Journal of Chromatography B, 878 (2010) 645–652

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Quantification of topotecan by liquid chromatography–mass spectrometry (LC–MS). Application to intestinal transport using rat everted gut sacs

Cécile Arellano[c,*], Peggy Gandia[b,c], Laure Bettuing[a], John Woodley[a], Etienne Chatelut[c]

[a] Faculté des Sciences Pharmaceutiques, Université Paul Sabatier Toulouse 3, 31062 Toulouse, France
[b] Laboratoire de Pharmacocinétique et Toxicologie Clinique, Institut Fédératif de Biologie, Hôpital Purpan, 31059 Toulouse, France
[c] EA3035 Clinique et Expérimentale de Médicaments Anticancereux, Institut Claudius Regaud, 31052 Toulouse, France

ARTICLE INFO

Article history:
Received 30 November 2009
Accepted 18 January 2010
Available online 25 January 2010

Keywords:
Topotecan
Ondansetron
LC–MS
Everted gut sacs
Keto-enol tautomerism

ABSTRACT

Topetecan is an important anti-cancer drug that has recently become available as an oral formulation. In order to study its intestinal absorption *in vitro* and a potential drug–drug interaction with the anti-emetic ondansetron, a sensitive and accurate method for the analysis of topotecan in biological media was required. We developed a liquid–liquid extraction method at pH 7.0–7.5 with a recovery of 85% and which took into account the complex chemical behaviour of topotecan related to the lactone opening and the keto-enol tautomerism. This enabled us to validate a new specific and sensitive LC–MS method of analysis, with satisfactory inter- and intra-day repeatability and accuracy. The method was applied to a study of topotecan uptake in rat everted gut sacs that showed that, despite being a P-glycoprotein substrate like topotecan, ondansetron did not interfere with topotecan uptake.

1. Introduction

Topotecan is a camptothecin analogue, a DNA topoisomerase I inhibitor that received marketing approval as an intravenous form for the treatment of ovarian cancer and relapsed small-cell lung cancer (SCLC) in 1996 and 1998 respectively. A capsule formulation for oral administration was recently approved (2007) for the treatment of relapsed small-cell lung cancer (SCLC) in patients who had a complete or partial response to first-line chemotherapy and who were at least 45 days from the end of that treatment. This oral form is particularly important for such patients because these capsules are the only oral single-agent chemotherapy approved for the treatment of SCLC after failure of first-line therapy. However, the oral treatment is characterized by a limited bioavailability (30–44%) and by extensive intra- and inter-patient variability, mainly attributed to the action of efflux the transport proteins P-glycoprotein (P-gp/ABCB1/MDR1), multi-resistance protein 2 (MRP2/ABCC2) and breast cancer resistance protein (BCRP/ABCG2) [1]. Our aim was to study the influence of the anti-emetic drug ondansetron, a known P-gp substrate, on the intestinal absorption of topotecan using the rat everted gut sac model *in vitro*. In fact, only sparse data are available concerning the oral administration of topotecan with drugs usually prescribed during chemotherapy. Ondansetron is given before the administration of a chemotherapy drug either 2 h before by the oral route or 30 min before by the intravenous route. At the present time, an interaction between orally administered ondansetron and oral topotecan has not been mooted but a drug interaction between these two drugs is possible as they are both substrates of the efflux transporter P-glycoprotein (P-gp) [1–5] which is highly expressed in the gut. The rat everted sac method requires the tissue to be incubated in tissue culture medium in the presence of the test drug, which is transported inside the sac and analysed at low concentrations in a small volume. Therefore, we needed a sensitive method for the quantification of topotecan in the gut sac. Several bioanalytical methods have been developed to quantify topotecan in biological matrices (plasma, tissue homogenates, urine, etc.) generally using liquid chromatography with fluorescence [6–9] or UV [10] detection to give sensitive methods. An LC-fluorescence technique was available in our laboratory to quantify topotecan in plasma after protein precipitation, but when we applied this technique to our samples, we observed a poor separation between topotecan and components of the matrix (TC199), including phenol red, making the method unsuitable for quantification. Moreover, some "intriguing findings" concerning topotecan stability in heart tissue were recently pointed out by Srinivas [11] who discussed whether there was a lack of topotecan stability due to the matrix or an analytical problem that was not detected using the LC-fluorescence system. In his opinion, these unexpected findings were more probably related to an analytical artefact than to a real stability problem, and he also suggested

* Corresponding author at: Université Paul Sabatier Toulouse 3, Faculté des Sciences Pharmaceutiques, 31062 Toulouse, France. Tel.: +33 562256885; fax: +33 562256885.
E-mail address: arellano@cict.fr (C. Arellano).

1570-0232/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2010.01.019

that the use of mass spectrometry detection should help in the detection of potential metabolites. Through our previous studies of intestinal absorption and metabolism of different pharmaceuticals [12–15], we have shown that the LC–MS technology available in our laboratory was suitable for the detection of metabolites during intestinal transport. To date there is only one report of an LC–MS method for the quantification of topotecan in plasma but without any details about the sample preparation and assay validation [16]. LC–MS has also been used for the identification of N-desmethyl topotecan in urine [6]. One of the major problems reported for topotecan quantification in biological matrices is the inter-conversion by ring opening of the lactone to the carboxylate form, which needs to be taken into account. In addition the lactone form is susceptible to keto-enol tautomerism. During the validation procedure for the quantification of topotecan in electron spray ionisation mass spectrometry (ESI-MS), we observed that the method was particularly hampered by the keto-enol tautomerism, and therefore we propose a procedure to overcome this as described in this paper.

2. Materials and methods

2.1. Chemicals

Topotecan hydrochloride was a generous gift from GlaxoSmithKline Laboratories. TC 199 (10× concentrated with Earle's salts), glutamine and ondansetron were purchased from Sigma–Aldrich (St Quentin Fallavier, France); acetic acid was from J.T. Backer (Phillipsburg, USA); methanol (SDS, France) and dichloromethane (Scharlau Chemie S.A., Spain) were of HPLC grade; ultrapure water was obtained using a Millipore Simplicity 185 apparatus.

2.2. LC–MS instrumentation and analytical conditions

Topotecan was measured by liquid chromatography with mass spectrometry detection (LC–MS) using an LC–MS Waters 2690 separation module interfaced to a ZQ mass spectrometer equipped with an electrospray ionisation (ESI) source (Waters, St Quentin, France) and managed by a Masslynx software package (version 3.5; Micromass, Manchester, UK). Analyses were run in positive mode: capillary and cone voltages at 2 kV and 30 V respectively; the temperature of the heated capillary at 120 °C and the desolvation temperature at 350 °C; the nitrogen nebulizing gas flow was set at 150 L/h, and the desolvation gas flow at 350 L/h. Isocratic elution was with a mobile phase of water/1% acetic acid/methanol (55:10:35, v/v/v) at 0.2 mL/min for a 10 min run time, in a temperature controlled room maintained at 24 °C. The topotecan retention time was around 4.8 min on a Waters Atlantis™ C18 (5 μm, 2.1 mm × 150 mm) column protected by a C18 guard column (Symmetry, 3.5 μm, 2.1 mm × 10 mm). The quantification was run in Single Ion Recording (SIR) mode by selecting the characteristic $[M+H]^+$ ion: $m/z = 422$. Chromatograms with UV detection were recorded in parallel for some analyses using a 2487 Waters dual detector (390 nm).

2.3. Gut sac preparation and incubation

Male Sprague–Dawley rats (221–240 g weight, Depré, Saint Doulchard, France) were used in our experiments. The animals were acclimatized for at least 3 days before sacrifice, and were handled according to EU Guidelines (Directive 86/609/EEC). Adult rats were starved for 24 h, humanely killed by a qualified person and the entire small intestine quickly excised and flushed through several times with NaCl solution (0.9%, w/v) at room temperature. The intestine was immediately placed in warm (37 °C), oxygenated TC 199 and 1–2 cm of the duodenum was slid onto a glass rod (2.5 mm diameter) and fastened with braided silk. The intestine was then gently everted over the rod, the first 2 cm cut off, and the everted intestine slid into fresh oxygenated medium. One end of the intestine was clamped and the whole length of the intestine was filled with fresh oxygenated medium with a 20 mL syringe. The intestine was sealed with a second clamp and the resulting large gut sac was divided into sacs of approximately 2.5 cm in length using braided silk sutures. The terminal few centimetres of ileum and duodenum were each discarded. The experiments were carried out in duplicate using the intestine from one rat each time. A stock solution of topotecan (1.2 mg/mL) was prepared in water (pH 3.5) and diluted just before incubation by addition of the appropriate quantity (from 0.01 to 0.2 mL) in TC 199 directly into the incubation Erlenmeyer flasks at 37 °C and the volume made up to 10 mL with TC 199, pre-gassed with 95% O_2/5% CO_2. The final topotecan concentrations for incubation were: 1.2, 2.4, 6, 12 and 24 μg/mL. After 15 min, an everted gut sac was added to each flask, the flasks stoppered with gas-tight silicon bungs and incubated for 60 min in an oscillating water bath (60 cycles/min). The same procedure was used to duplicate the set of topotecan solutions (between 1.2 and 24 μg/mL) containing ondansetron; 1 mL of a stock solution of ondansetron in TC 199 was added to give a final concentration of ondansetron of 16 μg/mL. After 60 min incubation, the sacs were removed, washed three times in saline and blotted dry. The sacs were then cut open and the serosal fluid drained into small tubes. Each sac was weighed before and after the serosal fluid collection to calculate the volume of medium inside the sac. Samples of the mucosal and serosal fluids were kept for extraction before the LC–MS analysis. From the volume of the sac contents the total quantity of topotecan present inside the sacs was calculated and the transport across the sac wall expressed as picomoles per mg weight of intestinal tissue.

2.4. Sample preparation

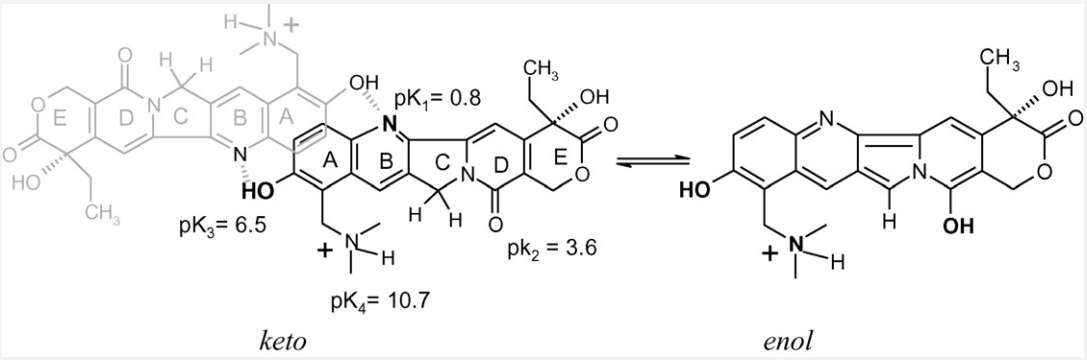
Topotecan was extracted from 1 mL of standard solutions (QC) in TC 199 (pH 7.2 ± 0.2) with 1.6 mL of dicholoromethane. After addition of the organic solvent, samples were vortexed for 2 min and the organic layer immediately collected. The solvent was evaporated under a nitrogen stream at 40 °C, and the resulting residue then dissolved in 1 mL of water acidified with acetic acid (pH around 3.8). The extraction procedure was carried out in under 15 min.

Samples of serosal and mucosal media were extracted with dichloromethane before analysis. The serosal content (approximately 0.5 mL) was first acidified with acetic acid (5 μL) and stored overnight at 4 °C in order to ensure that all the topotecan was in the lactone form (the pH was around 3.5–4). 100 μL of serosal samples were then diluted 10-fold in TC199 (around pH 7.2) to give a final volume of 1 mL and quickly extracted with 1.6 mL dichoromethane as described above for the QC. The residue was dissolved in 0.1 mL of acidic water for the incubation concentrations of 1.2, 2.4 and 6 μg/mL and in a final volume of 1 mL of acidic water for the upper concentrations.

2.5. Standard and quality control (QC) solutions of topotecan for validation

A stock solution of topotecan was prepared by dissolving 10 mg of topotecan hydrochloride in 10 mL of water acidified to pH 3.5 by addition of acetic acid and kept at 4 °C for at least 7 h before dilution. The stock solution was then initially diluted in water (pH 3.5) and then a second time in TC 199 (pH 7.2 ± 0.2) in order to have a final topotecan concentration of 1 μg/mL that was used for the preparation of calibration solutions at 5, 10, 50, 125, 200, 250 and

Table 1
Structural features of topotecan, ionisation sites and formation of dimers [11] and % of ionised forms in acidic pH calculated by the Henderson–Hasselbalch equation.



Ionisable function	pK_a	pH	% of ionised form NH^+	% of unionised form N
NR_2	0.8	3.3 4.0	0.32 0.06	99.68 99.94
Ionisable function	**pK_a**	**pH**	**% of ionised form >N-C=O (D ring)**	**% of unionised form >(N-C=O)H+**
>N-C=O	3.6	3.3 3.6 3.8 4.0	33.39 50.00 61.31 71.53	66.61 50.00 38.69 28.47
Ionisable function	**pK_a**	**pH**	**% of ionised form O–**	**% of unionised form OH**
OH (A ring)	6.5	3.3 4.0	0.06 0.32	99.94 99.68
Ionisable function	**pK_a**	**pH**	**% of ionised form NH_3^+**	**% of unionised form NH_2**
NH_2	10.4	3.3–4.0	100	0

300 ng/mL and for the quality controls (QCs: 25, 125, 250 ng/mL) by dilutions in TC 199. One millilitre of each QC solution was subsequently and immediately extracted with dichloromethane. Stock solutions were stored at 4 °C or at −18 °C in the case where they needed to be stored for more than 48 h. The stock solution could be stored at −18 °C and remained stable for several weeks.

2.6. Assay validation

The LC–MS method was validated in terms of sensitivity, specificity, linearity, recovery, accuracy and precision. Calibration curves ($n = 5$) were constructed by correlating the peak area as a function of the concentration of the spiked standard solutions (external standard). A least-square linear regression $1/X$ weighting was applied as it gave a better fit ($r^2 = 0.997$) compared with the regression analysis without weighting.

The accuracy (intra- and inter-day) was determined by calculating the % recovery (measured concentration/nominal concentration × 100) of quality control samples, and the percent relative standard deviation (RSD, %) was calculated as a measure of the intra- and inter-day precision. The extraction recovery at two QC levels (25 and 300 ng/mL) was determined by comparing the topotecan peak area from TC 199 (pH 7.2 ± 0.2) samples after extraction with those of standard samples at the same concentration prepared in acid water (pH 3.5) without extraction (stored at 4 °C for 8 h). Recovery was calculated from 5 replicates. The quantification limit was defined as the lowest concentration giving a signal-to-noise ratio greater than 10 (S/N > 10) with intra-day and inter-day precision and accuracy under 20% (RSD).

3. Results and discussion

3.1. Optimisation of the extraction and assay procedures

The rationale behind the extraction procedure resulted from the chemical properties of topotecan and the matrix used in the everted gut sac model. Sample pre-treatment was important to avoid co-elution of matrix components that could give ion suppression and decrease the sensitivity of the quantification [17,18]. Given the low protein concentration in the TC 199 medium used, protein precipitation, as often used in plasma, was ineffective in this medium. Therefore, we opted for a liquid–liquid procedure using dichloromethane, to remove salts and phenol red from the final samples.

Given its pK_a values [19] (see Table 1), topotecan is very slightly soluble in neutral aqueous media, and therefore the extraction efficiency with an organic solvent could be optimal for these conditions; the challenge being to keep the molecule in its lactone form to avoid a mixture of lactone and carboxylate forms with various hydrophilic/lipophilic properties. This optimisation was obtained if the extraction procedure was carried out in under 15 min giving satisfactory and reproducible extraction recoveries of over 85%. An uncontrolled and prolonged time of leaving the samples at neutral pH before extraction decreased the efficiency and increased the variability of extraction (data not shown).

The inter-conversion between the lactone and carboxylate forms of topotecan was established around a neutral pH (pH 6–7) [20]. In addition, a keto-enol equilibrium on the D ring and the formation of dimers has also been reported when the concentration of topotecan exceeded 10^{-5} M [19].

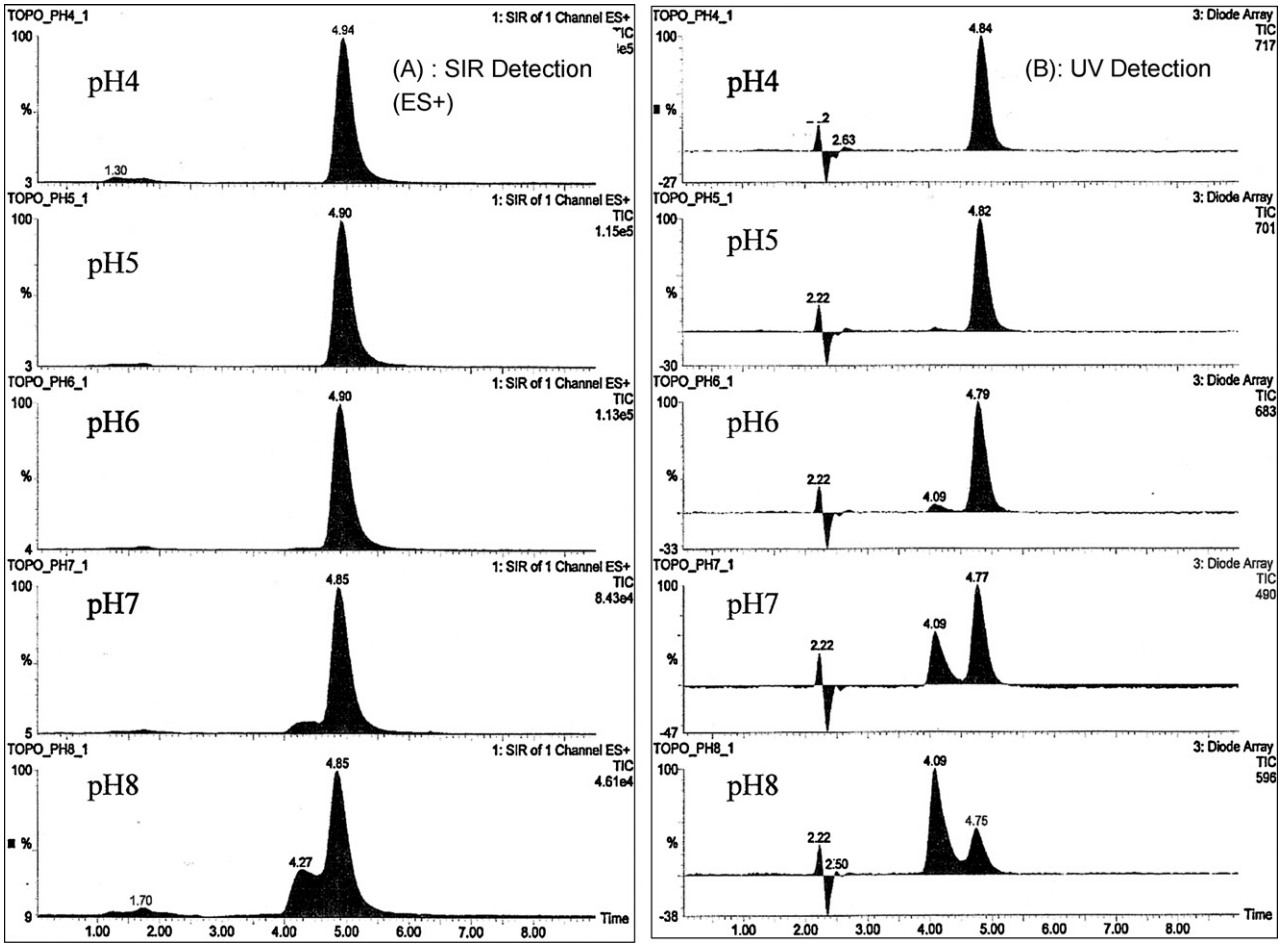


Fig. 1. Chromatogram of topotecan (120 ng/mL in water) at pH from 4 to 8. Left panel (A) ES^+ SIR detection: m/z 422; right panel (B) UV detection, 390 nm.

The properties of topotecan are closely related to the pH conditions (see Table 1) and have mainly been studied at moderately acidic and physiological pH, therefore, topotecan was reputed to be stable at low pH (pH 4) as the lactone form. The analytical conditions we used discriminated between the lactone and the carboxylate forms with retention times at 4.8 and 4.1 min respectively (Fig. 1). The peak observed at 4.1 min on the chromatogram using the SIR mode was attributed to the carboxylate form on the basis of the mass spectra showing peaks for $m/z = 440$ $[M+H]^+$ and $m/z = 422$ (loss of H_2O). At pH 8 the two forms coexist and the carboxylate peak (4.1 min) decreased when the solution was acidified to give only one peak due to the lactone form at pH 4. Moreover, according to the report of Strel'tsov et al. [19], the two forms absorb in the UV at 390 nm. Fig. 1 also highlights the relative peak intensity between the lactone (4.8 min) versus the carboxylate (4.1 min) peaks according to the detection method: the lactone form gave a lower response than the carboxylate with UV detection (390 nm) contrary to the result obtained with ESI-MS detection.

It was expected that the response obtained from a solution of topotecan in water without extraction would be easily reproduced to give a constant area for the peak corresponding to the lactone form at acidic pH. However, during our experiments, we observed that a solution of topotecan in water did not give a stable response in the LC–MS immediately after its preparation, even at acidic pH (3.5–4). When we prepared the stock solutions in acidic water followed by the suitable dilutions to check the extraction efficiency, we noted that the peak area obtained for topotecan varied with time and remained stable only several hours after the preparation (Fig. 2). It took around 7 h to obtain a signal with a stable peak area. After stabilisation and subsequent extraction, the samples were stable and could be stored overnight at room temperature without any impact on the results of the analysis. The phenomenon was also observed when we prepared a stock solution of topotecan at a concentration of 10^{-6} M (2.5 μg/mL) in water (pH 3.3) that did not favour the formation of dimers, and which was suitably diluted with acidic water to give final concentrations of 25 and 300 ng/mL before analysis. Furthermore, no additional peaks corresponding to dimers were detected, and therefore the existence of dimers in

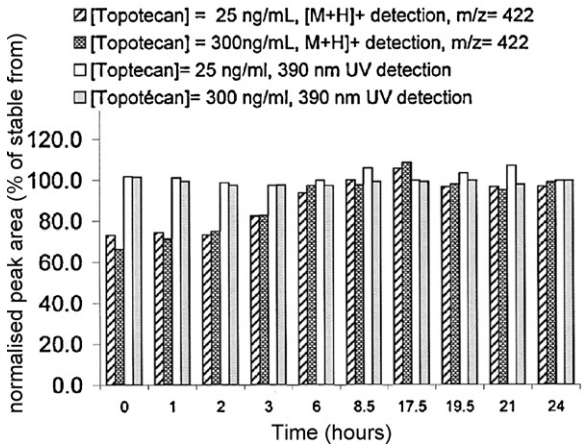


Fig. 2. LC–MS normalised peak area for topotecan in water at pH 3.3 (expressed as % of stable form) with time.

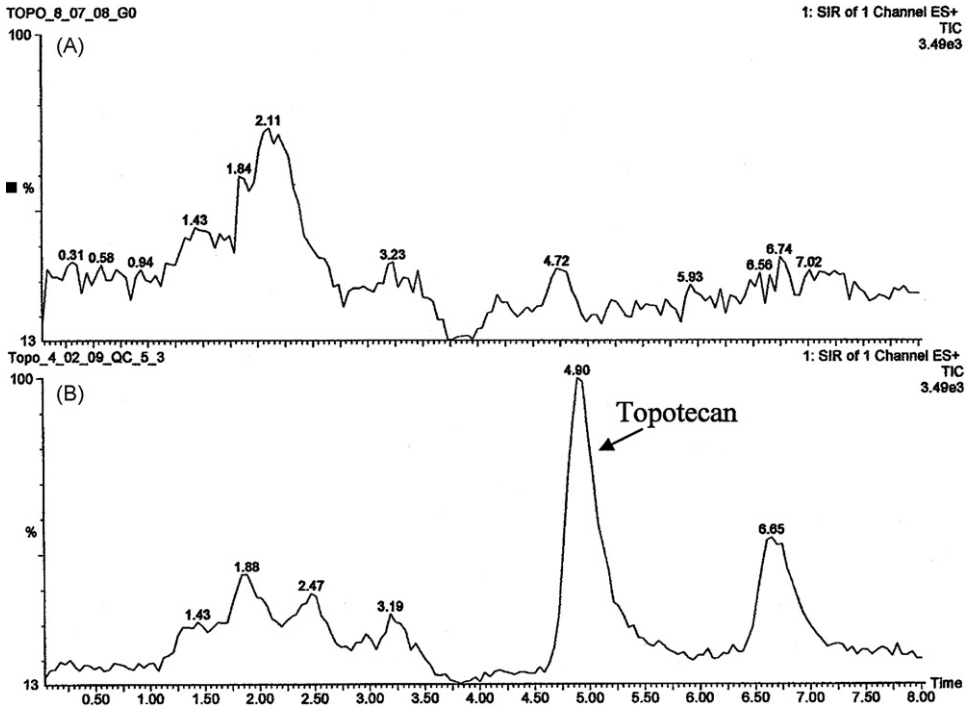


Fig. 3. Chromatogram recorded with blank sample (extraction of TC without topotecan) (A) and chromatogram of a QC sample with topotecan at 5 ng/mL (B).

the stock solution (concentration > 10^{-5} M) was consequently discounted, as was the presence of potential metabolites. In addition, the area relative to topotecan in the chromatograms resulting from the UV detection at 390 nm was constant in both cases and invariant with time, disproving the hypothesis of a slow dissolution of topotecan in water.

Another chemical property of topotecan is its ability to undergo a keto-enol equilibrium.

There are a few reports describing the influence of tautomerism on the quantification of compounds by ESI LC–MS [21–23], showing that accurate quantification was complicated by the presence of the different tautomers unavailable as standards. It was therefore possible to validate a suitable quantification method after reaching equilibrium, which could takes several hours (17 h at 56 °C [22]) or overnight at room temperature [23]. The analysis conditions used in our experiments enabled the lactone and the carboxylate forms of topotecan (Fig. 1) to be separated, but the keto and enol forms of the lactone were not discriminated in the chromatogram. Furthermore, we can deduce that the equilibrium profile shown in Fig. 2 reflects the equilibrium between the tautomers given that the response using peak area measurement with the $[M+H]^+$ ion was different for the two forms of the lactone (keto and enol), as previously reported for tetracycline derivatives [23]. In our case, the time required to reach equilibrium was around 7 h in acidic media at 4 °C (Fig. 2).

3.2. Analytical validation

We have validated an LC–MS method to quantify topotecan in a biological medium after a liquid–liquid extraction procedure. The sample preparation proposed was easy to carry out and efficient under conditions where the exposure of the samples at neutral pH was controlled and did not exceed 15 min. There was no peak observed at the same retention time as topotecan when TC 199 (without analytes, Fig. 3A) was tested after extraction, showing that the method was selective.

The quantification limit (LOQ) was defined as the lowest concentration giving a signal-to-noise ratio greater than 10 (S/N > 10) with intra-day and inter-day precision and accuracy under 20% (RDS). These criteria were met by a solution of topotecan at 5 ng/mL (0.05 ng injected) (Table 2) which was considered as the limit of quantification. This LOQ is sensitive enough for the quantification of our serosal samples from the everted sacs. In fact, at 5 ng/mL the S/N was greater than 10:1 (Fig. 3B) and it could be feasible to reduce the LOQ.

Table 2
Intra-day and inter-day repeatability and accuracy.

Nominal concentration (ng/mL)	Number of analysis	Measured concentration (ng/mL) mean ± RSD	Precision CV (%)	Accuracy recovery (%)
Intra-day				
LOQ				
5	4	5.6 ± 0.4	6.7	111.9
10	3	8.9 ± 1.5	16.8	89.2
125	3	118.7 ± 3.4	2.9	94.2
250	3	253.9 ± 5.3	2.1	101.2
Inter-day				
LOQ				
5	10	4.7 ± 0.77	17.8	94.3
10	10	9.5 ± 1.6	16.4	95.0
125	7	121.4 ± 1.6	3.5	97.1
250	10	256.9 ± 13.9	5.4	102.7

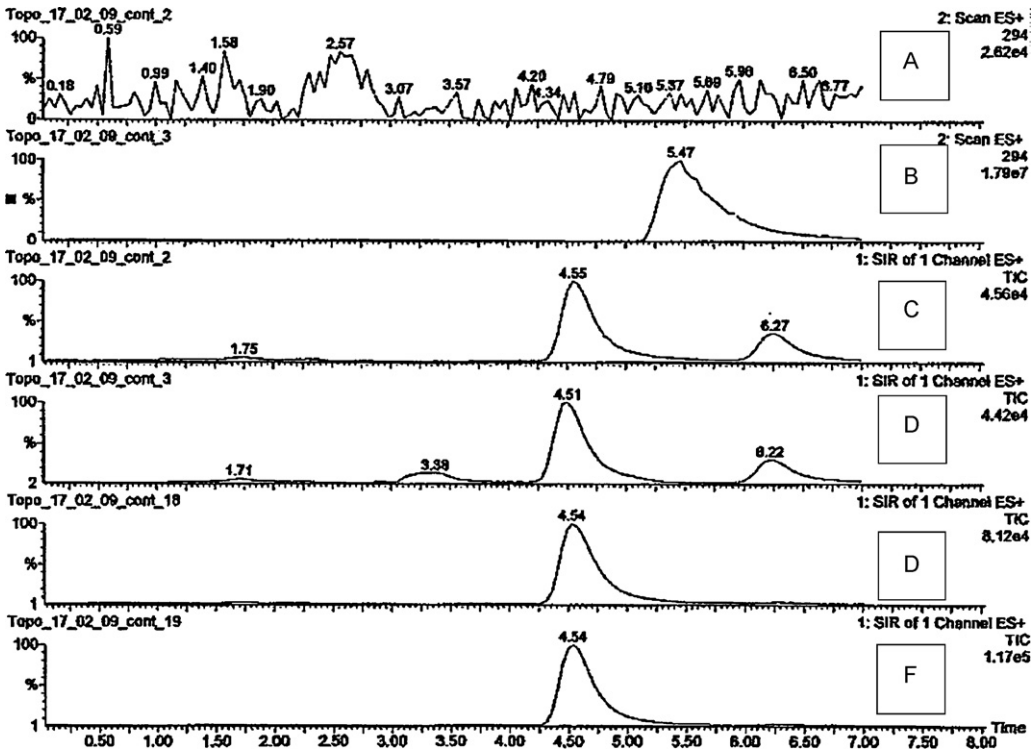


Fig. 4. Analysis of the serosal content from a gut sac incubated in TC containing topotecan with and without ondansetron (16 μg/mL): full scan mode selecting the *m*/*z* 294 $[M+H]^+$ ion with (A) 1.2 μg/mL topotecan alone (B) and with ondansetron. SIR mode (selecting *m*/*z* 422) with topotecan alone (C) 1.2 μg/mL, (E) 24 μg/mL and (D) with both topotecan (1.2 μg/mL) and ondansetron and (F) topotecan (24.2 μg/mL) and ondansetron.

The presence of ondansetron in the samples did not affect the assay of topotecan: the retention time of ondansetron was 5.5 min as shown in the chromatogram obtained by selecting its specific ion (*m*/*z* = 294) from the full scan recording of the analysis of a gut sac content incubated with both topotecan and ondansetron (Fig. 4, trace B). It can be noted that this peak was absent from the gut sac incubated without ondansetron (trace A). The mass spectra of both compounds are shown in Fig. 5: with a characteristic ion $[M+H]^+$ = 294; ondansetron (lower panel) was not detected in the SIR function used to quantify topotecan (upper panel) $[M+H]^+$ = 422. The chromatogram profile obtained was similar in the presence (Fig. 4D and F) or absence (Fig. 4C and E) of ondansetron as shown in the typical SIR chromatograms of the analysis of the gut sac content incubated with the lower (1.2 μg/mL) and the higher (24 μg/mL) concentrations of topotecan.

The linearity of the topotecan assay was studied between 0.5 and 300 ng/mL. In contrast to the solutions in water (pH 3.5), the solutions of topotecan in TC 199 (pH 7.2 ± 0.2) had to be used extemporaneously and extracted within 15 min to prepare the calibration curve (see Section 3.1) to avoid the opening of the lactone. As noted earlier, it was important to do the calibration using a topotecan stock solution stabilised for at least 7 h before use and kept at 4 °C. Under these conditions, the correlation between the area of the topotecan peak ($[M+H]^+$ ion) versus the concentration was linear between 0.5 and 300 ng/mL and repeatable with a mean slope (n = 5) of 269.11 ± 9.7 (CV = 3.6%), each calibration curve giving individually a correlation coefficient between 0.9975 and 0.9998. By comparison, the correlation coefficient resulting from the calibrations obtained when these conditions were not fulfilled (i.e. when dilutions were performed just after the preparation of the stock solution) was not satisfactory (mean r^2 = 0.9689; n = 5) and did not satisfy normal validation criterion [24]. The slopes were lower (151.8 ± 18.6; n = 5) and showed a greater variability (CV = 12%) as a result of the smaller area and the lack of equilibrium between the keto-enol forms of topotecan under these conditions. As our preliminary results were satisfactory in terms of linearity, precision and accuracy using an external standard for calibration, and given the difficulty of finding a suitable candidate, we decided to develop the quantification without an IS and the method was fully validated with this procedure.

The percentage relative standard deviation (% RSD) was calculated as an estimation of intra-day precision from three assays and the inter-day precision (% RSD) was evaluated from the measurements (n = 10) from 4 different days at low and high concentrations and 3 different days (n = 7) at medium concentration (Table 2). As can be seen in Table 2, the overall precision for the intra-day analysis ranged from 2.1 to 16.8% while the accuracy was from 89.2 to 101.2%; the inter-day assay precision ranged from 3.5 to 16.4% while the accuracy was from 95.2 to 102.7%. Thus, the method gave a satisfactory level of precision and accuracy to assay topotecan in TC 199.

3.3. *Influence of ondansetron on the intestinal absorption of topotecan*

The transporters P-glycoprotein (P-gp, MDR1, ABCB1), breast cancer resistance protein (BCRP, ABCG2), and multiple resistance protein 2 (MRP2, ABCC2) are all proteins located in the apical membranes of intestinal cells that transport absorbed drugs out of the cells back into the lumen of the intestine, and therefore they can diminish the absorption of a drug. They can also be the site of drug–drug interactions if two drugs are substrates for the same transporter. This can modify the bioavailability of a drug that may be altered in the presence of another compound, and with potentially important pharmacokinetic and pharmacological implications for patients. Ondansetron is an anti-emetic drug known to be a P-gp substrate [4,25] as is topotecan [2,26,27] and as ondansetron is often administered to patients before topetecan treatment it was important to know if there could be interaction between the two drugs at the transporter level. The LC–MS assay

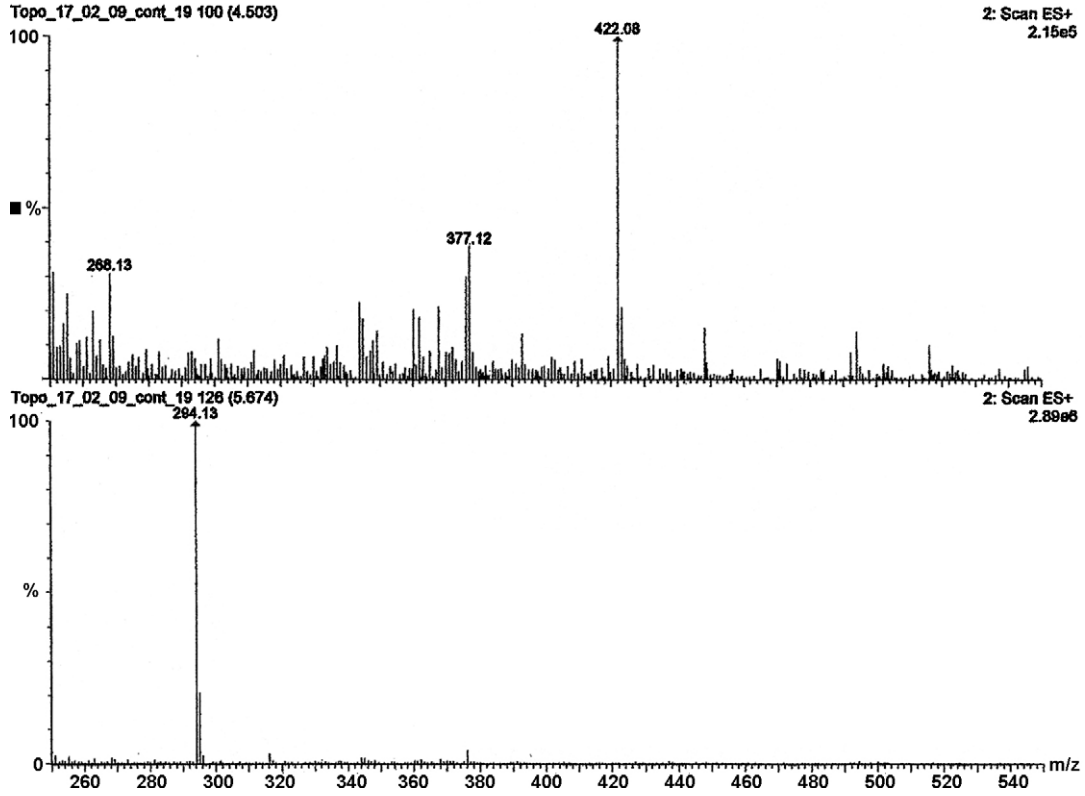


Fig. 5. Mass spectrum of topotecan (upper panel) and ondansetron (lower panel) both obtained from the analysis of a gut sac content incubated in TC containing 24 μg/mL topotecan in the presence of ondansetron (16 μg/mL).

of topotecan described above was applied to study the influence of ondansetron on the intestinal absorption of topotecan using everted gut sacs from rat intestine. We have previously shown that this *in vivo* model expresses P-gp as was demonstrated using specific transport markers like digoxin and inhibiting the transport with established P-gp inhibitors such as verapamil and quinidine [28,29]. The kinetic profile of topotecan absorption (without ondansetron) was linear ($r^2 = 0.991$) with concentrations between 1.2 and 24 μg/mL, a profile indicative of passive diffusion as the mechanism. Fig. 6 shows that the absorption of topotecan was not affected by ondansetron using an ondansetron concentration mimicking the intestinal concentration theoretically obtained in patients 2 h after its oral administration.

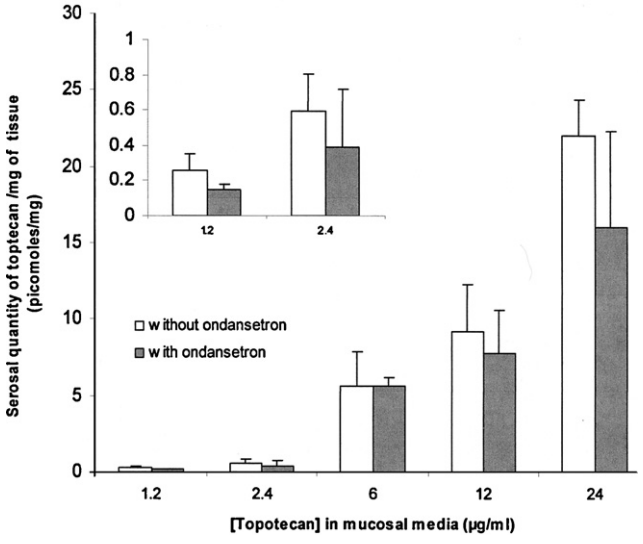


Fig. 6. Influence of ondansetron on topotecan absorption at different concentrations of topotecan in everted gut sacs expressed as pg per mg tissue weight. (Insert: magnification of the two lowest concentrations of topotecan.)

Two explanations may be proposed to explain this lack of significant effect. Firstly, as these two drugs are not reported to be inhibitors but only substrates with low affinity for P-gp [2,27,30], the concentration used may not be sufficient to induce any drug interaction when co-administered. Secondly, topotecan is a substrate BCRP and MRP2 [1,31] while it is not reported whether ondansetron is a substrate or inhibitor of these two transporters. To date, several drug interactions with topotecan related to BCRP have been reported *in vitro* and *in vivo* [32–34] and in clinical studies [35]. Thus the interaction of topotecan with BCRP probably has more significant consequences than its interaction with P-gp, and may therefore not be subject to interference by ondansetron if the latter does not interact with the BCRP transporter.

4. Conclusion

We have studied *in vitro* a potential interaction of topotecan with ondansetron using an everted gut sac model, and we have shown that the co-administration of ondansetron with topotecan did not interfere with its intestinal absorption. The quantification of topotecan was achieved with a new LC–MS analytical method. Topotecan has a complex behaviour in solution: it is sensitive to pH and undergoes a keto-enol equilibrium. There are few reports about the impact of such equilibria on the quantification of pharmaceuticals in LC–MS. It is very important to consider this property to obtain an accurate quantification in ESI LC–MS of compounds manifesting such tautomerism. To overcome the possible interference induced by this tautomerism, we proposed a procedure for sample preparation including a liquid–liquid extraction before LC–MS

analysis. The method was validated with a good level of sensitivity and accuracy, and was applied to samples in TC 199 media, but equally it could be extended to other matrices.

References

[1] H. Li, H.E. Jin, W. Kim, Y.H. Han, D.D. Kim, S.J. Chung, C.K. Shim, Pharm. Res. 25 (2008) 2601.
[2] A.H. Schinkel, E. Wagenaar, C.A. Mol, L. van Deemster, J. Clin. Invest. 97 (1996) 2517.
[3] C.J. Endres, P. Hsiao, F.S. Chung, J.D. Unadkat, Eur. J. Pharm. 27 (2006) 501.
[4] J.A. Scott, M. Wood, P. Flood, Anest. Analg. 103 (2006) 742.
[5] D. Balayssac, N. Authier, A. Cayre, F. Coudore, Toxicol. Lett. 156 (2005) 319.
[6] H. Rosing, V.M. Herben, D.M. van Gortel-van Zomeren, E. Hop, J.J. Kettenes-van den Bosch, W.W. ten Bokkel Huinink, J.H. Beijnen, Cancer Chemother. Pharmacol. 39 (1997) 498.
[7] N.A. de Vries, M. Ouwehand, T. Buckle, J.H. Beijnen, O. van Tellingen, Biomed. Chromatogr. 21 (2007) 1191.
[8] F. Bai, M.N. Kirstein, S.K. Hanna, L.C. Iacono, B. Johnston, C.F. Stewart, J. Chromatogr. B 784 (2003) 225.
[9] A.M. Vali, B. Shafaghi, S. Dadashzadeh, J. Chromatogr. B 818 (2005) 205.
[10] T. Bansal, M. Singh, G. Mishra, S. Talegaonkar, R.K. Khar, M. Jaggi, R. Mukherjee, J. Chromatogr. B 859 (2007) 261.
[11] N.R. Srinivas, Biomed. Chromatogr. 23 (2009) 447.
[12] C. Arellano, C. Philibert, O. Lacombe, J. Woodley, G. Houin, J. Chromatogr. B 807 (2004) 263.
[13] C. Arellano, C. Philibert, E.N. Dane a Yakan, C. Vachoux, O. Lacombe, J. Woodley, G. Houin, J. Chromatogr. B 819 (2005) 105.
[14] C. Arellano, C. Philibert, C. Vachoux, J. Woodley, G. Houin, J. Chromatogr. B 829 (2005) 50.
[15] C. Arellano, C. Philibert, C. Vachoux, J. Woodley, G. Houin, J. Pharm. Pharmaceut. Sci. 10 (2007) 26.
[16] S. Bardin, W. Guo, J.L. Johnson, S. Khan, A. Ahmad, J.X. Duggan, J. Ayoub, I. Ahmad, J. Chromatogr. A 1073 (2005) 249.
[17] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882.
[18] D.L. Buhrman, P.I. Price, P.J. Rudewicz, J. Am. Soc. Mass Spectrom. 7 (1996) 1099.
[19] S.A. Strel'tsov, S.L. Grokhovskii, I.A. Kudelina, V.A. Oleinikov, A.L. Zhuze, Mol. Biol. 35 (2001) 365.
[20] N. Sanna, G. Chillemi, A. Grandi, S. Castelli, A. Desideri, V. Barone, J. Am. Chem. Soc. 127 (2005) 15429.
[21] M. Cherlet, P. De Backer, S. Croubels, J. Chromatogr. A 1133 (2006) 135.
[22] M. Cherlet, S. Croubels, P. De Backer, J. Chromatogr. A 1102 (2006) 116.
[23] W.J. Blanchflower, R.J. McCracken, A.S. Haggan, D.G. Kennedy, J. Chromatogr. B 692 (1997) 351.
[24] FDA, in: Food and Drug Administration, Rockville, MD, 2002.
[25] I. Tamai, A. Saheki, R. Saitoh, Y. Sai, I. Yamada, A. Tsuji, J. Pharmacol. Exp. Ther. 283 (1997) 108.
[26] E.J. Jeong, X. Liu, X. Jia, J. Chen, M. Hu, Curr. Drug Metab. 6 (2005) 455.
[27] N.A. de Vries, J. Zhao, E. Kroon, T. Buckle, J.H. Beijnen, O. van Tellingen, Clin. Cancer Res. 13 (2007) 6440.
[28] L. Barthe, M. Bessouet, J.F. Woodley, G. Houin, Int. J. Pharm. 173 (1998) 255.
[29] G. Cornaire, J.F. Woodley, P. Herman, A. Cloarec, C. Arrellano, G. Houin, Int. J. Pharm. 278 (2004) 119.
[30] I.E. Kuppens, E.O. Witteveen, R.C. Jewell, S.A. Radema, E.M. Paul, S.G. Mangum, J.H. Beijnen, E.E. Voest, J.H. Schellens, Clin. Cancer Res. 13 (2007) 3276.
[31] U. Muenster, B. Grieshop, K. Ickenroth, M.J. Gnoth, Pharm. Res. 25 (2008) 2320.
[32] J.W. Jonker, J.W. Smit, R.F. Brinkhuis, M. Maliepaard, J.H. Beijnen, J.H. Schellens, A.H. Schinkel, J. Natl. Cancer Inst. 92 (2000) 1651.
[33] P. Breedveld, J.H. Beijnen, J.H. Schellens, Trends Pharm. Sci. 27 (2006) 17.
[34] R.L. Oostendorp, J.H. Beijnen, J.H. Schellens, Cancer Treat. Rev. 35 (2009) 137.
[35] C.M. Kruijtzer, J.H. Beijnen, H. Rosing, W.W. ten Bokkel Huinink, M. Schot, R.C. Jewell, E.M. Paul, J.H. Schellens, J. Clin. Oncol. 20 (2002) 2943.