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Selective and sensitive determination of lactone and hydroxy acid forms of camptothecin and two derivatives (CPT-11 and SN-38) by high-performance liquid chromatography with fluorescence detection

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ABSTRACT

A simple and rapid ion-pair high-performance liquid chromatographic (HPLC) method for the simultaneous determination of the lactone and hydroxy acid forms of camptothecin and its derivatives CPT-11 and SN-38 was developed. The method allows the hydroxy acid form to be eluted before the lactone form for each compound. The application of this method is limited by the minimum interval between subsequent injections and the void time for starting a measurement. However, it is possible to determine the lactone and hydroxy acid forms in aqueous solutions at the pH and the temperature near to biological conditions.

INTRODUCTION

Camptothecin is widely known as an antitumour alkaloid isolated from *Camptotheca acuminata* [1]. Recently, several derivatives of camptothecin were synthesized by Yakult (Tokyo, Japan) for medical application [2] and have been evaluated from the biological, physico-chemical and pharmaceutical standpoints [3]. The results indicated that a derivative of camptothecin, CPT-11 (I), was the most effective compound for antitumour purposes, and that SN-38 (II) was the main biologically active metabolite of I (Fig. 1) [3].

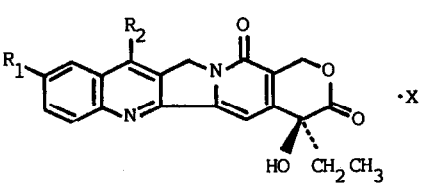

Fig. 1. Structures of camptothecin, CPT-11 (I) and SN-38 (II). Camptothecin: $R_1 = R_2 = H$, X = none. CPT-11: $R_1 = OCONC_5H_9NC_5H_{10}$, $R_2 = C_2H_5$, X = $HCl \cdot 3H_2O$. SN-38: $R_1 = OH$, $R_2 = C_2H_5$, X = H_2O.

The molecules of camptothecin, I and II all contain an α-hydroxy δ-lactone ring which is essential for their antitumour activity [4,5]. In general, the lactone ring is stable in acidic media but hydrolyses in neutral and/or alkaline media to form a hydroxy acid, and it is therefore important to clarify the effects of pH and temperature on the hydrolysis and lactonization of these compounds. Several kinetic studies of their hydrolysis and lactonization by means of acid–base titration [6,7], potentiometry [8], conductometry [7], spectrometry [9,10], high-performance liquid chromatography (HPLC) [11], etc., have been reported. However, these methods are insufficiently selective and insensitive for the purposes of our study.

This paper describes a rapid, selective and sensitive ion-pair HPLC method for the determination

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of the lactone and hydroxy acid forms of camptothecin, I and II in aqueous solutions.

EXPERIMENTAL

Materials and reagents

Camptothecin, I and II were obtained from Yakult. Sodium 1-heptanesulphonate for ion-pair HPLC was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Water was distilled and all other chemicals were of analytical-reagent grade. Britton–Robinson buffer solutions [12] (pH 4.0, 7.0 and 10.0) were used.

The HPLC column was a YMC (Kyoto, Japan) prepacked AM-312 C_{18} reversed-phase column (150 mm × 6 mm I.D., 5 μm). The mobile phase was a 1:1 mixture of methanol and pH 4.0, 0.1 *M* K_2HPO_4–H_3PO_4 buffer solution containing 3 m*M* (for I) or 6 m*M* (for camptothecin and II) sodium 1-heptanesulphonate.

Apparatus

A Hitachi Model 655 liquid chromatograph equipped with a JASCO (Tokyo, Japan) Model 820-FP intelligent spectrofluorometer and a Rheodyne (Cotati, CA, USA) Model 7125 injector with a 20-μl sampling loop was used. A Shimadzu (Kyoto, Japan) Chromatopac CR-4A integrator was used.

Analytical procedure

Samples were dissolved in the mobile phase to prepare solutions from about 20 to 1000 ng ml^{-1}. The solutions were subjected to HPLC at ambient temperature. The flow-rates of the mobile phase were 1.4 ml min^{-1} for I and 1.6 ml min^{-1} for camptothecin and II. The spectrofluorometer was automatically maintained at the maximum wavelength of each compounds, which are listed in Table I. The concentrations of the lactone and hydroxy acid were determined from the calibration graphs obtained by the peak-area method. The calibration

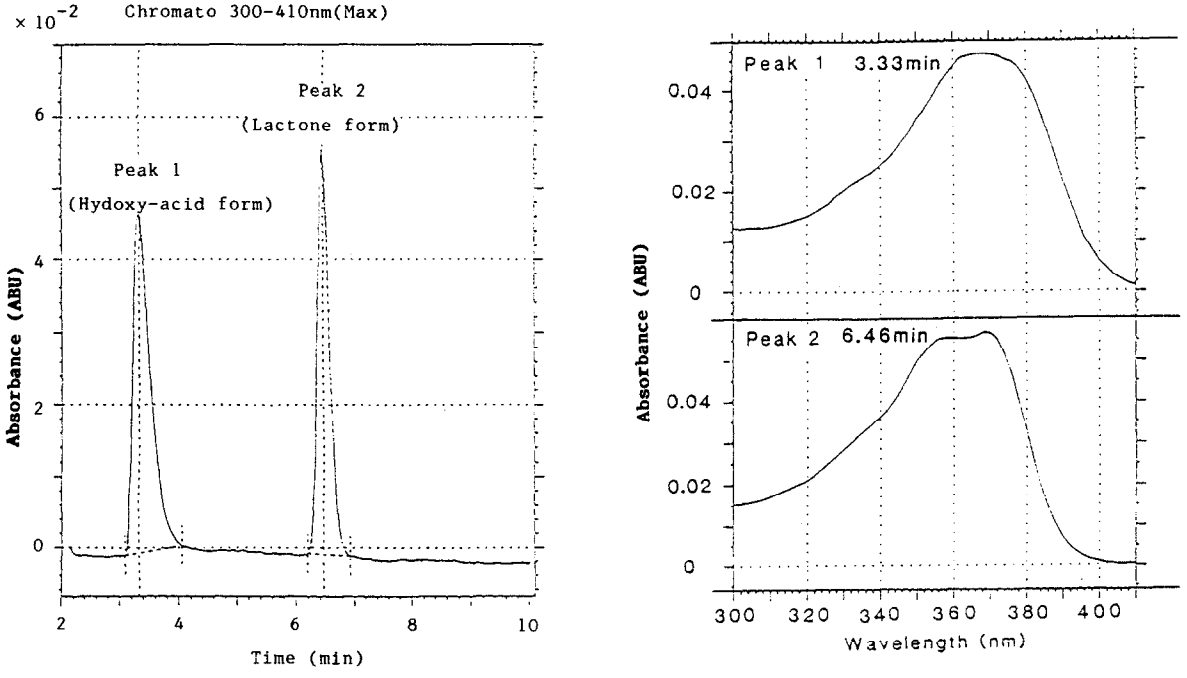

Fig. 2. Chromatogram of lactone and hydroxy acid forms of I and UV–VIS spectra of each peak measured with a multi-channel photodiode-array detector for HPLC. The sample was prepared from a 0.1% aqueous solution of I by dilution 20-fold with pH 7.0 Britton–Robinson buffer, and was stored at 35°C. HPLC was performed 1 h after diluting the sample. HPLC conditions: column, YMC AM-312 (ODS) (150 mm × 6 mm I.D.); temperature, ambient; eluent, methanol–0.1 *M*, pH 4.0 buffer solution containing 3 m*M* sodium 1-heptanesulphonate (50:50); flow-rate, 1.4 ml/min.

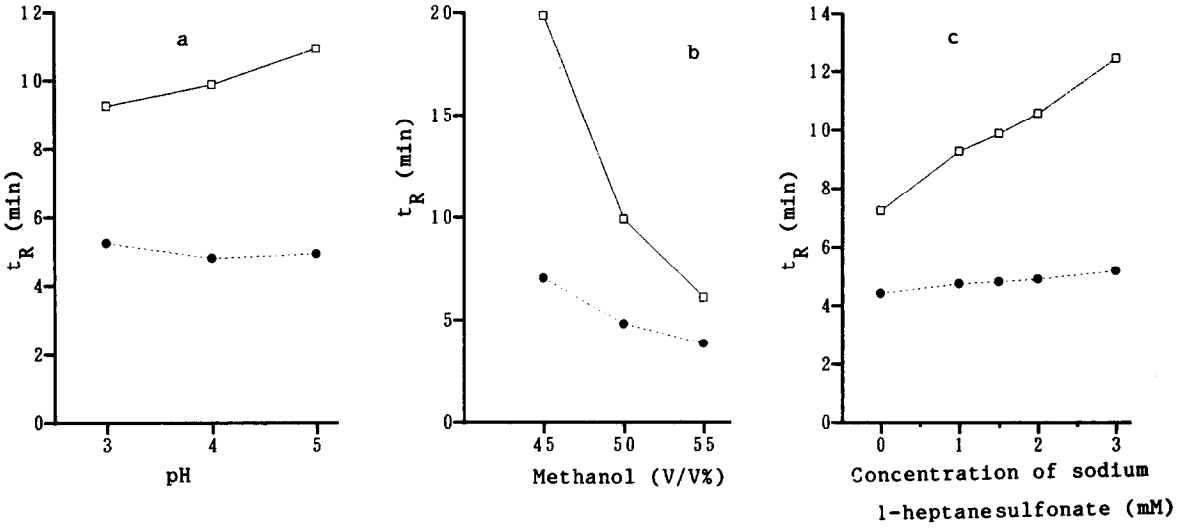

Fig. 3. Effects of (a) the pH of the aqueous phase, (b) the percentage (by volume) of methanol and (c) the concentration of sodium 1-heptanesulphonate in the mobile phase on the retention times of the hydroxy acid and lactone forms of I. HPLC conditions as in Fig. 2, except for the parameters evaluated and flow-rate (1.0 ml/min). □ = Lactone form; ● = hydroxy acid form.

graphs were obtained from standard sample solutions prepared by dissolving camptothecin, I or II in both pH 4.0 and pH 10.0 buffer solutions.

RESULTS AND DISCUSSION

Assignment of elution peaks

A preliminary HPLC separation of the lactone and hydroxy acid forms of I was carried out with a UV photodiode-array detector. Fig. 2 shows chromatograms and the UV spectra of the two elution peaks.

The two peaks eluting at 3.3 and 6.5 min were thought to represent the hydroxy acid and lactone, respectively. In order to confirm this, the UV spectra of the peaks were measured with a photodiode-array detector and were compared with those of the authentic samples. The results confirmed that the retention time of the hydroxy acid form was shorter than that of the lactone form.

Choice of mobile phase

In order to determine the optimum separation conditions, the mobile phase was examined. Fig. 3 shows the effects of pH, percentage (by volume) of methanol and concentration of sodium 1-heptanesulphonate in the mobile phase on the retention time (t_R).

The t_R of the hydroxy acid was slightly affected by these parameters, but that of the lactone was greatly affected by both the percentage of methanol and the concentration of sodium 1-heptanesulphonate. Similar elution patterns were found for camptothecin and II. The optimum HPLC conditions presented under Experimental were determined

TABLE I

MAXIMUM WAVELENGTHS OF EXCITATION AND EMISSION SPECTRA IN THE HPLC ELUENTS OF THE LACTONE AND HYDROXY ACID FORMS OF CAMPTOTHECIN, I AND II

Compound	Form	Maximum wavelength (nm)	
		Excitation	Emission
Camptothecin	Lactone	371	432
	Hydroxy acid	364	450
I	Lactone	372	431
	Hydroxy acid	374	444
II	Lactone	381	556
	Hydroxy acid	385	544

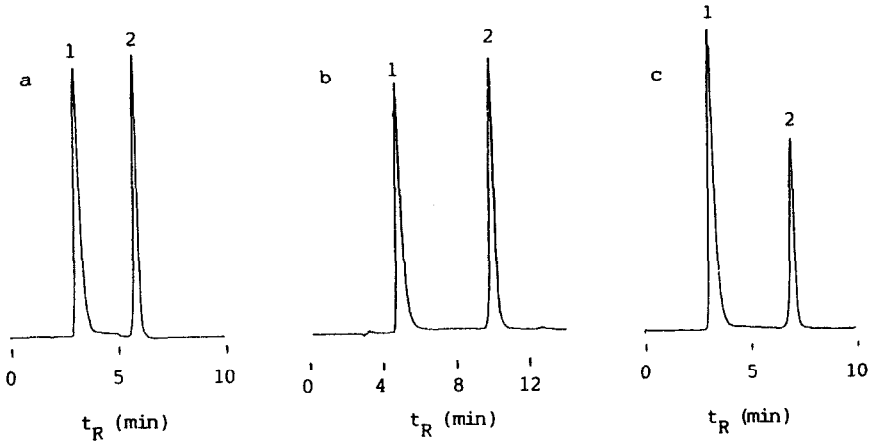

Fig. 4. Typical separation of the lactone and hydroxy acid forms of (a) camptothecin, (b) I and (c) II. For HPLC conditions, see Experimental. 1 = Hydroxy acid form; 2 = lactone form.

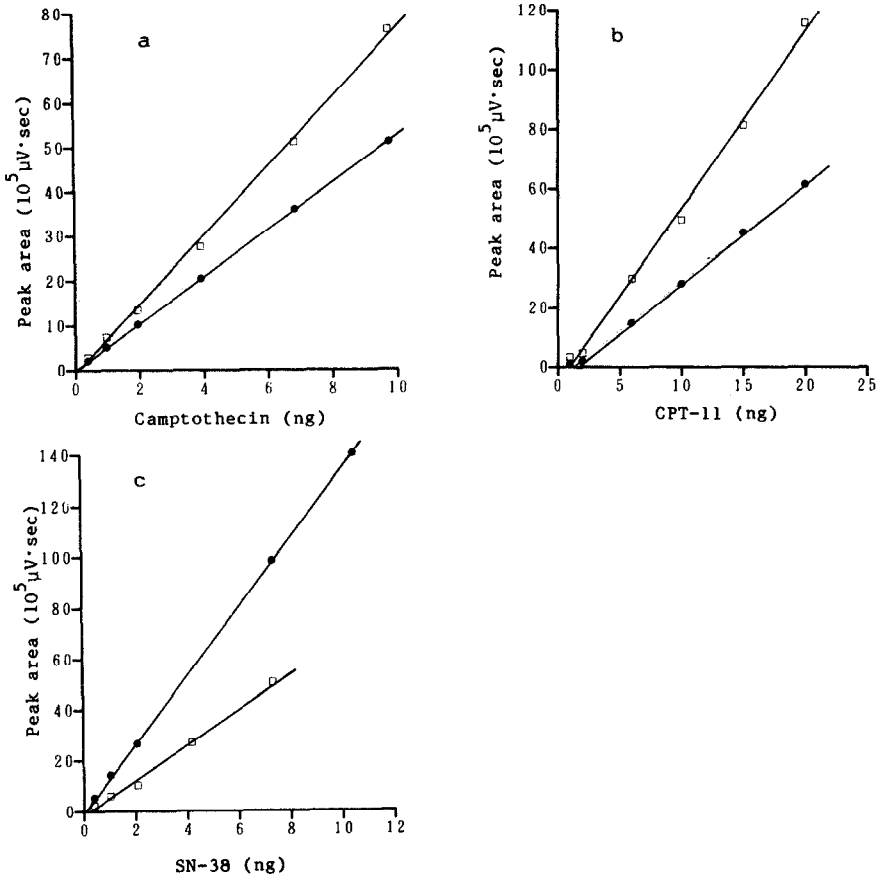

Fig. 5. Calibration graphs for the lactone and hydroxy acid forms of (a) camptothecin, (b) I and (c) II. Each peak area is the mean of two measurements. □ = Lactone form; ● = hydroxy acid form.

TABLE II

LIMIT OF DETECTION, LINEARITY RANGE AND PRECISION FOR THE LACTONE AND HYDROXY ACID FORMS OF CAMPTOTHECIN, I AND II

Compound	Form	Charged amount (ng)		Relative standard deviation (%)[a]	
		Limit of detection	Linearity range	Intra-assay	Inter-assay
Camptothecin	Lactone	0.05	0.4–10	1.0	1.8
	Hydroxy acid	0.1	0.4–10	0.6	2.0
I	Lactone	0.1	1–20	0.9	1.6
	Hydroxy acid	0.1	1–20	1.0	1.8
II	Lactone	0.1	0.4–7	1.0	2.0
	Hydroxy acid	0.2	0.4–10	0.7	1.7

[a] The intra- and inter-assay relative standard deviations were calculated from ten determinations and determinations on six different days, respectively.

from the analytical viewpoint, that is, from the peak shape, t_R and the separation factor.

Monitoring wavelength

Fluorescence detection was used for highly sensitive analysis. The maximum wavelenghts of the emission spectra and the excitation spectra were different from compound to compound, as shown in Table I.

The monitoring wavelengths for each compound were selected at the maximum emission and excitation wavelengths by programmed controlled switching.

Calibration graphs and precision

Fig. 4 shows typical chromatograms of the lactone and hydroxy acid forms for camptothecin, I and II, and Fig. 5 shows their calibration graphs.

The chromatograms showed sharp, symmetrical peaks for each compound, and the calibration graphs were linear. The results are summarized in Table II. The reproducibility among runs in one day was about 1% or less (relative standard deviation).

Application

The method was employed succesfully for kinetic studies of the hydrolysis and lactonization of camptothecin, I and II in aqueous solutions. Fig. 6 shows an example. The details will be presented in a later paper.

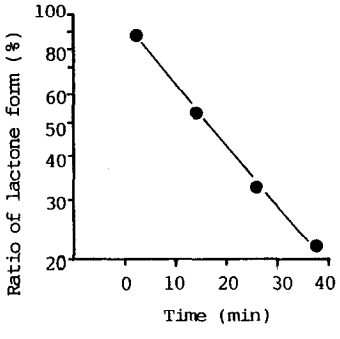

Fig. 6. Progress of the conversion reaction of the lactone form of I to the hydroxy acid form in an aqueous solution of pH 7.4 at 37°C.

REFERENCES

1 M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail and G. A. Sim, *J. Am. Chem. Soc.*, 88 (1966) 3888.
2 S. Sawada, S. Okajima, R. Aiyama, T. Yokokura, K. Yamaguchi and T. Miyasaka, *Chem. Pharm. Bull.*, 39 (1991) 1446.
3 Y. Kawato, M. Aonuma, Y. Hirota, H. Kuga and K. Sato, *Cancer Res.*, 51 (1991) 4187.
4 R. P. Hertzberg, M. J. Caranfa, K. G. Holden, D. R. Jakas, G. Gallagher, M. R. Mattern, S. M. Mong, J. O. Bartus, R. K. Johnson and W. D. Kingsbury, *J. Med. Chem.*, 32 (1989) 715.
5 M. C. Wani, P. E. Ronman, J. T. Lindly and M. E. Wall, *J. Med. Chem.*, 23 (1980) 554.
6 J. Grace and M. C. R. Symons, *J. Chem. Soc.*, (1961) 47.
7 M. L. Bender, H. Matsui, R. J. Thomas and S. W. Tobey, *J. Am. Chem. Soc.*, 83 (1961) 4193.
8 C. Galli, G. Illuminati, L. Mandolini and P. Tamborra, *J. Am. Chem. Soc.*, 99 (1977) 2591.
9 D. R. Storm and D. K. Koshland, Jr., *J. Am. Chem. Soc.*, 94 (1972) 5815.

10 M. C. Caswell and G. L. Schmir, *J. Am. Chem. Soc.*, 102 (1980) 4815.
11 J. H. Beijnen, J. J. M. Holthuis, H. G. Kerkdijk, O. A. G. J. van der Houwen, A. C. A. Paalman, A. Bult and W. J. M. Underberg, *Int. J. Pharm.*, 41 (1988) 169.
12 H. T. S. Britton and R. A. Robinson, *J. Chem. Soc.*, 458 (1931) 1456.