima Sanson¹, Suéllen Cristina Rennó Silva¹, Matheus Coutinho Gonçalves Martins¹, Alexandre Giusti-Paiva², Patrícia Penido Maia^{1*}, Isarita Martins¹

¹Faculty of Pharmacy, Laboratory of Toxicological Analysis, Federal University of Alfenas, ²Institute of Biomedical Sciences, Federal University of Alfenas

A liquid-liquid extraction (LLE) combined with high-performance liquid chromatography-diode array detection method for simultaneous analysis of four chemically and structurally different antineoplastic drugs (cyclophosphamide, doxorubicin, 5-fluorouracil and ifosfamide) was developed. The assay was performed by isocratic elution, with a C18 column (5 μm , 250 x 4.6 mm) and mobile phase constituted by water pH 4.0- acetonitrile-methanol (68:19:13, v/v/v), which allowed satisfactory separation of the compounds of interest. LLE, with ethyl acetate, was used for sample clean-up with recoveries ranging from 60 to 98%. The linear ranges were from 0.5 to 100 $\mu\text{g mL}^{-1}$, for doxorubicin and 1 to 100 $\mu\text{g mL}^{-1}$, for the other compounds. The relative standard deviations ranged from 5.5 to 17.7%. This method is a fast and simple alternative that can be used, simultaneously, for the determination of the four drugs in plasma, with a range enabling quantification of the drugs in pharmacokinetics, bioequivalence and therapeutic drug-monitoring studies.

Uniterms: Antineoplastic drugs/simultaneous determination in plasma. Liquid-liquid extraction. High-performance liquid chromatography/diode array detector.

Um método de extração líquido-líquido (ELL) combinado com cromatografia líquida de alta eficiência-detector de arranjo de diodos foi desenvolvido para análise simultânea de quatro fármacos antineoplásicos quimicamente e estruturalmente diferentes (ciclofosfamida, doxorubicina, fluoruracila e ifosfamida). O estudo foi realizado sob condições isocráticas, com coluna C18 (5 μm , 250 x 4.6 mm) e fase móvel constituída por água pH 4.0-acetonitrila-metanol (68:19:13, v/v/v), que permitiu separação satisfatória dos analitos de interesse. A ELL, com acetato de etila, foi utilizada para limpeza da amostra, com recuperação variando de 60 a 98%. As faixas foram lineares de 0,5 a 100 $\mu\text{g mL}^{-1}$ para doxorubicina e 1 a 100 $\mu\text{g mL}^{-1}$ para os outros compostos. O desvio padrão relativo variou de 5,5 a 17,7%. Este método é uma alternativa rápida e simples que pode ser usado, simultaneamente, para a determinação dos quatro fármacos em plasma, com uma faixa que permite quantificá-los em estudos de farmacocinética, bioequivalência e monitorização terapêutica.

Unitermos: Fármacos antineoplásicos/determinação simultânea em plasma. Extração líquido-líquido. Cromatografia líquida de alta eficiência/detector de arranjo de diodos.

INTRODUCTION

Antineoplastic agents are drugs used in chemotherapy which are not selective for cancer cells since they

also affect the growth and reproduction of healthy cells. According to the International Agency for Research on Cancer (IARC), at least nine alkylating cytostatic drugs are classified as carcinogenic to humans (Group 1). In addition, several drugs are classified by the IARC into Groups 2A and 2B (probably and possibly carcinogenic to humans, respectively). The optimal dose should produce a maximal anti-tumor effect with acceptable levels of toxicity.

*Correspondence: P. P. Maia, Laboratório de Análises Toxicológicas, Departamento de Análises Clínicas e Toxicológicas, Universidade Federal de Alfenas, Rua Gabriel Monteiro da Silva, 700, 37130.000 - Alfenas - MG, Brazil. E-mail: patpenido@unifal-mg.edu.br

ty. In clinical practice, these drugs frequently cause toxic effects that would be considered unacceptable. Although toxicity is commonly regarded as the most important effect to control, partly because it is easier to measure, the risk of under-dosing and reduced efficacy must also be considered. In most of the current chemotherapy protocols, antineoplastics are used as a combination of agents having different mechanisms of action in order to decrease the dose of each agent individually thus keeping the synergism of therapeutic effects and minimizing the toxic effects (Turci *et al.*, 2003)

Combination chemotherapy is administered in order to target as many types of cells in the tumor as possible. Selection of agents for combination chemotherapy regimens involves consideration of drug-specific factors such as mechanism of action, antitumor activity, and toxicity profile. Drugs that possess minimally overlapping mechanisms of action and toxicities are combined when possible (Kamil *et al.*, 2010).

Nevertheless, as a consequence, patients may be exposed to a wide variety of substances and few methods have been described for the simultaneous determination of these drugs in plasma (Roberts *et al.*, 2006; Turci *et al.*, 2002; Kaestner, Sewell, 2007). In the present study, a single method was developed to determine four substances with toxicological significance in plasma.

Cyclophosphamide (CP), 2-bis(2-chloroethyl) aminotetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide, is one of the most commonly used antineoplastic alkylating agents. It has also been used due to its immunosuppressive properties in the treatment of autoimmune diseases as well as in renal and bone marrow transplants. CP has been classified as a human carcinogen (Group 1) by the IARC. Ifosfamide (IF), 3-(2-chloroethyl)-2-[(2-chloro-ethyl) amino] tetrahydro-2H-1,3,2-oxazaphosphorin-2-oxide, is a structural analogue of CP having alkylating activity after enzymatic activation to cytotoxic compounds. IF differs from CP in the transfer of one 2-chloroethyl group from the extracyclic to the endocyclic nitrogen. This results in differences in pharmacological and toxicological properties. Although IF has been shown to be carcinogenic in animals, there is no evidence of its carcinogenicity in humans and it is therefore included by the IARC in Group 3. Both cyclophosphamide and ifosfamide are widely used in the treatment of non-Hodgkin lymphomas and a variety of bone and soft tissue sarcomas. The pharmacological profile of these drugs is important and, in order to gather this information, reliable methods for the determination of oxazaphosphorines and their metabolites are necessary when determining optimal dosages (Turci *et al.*, 2003; Ekhart *et al.*, 2007; Baumann, Preiss, 2001).

Doxorubicin (DOX) is classified as an anthracycline, a glycosidic derivative of tetra-hydronaphthacene that belongs to the tetracycline class of antibiotics. The antineoplastic activity of these drugs has been mainly attributed to their strong interactions with DNA in target cells. These drugs can deform DNA structure and terminate its biological function. Furthermore, they can be reduced to their semiquinone form by biological reducing agents, such as NADH and NADPH. Free radicals can be generated, which can attack cell components, such as membrane and DNA, and halt cell growth. According to the IARC, these agents are probably carcinogenic to humans (Group 2A). There is inadequate evidence of carcinogenicity in humans, but sufficient evidence of carcinogenicity in animals (Turci *et al.*, 2003; Ekhart *et al.*, 2007).

The drug 5-fluorouracil (5-FU), 5-fluoropyrimidine-2,4-dione, is an antimetabolite frequently used in the treatment of malignancies including cancers of the gastrointestinal tract, lung, and breast. It acts as a competitor of pyrimidine and needs to be converted to the nucleotide level so as to exert its effect. 5-FU is classified as Group 3 (not classifiable as to carcinogenicity to humans) by the IARC based on inadequate evidence of carcinogenicity in humans and animals (Turci *et al.*, 2003).

Several methods have been described in literature for environmental and biological monitoring of antineoplastics. The majority of the papers have described methods employing HPLC, using various detector types. Anthracyclines and 5-FU can be determined by HPLC with fluorescence and UV detection, respectively. Sample extracts were injected into a C18 reversed-phase column with a proportion of organic solvent and buffer as mobile phase. Determination of oxazaphosphorines in serum, blood or plasma has been demonstrated by using HPLC with detection at low UV wavelengths. The sample preparation techniques commonly used involve liquid-liquid extraction (LLE) and solid phase extraction (SPE) (Boos *et al.*, 1994; Burton, James, 1988; Hardy, Erlichman, Soldin, 1984; Margison *et al.*, 1986; May-Manke *et al.*, 1999; Rustum, Hoffman, 1987; Sottani *et al.*, 2005; Martins *et al.*, 2009).

Since HPLC assays are inexpensive and widely used, they represent a good choice for the analysis of antineoplastic drugs. Moreover, LLE is still the sample preparation technique most employed and used in routine analysis. The purpose of this investigation was to develop a single analytical LLE-HPLC-UV method for the simultaneous determination of CP, IF, 5-FU and DOX in plasma samples.

MATERIALS AND METHODS

Instrumental and operating conditions

The HPLC equipment consisted of a Shimadzu LC-10ATvp (Kyoto, Japan) gradient system equipped with a Shimadzu SIL-10AF (Kyoto, Japan) auto-injector with a 50 μL loop. The column oven used was a Shimadzu CTO-10ASvp (Kyoto, Japan) operated at 25 °C. Firstly, the detection was performed with a Shimadzu SPD-M10Avp (Kyoto, Japan) diode array detector and the quantification was performed with a Shimadzu SPD-10Avp (Kyoto, Japan) UV detector at 195 nm and at 265 nm. Separation was achieved by isocratic elution carried out with a mobile phase constituted of water adjusted to pH 4 (with hydrochloric acid)- acetonitrile- methanol (68:19:13, v/v/v), at a flow rate of 0.6 mL min⁻¹ for 8 min and then 1.0 mL min⁻¹, with a Shimpak CLC-ODS (250 x 4.6 mm, 5 μm) column protected by a similar guard-column (40 x 4.6 mm). Data acquisition and treatment were performed by Class-VP software (Shimadzu).

Standards and reagents

Cyclophosphamide (CP), 5-fluorouracil (5-FU) and ifosfamide (IF) were purchased from Sigma-Aldrich Chemical Company (St. Louis, USA); doxorubicin (DOX) was purchased from Fluka (Buchs, Switzerland). Acetonitrile and methanol (HPLC grade) were obtained from Mallinckrodt® (Paris, USA). Other chemicals that were used include the following: hydrochloric acid, isopropyl alcohol, chloroform, ethyl ether and ethyl acetate from Vetec (Rio de Janeiro, Brazil). Water was distilled and purified by using a Millipore® Milli-Q Plus system (São Paulo, Brazil).

Standard solutions

Stock standard solutions were prepared by dissolution of each drug in methanol to obtain a concentration of 1 mg mL⁻¹. These solutions were stored at -20 °C and prepared weekly. The working solutions were prepared each day by making a 10-fold dilution of the stock solution in methanol.

Sample preparation

Aliquots of 1.0 mL of blank plasma spiked with the drugs (CP, IF, DOX and 5-FU) were extracted with 5.0 mL of ethyl acetate. The tubes were submitted to shaking for 15 minutes at 50 rpm and then centrifuged for 10 minutes

at 4000 x g. The upper organic phases were transferred to conical tubes and separated, and the aqueous layer re-extracted with 5.0 mL of ethyl acetate. The organic phases were combined and evaporated at 40 °C under a gentle stream of nitrogen. The residues were reconstituted in 200 μL mobile phase and 50 μL were injected into the HPLC system.

Confidence parameters

Validation of this study was in compliance with FDA guidelines (FDA, 2001). The following parameters were assayed: robustness, linearity, lower limit of quantification (LOQ), precision and recovery.

Robustness was performed at middle level (20 $\mu\text{g mL}^{-1}$) and was explored using mobile phase flow rate and column temperature. Linearity was tested by examination of a plot of residues produced by linear regression of the responses on the amounts of the analytes in a calibration set, between 1 and 100 $\mu\text{g mL}^{-1}$, in six replicates for each level. A calibration curve was generated in duplicate for each analytical run and consisted of a blank and six non-zero samples covering the expected range, including LOQ.

LOQ was obtained by the successive dilutions to determine the lowest concentration with accuracy and precision, as 10% RSD (relative standard deviation), with a signal-to-noise ratio of 10:1.

Precision of the method was evaluated by within-day (n=3) assay using plasma spiked with the drugs at LOQ, middle and high level, during a single analytical run. The results obtained for precision were expressed as relative standard deviation (RSD %). Recovery was determined by five replicate analyses of samples after spiking with a known mass of the analyte, in the non-contaminated samples. The results were compared with those obtained when the analyte was spiked after the clean-up procedure of the sample.

Method application

To assess the applicability of the validated method, the level of the drugs in plasma were measured after administration of the drugs at 10 mg/kg in Male Wistar rats. The animals (n=5), weighing 300 \pm 20 g, were kept under standardized conditions, according to institutional guidelines. Blood samples were collected (jugular vein cannulation) at 0 min, 15 min, 3, 6, 12 and 24 hours after intravenous administration of the drugs in saline. The samples were centrifuged for 15 min at 2000 x g and the plasma was stored at -20 °C until analysis.

RESULTS AND DISCUSSION

The HPLC method appears to be the most feasible for attaining the lower limit of detection and quantification when used for detection of multiple antineoplastics (Larson, Khazaeli, Dillon, 2003). Since patients are exposed to a wide variety of drugs, it is necessary to develop methods able to quantify multiple agents, with a single sampling. Although there is a large demand for analytical methods that simultaneously determine various compounds in biological and non biological matrices, current literature presents methods for an individual agent only, or for chemically similar groups. In this study, the aim was to develop a method able to determine the presence of four structurally different drugs (Figure 1), extensively used in clinical practice, in a single analysis. This enables more comprehensive monitoring of the therapeutic treatment to be carried out.

Optimized chromatographic conditions

The diode array detector was able to analyze, simultaneously, the extract sample at various wavelengths. In fact, this property increased the selectivity of the method since the similarity of the peaks, in the retention time of the analytes (Table I), are compared with the second

derivative spectrum, in a library developed with standard solutions (Figure 1).

The HPLC behavior of CP, IF, 5-FU and DOX extracted from human plasma was isocratically examined by using a C-18 analytical column. Several mobile phases were tested and it was found that water adjusted to pH 4- acetonitrile-methanol (68:19:13, v/v/v) at a flow rate of 0.6 mL min⁻¹ for 8 min and 1.0 mL min⁻¹ allowed satisfactory separation of the compounds of interest. The method robustness was demonstrated since the response of the analytes remained constant when one percent variation in the organic composition of the mobile phase, in the flow rate of mobile phase and in the column temperature was applied to the chromatographic conditions.

Before performing validation experiments, system suitability was evaluated. These tests are used to verify if the resolution and repeatability of the system are adequate for the analysis and are used for checking system performance (Shabir, 2003).

Parameters such as plate count, tailing factors and resolution were determined and compared against the specifications, as demonstrated in Table I. It is possible to observe that the system was suitable since the results of the test were considered satisfactory, according to Shabir (2003), who reported an acceptable range of plate count > 2000, resolution > 2.0, tailing factor between 0.5 and 2.0

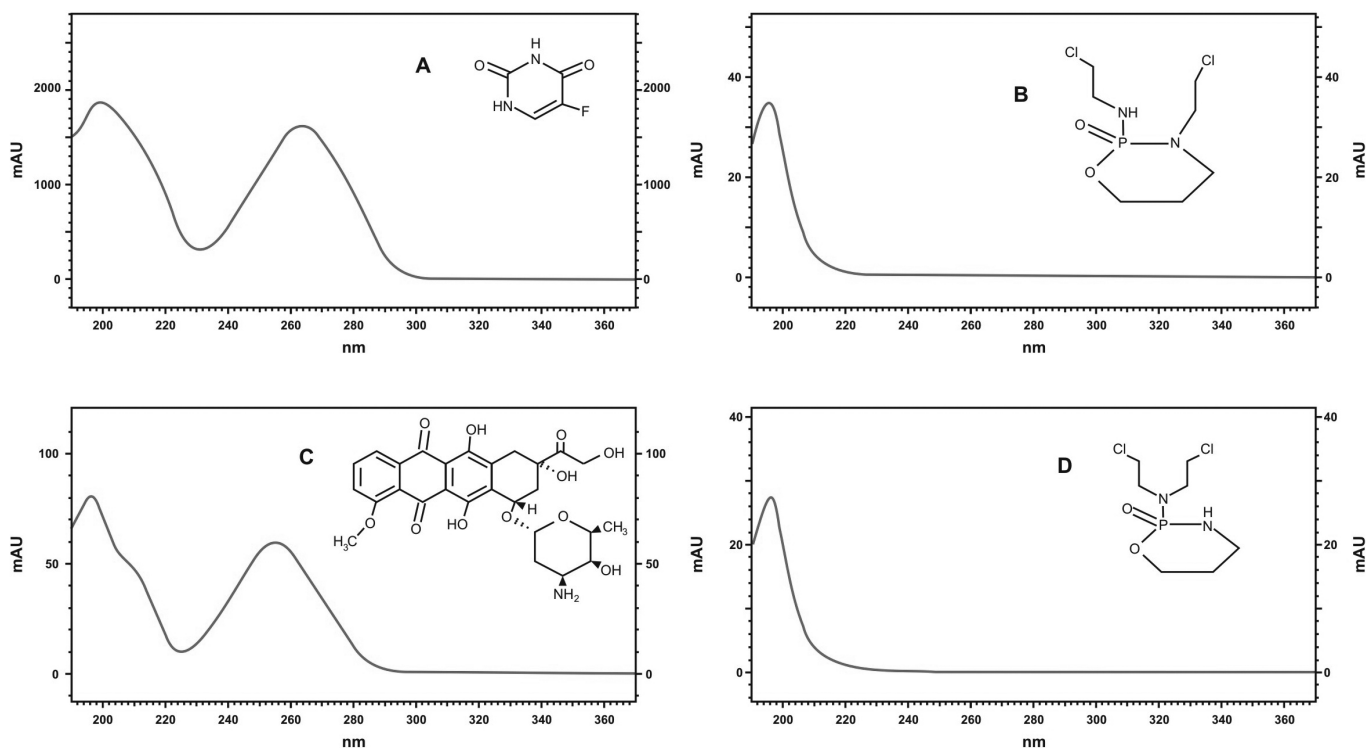


FIGURE 1 - Spectra and chemical structures of antineoplastic drugs: (A) fluorouracil, (B) ifosfamide, (C) doxorubicin and (D) cyclophosphamide.

TABLE I - System suitability parameters* for HPLC-UV method evaluated for simultaneous determination of fluorouracil, ifosfamide, cyclophosphamide and doxorubicin

Analyte	Retention time (min)	Plate count (N)	Resolution (Rs)	Tailing factor (T)	Retention factor (k)
5-FU	5.0	3456	-	1.5	4
IF	15.9	27719	11.9	1.2	15
DOX	16.3	28566	0.4	1.7	17
CP	18.5	26807	2.0	1.5	16

*Reference values: $N \geq 2000$; $R_s \geq 2$; $0.5 \leq T \leq 2$; $k > 2$ (Shabir, 2003)

and $k > 2$ (Shabir, 2003). Although the values of resolution between the peaks of DOX and IF was 0.4, these analytes were read at different wavelengths.

Optimized sample treatment

To clean-up samples, a number of experiments were then conducted to optimize the extraction efficacy. In order to find the most efficient extraction procedure for the analytes studied using LLE, different conditions were compared.

Liquid-liquid extraction is simple and a large number of solvents, pure and commercially available, can be used which provide a wide range of solubility and selectivity. Furthermore, the proteins present in the samples are denatured, eliminating the contamination of the chromatographic column (Queiroz, Collins, Jardim, 2001). Coe *et al.* (1996) reported an LLE procedure for the analysis of fluorouracil in plasma samples, using the pH adjustment to 6.0 by adding acetate buffer, followed by a saturated solution of sodium sulfate and 7.0 mL of ethyl acetate, with around 46% of recovery (Coe *et al.*, 1996). For the ifosfamide and cyclophosphamide isomers, Turci *et al.* (2002) proposed an LLE for urine samples in which 5 mL of sample was adjusted to pH 7, then extracted three times with 10 mL of ethyl acetate (Turci *et al.*, 2002). A percentage of 85% for both analytes was obtained. Kümmerle *et al.* (2003) proposed an LLE for DOX using 500 μ L of biological fluid, pre-treated with a precipitation of the protein, then 250 μ L of acetone and 50 μ L of an aqueous solution of zinc sulfate 70% as a solvent extractor were added (Kümmerle *et al.*, 2003). In our study, the principles of liquid-liquid extraction were optimized according to the following steps: type and volume of extractor solvent, shaking velocity, number of extractions totaling 10 mL and salting-out effect.

Various extractor solvents were tested alone and as a mixture of solvents. The response of the analytes was variable, as shown in Figure 2, and a satisfactory percentage of recovery for the four analytes was obtained with ethyl

acetate. This solvent was the same used in the method of extraction by Turci *et al.* and Coe *et al.* for the analytes ifosfamide and cyclophosphamide, and 5-fluorouracil, respectively (Coe *et al.*, 1996; Turci *et al.*, 2002). The volume of the solvent of 10 mL (Figure 2) was considered optimum since a balance between the efficiency of the extraction and the environmental implications were considered in the present study.

Shaking velocity was tested at speeds of 50 and 70 rpm in an automatic horizontal shaker. As demonstrated in Figure 2, with the rate of 70 rpm a slightly higher percentage of recovery was observed, but this speed caused significant solvent extractor loss and increased interference in the extract. Thus, the 50 rpm rate was considered optimum.

If a component has a low coefficient of distribution between an organic solvent and water, one or more simple extractions will not remove a lot of the components from the water. This distribution coefficient can be changed by adding salt to water. Sometimes, the difference in solubility is dramatic, since organic compounds are more soluble in pure water than in water with salt. Thus, a simple dissolution of salt in the aqueous layer can increase the distribution of an organic compound in an organic solvent; an effect commonly known as salting-out (Queiroz *et al.*, 1995). In the present study the following salts were tested: NaCl, Na₂SO₄ and (NH₄)₂SO₄. However, the addition of salt did not increase the percentage of recovery.

The solid phase extraction was also assessed in order to obtain a selective separation, purification and concentration of compounds present in the complex matrices. According to the literature (Baumann *et al.*, 1999; Micoli *et al.*, 2001; Minoia, Perbelini, 2000), the initial sorbent tested was octadecilsilane (C18) in a cartridge. However, it was not possible to optimize a condition to allow the simultaneous extraction of the four analytes, with a satisfactory percentage of recovery. Therefore, the styrene/divinylbenzene copolymer as sorbent was tested, under different conditions for steps of conditioning, sample pH, washing and elution. But again, these tests did not result in an optimum condition for the agents studied. This

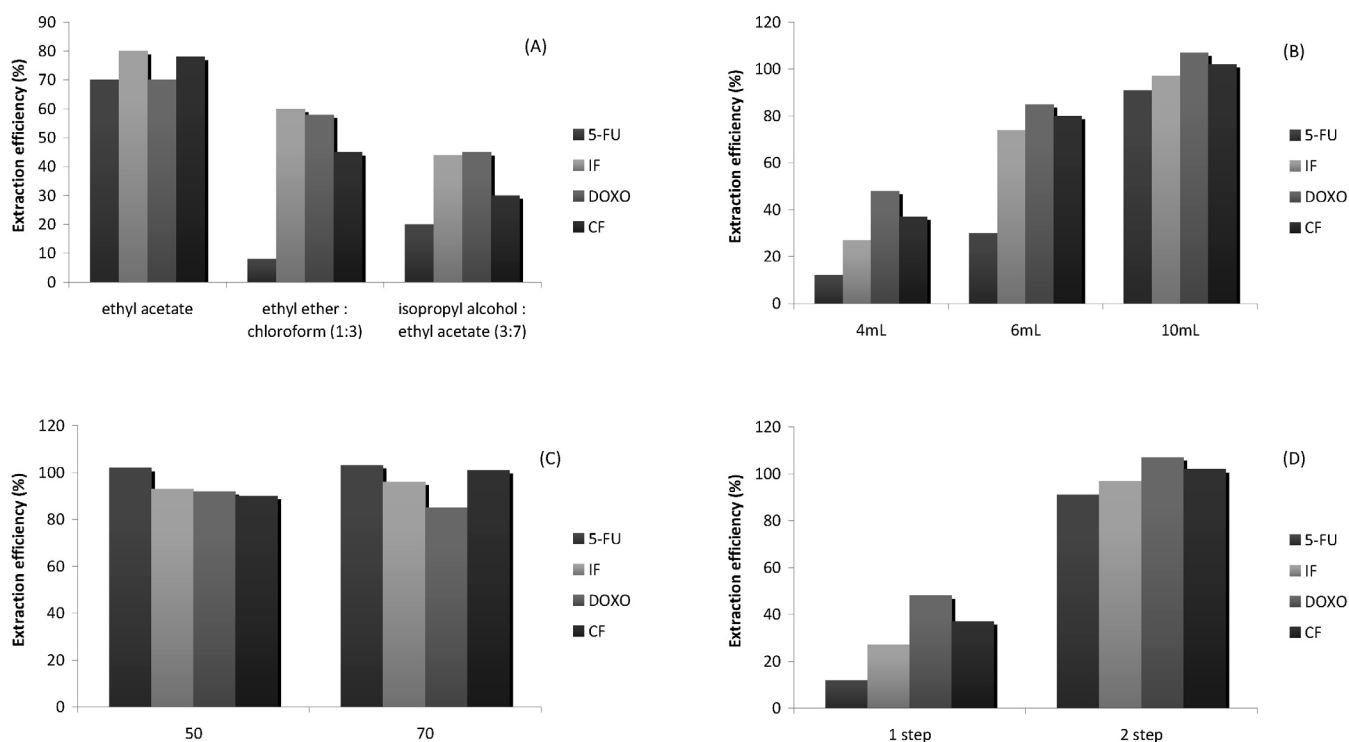


FIGURE 2 - Results expressed as extraction efficiency (%) of fluorouracil (5-FU), ifosfamide (IF), cyclophosphamide (CP) and doxorubicin (DOX), in the LLE studies of type (A) and solvent extractor volume (B), shaking velocity (C) and number of extractions totaling 10 mL (D).

negative result can be explained by the different physico-chemical characteristics of anticancer substances, such as solubility and pKa, factors that interfere with the SPE. Finally, silica and florisil, home-made cartridges, were tested in the matrix clean-up. These tests also produced no satisfactory results. Therefore, LLE was applied in the plasma samples and in Figure 3, it is possible to observe

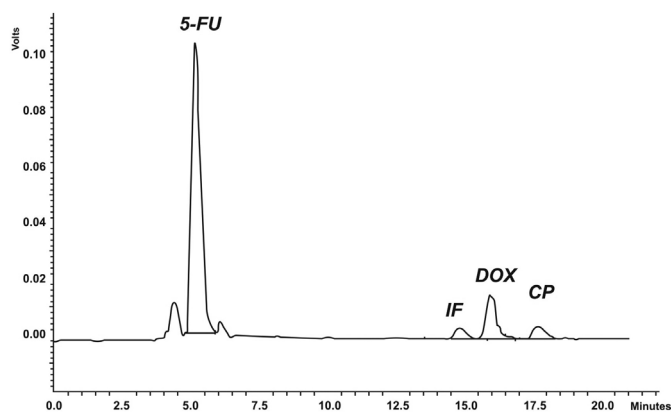


FIGURE 3 - Typical HPLC chromatograms, under optimal conditions evaluated: $20 \mu\text{g mL}^{-1}$ of fluorouracil (5-FU), ifosfamide (IF), doxorubicin (DOX) and cyclophosphamide (CP) extracted from plasma samples.

the typical chromatograms of extracted plasma samples at a fortification level of $20 \mu\text{g mL}^{-1}$ for IF, CF, 5-FU and DOX by using the optimized HPLC conditions.

Confidence parameters

Calibration curves were obtained by plotting the peak area and resulted in a linear response over the range studied. The results of the linearity test are presented in Table II. The calibration curves were linear from $1\text{-}100 \mu\text{g mL}^{-1}$.

The method was linear in the range of $1 \mu\text{g mL}^{-1}$ to $100 \mu\text{g mL}^{-1}$ for the drugs 5-FU, IF and CF. For doxorubicin, the method was linear in the concentration range of $0.5 \mu\text{g mL}^{-1}$ to $100 \mu\text{g mL}^{-1}$. The sensitivity of the method, assessed by the value of the slope, shows that for IF, CF and DOX the method provides similar sensitivity. For 5-FU, the method demonstrated a higher sensitivity, since the value of the slope for this analyte is about 20 times larger compared to other compounds studied.

The LOQ value was $1 \mu\text{g mL}^{-1}$ for the analytes, except for DOX that was $0.5 \mu\text{g mL}^{-1}$. Thus, with these values it is possible to quantify the analytes in plasma samples (Table III) after a therapeutic dose (Goodman, Gilman, 2006). The results are in agreement with the

TABLE II - Analytical curve parameters for simultaneous determination of fluorouracil (5-FU), ifosfamide (IF), doxorubicin (DOX) and cyclophosphamide (CP) by HPLC-DAD-UV

Parameters	5-FU	IF	DOX	CP
Range ($\mu\text{g mL}^{-1}$)	1- 100	1-100	0.5-100	1-100
Correlation coefficient (r)	0.992	0.995	0.998	0.991
Slope	633472	31722	38117	30413
Intercept	20300	67466	42577	-14040

TABLE III - Mean plasmatic concentration of 5-fluorouracil (5-FU), ifosfamide (IF), doxorubicin (DOX) and cyclophosphamide (CP), according to Goodman & Gilman, 2006.

Drug	Mean plasmatic concentration
5-FU	Intravenous administration: $46 \mu\text{g mL}^{-1}$
IF	Intravenous administration: $53.0 \mu\text{g mL}^{-1}$ Oral administration: $52.2 \mu\text{g mL}^{-1}$
DOX	Intravenous administration: $33.7 \mu\text{g mL}^{-1}$
CP	Intravenous administration: $0.49 \mu\text{g mL}^{-1}$

study conducted by Martins et al. 2009 that evaluated a range of 1- $150 \mu\text{g mL}^{-1}$, for simultaneous determination of IF and CP.

The repeatability of the optimized method was calculated by the relative standard deviation (RSD%) and was expressed as the following equation: $\text{RSD}\% = (\text{SD} / \text{mean measured concentration}) \times 100$, and SD is the standard deviation (Table IV). The efficiency of the extraction obtained after the optimized LLE was satisfactory for the drugs studied, as summarized in Table IV.

TABLE IV - Precision and recovery of the method for simultaneous analysis of fluorouracil (5-FU), ifosfamide (IF), doxorubicin (DOX) and cyclophosphamide (CP) by LLE-HPLC-UV in plasma

	Level ($\mu\text{g mL}^{-1}$)		
	1	20	100
5-FU			
RSD %	13.4	11.3	5.7
recovery (%)	74	85	91
IF			
intra-assay (RSD%)	17.7	5.5	9.8
recovery (%)	91	93	96
DOX			
intra-assay (RSD%)	17.6	14.1	8.6
recovery (%)	81	88	97
CP			
intra-assay (RSD%)	8.6	12.5	7.5
recovery (%)	60	90	98

Recoveries near 100% were in compliance with the FDA guide (FDA, 2001). The results obtained with the optimized LLE can be considered satisfactory since the method was precise and accurate. The results were compared with those in the literature, such as the study of Coe *et al.* (1996) that obtained, for 5-FU, an average recovery of 46% and Turci *et al.* (2002), that reported a recovery of 85% for IF and CP in urine, but using a total volume of 30 mL of solvent extractor (Coe *et al.*, 1996; Turci *et al.*, 2002).

Method application

In order to demonstrate the applicability of the validated method the drugs were administered to rats. First, a calibration curve for each drug was obtained by adding 5-FU, IF and CP to aliquots of a pool of plasma of rats at concentrations of 1, 20 and $100 \mu\text{g mL}^{-1}$, and DOX at concentrations of 0.5, 20 and $100 \mu\text{g mL}^{-1}$. These samples were submitted to the method, then the signals obtained were plotted to obtain the analytical curves and linearity values. To check the influence of the matrix, a correlation test was performed comparing the standard curves obtained for each analyte in human plasma to curves obtained in plasma from rats. A correlation between the curves ($r > 0.85$) was observed, confirming that the method validated in human plasma can be used for plasma of animals.

The samples were obtained through the administration of a single intravenous infusion (2 mL) of saline containing antineoplastic agents studied in therapeutic concentration. We also obtained a sample blank by injecting only 2 mL of saline without drugs. The blood samples were then collected at the timepoints of 15 minutes, 3, 6, 12 and 24 hours after the infusion. After separation of plasma, it was submitted to the optimized LLE and the data were used to obtain the kinetic curve of each drug. In Figure 4 it can be observed that the maximum concentration of antineoplastics studied was obtained from the first collection time (15 minutes), as expected for an intravenous infusion. For DOX, a kinetic curve was not constructed since the plasma concentration was only quantified at the first timepoint collection.

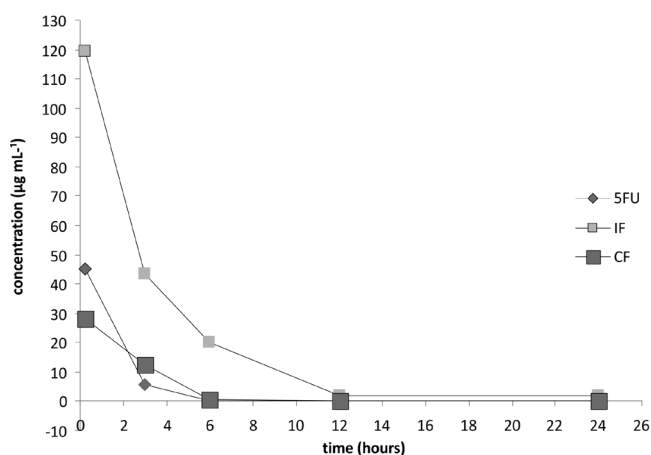


FIGURE 4 - Kinetic curve of antineoplastic drugs 5-fluorouracil (-5-FU), ifosfamide (IF) and cyclophosphamide (CP), obtained after a single intravenous infusion in rats.

CONCLUSIONS

In the present study, an LLE-HPLC-DAD method for the simultaneous analysis of four chemically and structurally different antineoplastic drugs was developed. This method is simple, fast and presented values of linearity, robustness, precision and efficiency of extraction in the range evaluated. Thus, the features of the proposed method to determine the antineoplastic agents fluorouracil, ifosfamide and cyclophosphamide in plasma, i.e., good analytical performance, simplicity and low cost of the technique, make this approach very convenient for application in therapeutic drug monitoring and pharmacokinetic studies. And, for doxorubicin, it can be applied in the diagnosis of poisoning, since it can detect/quantify concentrations above the therapeutic range.

ACKNOWLEDGEMENTS

This research was supported by the National Council for Scientific and Technological Development (CNPq)/Brazil (grant from MCT-CNPq 54/2005, n° 402630/2005), and by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)/Brazil (process number CDS-APQ-4487-4.04/07).

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Received for publication on 13rd July 2010

Accepted for publication on 10th December 2010