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Short communication

Simultaneous determination of a camptothecin derivative, used as an anticancer drug, and its photodegradation products by high-performance liquid chromatography

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Abstract

A simple and rapid ion-pair high-performance liquid chromatographic method using a polymer-based column bonded octadecyl group was developed for the simultaneous determination of the anticancer drug CPT-11 and its three main photodegradation products. The analytes were detected by ultraviolet absorption at 254 nm.

Keywords: Pharmaceutical analysis; Photodegradation; CPT-11; Camptothecin

1. Introduction

CPT-11 (I) is an anticancer drug produced by Yakult (Tokyo, Japan) [1], and is a derivative of camptothecin, an antitumor alkaloid isolated from *Camptotheca acuminata* [2]. Lown and Chen [3] investigated the interaction between deoxyribonucleic acid and photoactivated camptothecin and identified the chemical structure of the photodegradation product. There is as yet no report concerning the photodegradation pattern of camptothecin and its derivatives.

In a previous paper, we isolated and purified three main photodegradation products of I and determined their chemical structures [4]. We have established a new HPLC method involving the use of an eluent free from methanol, to investigate the photodegradation pattern of I, because one of the photodegradation products is readily reacted with methanol. This paper describes the new HPLC method and an application of the method.

2. Experimental

2.1. Chemicals and reagents

Compound I was obtained from Yakult (Tokyo, Japan), sodium 1-heptanesulfonate for ion-pair chromatography was from Tokyo Kasei Kogyo (Tokyo, Japan). Water was distilled and all other chemicals

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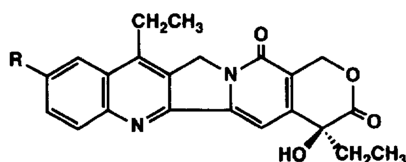
were of reagent grade. The three photodegradation products of I, D-1 (II), D-2 (III) and D-3 (IV), were those isolated in a previous study [4]. The chemical structures of I, II, III and IV are shown in Fig. 1

2.2. HPLC

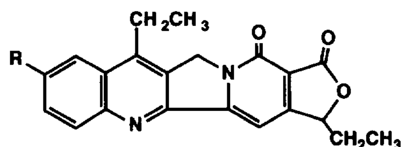
HPLC was performed using a solvent delivery system (Model 655; Hitachi, Tokyo, Japan), an ultraviolet detector (Model 638; Hitachi, Tokyo, Japan) and an auto-sampler (Model AS48; Tosoh, Tokyo, Japan). Data processing of HPLC was carried out with an integrator (Model C-R4A; Shimadzu, Kyoto, Japan).

The HPLC column used was a polyvinyl alcohol polymer-based one bonded with an octadecyl group (5 μm), 15 cm×6 mm I.D., (Asahipak ODP-50; Asahikasei, Tokyo, Japan)[1]. The eluent was a 3:1 (v/v) mixture of a 0.1 *M* K_2HPO_4–H_3PO_4 buffer solution, pH 3.0, containing 4 m*M* sodium 1-heptanesulfonate and acetonitrile.

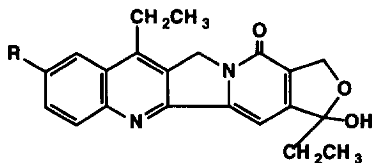
a) CPT-11



b) D-1



c) D-2



d) D-3

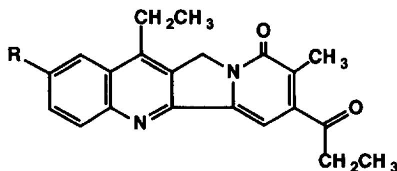


Fig. 1. Chemical structures of CPT-11 (I), D-1 (II), D-2 (III) and D-3 (IV). R=$OCONC_5H_9NC_5H_{10}$.

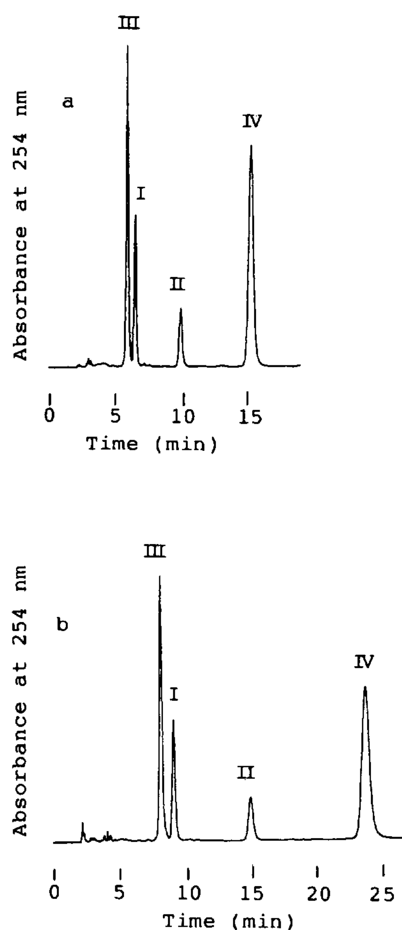


Fig. 2. Typical chromatograms of I, II, III and IV without added eluent (a) or with 4 m*M* sodium 1-heptanesulfonate (b). HPLC conditions are as described in Section 2 except that there is no ion-pair reagent for (a). The sample is as described in Table 1.

2.3. Analytical procedure

Samples were dissolved in the eluent to prepare solutions from about 2 to 90 μg ml^{-1}. The HPLC separation was performed at 30°C, and the flow-rate

[1] The product is currently available from Showa-Denko K.K. (Tokyo, Japan).

Table 1
Separation behavior of I, II, III and IV on several ODS columns

Column	Capacity factor				Resolution between III and I
	III	I	II	IV	
Waters-Bondasphere C_{18} 15 cm×3.9 mm I.D.	4.35	4.74	9.97	17.9	1.02
Shiseido Capcell Pak C_{18} (SG) 15 cm×4.6 mm I.D.	2.14	2.36	4.77	8.89	0.88
Tosho ODS-80TM 15 cm×4.6 mm I.D.	5.14	5.48	10.6	19.0	0.91
Asahi Kasei Asahipak ODP-50 15 cm×6.0 mm I.D.	1.87	2.17	3.84	6.34	1.58
Gaskuro Kogyo Inertsil ODS 15 cm×4.6 mm I.D.	4.59	4.96	10.1	18.1	0.89

HPLC conditions: Eluent, 0.1 *M* phosphate buffer solution, pH 3.0–acetonitrile (3:1, v/v); Flow-rate, 1.0 ml min^{-1}; Detection, 254 nm, 0.16 AUFS.
Sample: The mixture of I (ca. 8.9 μg/ml), II (ca. 6.0 μg/ml), III (25 μg/ml) and IV (33 μg/ml).

was 1.0 ml min^{-1}. The detection wavelength was set at 254 nm and the sample injection volume was 20 μl.

3. Results and discussion

3.1. Choice of mobile phase

Compound III, one of the main photodegradation products of I, easily reacts with methanol [4]. Therefore, the HPLC method presented previously could not be applied to separate I and its photodegradation products II, III and IV. The assessment of HPLC conditions was performed using some eluents, adjusted to pHs of 3.0, 4.0 and 4.5, consisted of phosphate buffer solution–acetonitrile (3:1, v/v). The mixture of I and three main photodegradation products was subjected to HPLC on a YMC prepacked column AM-312 C_{18} (5 μm) 15 cm×6 mm I.D. The same column was previously used for determination of the lactone and hydroxy acid forms of I [5]. Under all pH conditions, III, I, II and IV were eluted in this order, with poor separation between III and I.

3.2. Choice of column

Next, the same separation tests were carried out on several ODS columns using the eluent of buffer solution, pH 3.0–acetonitrile (3:1, v/v), which showed the highest separation on YMC AM-312. The capacity factors and resolutions on these ODS columns are listed in Table 1. Asahipak ODP-50 was

Table 2
Limit of detection, linearity range, precision and calibration equations for I, II, III and IV

Compound	Charged amount (ng)		Precision range	Calibration equation[a] Relative standard deviation (%)[b]
	Limit of detection	Linearity		
I	1.4	125–1879	1.6	$Y=1282X+1302$ (r=0.99993)
II	0.7	44–666	1.3	$Y=1068X+270$ (r=0.99987)
III	2.4	56–840	1.5	$Y=902X-1666$ (r=0.99994)
IV	0.6	64–960	1.4	$Y=1098X+198$ (r=0.99989)

[a] X and Y in each equation represent the charged amount (ng) and peak area (μV s), respectively.
[b] The relative standard deviations were calculated from ten determinations.

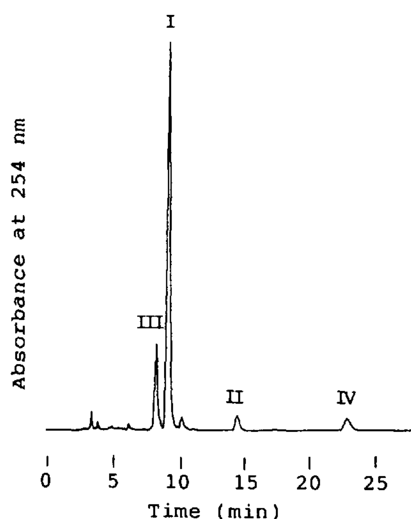


Fig. 3. Chromatogram of the buffer solution (pH 7.0) of I (4.0 mg/ml) after irradiation of 300 000 lx·h with a white fluorescent lamp. HPLC conditions are as described in Section 2. The sample was diluted 50 times with eluent before injection.

found to be the most suitable column for compounds I–IV in this respect.

Fig. 2 shows typical chromatograms obtained

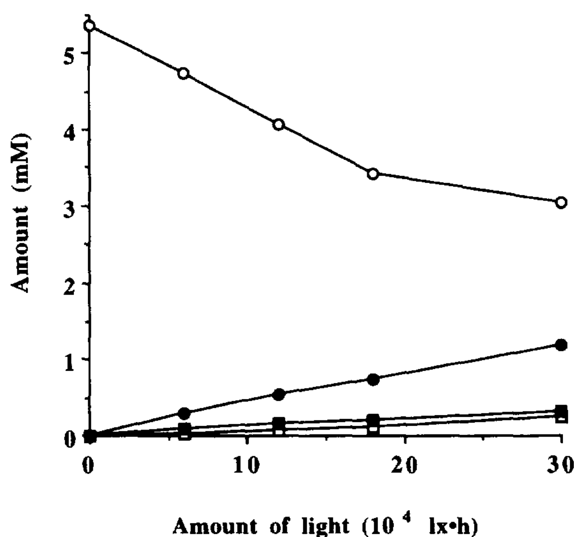


Fig. 4. Time courses of the residual amount of I and the formed amount of photodegradation products II, III and IV in the buffer solution of I (pH 7.0, 4.0 mg/ml) irradiated with a white fluorescent lamp. (○) I, (□) II, (●) III, (■) IV.

with or without ion-pair reagent. By adding 4 m*M* sodium 1-heptanesulfonate to the aqueous buffer solution, the resolution between III and I was improved to 1.78 from 1.58.

The detection wavelength selected was 254 nm (maximum absorption of CPT-11), since its photodegradation products have nearly the same ultraviolet absorption spectra [6]. These results led to the HPLC conditions described in Section 2.

3.3. Calibration graphs and precision

All calibration graphs of I, II, III and IV showed very good linearity (r>0.9998) and passed almost through the point of origin. The results are summarized in Table 2. The relative standard deviation values at 44–125 ng injected were ca. 1.5% (n=10) for all analytes.

3.4. Application

This HPLC method was employed successfully for kinetic studies of the photodegradation of I in aqueous solutions. Fig. 3 shows the chromatogram of the buffer solution (pH 7.0) of I after irradiation of 300 000 lx·h with a white fluorescent lamp and Fig. 4 shows the time courses of all analytes that were obtained under the above experimental conditions. The details will be presented in a later paper [7].

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