ELSEVIER

Journal of Chromatography B, 764 (2001) 49–58

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Review

Antitumor drugs possessing topoisomerase I inhibition: applicable separation methods

Toshihiro Oguma*

Drug Metabolism and Physicochemical Property Research Laboratory, Daiichi Pharmaceutical, 1-16-13 Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan

Abstract

Separation methods for antitumor drugs capable of topoisomerase I inhibition were reviewed in this study. Camptothecin (CPT) its related analogues seemed to be promising anticancer drugs that exhibit topoisomerase I inhibition. This group of compounds contain a closed α-hydroxy-δ-lactone ring (lactone form) that can undergo reversible hydrolysis to form the open-ring form (carboxylate form). In vitro pharmacological study showed that the antitumor activity of the lactone form was higher than that of the carboxylate form. Thus a quantitative method to separate these two forms is important to evaluate the pharmacokinetics and pharmacodynamics of these compounds. Nevertheless, current separation methods are complicated by the pH-dependent instability of the lactone moiety. High-performance liquid chromatography (HPLC) coupled with fluorometric detection has been widely used for the quantitation of the drug as the intact lactone form or as the total lactone carboxylate forms in biological matrices. In this report we reviewed current applicable chromatographic techniques for further bioanalytical studies of CPT derivatives including sample preparations, HPLC columns, mobile phases and additives.

Keywords: Reviews; Topoisomerase I inhibitors; Enzymes

Contents

*Tel.: +81-3-3680-0151; fax: +81-3-5696-8332.
E-mail address: ogumab0g@daiichipharm.co.jp (T. Oguma).

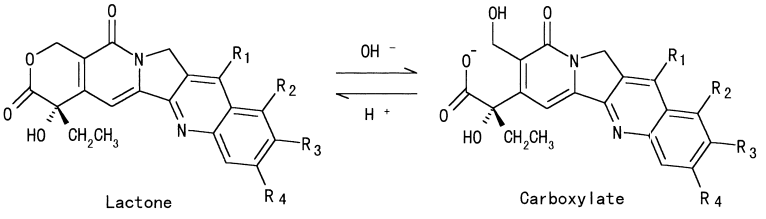
0378-4347/01/$ – see front matter
PII: S0378-4347(01)00380-2

1. Introduction

Camptothecin (CPT) was initially isolated from the Chinese tree, *Camptotheca acuminata* and its structure was identified by Wall et al. [1]. It inhibited type I DNA topoisomerase (topoisomerase I) [2–4] and showed strong antitumor effects against several experimental tumors [5], but failed to show any meaningful antitumor activity and caused severe and unpredictable cystitis during clinical trials in the early 1970s [6,7].

Subsequent efforts to find CPT derivatives with higher antitumor activity and less toxicity led to the discovery of irinotecan (CPT-11), topotecan (TPT), 9-aminocamptothecin (9-AC), 9-nitrocamptothecin, 10-hydroxycamptothecin (HCPT), lurtotecan (LRT) and DX-8951f (exatecan) (Fig. 1). CPT-11 and TPT were subsequently approved to treat colorectal and ovarian cancers, respectively.

These CPT derivatives contain a lactone ring in its molecule that can undergo reversible hydrolysis to form the carboxylate form at physiological pH. In vitro and in vivo studies, showed that the antitumor activity of the lactone form was higher than that of the carboxylate form. Therefore, analytical methods that could measure the lactone and carboxylate forms separately were required to further analyses the pharmacokinetics and pharmacodynamics of this



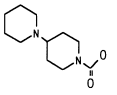
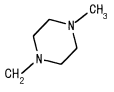
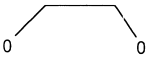
Compounds	Substitute Group R_1	R_2	R_3	R_4
Camptothecin	H	H	H	H
Irinotecan	CH_2CH_3	H		H
SN-38	CH_2CH_3	H	OH	H
Topotecan	H		OH	H
9-Aminocamptothecin	H	NH_2	H	H
10-Hydroxycamptothecin	H	H	OH	H
Lurtotecan		H		
Exatecan	$CHNH_2$	CH_2	CH_3	F

Fig. 1. Chemical structures of the lactone and carboxylate forms of camptothecin and its analogues.

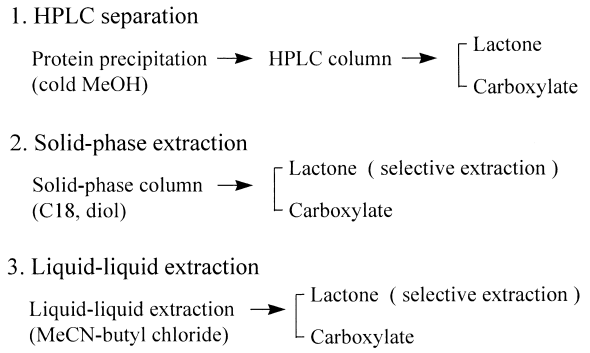


Fig. 2. Separation methods for the lactone and carboxylate forms of camptothecin and its analogues.

class of drugs. Several analytical methods using high-performance liquid chromatography (HPLC) have been developed for the determination of only the lactone form or for the simultaneous determination of the lactone and carboxylate forms (Fig. 2). Proper and rapid sample handling throughout the assay was needed to obtain precise drug concentrations. In contract, many conventional methods using HPLC have been developed and used routinely for the determination of the total lactone and carboxylate forms. The most currently used analytical method for CPT derivatives assay is HPLC coupled with fluorometric detection. This fluorometric detection method showed high sensitivity. Many useful methods have been developed one after the other and their analytical methodology have been ascertained by method validation studies. Many of them have reached the stage of practical routine work and successfully applied to preclinical and clinical studies for pharmacokinetic evaluation of these drugs. A review article by Loos et al. [8] was very useful to help understand the details of TPT, CPT-11, 9-AC and LRT assay methods.

2. Separation methods for lactone and carboxylate forms

2.1. HPLC separation

Sample preparation which involved protein precipitation with organic solvents followed by centrifugation and direct injection into the HPLC system (C_{18} reversed-phase columns) are routinely used to achieve separation of the lactone and carboxylate forms. The mobile phase, which contained ion-pairing reagents such as tetrabutylammonium phosphate and analogues, produced sharp peaks and simultaneously separated the lactone and carboxylate forms of the compound. In general, ice-cold methanol (MeOH) was the chosen organic solvent for precipitation of plasma proteins. The lactone form of CPT-11 in MeOH extracts have been reported to be stable at −30°C for 5 days [9] and that of TPT at −70°C for 15 months [10]. CPT-11 is a prodrug which can be hydrolyzed to its active SN-38 (7-ethyl-10-hydroxy-camptothecin). Simultaneous methods must be able to separate the lactone and carboxylate forms of both CPT-11 and SN-38 in one single run. HPLC methods using cationic ionpair reagents made it possible to separate all four components. In this separation method, the concentrating of MeOH extracts is unable to increase sensitivity due to the instability of the lactone ring. It is also laborious to extract the lactone form rapidly and directly after collection of each individual plasma sample. The lower limit of quantitation (LLQ) in plasma were established at 1–10 ng/ml for the lactone form of CPT-11 [9,11,12,14] and 0.5–5 ng/ml for the lactone form of SN-38 [9,11–14], respectively. The LLQs for the lactone form of other CPT analogues were 0.05–5 ng/ml for TPT [10,15–19], 5 ng/ml for 9-AC [20], 2 ng/ml for HCPT [21] and 0.5 ng/ml for CPT [22–24], respectively.

2.2. Solid-phase extraction

Solid-phase extraction (SPE) techniques were used to separate the lactone form from the carboxylate form by stepwise elution method, where the carboxylate form was completely removed in the first eluted fraction (e.g. 50% MeOH for exatecan), and only the lactone form was eluted during the final elution (e.g. acidified MeOH for exatecan). A C_{18} solid-phase cartridge was used for the extraction of the lactone form from plasma sample except for LRT, which used a diol (2-OH) solid-phase cartridge [25]. SPE enabled increased sensitivity due to a concentrating step in the procedure. The volume of plasma could be increased in this method because interfering endogenous materials were removed from the plasma.

The LLQs of the lactone form in plasma were established at 0.09–0.2 ng/ml for 9-AC [26,27], 0.05 ng/ml for LRT [25], 0.2–3 ng/ml for exatecan (Fig. 3) [28,29] and 2 ng/ml for CPT [30]. This SPE method was relatively convenient for the determination of the lactone form because it was possible to stabilize the lactone form by direct freezing of the plasma sample at clinical sites. As for exatecan, the lactone form in human plasma was stable after storage at −80°C for 6 months. However, the lactone form was unstable at −20°C, suggesting change of the lactone form to the carboxylate form [28]. The plasma samples were stored below −80°C in a container with liquid nitrogen at clinical sites and delivered to the laboratory where plasma samples were assayed. This simultaneous determination of the lactone and carboxylate forms was not possible with this separation method due to the fact that this method required separate analyses for the lactone and total lactone plus carboxylate forms. The other major disadvantage of this methodology was the need for relatively large plasma volumes.

2.3. Liquid–liquid extraction

The liquid–liquid extraction (LLE) method which only required a one-step solvent extraction of the lactone form was sensitive and convenient. After the samples were thawed, the lactone form was extracted with an organic phase consisting of acetonitrile–butylchloride (1:4, v/v), while the carboxylate form remained in the aqueous phase. Selinger et al. [31] developed a method for the determination of the lactone form of LRT using whole blood instead of plasma. Since a plasma preparation step was not needed, this method was rapid with simple sample handling at the clinical sites. LLE enabled increased sensitivity due to an extract concentration step in the method similar to the solid-phase extraction method. The LLQs of the lactone form in plasma were established at 0.5 ng/ml for CPT-11 and SN-38 [32], 0.05 ng/ml for 9-AC [33] and 0.15 ng/ml for LRT [31].

3. Analysis of total lactone and carboxylate forms

3.1. HPLC methods

CPT analogues have strong fluorescence characteristics. Thus, relatively low concentrations of these compounds could be measured in biological matrices without laborious precolumn derivatization tech-

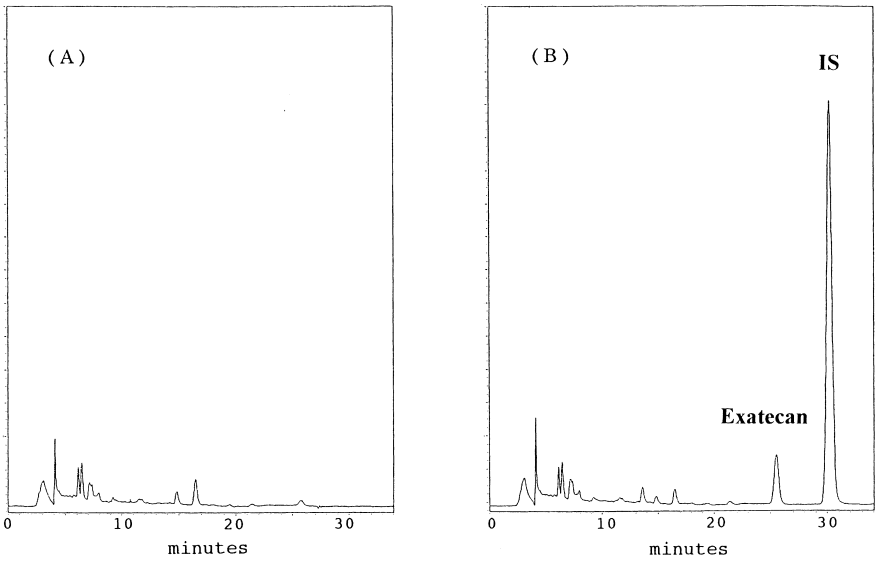


Fig. 3. Representative chromatograms of plasma extracts obtained with (A) blank human plasma and (B) blank human plasma spiked with the lactone form of exatecan (0.186 ng/ml) and the internal standard (I.S.).

niques. In general, HPLC methods using fluorescence detection have been widely used to determine the concentration of CPT analogues.

Numerous HPLC methods have been developed and used for the determination of total concentration of lactone plus carboxylate forms of CPT and its analogues. Extraction methods using protein precipitation with acidified MeOH or SPE columns have been commonly used in the pretreatment procedures of these biological samples. These methods have the advantages of simple pretreatment of plasma and multiple monitoring. Several methods for the determination of total levels of CPT-11 and SN-38 have been described. The LLQs of the total levels in plasma were established at 1–30 ng/ml for CPT-11 [34–41] and 0.0039–20 ng/ml for SN-38 [34–43].

Sparreboom et al. [39] developed a method for the simultaneous determination of CPT-11, SN-38, its β-glucuronide derivative (SN-38G) and 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (PRP 121056A; APC) in human plasma, urine and feces. This method involved a protein precipitation step to clean-up the sample followed by direct injection into the HPLC system without incubation of the plasma sample with β-glucuronidase. The LLQs of total levels in human plasma were established at 10 ng/ml for SN-38G and APC.

Kurita et al. [40] have described a rapid and simple method for the simultaneous determination of CPT-11, SN-38 and SN-38G in rat plasma using a fully automated on-line SPE system, PROSPEKT. This method enabled the processing of many samples within a relatively short time period and the direct determination of SN-38G. The LLQs of total levels in rat plasma were established at 5 ng/ml for CPT-11 and SN-38, 2.5 ng/ml for SN-38G.

Fraier et al. [44] developed an Oasis™ SPE method using 96-well plates which involved the analysis of water-soluble polymer-bound CPT conjugate (MAG–CPT) and free CPT in dog plasma. This method enabled the processing of many samples with high efficiency. The LLQs of the method were 1.17 ng/ml for CPT and 103.10 ng/ml (as CPT equivalent) for MAG–CPT using 0.1 and 0.05 ml of plasma.

A method for the determination of total LRT levels in human plasma and urine after administration of liposomal LRT was developed by Loos et al. [45]. This method enabled the determination of total drug levels using a sample clean-up procedure that disrupts the liposomes. The LLQs of total levels were established at 1 ng/ml in plasma. In this method, the native fluorescence intensity of LRT was increased by the postcolumn exposure to UV light (254 nm) in a photochemical reaction unit. The LLQs of total levels were established at 0.5 ng/ml in urine.

3.2. HPLC–MS methods

HPLC–MS is a flexible technique and has developed rapidly as routine methods for bioanalytical studies. Recently, several metabolites of CPT-11 in human plasma and urine were identified by HPLC–MS–MS using the atmospheric pressure chemical ionization (APCI) mode [46]. A method for the detection of the photodegradation products (PDPs) of CPT-11 in human plasma and urine was developed using HPLC–MS in APCI mode. This method was then used in the screening of pharmaceutical and biological samples for the presence of PDPs which were found to be present in the starting clinical solution as trace contaminants [47].

Ragot et al. [48] have described a sensitive and highly specific method for the simultaneous determination of CPT-11 and SN-38 in human serum using HPLC–MS in electrospray ionization (ESI) mode. This method enabled wide linearity ranges up to 10 000 ng/ml for CPT-11 and up to 100 ng/ml for SN-38. The LLQs were established at 10 ng/ml for CPT-11 and 0.5 ng/ml for SN-38. These sensitivities using a mass spectrometer were similar to those previously obtained with HPLC techniques with fluorescence detection.

A simultaneous method for exatecan and its 4-hydroxymethyl metabolite (UM-1) in human plasma and urine was developed and validated by HPLC–MS–MS using the APCI mode [49]. A similar HPLC method using fluorescence detection could not be developed due to the presence of interfering peaks corresponding to the elution time of UM-1. The LLQs of DX-8951 and UM-1 were 0.1 ng/ml in plasma and 1 ng/ml in urine.

There is no doubt that HPLC–MS methods will

progress further and many highly sensitive methods using HPLC–MS will be developed in the future.

3.3. Other methods

Burke et al. [50] developed a fluorescence detection method to measure TPT in human plasma and whole blood using two-photon excitation at 730 or 820 nm. The lower limits of detection were about 21 and 420 ng/ml in plasma and whole blood, respectively. Since skin, blood and tissues are translucent at long wavelength, this indicated the possibility of using long-wavelength two-photon excitation for direct measurements in whole blood or noninvasive detection through skin.

Recently, two sensitive and specific enzyme-linked immunosorbent assays (ELISAs) for the determination of total CPT-11 and SN-38 in rat serum were developed [51]. An enzyme marker was prepared by labeling the drugs with horseradish peroxidase. The LLQs were established at 16 pg/ml for CPT-11 and 160 pg/ml for SN-38. Furthermore, another highly sensitive ELISA for the determination of the lactone form of TPT was recently developed with an LLQ of 80 pg/ml in rat serum [52]. An enzyme marker was prepared by labeling the drugs with β-D-galactosidase. The ELISA method appeared to be highly specific to the lactone form, because the specific antibody and recognized the lactone moiety of TPT. These ELISA methods might be eventually specific and sensitive enough to quantify CPT-11, SN-38 and TPT in human samples, which could be used in therapeutic drug monitoring and clinical pharmacokinetic studies.

4. Conclusions

In this review, we have reported current separation methods for comptothecin derivatives which are antitumor drugs possessing topoisomerase I inhibition. Due to tremendous efforts, numerous sensitive and specific methods for the separation of the lactone and carboxylate forms have been developed as shown in Table 1.

For lactone only measurements, the samples were processed using cold methanol deproteinization, SPE or LLE techniques. The LLE method is the most effective and simple because the lactone form can be extracted directly at the clinical sites. Reversed-phase HPLC methods with fluorescence detection have been well represented and showed LLQs of sub-nanogram levels in human plasma. The applications of HPLC–MS increased the selectivity but not sensitivity. As technical advances in mass spectrometry continue, more improvements and new applications can be expected in the future. Until now the advantages of ELISA methods have not been exploited except for two methods established by Saita et al. [51,52], and wider applications can be expected due to their low price and ease of operation.

Studies of CPT analogues have already established the potential of DNA topoisomerase I as an effective therapeutic target for cancer chemotherapy. Additional CPT analogues or related compounds which exhibit high anticancer activity and less toxicity are likely to emerge for clinical development in the future. It has been suggested that more sensitive methods using smaller sample volumes are required for routine clinical therapeutic drug monitoring and clinical pharmacokinetic studies.

5. Nomenclature

CPT	Camptothecin
CPT-11	Irinotecan, Campto®, Camptosar®, 7 - Ethyl - 10 - [4 - (piperidino) - 1-piperidino] - carbonyloxycamptothecin
SN-38	7-Ethyl-10-hydroxycamptothecin
SN-38G	7-Ethyl-10-hydroxycamptothecin glucuronide
APC	7-Ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin
TPT	Topotecan, Hycamtin®, SKF 104864, NSC 609669, (*S*)-9-dimethylaminomethyl-10-hydroxycamptothecin
9-AC	9-Aminocamptothecin
HCPT	10-Hydroxycamptothecin
LRT	GI147211, 7-(4-methylpiperazinomethylene) - 10,11 - ethylenedioxy-20(*S*)-camptothecin

Table 1
Analytical methods for the determination of lactone and carboxylate forms of camptothecin and its analogues

Reference	Analyte		Sample	Sample preparation	HPLC column (particle size in μm, length in cm×I.D. in mm)	Mobile phase flow-rate (ml/min)	Detection		LLQ (ng/ml)
							Ex. (nm)	Em. (nm)	
Rothenberg et al. [11]	CPT-11	Lactone, Carbox	Plasma (human)	Protein precipitation (cold MeOH)	μBondapak-C_{18} (10, 30×3.9) and a guard column	MeOH–sodium phosphate with 3 m*M* heptanesulfonic acid (pH 4.0), 65:35 (0.8)	375	430	2[a]
	SN-38	Lactone, Carbox	Plasma (human)	Protein precipitation (cold MeOH)	Nova-pak C_{18} (4, 15×3.9)	MeCN–water (pH 6.14), 1:3 (1)	375	566	2[a]
Rivory et al. [12]	CPT-11 SN-38	Lactone, Carbox	Plasma (human)	Protein precipitation (cold MeOH–MeCN, 1:1)	Nova-pak Radial-Pak C_{18} (4, 10×5) and a guard column	MeCN–75 m*M* ammonium acetate (pH 6.4), 22:78 (1), containing 5 m*M* tetrabutylammonium phosphate	355	515	10 2
Kaneda et al. [13]	SN-38	Lactone, Carbox	Plasma (human)	Protein precipitation (cold MeOH)	Puresil C_{18} (5, 15×4.6) and a guard column	MeCN–100 m*M* ammonium acetate (pH 5.5), 3:7 (1), containing 20 m*M* tetrapentylammonium bromide	380	540	5
Herben et al. [9]	CPT-11 SN-38	Lactone, Carbox	Plasma (human)	Protein precipitation (cold MeOH–MeCN, 1:1)	Zorbax SB-C_{18} (3.5, 15×4.6) and a guard column	MeCN–100 m*M* ammonium acetate (pH 6.4)–triethylamine, 156:800:1 (1.5), containing 5 m*M* tetrabutylammonium phosphate	375 385	460 525	1 0.5
Chollet et al. [14]	CPT-11 SN-38	Lactone, Carbox	Plasma (dog, rat)	Protein precipitation (cold MeOH)	Symmetry C_{18} (5, 15×3.9) and a guard column	75 m*M* ammonium acetate–7.5 m*M* tetrabutylammonium bromide (pH 6.4)–MeCN, gradient (1.8)	362 375	425 560	5.9 2.4
Beijnen et al. [15]	TPT	Lactone, Carbox	Plasma (human)	Protein precipitation (cold MeOH)	LiChrosorb RP-18 (5, 12.5×4) and a guard column	MeOH–water–1 *M* phosphate buffer (pH 6.0)–250 m*M* sodium dioctylsulphosuccinate-triethylamine, 325:215:11.5:20:1.5 (1)	381	527	5
Rosing et al. [16]	TPT	Lactone	Plasma (human)	Protein precipitation (cold MeOH)	Zorbax SB-C_{18} (3.5, 7.5×4.6) and a guard column	MeOH–100 m*M* hexane sulfonic acid in MeOH–10 m*M* *N,N,N′,N′*-tetramethylethylenediamine (pH 6.0), 25:10:65 (1)	361	527	0.05
		Total	Plasma (human)	Protein precipitation (cold MeOH–acidification)			361	527	0.05
Rosing et al. [17]	TPT	Lactone	Plasma (rat, dog)	Protein precipitation (cold MeOH)	Zorbax SB-C_{18} (3.5, 7.5×4.6) and a guard column	MeOH–100 m*M* hexane sulfonic acid in MeOH–10 m*M* *N,N,N′,N′* tetramethylethylenediamine (pH 6.0), 25:10:65 (1)	361	527	Rat: 0.1 Dog: 0.2
		Total	Plasma (rat, dog)	Protein precipitation (cold MeOH–acidification)			361	527	Rat: 0.1 Dog: 0.2
Loos et al. [18]	TPT	Lactone, Carbox	Plasma (human)	Protein precipitation (cold MeOH)	Shandon Hypersil BDS C_{18} (3, 10×3) and a guard column	10 m*M* potassium dihydrogenphosphate containing 25% MeOH and 0.2% triethylamine, (pH 6.0) (0.7)	381	525	0.1
		Total	Urine (human)	Acidification (orthophosphoric acid)	Shandon Hypersil BDS C_{18} (3, 10×3) and a guard column	10 m*M* potassium dihydrogenphosphate containing 20% MeOH and 0.2% triethylamine, (pH 6.0) (1)	381	525	10
Warner et al. [19]	TPT	Lactone, Carbox	Plasma (human)	Protein precipitation (cold MeOH)	NovaPak-C_{18} (4, 15×3.9) and a guard column	MeCN–3% triethylamine acetate buffer (pH 5.5), 12:88 (1)	390	520	0.5
							350–470	510–650	0.75
Rosing et al. [10][b]	TPT	Lactone	Plasma (human)	Protein precipitation (cold MeOH)	Zorbax SB-C_{18} (3.5, 7.5×4.6) and a guard column	MeOH–75 m*M* potassium dihydrogen phosphate containing 0.2% triethylamine (pH 6.0), 27.5:72.5 (1)	380	527	0.1
		Total	Plasma (human)	Protein precipitation (cold MeOH–acidification)			380	527	0.1
			Urine (human)	Acidification (phosphoric acid)			380	527	25
			Feces (human)	Extraction with MeCN –ammonium acetate			380	527	300[c]

Table 1. Continued

Reference	Analyte		Sample	Sample preparation	HPLC column (particle size in μm, length in cm×I.D. in mm)	Mobile phase flow-rate (ml/min)	Detection		LLQ (ng/ml)
							Ex. (nm)	Em. (nm)	
Supko et al. [20]	9-AC	Lactone	Plasma (human)	Protein precipitation (cold MeOH)	Ultrasphere ODS (5, 25×4.6) and a guard column	MeCN–MeOH–100 m*M* ammonium acetate (pH 5.5), 23:10:67 (1), postcolumn acidification by 300 m*M* trifluoroacetic acid (0.3)	352	418	5
		Total	Plasma (human)	Protein precipitation (acidification–cold MeOH)			352	418	5
Li et al. [21]	HCPT	Lactone, Carbox	Plasma Urine Feces Tissue (rat)	Protein precipitation (cold MeOH–MeCN, 1:1)	RP C_{18} (5, 25×4.6) and a guard column	MeCN–75 m*M* ammonium acetate (pH 6.4), 3:7, containing 5 m*M* tetrabutylammonium phosphate (0.8)	363	550	2 2 2[c] 10
Supko [22]	CPT	Lactone	Plasma (human)	Protein precipitation (cold MeOH)	Ultrasphere ODS (5, 25×4.6) and a guard column	MeCN–100 m*M* ammonium acetate (pH 5.5), 28:72, containing 1 m*M* sodium dodecyl sulfate (1)	347	418	0.5
		Total	Plasma (human)	Protein precipitation (acidification–cold MeOH)			347	418	0.5
Scott et al. [23]	CPT	Lactone, Carbox	Plasma (rat)	Protein precipitation (cold MeCN)	Hypersil ODS C_{18} (5, 15×4.2) and a guard column	MeCN–25 m*M* phosphate buffer (pH 6.5), 27:73, containing 5 m*M* tetrabutylammonium dihydrogen phosphate (2)	370	435	1[a]
Beijnen et al. [24]	CPT	Lactone, Carbox	Plasma (rat)	Protein precipitation (cold MeOH)	LiChrosorb RP-18 (5, 12.5×4) and a guard column	MeOH–5 m*M* phosphate buffer (pH 6.5)–300 m*M* tetrabutylammonium phosphate, 500:500:15 (1)	369	426	1[a]
Takimoto et al. [26]	9-AC	Lactone	Plasma (human)	SPE (C_{18}) (25% MeOH–75% MeOH/KH_2PO_4)[d]	Ultrasphere ODS (5, 25×4.6) and a guard column	MeOH–25 m*M* KH_2PO_4 (pH 2.55), 45:55 (1)	365	440	0.09
		Total	Plasma (human)	Acidification, SPE (C_{18}) (25% MeOH–75% MeOH/KH_2PO_4)[d]			365	440	0.9
Gijn et al. [27]	9-AC	Lactone	Plasma (human)	Protein precipitation (cold MeOH) SPE (C_{18}) (25% MeOH–MeOH)[d]	Zorbax SB-C_{18} (3.5, 7.5×4.6) and a guard column	MeOH–water (pH 2.2), 3:6 (1)	370	450	0.2
		Total	Plasma (human)	Protein precipitation (cold MeOH–acidification)			370	450	0.2
Stafford et al. [25]	LRT	Lactone	Plasma (dog)	SPE (diol) (40% MeOH–95% MeOH)[d]	BDS Hypersil C_8 (5, 25×4.6) and a guard column	MeOH–100 m*M* ammonium acetate (pH 4.0), 25:75 (1)	378	420	0.05
		Total	Plasma (dog)	Acidification SPE (C_{18}) (MeOH)	BDS Hypersil C_8 (5, 25×4.6) and a guard column	MeOH–100 m*M* ammonium acetate (pH 5.5), 24:76 (1)	378	420	0.1
Oguma et al. [28]	Exatecan	Lactone	Plasma (human)	SPE (C_{18}) (50% MeOH–MeOH/HCl)[d]	TSKgel ODS-80Ts (5, 25×4.6)	MeCN–50 m*M* KH_2PO_4 (pH 3.0), 18:82 (1)	365	445	0.2
		Total	Plasma (human)	Acidification SPE (C_{18}) (MeOH–HCl)			365	445	0.2

Table 1. Continued

Reference	Analyte		Sample	Sample preparation	HPLC column (particle size in μm, length in cm×I.D. in mm)	Mobile phase flow-rate (ml/min)	Detection		LLQ (ng/ml)
							Ex. (nm)	Em. (nm)	
Oguma et al. [29]	Exatecan	Lactone	Plasma (mouse)	SPE (C_{18}) (50% MeOH–MeOH/HCl)[d]	Puresil C_{18} (5, 15×4.6)	MeCN–50 m*M* KH_2PO_4 (pH 3.0), 18:82 (1)	365	445	3
		Total	Plasma (mouse)	Acidification SPE (C_{18}) (MeOH/HCl)			365	445	3
Ahmed et al. [30]	CPT	Lactone	Plasma (human)	SPE (C_{18}) (20% MeOH–75% MeOH/KH_2PO_4)[d]	μBondapak-C_{18} (10, 30×3.9) and a guard column	MeCN–25 m*M* KH_2PO_4 containing 1 m*M* sodium heptanesulphonate (pH 4.8), 35:65 (0.85)	360	440	2
		Total	Plasma (human)	Protein precipitation (cold MeOH–acidification)			360	440	2
Bruijn et al. [32]	CPT-11	Lactone	Plasma (human)	LLE (MeCN–butyl chloride, 1:4)	Hypersil ODS (5, 10×4.6) and a guard column	MeOH–100 m*M* ammonium acetate containing 10 m*M* tetrabutylammonium sulfate (pH 5.50), 4:6 (1)	355	515	0.5
	SN-38						355	515	0.5
	CPT-11	Total	Plasma (human)	Protein precipitation (cold MeOH–water–perchloric acid, 20:20:1)	Hypersil ODS (5, 10×4.6) and a guard column	MeOH–100 m*M* ammonium acetate containing 10 m*M* tetrabutylammonium sulfate (pH 5.50), 35:65 (1)	355	515	2
	SN-38						355	515	2
Loos et al. [33]	9-AC	Lactone	Plasma (human)	LLE (MeCN–butyl chloride, 1:4)	Inertsil ODS-80A (5, 15×4.6) and a guard column	MeOH–water (pH 2.2), 4:6 (1)	370	450	0.05
	9-AC	Total	Plasma (human)	Protein precipitation (MeOH–5% perchloric acid, 1:1)	Inertsil ODS-80A (5, 15×4.6) and a guard column	MeOH–water (pH 2.1), 32.5:67.5 (1)	370	450	0.1
Selinger et al. [31]	LRT	Lactone	Blood (human)	LLE (MeCN–butyl chloride, 1:4)	BDS Hypersil C_8 (5, 25×4.6) and a guard column	MeCN–133 m*M* ammonium acetate (pH 5.5), 1:3 (1)	378	420	0.15

Lactone, lactone form; Carbox, carboxylate form; Total, lactone plus carboxylate forms; Ex., excitation wavelength; Em., emission wavelength; LLQ, lower limit of quantitation; MeOH, methanol; MeCN, acetonitrile.

[a] Lowest concentration on calibration curve.

[b] Simultaneous determination of *N*-desmethyltopotecan.

[c] Concentration in ng/g feces.

[d] A, B: Carboxylate form is removed by solution A from solid-phase column and lactone form is extracted using solution B.

HPLC	High-performance liquid chromatography
HPLC–MS	High-performance liquid chromatography–mass spectrometry
HPLC–MS–MS	High-performance liquid chromatography–mass spectrometry–mass spectrometry
APCI	Atmospheric pressure chemical ionization
ESI	Electrospray ionization
ELISA	Enzyme-linked immunosorbent assay
LLQ	Lower limit of quantitation
MeOH	Methanol
MeCN	Acetonitrile

References

[1] M.E. Wall, M.C. Wani, C.E. Cook, K.H. Palmar, A.T. McPhail, G.A. Sim, J. Am. Chem. Soc. 88 (1966) 3888.

[2] Y.-H. Hsiang, R. Hertzberg, S. Hecht, L.F. Liu, J. Biol. Chem. 260 (1985) 14873.

[3] Y.-H. Hsiang, L.F. Liu, Cancer Res. 48 (1988) 1722.

[4] R.P. Hertzberg, M.J. Caranfa, S.M. Hecht, Biochemistry 28 (1989) 4629.

[5] R.C. Gallo, J. Whang-Peng, R.H. Adamson, J. Natl. Cancer Inst. 46 (1971) 789.

[6] J.A. Gottlieb, A.M. Guarino, J.B. Call, V.T. Oliverio, J.B. Block, Cancer Chemother. Rep. 54 (1970) 461.

[7] F.M. Muggia, P.J. Creaven, H.H. Hansen, M.H. Cohen, O.S. Selawry, Cancer Chemother. Rep. 56 (1972) 515.

[8] W.J. Loos, P.D. Bruijn, J. Verweij, A. Sparreboom, Anti-Cancer Drugs 11 (2000) 315.

[9] V.M.M. Herben, D. Mazee, D.M. Van Zomeren, S. Zeedijk, H. Rosing, J.H.M. Schellens, W.W. Ten Bokkel Huinink, J.H. Beijnen, J. Liq. Chromatogr. Rel. Technol. 21 (1998) 1541.
[10] H. Rosing, D.M. Van Zomeran, E. Doyle, W.W. Ten Bokkel Huinink, J.H.M. Scellens, A. Bult, J.H. Beijnen, J. Chromatogr. 727 (1999) 191.
[11] M.L. Rothenberg, J.G. Kuhn, H.A. Burris III, J. Nelson, J.R. Eckardt, M. Tristan Morales, S.G. Hilsenbeck, G.R. Weiss, L.S. Smith, G.I. Rodriguez, M.K. Rock, D.D. Von Hoff, J. Clin. Oncol. 11 (1993) 2194.
[12] L.P. Rivory, J. Robert, J. Chromatogr. 661 (1994) 133.
[13] N. Kaneda, Y. Hosokawa, T. Yokokura, Biol. Pharm. Bull. 20 (1997) 815.
[14] D.F. Chollet, L. Goumaz, A. Renard, G. Montay, L. Vernillet, V. Arnera, D.J. Mazzo, J. Chromatogr. 718 (1998) 163.
[15] J.H. Beijnen, B.R. Smith, W.J. Keijer, R. Van Gijn, W.W. Ten Bokkel Huinink, L.Th. Vlasveld, S. Rodenhuis, W.J.M. Underberg, J. Pharm. Biomed. Anal. 8 (1990) 789.
[16] H. Rosing, E. Doyle, B.E. Davies, J.H. Beijnen, J. Chromatogr. 668 (1995) 107.
[17] H. Rosing, E. Doyle, J.H. Beijnen, J. Pharm. Biomed. Anal. 15 (1996) 279.
[18] W.J. Loos, G. Stoter, J. Verweij, J.H.M. Schellens, J. Chromatogr. 678 (1996) 309.
[19] D.L. Warner, T.G. Burke, J. Liq. Chromatogr. Rel. Technol. 20 (1997) 1523.
[20] J.G. Supko, L. Malspeis, J. Liq. Chromatogr. 15 (1992) 3261.
[21] Y.-F. Li, R. Zhang, J. Chromatogr. 686 (1996) 257.
[22] J.G. Supko, L. Malspeis, J. Liq. Chromatogr. 14 (1991) 1779.
[23] D.O. Scott, D.S. Bindra, V.J. Stella, Pharm. Res. 10 (1993) 1451.
[24] J.H. Beijnen, H. Rosing, W.W. Ten Bokkel Huinink, H.M. Pinedo, J. Chromatogr. 617 (1993) 111.
[25] C.G. Stafford, R.L. St. Claire III, J. Chromatogr. 663 (1995) 119.
[26] C.H. Takimoto, R.W. Klecker, W.L. Dahut, L.K. Yee, J.M. Strong, C.J. Allegra, J.L. Grem, J. Chromatogr. 655 (1994) 97.
[27] R. Van Gijn, V.M.M. Herben, M.J.X. Hillebrand, W.W. Ten Bokkel Huinink, A. Bult, J.H. Beijnen, J. Pharm. Biomed. Anal. 17 (1998) 1257.
[28] T. Oguma, Y. Ohshima, M. Nakaoka, J. Chromatogr. 740 (2000) 237.
[29] T. Oguma, M. Yamada, T. Konno, K. Inukai, M. Nakaoka, Biol. Pharm. Bull. 24 (2001) 176.
[30] F. Ahmed, V. Vyas, A. Saleem, X.-G. Li, R. Zamek, A. Cornfield, P. Haluska, N. Ibrahim, E.H. Rubin, E. Gupta, J. Chromatogr. 707 (1998) 227.
[31] K. Selinger, G. Smith, S. Depee, C. Aureche, J. Pharm. Biomed. Anal. 13 (1995) 1521.
[32] P.D. Bruijn, J. Verweij, W.J. Loos, K. Nooter, G. Stoter, A. Sparreboom, J. Chromatogr. 698 (1997) 277.
[33] W.J. Loos, A. Sparreboom, J. Verweij, K. Nooter, G. Stoter, J.H.M. Schellens, J. Chromatogr. 694 (1997) 435.
[34] N. Kaneda, H. Nagata, T. Furuta, T. Yokokura, Cancer Res. 50 (1990) 1715.
[35] N. Kaneda, T. Yokokura, Cancer Res. 50 (1990) 1721.
[36] I. Barilero, D. Gandia, J.-P. Armand, A. Mathieu-Boué, M. Ré, A. Gouyette, G.G. Chabot, J. Chromatogr. 575 (1992) 275.
[37] S. Tanaka, Y. Yoshida, W. Suzuki, K. Sudo, H. Hakusui, Jpn. Pharmacol. Ther. 22 (1994) 163.
[38] H. Sumiyoshi, Y. Fujiwara, T. Ohune, N. Yamaoka, K. Tamura, M. Yamakido, J. Chromatogr. 670 (1995) 309.
[39] A. Sparreboom, P.D. Bruijn, M.J.A. De Jonge, W.J. Loos, G. Stoter, J. Verweij, K. Nooter, J. Chromatogr. 712 (1998) 225.
[40] A. Kurita, N. Kaneda, J. Chromatogr. 724 (1999) 335.
[41] J. Escoriaza, A. Aldaz, C. Castellanos, E. Calvo, J. Giráldez, J. Chromatogr. 740 (2000) 159.
[42] L.P. Rivory, M. Findlay, S. Clarke, J. Bishop, J. Chromatogr. 714 (1998) 355.
[43] P.D. Bruijn, M.J.A. De Jonge, J. Verweij, W.J. Loos, K. Nooter, G. Stoter, A. Sparreboom, Anal. Biochem. 269 (1999) 174.
[44] D. Fraier, E. Frigerio, G. Brianceschi, M. Casati, A. Benecchi, C. James, J. Pharm. Biomed. Anal. 22 (2000) 505.
[45] W.J. Loos, D. Kehrer, E. Brouwer, J. Verweij, P. De Bruijn, M. Hamilton, S. Gill, K. Nooter, G. Stoter, A. Sparreboom, J. Chromatogr. 738 (2000) 155.
[46] H.M. Dodds, M.-C. Haaz, J.-F. Riou, J. Robert, L.P. Rivory, J. Pharmacol. Exp. Ther 286 (1998) 578.
[47] H.M. Dodds, J. Robert, L.P. Rivory, J. Pharm. Biomed. Anal. 17 (1998) 785.
[48] S. Ragot, P. Marquet, F. Lachâtre, A. Rousseau, E. Lacassie, J.M. Gaulier, J.L. Dupuy, G. Lachâtre, J. Chromatogr. 736 (1999) 175.
[49] T. Oguma, T. Konno, A. Inaba, M. Nakaoka, Biomed. Chromatogr. 15 (2001) 108.
[50] T.G. Burke, H. Malak, I. Gryczynski, Z. Mi, J.R. Lakowicz, Anal. Biochem. 242 (1996) 266.
[51] T. Saita, H. Fujito, M. Mori, Biol. Pharm. Bull. 23 (2000) 911.
[52] T. Saita, H. Fujito, M. Mori, Biol. Pharm. Bull. 24 (2001) 321.