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Simple and versatile high-performance liquid chromatographic method for the simultaneous quantitation of the lactone and carboxylate forms of camptothecin anticancer drugs

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Abstract

The well documented hydrolysis of the α-hydroxy-δ-lactone ring moiety in camptothecin and related analogues is routinely monitored using high-performance liquid chromatography (HPLC). Previous HPLC separations of the lactone and carboxylate forms of camptothecins have often required mobile phases containing three to four components; ion-pairing reagent to provide adequate retention of the carboxylate form of the drug; buffer to control the ionic strength and pH of the mobile phase; acetonitrile to control the retention of the lactone form and, in some instances, sodium dodecyl sulfate to reduce peak tailing. Because of the complexity of the mobile phases employed, development of these assays can be a laborious process, requiring re-optimization for each new analogue. In this study, we have developed a simple HPLC methodology for the simultaneous separation of the lactone and carboxylate forms of numerous camptothecin analogues. The mobile phase employed includes only triethylamine acetate (TEAA) buffer and acetonitrile. In this application, triethylamine serves multiple roles; as the ion-pairing reagent, as a masking agent for underivatized silanols and as the major buffer component. By altering only the composition of TEAA buffer with respect to acetonitrile, method development becomes a more streamlined and time efficient process. In this publication, we present the simultaneous separation of the lactone and carboxylate forms of camptothecin and four related analogues, namely, topotecan, GI147211, 10-aminocamptothecin and the CPT-11–SN-38 drug–metabolite pair. It is proposed that this new mobile phase, consisting of only triethylamine acetate buffer and acetonitrile, can be used for the analysis of the several camptothecin derivatives presently in clinical trials as well as the numerous other analogues in preclinical development.

Keywords: Camptothecin

1. Introduction

Since its isolation in the mid 1960s, camptothecin [1] and its related analogues have been studied as potential anti-cancer drugs. Camptothecin has an α-hydroxy-δ-lactone ring moiety that can be hydrolyzed to form a water-soluble carboxylate form of the drug (Table 1). Early evidence suggested that the two forms of the drug possessed similar cytotoxicity [1,2], therefore, 20-*S*-camptothecin was introduced into clinical trials in the early 1970s as the water-soluble sodium carboxylate salt. Due to the high toxicity and low activity of the carboxylate form of the drug, the clinical trials were discontinued in 1972

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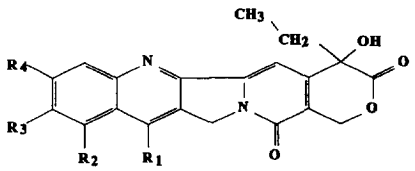
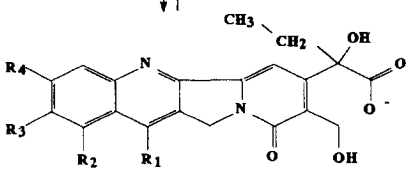
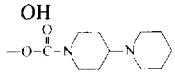
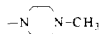
Table 1
Chemical structure of camptothecin and camptothecin-related analogues



Lactone



Carboxylate

Compound	R_1	R_2	R_3	R_4
Camptothecin	H	H	H	H
Topotecan	H	$CH_2NH(CH_3)$	OH	H
CPT-11	C_2H_5	H		H
SN-38	C_2H_5	H	OH	H
10-Amino-CPT	H	H	NH_2H	
GI147211		H	$-O-CH_2-CH_2-O-$	

[3–6]. It has since been shown that topoisomerase I is the target of camptothecins [7,8] and that an intact lactone ring is necessary for biological activity [9]. Following these discoveries, interest in camptothecin and its analogues has been rekindled.

Pharmacokinetic and pharmacodynamic studies for any drug under clinical development are important in order to optimize the therapeutic activity of the agent. Such studies are of greater interest, however, in the case of the camptothecins, because of their dynamic nature. Understanding the pharmacokinetic and pharmacodynamic parameters of lactone ring opening requires methodologies that allow quantitation of the intact lactone species, total drug concentrations and preferably, carboxylate levels as well. High-performance liquid chromatography (HPLC) using a C_{18} column is routinely used to achieve separation and quantitation. Several methods have been described for the camptothecins involving quantitation of only the lactone form [10–15] or for the simultaneous determination of the lactone and carboxylate forms [16–22]. Method development for HPLC analyses of camptothecins can be very difficult, however, due to the numerous variables that must be considered. In the simplest case of the lactone only separation, two or three components are normally present in the mobile phase. A buffer is used to control the pH of the mobile phase, while acetonitrile is used to control retention of the lactone form of the analogue. A third component is also frequently added to control peak tailing due to interactions between the analyte and underivatized silanols on the column wall. At pH values above 3.5, silanols act as weak acids that have a strong interaction with basic or amino-containing compounds [23]. This interaction results in a dual retention process, which produces peak tailing. Pronounced peak tailing makes quantitation more difficult and less accurate. The addition of a positively charged substance to the mobile phase counteracts this problem by interacting with the underivatized silanols and essentially "coating" the column [23]. In the past, HPLC methods for camptothecin separations have included substances such as sodium dodecyl

sulfate (SDS) in the mobile phase to improve peak shape [12]. For the more accurate and less laborious simultaneous determination of the lactone and carboxylate forms of a camptothecin, an additional component, namely an ion-pairing agent, must also be employed. Without this ion-pairing agent, the chromatographic behavior of the charged carboxylate species is such that it will not be retained on the C_{18} column to an appreciable extent (i.e. the carboxylate elutes in the void volume), precluding simultaneous separation of the lactone and carboxylate form of the drug. The ion-pairing agent tends to mask the charge of the camptothecin carboxylate species, increasing its affinity for the column [24]. Common ion-pairing reagents such as tetrabutylammonium phosphate are normally added to a buffered mobile phase to facilitate the separation.

Method development for the analysis of camptothecins using HPLC can therefore be very time-consuming and complicated. Normally the following three or four parameters must be optimized for a successful separation: Type and concentration of ion-pairing reagent; type, concentration and pH of buffer; amount of organic modifier and concentration of SDS or other agent capable of masking silanols. In this study, we document a HPLC methodology for the simultaneous separation of the lactone and carboxylate forms of numerous camptothecin analogues, employing only a simple two-component mobile phase system consisting of triethylamine acetate (TEAA) buffer and acetonitrile. In this application, triethylamine acts as the ion-pairing agent, masks underivatized silanols and is the major buffer component. By altering only the composition of the TEAA buffer with respect to acetonitrile (rather than three or four separate components), method development time is markedly reduced. We have used this methodology for the simultaneous separation of the lactone and carboxylate forms of the following camptothecins, currently in clinical trials; camptothecin, GI147211, topotecan and the CPT-11–SN-38 drug–metabolite pair. We have also separated a new camptothecin related analogue, namely 10-aminocamptothecin, which has not yet been introduced into clinical trials. To ensure the applicability of this method to relevant clinical studies, GI147211 has been analyzed in the presence of whole blood and topotecan has been analyzed in the presence of plasma. It is proposed that this new mobile phase, consisting of TEAA buffer and acetonitrile, can be easily used and optimized for the analysis of existing camptothecin-related analogues, as well as many newly synthesized analogues now under preclinical development.

2. Experimental

2.1. Chemicals and HPLC reagents

Samples of CPT-11 and SN-38 were generously donated by Pharmacia and Upjohn (Kalamazoo, MI, USA) and Yakult Honsha Pharmaceutical (Tokyo, Japan). We gratefully acknowledge the National Cancer Institute, Division of Cancer Treatment (Bethesda, MD, USA) for providing samples of topotecan. GI147211 was graciously donated by Glaxo (Research Triangle Park, NC, USA). Samples of 10-aminocamptothecin and camptothecin were generously provided by the laboratories of Drs. Monroe Wall and Mansukh Wani (Research Triangle Institute). All of the camptothecins were in the 20*S* configuration. HPLC-grade acetonitrile and triethylamine were purchased from Fisher Scientific (Fair Lawn, NJ, USA). High purity water was provided by a Milli-Q UV Plus purification system (Millipore, Bedford, MA, USA). Unless noted, all other chemicals were of reagent grade and used without further purification. Stock solutions of each drug were prepared in A.C.S. spectrophotometric grade dimethylsulfoxide (DMSO; Aldrich, Milwaukee, WI, USA) at a concentration of $2 \cdot 10^{-3}$ *M* and stored in the dark at 4°C until use. Phosphate buffered saline (PBS) refers to an aqueous solution of 8 m*M* dibasic sodium phosphate (Na_2HPO_4), 1 m*M* potassium phosphate monobasic crystals (KH_2PO_4), 137 m*M* sodium chloride (NaCl) and 3 m*M* potassium chloride (KCl). In all analyses, the mobile phase consisted of TEAA buffer (1–3% triethylamine in water (v/v), adjusted to pH 5.5 with glacial acetic acid) and acetonitrile. The ratio of acetonitrile–buffer (v/v) was adjusted to achieve the desired retention characteristics of the analytes for each separation. All mobile phases were filtered and vacuum degassed using sonication prior to use.

2.2. HPLC apparatus

The isocratic HPLC system utilized consisted of a Waters 501 HPLC pump (Millipore), a Rheodyne injector (Rainin Instruments, Woburn, MA, USA) fitted with a 50- or 100-μl sample loop, and a Gilson Model 121 fluorescence detector (Gilson Medical Electronics, Middleton, WI, USA) equipped with filters. In all instances, excitation from 305 to 395 nm was employed, except in the case of topotecan, where excitation from 350 to 470 nm was used. Emission from 510 to 650 nm was used for the analysis of topotecan, 10-aminocamptothecin, SN-38 and the simultaneous CPT-11–SN-38 separation. Emission from 430 to 470 nm was employed for the analysis of camptothecin, GI147211 and CPT-11. Separations were carried out at ambient temperature using a Waters NovaPak-C_{18} 4 μm particle-size reversed-phase 150×3.9 mm I.D. column, preceded by a Waters Guard-Pak C_{18} Nova-Pak precolumn insert (Millipore). In all instances, a flow-rate of 1.0 ml/min was employed. Fluorescence output signal was monitored and integrated using Millennium 2010 Chromatography Manager software (Millipore) on a 386 IBM computer. Each separation was performed five times with average retention times being reported for the carboxylate and lactone forms of each drug.

2.3. Sample preparation in PBS

A stock solution containing 2 m*M* of the drug of interest in DMSO was prepared and stored at −20°C. Each day, a 50-μl aliquot of this stock solution was added to 50 μl of PBS, pH 3.0, for lactone or PBS, pH 10.0, for carboxylate, in order to form 1 m*M* carboxylate and 1 m*M* lactone standard solutions, respectively. These 1 m*M* solutions were allowed to stand at room temperature for 30 min to ensure equilibration. The following sequential dilutions were performed before injection of the analyte onto the column. A 20-μl volume of each of the 1 m*M* standard solutions was added to 1.96 ml of PBS, pH 7.4, to form a 20 μ*M* solution containing equal amounts of carboxylate and lactone (10 μ*M* each). A 20-μl volume of this 20 μ*M* solution was immediately added to 1.98 ml of PBS, pH 7.4, vortex-mixed and injected onto the column as a 200 n*M* solution.

Standards containing either carboxylate or lactone were prepared by the addition of a 40-μl volume of the appropriate 1 m*M* standard solution to 1.96 ml PBS of the appropriate pH (3.0 or 10.0, respectively) to form a 20 μ*M* solution. A 20-μl volume of this 20 μ*M* solution was then added to 1.98 ml of PBS of the appropriate pH and immediately injected onto the column as a 200 n*M* solution.

2.4. Sample preparation of GI147211 in whole blood

Whole human blood was obtained from a healthy female donor by drawing blood into sterile vacutainers containing heparin, to prevent clot formation. A stock solution containing 500 μ*M* GI147211 in DMSO was prepared and stored at −20°C. A 20-μl volume of the stock solution was added to 10 ml of whole blood that had previously been incubated at 37°C for 30 min to form a 1 μ*M* solution. At each respective time interval, a 50-μl volume was removed from the incubation tube and added to 200 μl of ice-cold methanol (−20°C), vortex-mixed for 20 s and centrifuged at 8000 *g* for 1 min. The supernatant was directly injected onto the HPLC column. Aliquots were taken and HPLC analyses was performed at times of 1, 10, 20, 30, 60, 120 and 180 min, respectively. Percentage recovery of GI147211 lactone and carboxylate from blood was calculated as shown in Eq. (1).

$$\text{Percentage recovery} = 100 \times \frac{\text{average peak area in blood}}{\text{average peak area in PBS}} \quad (1)$$

The percentage recovery of drug using the aforementioned methanol extraction method was determined to be 78.6±7.4 for the carboxylate and 66.8±4.8 for the lactone.

2.5. Sample preparation of topotecan in plasma

Outdated human plasma was obtained from Central Kentucky Blood Center (Lexington, KY, USA) and stored at −20°C until use. A stock solution containing 100 μ*M* topotecan in DMSO was prepared and stored at −20°C. A 50-μl volume of the

stock solution was added to 407.5 μl of PBS (pH 3.0 or 10.0) to form 5000 ng/ml solutions of topotecan carboxylate and lactone. This solution was serially diluted with PBS of the appropriate pH to form 50 ng/ml solutions. A 50-μl volume of both the carboxylate and the lactone solution was added to 900 μl of plasma to form a sample containing 2.5 ng/ml of topotecan carboxylate and 2.5 ng/ml of topotecan lactone. A 200-μl volume of the plasma solution was added to 800 μl of ice-cold methanol (−20°C), vortex-mixed for 20 s and centrifuged at 8000 *g* for 1 min. The supernatant was diluted 1:1 (v/v) with TEA buffer and 100 μl were injected directly onto the HPLC column.

3. Results and discussion

3.1. HPLC analysis of camptothecin

Initial HPLC methodologies utilizing fluorescence detection for camptothecin analysis were devised so that only the lactone species of camptothecin was retained on the C_{18} column, with the carboxylate species being eluted in the void volume [11,12]. The mobile phases employed in these early studies included the potassium phosphate buffer–methanol system of Loh and Ahmed [11] and an ammonium acetate buffer–acetonitrile–SDS system described by Supko and Malspeis [12]. Although HPLC method development and mobile phase selection is somewhat simpler for these lactone-specific separations, two injections and an internal standard are required for determination of total drug or the carboxylate species present. The first injection is performed to determine the amount of lactone species present in the sample. This is done by a ratio method, which relates lactone peak area to internal standard peak area. The sample is then acidified (driving the lactone–carboxylate equilibrium very far to the left) and injected again. The ratio of lactone peak area to internal standard peak area of this injection is designated as the amount of total drug in the sample. The amount of lactone can then be compared with the amount of total drug to determine indirectly the amount of carboxylate species present. More recent HPLC methodologies using ion-pairing to obtain a simultaneous separation of the lactone and carboxylate species of camptothecin have been described [19–21]. In these studies, an ion-pairing agent such as tetrabutylammonium phosphate is added to the mobile phase. This ion-pairing agent changes the chromatographic behavior of the carboxylate so that it exhibits sufficient retention on the C_{18} column to allow for separation of the carboxylate species from the void volume.

In the present study, we have used a mobile phase composed of only TEAA buffer and acetonitrile to simultaneously separate the carboxylate and lactone forms of camptothecin in less than 10 min. As is evident from Fig. 1, this mobile phase provides adequate retention of both the carboxylate (2.2 min) and the lactone (8.7 min) camptothecin species, while controlling the tailing due to analyte interactions with underivatized silanols. The specific mobile phase content for this separation was 23:77 (v/v) acetonitrile–TEAA buffer (1%, v/v), pH 5.5.

3.2. HPLC analysis of topotecan

Past HPLC methodologies using fluorescence detection for topotecan have been performed for the

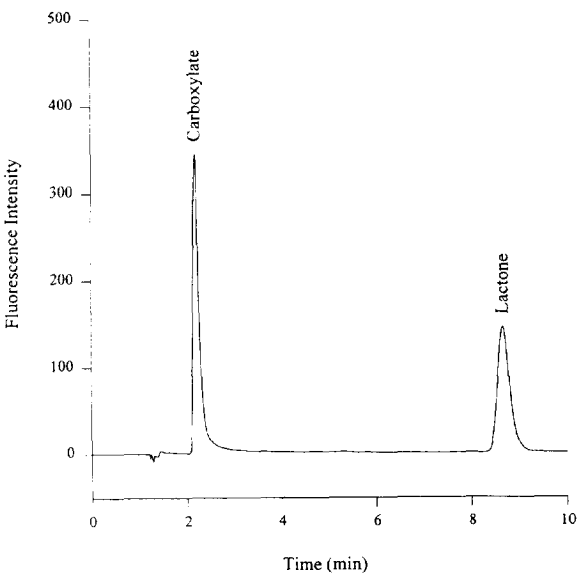


Fig. 1. HPLC separation of camptothecin carboxylate and lactone species. The mobile phase consists of 23:77 (v/v) acetonitrile–triethylamine acetate buffer (1%, v/v), pH 5.5. Fluorescence detection wavelengths are 305–395 nm (excitation) and 430–470 nm (emission).

simultaneous separation of the carboxylate and lactone species [16–18]. The mobile phase employed by Rosing et al. [16] consists of a methanol–hexane-1-sulfonic acid in methanol–N,N,N′,N′-tetramethylethylenediamine buffer, while that of Beijnen et al. [17] consists of dioctyl sulfosuccinate–sodium phosphate buffer–triethylamine–methanol. A more recent report by Loos et al. [18] employs a potassium dihydrogenphosphate–methanol–triethylamine mobile phase. Re-optimization of conditions with routine column changes and daily preparation of the complex mobile phases described above (containing three to four components) makes these mobile phases unattractive for routine use.

Our simplified mobile phase, composed of only TEAA buffer and acetonitrile, has been used to simultaneously separate the carboxylate and lactone species of topotecan in less than 8 min (Fig. 2). Topotecan contains a dimethylaminomethyl group that can potentially interact with residual silanols. Therefore, to further minimize peak tailing, the percentage of triethylamine present in the buffer was increased from 1 to 2% (v/v). As shown in Fig. 2A,B, this increase in triethylamine content did significantly improve the peak shapes of the lactone and carboxylate analytes. The specific mobile phase for the optimum separation was 12:88 (v/v) acetonitrile–TEAA buffer (2%, v/v), pH 5.5. Using this specific mobile phase composition, the carboxylate form elutes in 2.2 min while the lactone elutes in 6.8 min.

3.3. HPLC analysis of CPT-11 and SN-38

The development of clinically useful HPLC methodologies for the separation of CPT-11 presents a

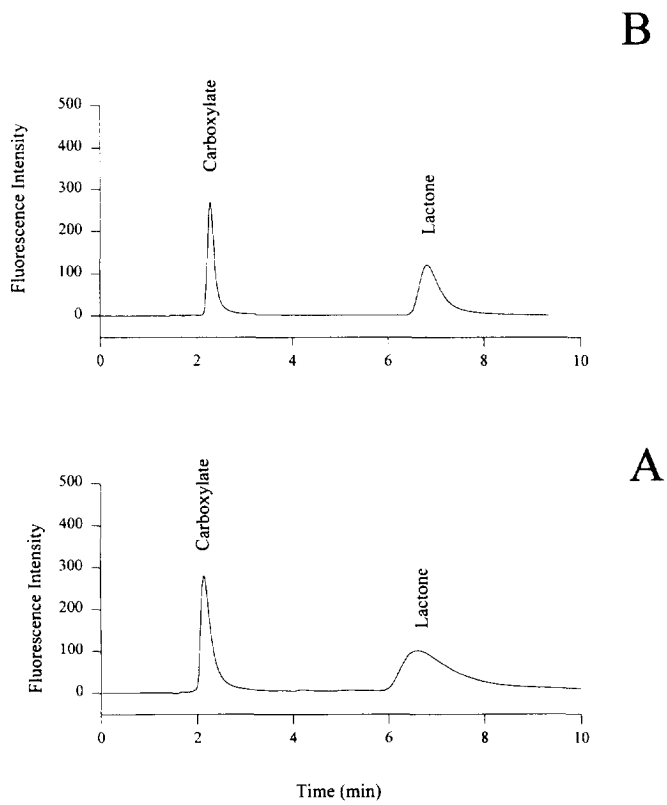


Fig. 2. HPLC separation of topotecan carboxylate and lactone species. Fluorescence detection wavelengths are 350–470 nm (excitation) and 510–650 nm (emission). (A) HPLC separation of topotecan using 1% triethylamine acetate buffer. Mobile phase conditions are 14:86 (v/v) acetonitrile–triethylamine acetate buffer (1%, v/v), pH 5.5. (B) HPLC separation of topotecan using 2% triethylamine acetate buffer. Mobile phase conditions are 12:88 (v/v) acetonitrile–triethylamine acetate buffer (2%, v/v), pH 5.5.

more difficult challenge as CPT-11 is a prodrug for the biologically active SN-38 agent. Although initial methods resolved only the intact lactone form of CPT-11 and SN-38 [15], relevant HPLC methods should be able to separate lactone and carboxylate species of both drugs (four components in total) in a reasonable amount of time. A simultaneous method employed by Rivory and Robert [22] consists of an ammonium acetate buffer–acetonitrile–tetrabutylammonium phosphate mobile phase using a traditional C_{18} column. Although all four components were separated under these conditions, significant peak tailing precluded baseline separation.

For the simultaneous separation of CPT-11 and SN-38, a mobile phase composed of only TEAA buffer and acetonitrile is once again used, with the separation being performed in less than 13 min (Fig. 3). A mobile phase of 20:80 (v/v) acetonitrile–TEAA buffer (3%, v/v), pH 5.5, provides optimum peak shape and separation of all four components. The separation of the lactone and carboxylate species of CPT-11 and SN-38 are shown individually in Fig. 4A,B. The mobile phase conditions employed result in retention times of 2.3 min for CPT-11 carboxylate, 2.8 min for SN-38 carboxylate, 8.4 min for CPT-11 lactone and 11.9 min for SN-38 lactone. As plasma levels of SN-38 are significantly lower than those of CPT-11 [15], fluorescence detection with excitation from 305 to 395 nm and emission from 510 to 650 nm was used for the separation of CPT-11 and SN-38 as well as for the separation of lactone and carboxylate forms of only SN-38. For enhanced sensitivity when analyzing only CPT-11, detection with excitation from 305 to 395 nm and emission from 430 to 470 nm was employed.

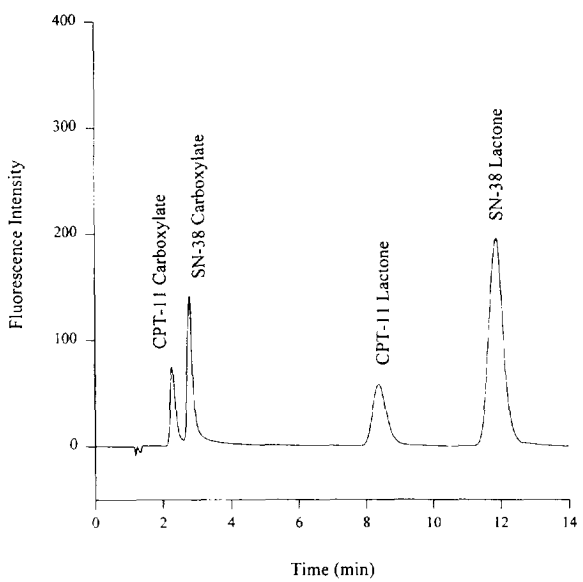


Fig. 3. Simultaneous HPLC separation of CPT-11 and SN-38 carboxylate and lactone forms. The mobile phase consists of 20:80 (v/v) acetonitrile–triethylamine acetate buffer (3%, v/v), pH 5.5. Fluorescence detection wavelengths are 305–395 nm (excitation) and 510–650 nm (emission).

3.4. HPLC analysis of GI147211

Recently, HPLC methodologies using fluorescence detection have been developed for the determination of the lactone species of GI147211 [13,14]. Both of these methods employ a traditional ammonium acetate buffer–acetonitrile mobile phase. To date, no methods have been published for the simultaneous separation of the carboxylate and lactone forms of GI147211.

In the case of GI147211, the TEAA buffer–acetonitrile mobile phase simultaneously separates the carboxylate and lactone species in less than 10 min (Fig. 5). The specific mobile phase content for this separation is 19:81 (v/v) acetonitrile–TEAA buffer (1%, v/v), pH 5.5. These conditions provide adequate retention of both the carboxylate (2.5 min) and lactone (8.5 min) species.

3.5. HPLC analysis of 10-aminocamptothecin

To further demonstrate the potential versatility of this newly developed method, an analogue that had not been separated previously using HPLC in this laboratory and for which no published HPLC methods were available, was studied. The analogue chosen for this study was 10-aminocamptothecin. The mobile phase was optimized for 10-aminocamptothecin and consisted of 17:83 (v/v) acetonitrile–TEAA buffer (1%, v/v), pH 5.5. Using this method, the carboxylate and lactone species were simultaneously separated in less than 12 min. As seen in Fig. 6, these conditions provide retention of both the carboxylate (2.7 min) and lactone (9.8 min) species.

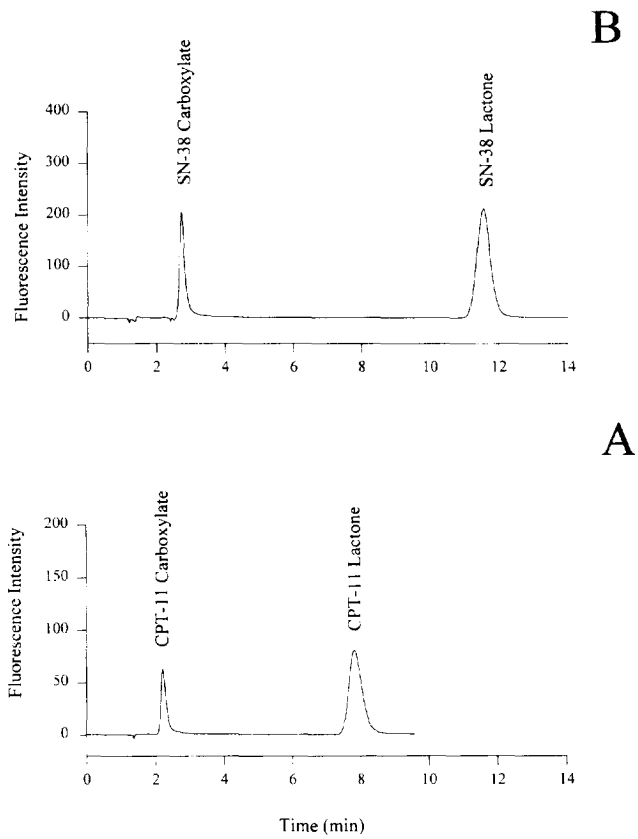


Fig. 4. (A) HPLC separation of only CPT-11 carboxylate and lactone forms. The mobile phase consists of 20:80 (v/v) acetonitrile–triethylamine acetate buffer (3%, v/v), pH 5.5. Fluorescence detection wavelengths are 305–395 nm (excitation) and 430–470 nm (emission). (B) HPLC separation of only SN-38 carboxylate and lactone forms. The mobile phase consists of 20:80 (v/v) acetonitrile–triethylamine acetate buffer (3%, v/v), pH 5.5. Fluorescence detection wavelengths are 305–395 nm (excitation) and 510–650 nm (emission).

3.6. HPLC analysis of GI147211 in whole blood

As mentioned previously, HPLC analysis is routinely used to monitor the kinetics of lactone ring opening for many of the camptothecin related drugs. Shown in Fig. 7 are chromatograms depicting the ring opening over time for GI147211 incubated in whole blood at 37°C. As is evident from the figure, it is possible to quantitate both the lactone and carboxylate forms of GI147211 during the same chromatographic run. The first peak represents components of whole blood that elute in the void volume. The second peak is the carboxylate form of GI147211, while the third peak is the lactone form of GI147211. As can be seen from the chromatograms, the blood matrix does not interfere with the resolution or quantitation of the resulting carboxylate and lactone peaks. It is postulated that many other camptothecin-related analogues, including ones separated in this study, can be quantified in blood or blood components with even greater accuracy than GI147211, as their native fluorescence is typically ten times higher than that of GI147211.

3.7. HPLC analysis of topotecan in plasma

HPLC is also frequently used to monitor the kinetics of lactone ring opening in human plasma during clinical trials. Applications for clinical plasma determinations should be able to detect topotecan levels in plasma at below 5 ng/ml. The analysis of a plasma blank sample (A) and of plasma spiked with

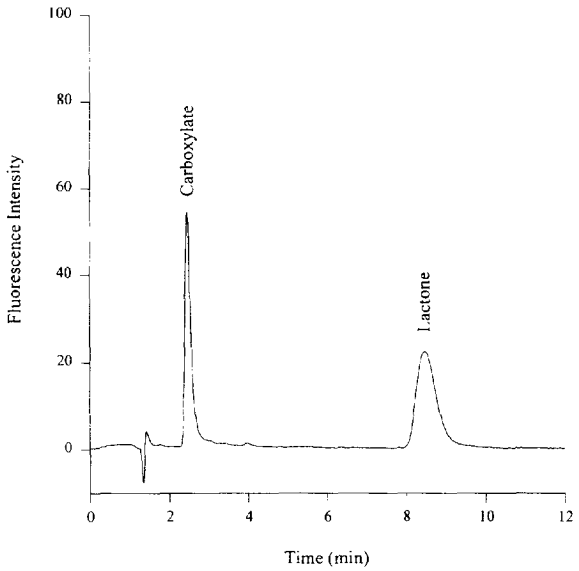


Fig. 5. HPLC separation of GI147211 carboxylate and lactone species. The mobile phase consists of 19:81 (v/v) acetonitrile–triethylamine acetate buffer (1%, v/v), pH 5.5. Fluorescence detection wavelengths are 305–395 nm (excitation) and 430–470 nm (emission).

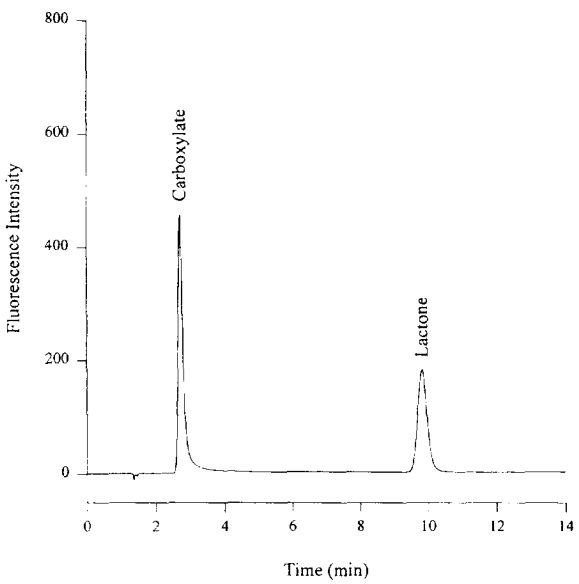


Fig. 6. HPLC separation of 10-aminocamptothecin carboxylate and lactone species. Mobile phase is 17:83 (v/v) acetonitrile–triethylamine acetate buffer (1%, v/v), pH 5.5. Fluorescence detection wavelengths are 305–395 nm (excitation) and 510–650 nm (emission).

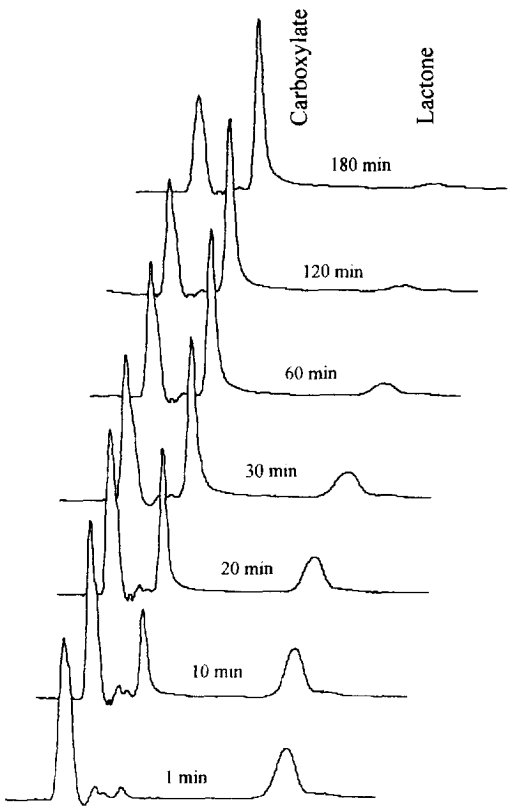


Fig. 7. Chromatographic analysis of GI147211 in whole blood over time when incubated at 37°C. The blood matrix components eluted at 1.5 min in the void volume, while GI147211 carboxylate eluted at 2.5 min and GI147211 lactone eluted at 8.5 min. At the indicated time intervals, a 50-μl volume of 1 μ*M* GI147211 in whole blood is added to 200 μl of ice-cold methanol (−20°C), vortex-mixed for 20 s and centrifuged at 8000 *g* for 1 min. The supernatant is directly injected onto the HPLC column. The mobile phase consists of 19:81 (v/v) acetonitrile–triethylamine acetate buffer (1%, v/v), pH 5.5. Fluorescence detection wavelengths are 305–395 nm (excitation) and 430–470 nm (emission).

2.5 ng/ml of topotecan carboxylate and 2.5 ng/ml of topotecan lactone (B) are shown in Fig. 8. Both topotecan carboxylate and lactone can be accurately quantitated at this concentration, despite the broad appearance of the lactone peak. The plasma matrix does not interfere with the detection of the carboxylate or lactone peaks, even at this low concentration. In the case of other camptothecin analogues studied using slightly different mobile phases, the plasma matrix peaks are also separable from the analytes.

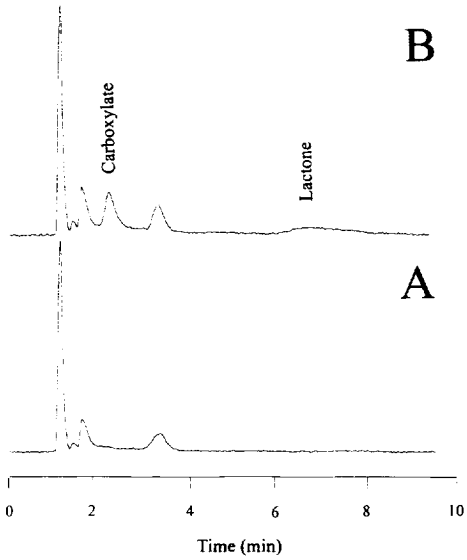


Fig. 8. (A) HPLC separation of plasma blank sample. (B) HPLC separation of topotecan in plasma. The plasma matrix yields one peak with a retention time of 3.5 min, which is not eluted in the void volume. Topotecan carboxylate eluted at 2.3 min and topotecan lactone eluted at 6.8 min. Fluorescence detection wavelengths are 350–470 nm (excitation) and 510–650 nm (emission). Mobile phase conditions are 12:88 (v/v) acetonitrile–triethylamine acetate buffer (2%, v/v), pH 5.5.

4. Conclusions

We have developed simple HPLC methods for the simultaneous separation of the lactone and carboxylate forms of numerous camptothecin-related analogues, in which the mobile phase includes only TEAA buffer and acetonitrile. In this application, triethylamine acts as the ion-pairing reagent, masks underivatized silanols and also serves as the major buffer component. By altering only the composition of TEAA buffer with respect to acetonitrile, method development time is reduced markedly. The simultaneous separation of the lactone and carboxylate forms of camptothecin, topotecan, GI147211, 10-aminocamptothecin and the CPT-11–SN-38 drug–metabolite pair have been performed. It has also been shown that this new mobile phase, consisting of only TEAA buffer and acetonitrile, can be utilized for the kinetic analysis of the ring opening of GI147211 in human whole blood and of topotecan in plasma.

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