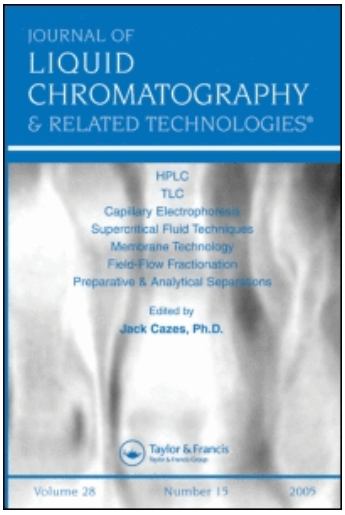

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SENSITIVE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC FLUORESCENCE DETERMINATION OF TOPOTECAN IN HUMAN PLASMA AND PAROTID SALIVA

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ABSTRACT

A sensitive and specific high performance liquid chromatographic (HPLC) method with fluorescence detection was developed and validated for the determination of topotecan, as lactone form and total lactone plus carboxylate forms, in human plasma and parotid saliva samples. The sample pretreatment procedure involved a simple protein precipitation with cold methanol to quantify the lactone form, or a protein precipitation with methanol and acidification with perchloric acid (to convert the lactone ring-opened form into its lactone form quantitatively), to quantify topotecan as a total of lactone and carboxylate forms. The supernatant was analyzed by HPLC using a Zorbax SB-C_{18} column and a mobile phase containing 0.01 M N,N,N',N'-tetramethylethylenediamine (TEMED) in water (pH 6)-0.1 M hexane-1-sulfonic acid in methanol-methanol (62:10:28, v/v/v).

The detection was performed at 361 nm for excitation and 527 nm for emission. The assay showed linearity in the tested range of 0.1- 75 ng mL^{-1}. The limit of quantitation was 0.05 ng mL^{-1}. Precision expressed as %RSD was in the range 0.4 to 17% (limit

of quantitation). Accuracy ranged from 85 to 109%. Extraction recovery from plasma or parotid saliva averaged 90%. In this paper, the stability of topotecan as its lactone form in plasma, blood, and methanolic extracts was tested under various conditions. Particularly interesting was the higher stability of the lactone form in the whole blood. The method's ability to quantify topotecan with precision, accuracy, and sensitivity makes it useful in pharmacokinetic studies.

INTRODUCTION

Topotecan ([S]-9-dimethylaminomethyl-10-hydroxy-camptothecin) is a semisynthetic hydrophilic camptothecin analog which especially inhibits the intranuclear enzyme topoisomerase I.[1-3] This drug has shown anticancer activity against leukemias and a variety of solid tumors in clinical trials (colon cancer, breast cancer, esophagus cancer, neuroblastoma, and ovarian cancer).[4-7] Today, this drug is registered in Europe, since 1996, in the treatment of advanced metastatic ovarian cancer after failure with previous first line chemotherapy.

Topotecan exists as a pentacyclic structure with a lactone moiety in the terminal or E ring (Figure 1). In aqueous solution, the lactone undergoes rapid and reversible pH-dependent hydrolysis to the hydroxy-acid, the predominant form at physiologic pH.[8-10] Only the closed lactone form of the drug inhibits topoisomerase I.

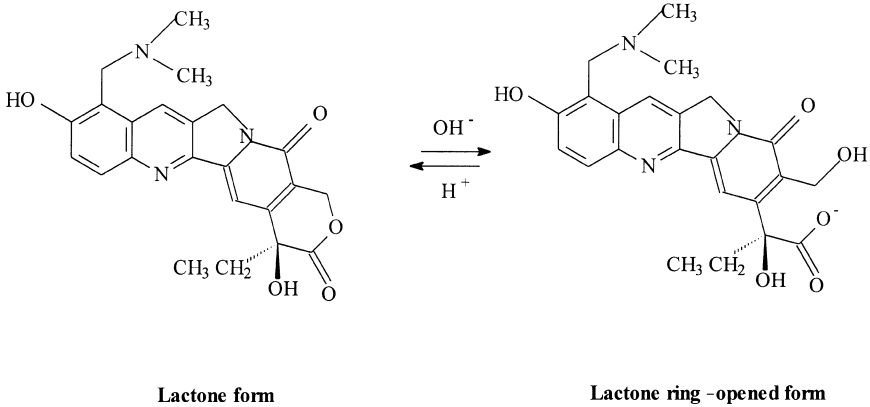

Figure 1. Equilibrium reaction between topotecan (A) and its lactone ring-opened form (B).

Several high performance liquid chromatographic (HPLC) methods have been developed to quantify topotecan in human plasma.[3,11-14] These methods involved the quantification of the lactone form of topotecan (with acidification to quantify the lactone plus carboxylate forms, or without acidification to quantify the lactone form only),[3,14] or the simultaneous analysis of the lactone and the carboxylate forms.[11,12,13] In a recent paper, Rosing et al.[14] developed a method for the simultaneous determination of topotecan and its metabolite *N*-desmethyltopotecan as their lactone forms in human plasma, urine, and feces. However, no bio-analytical assays have been fully validated for the determination of topotecan in parotid saliva. All these methods involved a protein precipitation step with cold methanol and subsequent injection of the supernatant onto the column to quantify the lactone form. For the determination of topotecan as total of the lactone and carboxylate forms, the matrix was acidified to convert the lactone ring-opened form into the lactone form.

This paper describes a rapid, specific, reliable, and sensitive analytical method to quantify topotecan (lactone form or lactone plus carboxylate forms) in human plasma and parotid saliva. The HPLC method presented here, was derived from that of Rosing et al.[3] in human plasma and modified. This method was validated according to validation procedures, parameters, and acceptance criteria based on USP XXIII guidelines[15-17] and recommendations of Shah et al.[16] This method was used to determine topotecan in plasma and saliva samples in patients with advanced malignant ovarian cancer.

EXPERIMENTAL

Materials and Reagents

Topotecan (hydrochloride salt, SKF 104864-A) was purchased from SmithKline Beecham Pharmaceuticals (Nanterre, France). Methanol (Carlo Erba Val de Reuil, France), hexane-1-sulfonic acid, and N,N,N',N'-tetramethylethylenediamine (TEMED) (Sigma, St Louis, MO, USA), perchloric and orthophosphoric acids (Merck, Darmstadt, Germany) were all of analytical-reagent grade. 7% perchloric acid was prepared by dilution in purified water (Laboratoires Fandre, Ludres, France).

The standard solution of topotecan was prepared in purified water at the concentration of 1 mg mL^{-1}. A working solution (10 µg mL^{-1}) was prepared in purified water and stored at –80°C. Subsequent dilutions were performed, extemporaneously, by successive 10-fold dilutions in methanol; they were used to prepare calibration curves and quality control (QC) samples. An unextracted working standard solution (5 ng mL^{-1}) in methanol was prepared daily to check the resolution of the chromatographic system. All manipulations were performed under laminar airflow.

For the validation of the method, human plasma (Centre de Transfusion Sanguine, Montpellier, France) and parotid saliva were obtained from pooled samples collected from healthy volunteers. They were stored at -20°C before use.

Instrumentation

The chromatographic system consisted of a Shimadzu model LC9A pump (Kyoto, Japan), a Shimadzu model RF-10Axl fluorescence detector, a Rheodyne loading valve (model 7010), fitted with a 100-µL sample loop (Touzart et Matignon, Paris, France), and a Shimadzu integrator model C-R5A (chart speed, 5 mm min^{-1}). The excitation wavelength was 361 nm and the emission wavelength was 527 nm. HPLC separation was performed on a stainless-steel Zorbax SB-C_{18} analytical column (75 x 4.6 mm I.D.), packed with 3.5 µm diameter particles (Touzart et Matignon, Paris, France). A guard column (Brownlee RP_{18}, 15 x 3.2 mm I.D., 7 µm particle size; Touzart et Matignon) was placed just before the inlet of the analytical column to reduce its contamination.

Chromatographic Conditions

The eluent was pumped through the column at a flow rate of 1 mL min^{-1} and consisted of a mixture of 0.01 M TEMED in water (the pH was adjusted to 6 with orthophosphoric acid)-0.1 M hexane-1-sulfonic acid in methanol-methanol (62:10:28, v/v/v). This mobile phase was filtered through a 0.45-µm HV filter (Millipore, Bedford, MA, U.S.A.) then deaerated ultrasonically prior to use. The volume injected was 100 µL. Chromatography was performed at ambient temperature (20°C).

Analytical Procedure

Calibration Curves and Quality Control Samples

In 5 mL glass tubes, appropriate volumes of topotecan working solutions in methanol were added to plasma or parotid saliva samples to obtain calibration standards at the concentrations of 0.1, 0.25, 0.5, 0.7, 1, 2.5, 5, 7.5, 10, 25, 50, and 75 ng mL^{-1}.

QC samples were prepared at the concentrations of 0.05, 0.75, 2, 35, and 65 ng mL^{-1}.

The calibration curve and five QC samples were run with every set of twenty unknown samples.

Sample Pretreatment

Lactone Form

For the determination of the analyte as its lactone form, sample preparation was performed on an ice-water bath. In a polypropylene container of 1.5 mL, to 0.1 mL of plasma, 0.2 mL of absolute methanol (-20°C) was immediately added. Proteins were denatured during 15 s of full speed Vortex mixing, then all the vials were centrifuged at 9500g for 4 min. The clear supernatant was diluted 1:1 (v/v) with distilled water and a volume of 100 µL was injected onto the column.

Lactone plus Carboxylate Forms

In a polypropylene container of 1.5 mL, to 0.1 mL of plasma (or parotid saliva), 0.1 mL of absolute methanol (-20°C) and 0.1 mL of 7% perchloric acid were added and proteins were denatured during 15 s of full speed Vortex mixing. Then all the vials were centrifuged at 9500g for 4 min. The clear supernatant was diluted 1:1 (v/v) with distilled water then 100 µL were injected into the column.

Data Analysis

The peak areas were plotted against theoretical concentrations.

Standard calibration curves were obtained from unweighted least-squares linear regression analysis of the data (formula: y = a + bx; where x = concentration and y = peak area). Three different calibrations curves were constructed (0.1 to 1; 1 to 10; and 7.5 to 75 ng mL^{-1}). The detector was set at a sensitivity of 1 and at a gain of 1 for the calibration curve between 0.1 and 1 ng mL^{-1}; for the two other standard curves it was set at a sensitivity of 2 and at a gain of 2. The resulting slopes and intercepts were used to obtain concentration values for that day's quality control samples and unknown samples.

The linearity of the method was confirmed by comparing the slopes and the intercepts of linear calibration curves with zero, and the correlation coefficients with 1. In addition, the normal distribution of the residuals (difference between nominal and back-calculated concentrations) was verified. Moreover, to compare the back-calculated concentrations (C_{TEST}) to the theoretical concentrations (C_{REF}), the Bias (or mean predictor error) was computed as follows:

$$\text{Bias} = \frac{1}{n}\sum_{i=1}^{i=n}\left[C_{TEST(i)} - C_{REF(i)}\right]$$

with n the number residual values.

The 95% confidence interval for bias was also computed.

Selectivity

The specificity of the method was investigated by analyzing ten different batches of blank human plasma and parotid saliva samples from healthy volunteers to determine whether endogenous constituents coeluted with topotecan. The retention times of endogenous compounds in the matrix were compared with that of topotecan.

Plasma and parotid saliva samples from patients receiving other drugs were analyzed for interference. The following drugs were checked: granisetron, ondansetron, tropisetron, alizapride, zolpidem, tianeptine, methylprednisolone, fluoxetine, dextropropoxyphene, paracetamol, enoxaparin, nifedipine, bromazepam, lorazepam, chlorazepate, levothyroxine, zopiclone, tinzaparin, cetirizine, simvastatin, famotidine, glibenclamide, omeprazole.

Precision and Accuracy

Intraday and between-day precisions of the assay were assessed by performing replicate analyses of QC samples in plasma or saliva against a calibration curve. The procedure was repeated on different days, on the same spiked standards, to determine between-day repeatability. Intraday repeatability was determined by treating spiked samples, in replicate, the same day.

The accuracy was evaluated as [mean found concentration/theoretical concentration] x 100. Precision was given by the percent relative standard deviation (RSD).

Extraction Recovery

The extraction efficiency (recovery) was determined two times at five concentration levels (10, 25, 50, 75, and 100 ng mL^{-1}). The recovery was determined by comparing peak areas from plasma or parotid saliva samples spiked with known amounts of drug before protein precipitation versus peak areas of the same concentrations added to the supernatant after protein precipitation.

Determination of the Limit of Quantitation (LOQ)

The LOQ estimated on QC samples was defined as the lowest drug concentration which can be determined with a RSD $\leq$ 20% and an accuracy between 100 $\pm$ 20% on a day-to-day basis.[15-17]

Stability Study

The stability of the lactone form of topotecan was studied using QC samples at concentrations of 7.5, 40, and 75 ng mL^{-1}. Three replicates were analysed at every time point. Results are expressed as percent recovery of initial topotecan concentration.

In plasma, the short-term stability was assessed after 0.25, 0.5, 0.75, 1, 1.5, and 2 h of storage at both ordinary laboratory conditions (20°C and daylight exposure) and at 4°C. Stability assays were also carried out in the whole blood. After sample preparation, the blood QC samples were centrifuged at 1500 g for 10 min after various storage periods (0, 0.25, 0.5, 0.75, 1, 1.5, 2, and 4 h at 20°C; 0, 1, 2, 4, 6, 12, and 16 h at 4°C). At the obtained plasma, cold methanol was immediately added, then the assay procedure was completed as described above.

The stability of the drug in frozen plasma samples (-20°C) was determined by periodic analysis over a span of one month. Samples were analyzed immediately after preparation (reference values), and after storage. Prior to their analysis, cold methanol was immediately added to each sample then samples were brought to room temperature and well vortex- mixed.

The stability of the lactone form of topotecan in the methanol extract originating from plasma spiked with topotecan was also investigated, at 20, 4, and -20°C.

Stability was defined as <10% loss of initial drug concentration.

Pharmacokinetic Study

This method was used for the quantification of topotecan (lactone plus carboxylate forms) in plasma and parotid saliva during a pharmacokinetic study in patients with advanced metastatic ovarian cancer. The study protocol was reviewed and approved by the institutional review board. Topotecan was administered by a 30-min continuous infusion (0.5 to 1.5 mg m^{-2} day^{-1} for 5 successive days). Special attention was paid to sample handling. Blood collected in heparinized tubes and parotid saliva samples were drawn before drug administration (T0), 5 min before the end of infusion, and 30 min, 1, 2, 4, and 8 h after the end of infusion. Blood samples were centrifuged (1500g for 10 min) at 4°C. A 1 mL aliquot of plasma was transferred to another tube. Parotid saliva samples were centrifuged at 9500g for 4 min before storage. Plasma and parotid saliva samples were stored at –80°C until assay. Pharmacokinetic parameters were calculated using the Pk-fit software.[18]

RESULTS

Retention Times and Specificity

Observed retention time was 7.7 min (capacity factor = 6.69). No peaks interfered at the retention time of topotecan (Figures 2a and 3a). No interference was found with all drugs tested that could be co-administered.

Representative chromatograms are shown in Figures 2 and 3.

Linearity

Without Acidification of the Sample

Inter-assay repeatability was determined for calibration curves prepared on different days (n = 6) in plasma. Linear relationships were obtained between the peak area of topotecan and the concentration [$y = 103024 (\pm 10777) x + 2405 (\pm 1512)$, $r^2 = 0.996$ (CV = 0.34%) at low concentrations; $y = 23483 (\pm 1929) x + 2683 (\pm 4176)$, $r^2 = 0.992$ (0.46%) at middle concentrations, and $y = 24254 (\pm 430) x - 14106 (\pm 17516)$, $r^2 = 0.998$ (0.16%) at high concentrations].

With Acidification of the Sample

Inter-assay repeatability was determined for calibration curves prepared on different days (n = 6) in plasma and parotid saliva. In plasma, linear relationships were obtained between the peak area of topotecan and the concentration [$y = 93354 (\pm 646.7) x + 786.9 (\pm 866.0)$, $r^2 = 0.998$ (CV = 0.13%) at low concentrations; $y = 23846 (\pm 3396) x + 820.9 (\pm 1064.3)$, $r^2 = 0.996$ (0.27%) at middle concentrations, and $y = 25865 (\pm 1027) x - 16061 (\pm 20032)$, $r^2 = 0.996$ (0.41%) at high concentrations]. In parotid saliva, for the three calibration curves, results are as follows: $y = 99336 (\pm 7666) x + 4663 (\pm 1546)$, $r^2 = 0.994$ (CV = 0.26%); $y = 26758 (\pm 1321) x - 2202 (\pm 4177)$, $r^2 = 0.999$ (0.027%); $y = 28260 (\pm 1145) x - 20301 (\pm 7851)$, $r = 0.999$ (0.054%), respectively.

For each point of calibration standards, the concentrations were back-calculated from the equation of the linear regression curves (experimental concentrations) and the per cent relative standard deviations (RSD %) were computed. Results are presented in Table 1. A linear regression of the back-calculated concentrations versus the nominal ones provided a unit slope and an intercept equal to 0 (Student's *t*-test). The distribution of the residuals (difference between nominal and back-calculated concentrations) shows random variations, the number of positive and negative values being approximately equal. Moreover, they were normally distributed and centred around zero. The bias (0.0093 in plasma and -1.09×10^{-5} in parotid saliva) was not statistically

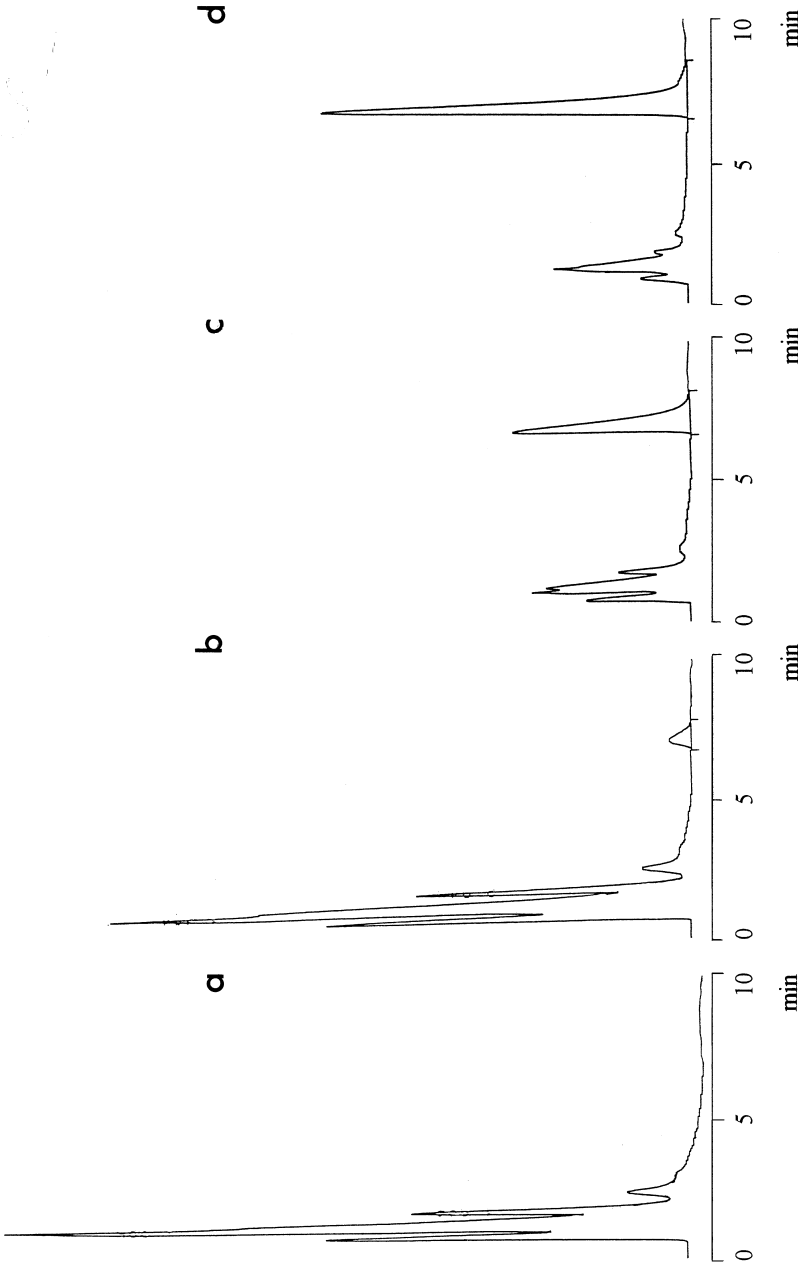

Figure 2. Chromatograms of blank plasma (a); plasma spiked with 0.5 ng mL^{-1} of topotecan (b); plasma spiked with 10 ng mL^{-1} of topotecan; (c); plasma sample of a patient treated with topotecan (1.5 mg m^{-2}) collected 1.5 hour after drug administration on day 1 of treatment (topotecan: 39.1 ng mL^{-1}) (d). For chromatographic conditions see text.

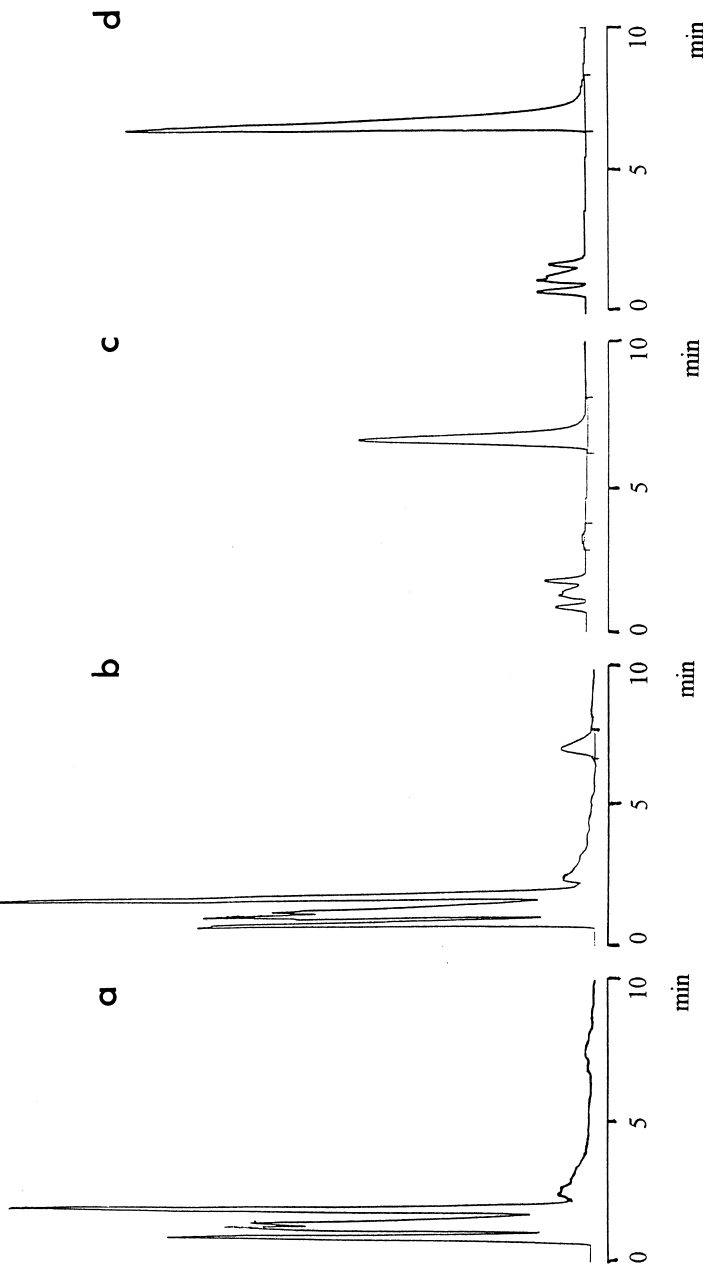

Figure 3. Chromatograms of blank parotid saliva (a); parotid saliva spiked with 0.5 ng mL^{-1} of topotecan (b); parotid saliva spiked with 10 ng mL^{-1} of topotecan (c); parotid saliva sample of a patient treated with topotecan (1.5 mg m^{-2}) collected 1.5 hour after drug administration on day 1 of treatment (topotecan: 44.9 ng mL^{-1}) (d). For chromatographic conditions see text.

Table 1

Inter-Assay Reproducibility of the HPLC Analysis

Theoretical Concentrations (ng mL^{-1})	Back-Calculated Concentrations (ng mL^{-1})	RSD (%)	Recovery (%)
	Plasma without Acidification		
0.1	0.0940	3.79	93.9
0.25	0.267	10.3	106.8
0.5	0.483	5.56	96.6
0.7	0.704	5.49	100.6
1	1.00	3.12	100.2
2.5	2.49	4.52	99.8
5	4.79	7.22	95.8
7.5	7.63	6.07	101.7
10	9.97	2.02	99.7
25	24.7	3.35	98.8
50	49.2	3.52	98.5
75	75.5	1.46	100.6
	Plasma with Acidification		
0.1	0.104	6.54	104.2
0.25	0.213	3.24	85.3
0.5	0.461	7.48	92.2
0.7	0.653	1.98	93.2
1	0.970	9.68	97.0
2.5	2.51	3.58	100.2
5	5.25	7.86	105.1
7.5	7.59	4.50	101.2
10	9.71	10.65	97.1
25	25.20	5.63	100.8
50	49.95	5.94	99.9
75	74.77	2.68	99.7
	Parotid Saliva with Acidification		
0.1	0.096	9.93	96.2
0.25	0.261	7.43	104.6
0.5	0.499	8.41	99.8
0.7	0.685	4.83	97.8
1	1.01	8.11	101.0
2.5	2.46	2.86	98.4
5	4.93	1.62	98.6
10	10.14	2.40	101.4
25	24.96	0.36	99.8
50	49.94	2.28	99.9
75	75.19	0.94	100.3

different from zero (Student's *t*-test) and the 95% confidence interval included the zero value (-0.203-0.221 in plasma, -0.084-0.084 in parotid saliva).

Precision and Accuracy

For concentrations of calibration standards (0.1-75 ng mL^{-1}), the accuracy ranged from 85.3 to 107% (Table 1). Precision around the mean value did not exceed 11%.

Using QC samples, the results for accuracy, intraday, and between-day precision are presented in Table 2.

Extraction Recovery

In plasma, the mean recovery (n = 10) averaged 89.5 ± 3.6%. In parotid saliva (n = 10), recovery was 90.4 ± 6.3%. The extraction efficiency is not statistically different over the range of concentrations studied.

Limit of Quantitation and Limit of Detection

The limit of quantitation was 0.05 ng mL^{-1} in plasma and parotid saliva. Using QC samples, RSD did not exceed 17% and accuracy was 95-111%.

Stability

The percent recovery is not statistically different over the range of concentrations studied.

Stability results in plasma and blood, are given in Table 3 and presented in Figure 4. After storage at 20 and 4°C, a monoexponential decline in drug concentration occurred. In plasma, the corresponding half-life values averaged 1.2 h at 20°C and 5 h at 4°C. The stability of the lactone form is higher in blood than in plasma, the half-life values were 10 and 64 h, respectively.

In plasma frozen at –20°C, losses ≤ 10% were observed after 3 days; after 8 days a 20% decrease in all concentrations tested was observed.

In methanolic extracts (Figure 4), after sample pretreatment, stability assays indicated that, at 20°C, a monoexponential decline in drug concentration occurred with a half-life of 5.6 h. After 30 min, the percent recovery averaged 90% (CV = 2.7%); a mean of 15% decrease in concentrations was observed after 1 hour. Losses were decelerated at + 4°C, the half-life averaged 24 h; a

Table 2

Intra-Day and Interday Precision and Accuracy of the HPLC Method

Theoretical Concentrations (ng mL^{-1})	Back-Calculated Concentrations (ng mL^{-1})	RSD (%)	Recovery (%)
	Within-Day (n=6)		
	Plasma with Acidification		
0.05	0.0544	13.8	108.7
0.75	0.796	7.42	106.1
2	1.76	3.33	88.0
35	32.2	3.02	92.0
65	63.3	2.13	97.4
	Parotid Saliva with Acidification		
0.05	0.0476	17.0	95.2
0.75	0.737	6.63	98.3
2	1.94	4.31	97.0
35	37.9	3.05	108.3
65	66.2	4.29	101.8
	Between-Day (n=7)		
	Plasma without Acidification		
0.05	0.05	7.5	100.0
0.75	0.80	6.6	106.7
2	2.03	0.16	101.5
35	37.8	1.16	108.0
65	70.4	1.64	108.3
	Plasma with Acidification		
0.05	0.0536	8.73	107.3
0.75	0.757	4.22	101.0
2	1.79	5.70	89.3
35	34.27	4.02	97.9
65	63.34	2.55	97.5
	Parotid Saliva with Acidification		
0.05	0.056	10.7	111.4
0.75	0.768	5.85	102.4
2	1.92	4.31	95.9
35	36.77	3.05	105.1
65	67.40	4.29	103.7

Table 3

Mean Percent Recoveries After Storage at 20 and 4°C

	Percent Recovery (Mean ± SD)			
	Plasma		Blood	
	20°C	4°C	20°C	4°C
15 min	84.2 ± 1.5	87.9 ± 2.5	95.8 ± 2.6	---
30 min	72.1 ± 2.1	85.7 ± 2.3	92.3 ± 3.3	---
45 min	61.5 ± 3.5	82.3 ± 2.6	92.1 ± 2.5	
60 min	54.3 ± 1.2	80.9 ± 3.5	90.4 ± 5.8	96.6 ± 0.7
90 min	41.7 ± 1.6	75.9 ± 6.4	87.8 ± 5.1	---
120 min	30.5 ± 2.7	70.4 ± 5.7	85.3 ± 4.03	94.6 ± 1.8
4 h	---	---	73.1 ± 6.9	89.8 ± 8.3
6 h	---	---	---	88.8 ± 4.4
12 h	---	---	---	85.9 ± 1.4
16 h	---	---	---	80.7 ± 5.7

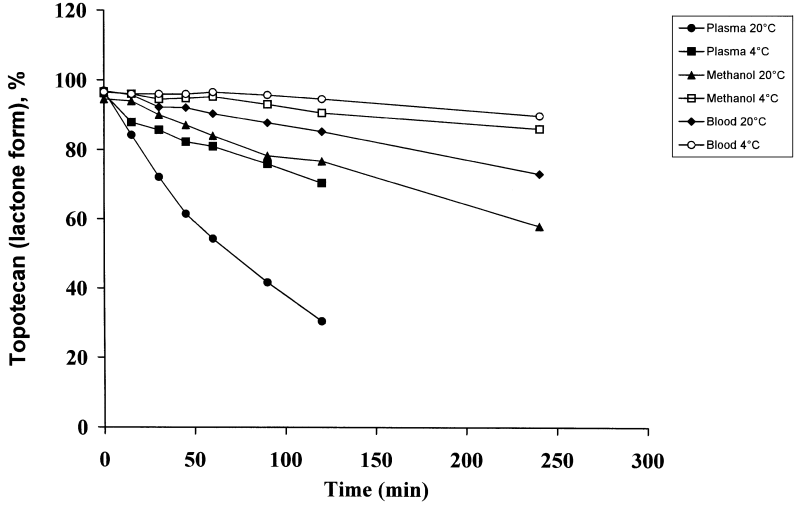

Figure 4. Stability of the lactone form in plasma, blood, and methanolic extracts at different temperatures.

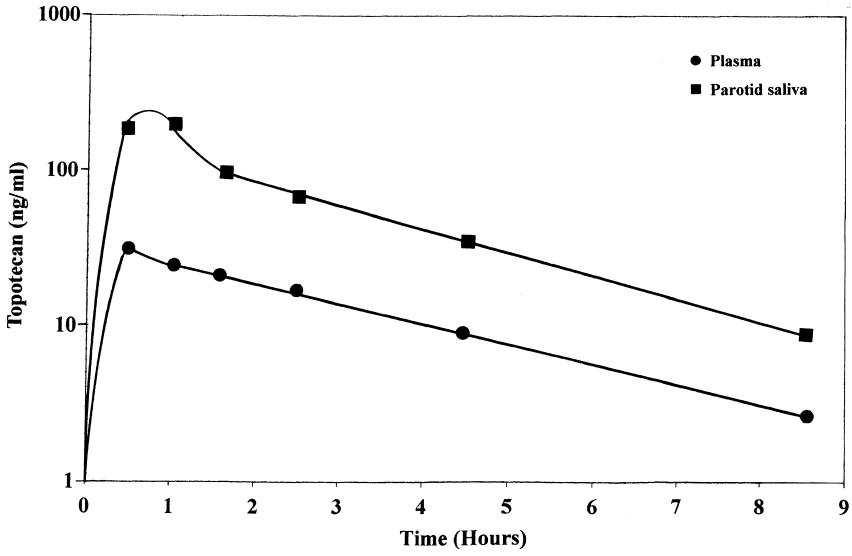

Figure 5. Plasma and parotid saliva concentration-time profile of topotecan after intravenous infusion of topotecan (1.12 mg m^{-2} given over 30 min) to a patient with advanced metastatic ovarian cancer.

mean of 15% decrease in concentrations was observed after 6 hours. In methanolic extracts frozen at – 20°C, the lactone form was stable during 10 days; a significant decrease ranging from 12 to 31% was observed after 15 days.

Pharmacokinetic Study

Figure 5 shows the plasma and parotid saliva concentration versus time profiles of topotecan in a representative patient. Plasma and saliva pharmacokinetics could be described using a bi-exponential pattern. The half-life of the terminal part of the curve was 2.2 h for plasma; it was of the same order of magnitude than that determined from salivary data (1.85 h). For this patient, high topotecan concentrations were found in parotid saliva. The ratio $AUC_{saliva}/AUC_{plasma}$ (AUC being the area under plasma (or saliva) concentration versus time curve) was 5.5.

For this pharmacokinetic study, patient underwent pharmacokinetic evaluation during one to three cycles of treatment, depending on the patient. From the first 10 patients included (15 courses), a high correlation was found

between saliva concentrations and plasma concentrations ($r = 0.76$, $p<0.001$). Moreover, for patients having more than one pharmacokinetic evaluation, the ratios, saliva concentrations / plasma concentrations remain constant.

DISCUSSION AND CONCLUSION

In this paper, we describe an HPLC method to quantify the lactone form of topotecan or topotecan as total lactone plus carboxylate forms in human plasma and parotid saliva samples. This method requires small sample volumes (0.1 mL) and enables rapid analysis of topotecan in plasma. Its advantages are: i) it requires a simple protein precipitation, ii) its ease and speed of execution without sacrifice of selectivity, and iii) its low cost. Moreover, this method can be used to quantify the lactone form or both lactone and carboxylate forms in biological samples using the same standard curves. Indeed, the coefficients and the exponents of the linear regression lines, with or without acidification of the medium, did not differ statistically.

Assay performance was assessed both on the basis of the statistical characteristics of individual calibration lines and from the results of quality control samples. Moreover, the specificity of this method was verified using a variety of drugs that could be co-administered to the patients. The limit of quantitation of 0.05 ng mL^{-1} in plasma and parotid saliva, was lower than that reported by Beijnen et al. (detection limit, 0.2 ng mL^{-1}),[13] Loos et al. (limit of quantitation, 0.1 ng mL^{-1} in plasma and 10 ng mL^{-1} in urine),[12] and Rosing et al. (limit of quantitation, 0.1 ng mL^{-1} in plasma, 25 ng mL^{-1} in urine and 0.3 $\mu g\ g^{-1}$ in feces).[14] In this paper, the stability of topotecan as its lactone form in plasma, blood, and methanolic extracts was tested under various conditions. Three different concentrations were studied, results indicated that rapid transformation into the carboxylate form occurred in plasma and methanolic extracts, while higher stability was found in the whole blood. The present method validation results indicate that the performance characteristics of the method fulfilled the requirements for a sufficiently accurate and precise assay method to carry out pharmacokinetic studies.

This method has been used to quantify topotecan in plasma and saliva samples collected during a pharmacokinetic study in patients with advanced metastatic ovarian cancer. Today, ten patients (1 to 3 courses per patient) have been included in the study. A high correlation ($r = 0.76$) of saliva concentrations with plasma concentrations was found; moreover, the ratios saliva concentrations / plasma concentrations remain constant, for one patient, over the successive courses of chemotherapy. These results did not support the findings of van Warmerdam et al.,[19] indeed, these authors investigating plasma/saliva ratios from 15 patients concluded that saliva is not a reliable matrix for topotecan analysis, since no correlations, saliva concentrations versus plasma con-

centrations, were found. The results obtained in the present pharmacokinetic study will be confirmed using a larger population of patients.

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