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Short communication

Development of a sensitive liquid chromatography–electrospray ionization tandem mass spectrometry method for the measurement of 7-cyanoquinocarcinol in human plasma

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Abstract

A sensitive method for the determination of an anti-cancer agent, DX-52-1 (7-cyanoquinocarcinol, I) and quinocarmycin (II) which is formed from I either by metabolism or degradation, in human plasma has been developed utilising liquid chromatography electrospray–ionization tandem mass spectrometry (LC–ESI-MS–MS). The procedure involves solid-phase extraction at pH 2 and low temperature (4–6°C) to prevent the decomposition of I to II, the separation by reversed-phase HPLC and the multiple reaction monitoring (MRM) by ESI-MS–MS. The mean precision and accuracy at the lower limit of quantitation (LLOQ) of I, 0.25 ng ml^{-1}, were 8.7% and −10.8%, respectively. Since an interfering peak eluting slightly earlier than II was observed on the HPLC of blank plasma, the LLOQ of II was set at 5 ng ml^{-1} where the mean precision and accuracy were 15.6% and −9.8%. The results suggested that the method is useful for the simultaneous monitoring of Iand II in the clinical trials of I. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: 7-Cyanoquinocarcinol

1. Introduction

DX-52-1 (7-cyanoquinocarcinol, I, Fig. 1) is an analogue of quinocarmycin (II, Fig. 1) [1,2], newly synthesised in Kyowa Hakko Kogyo (Tokyo, Japan) [3,4]. It demonstrated specific activity for melanomas in the National Cancer Institute (Bethesda, MD, U.S.A) in vitro human tumour cell line screen and is now being developed to clinical trials [5].

Since I is gradually degraded to II in aqueous solutions (unpublished data) an analytical method which provides simultaneous determination of I and its active metabolite (degradation product), II, is needed for pharmacokinetic analysis of I in clinical trials. Owing to their low UV extinction coefficients, an HPLC–UV method did not provide sufficient sensitivity.

Recently electrospray liquid chromatography–tandem mass spectrometry (LC–ESI-MS–MS) has come to be used widely in the determination of drugs in biological fluids because of its high sensitivity and selectivity as well as its applicability to compounds

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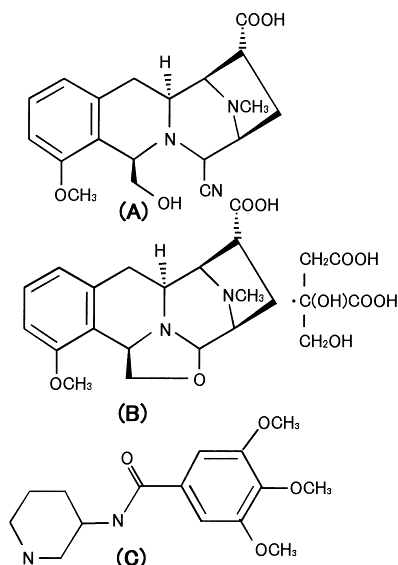

Fig. 1. Structures of (A) DX- 52-1 (I, MW=358) (B) quinocarmycin (II, MW=331) and (C) (±)-3,4,5-trimethoxy-*N*-3-pyperidinyl benzamide (internal standard, I.S. MW=295).

with low UV absorption [6–8]. Thus we tried to apply LC–ESI-MS–MS to the analysis of I and II and established a method applicable to their simultaneous determination in human plasma, which is described in the present report.

2. Experimental

2.1. Chemicals

Compounds I, II and (±)-3,4,5-trimethoxy-*N*-3-pyperidinyl benzamide (internal standard, I.S., Fig. 1) were synthesised in Kyowa Hakko Kogyo (Tokyo, Japan). HPLC-grade acetonitrile was purchased from Kanto Kagaku (Tokyo, Japan). Purified water was produced using a Milli-Q SP apparatus from Millipore (Bedford, MA, USA). Disposable solid-phase extraction cartridges (Sep-Pak® C_{18}; Classic Cartridge, Short Body, 0.85 ml/filled cartridge) was also purchased from Millipore. Other chemicals and solvents were of reagent grade commercially available.

2.2. Stock solutions

Compounds I and II were dissolved in HPLC-grade acetonitrile and Milli-Q water respectively at a concentration of 1 $mg\,ml^{-1}$ and stored at −70°C. The I.S. was dissolved in methanol at a concentration of 0.5 $mg\,ml^{-1}$ and stored at −70°C. The I.S. working solution was prepared by diluting it with 500 m*M* formic acid to 0.2 $\mu g\,ml^{-1}$.

2.3. Sample preparation

Human blood was obtained from healthy male volunteers. About 50 ml of blood was sampled into a heparinized injection cylinder and centrifuged (2000 *g*, 4°C, 15 min) to obtain plasma. The plasma was stored at −20°C until analysis. The calibration samples of I were prepared by diluting the stock solution with a 20-fold volume of 10 m*M* ammonium formate (pH 3.0) and subsequently diluted with human blank plasma to concentrations of 0.25–50 $ng\,ml^{-1}$. The caliblation samples of II was prepared by diluting the stock solution with a 25-fold volume of 10 m*M* ammonium formate (pH 3.0) and subsequently diluted with human blank plasma to concentrations of 5–100 $ng\,ml^{-1}$.

2.4. Sample work-up

Plasma or calibration sample (500 μl each) was mixed with 1.5 ml of 500 m*M* formic acid and 25 μl of the I.S. working solution, and was passed under gravity through a Sep-Pak® C_{18} cartridge conditioned previously with 5 ml of acetonitrile, 5 ml of milli-Q water and 5 ml of 500 m*M* formic acid successively. The cartridge was then washed with 5 ml of 500 m*M* formic acid, flushed with air and eluted with 3 ml 10 m*M* ammonium formate (pH 3.0)–acetonitrile (8:2, v/v). The eluent was filtered through a membrane (UFC40GV25, Millipore) under centrifugation (2000 *g*, 5 min, two times). The filtrate was freeze-dried (FLEXI-DRY™, FTS® Systems Inc, Stone Ridge, NY, USA) overnight at −70°C and 13.3 Pa, reconstituted in 100 μl of acetonitrile and freeze-dried again under the same conditions to increase the recovery. The residue was

reconstituted in 50 μl of 10 m*M* ammonium formate (pH 3.0) and an aliquot (20 μl) was injected into the LC–ESI-MS–MS. Since conversion of I to II is suppressed by lowering the temperature (particularly below 10°C, unpublished data) the preparation was performed in a refrigerated room (4–6°C) or in an ice bath.

2.5. LC–ESI-MS–MS analysis

LC–ESI-MS–MS analysis was performed with a Micromass (Altrincham, Chesire, UK) Quattro mass spectrometer equipped with an electrospray interface and a MassLynx data system. The Quattro was coupled to a Gulliver HPLC system (Jasco, Hachioji, Tokyo, Japan) equipped with two PU 980 pumps and a Model 7125 injector (Rheodyne, Cotati, CA, USA). The HPLC columns used were, Develosil ODS HG-5 (5 μm, 150×2.0 mm) and the Develosil ODS HG-5 guard column (5 μm, 150×2.0 mm) purchased from Nomura Kagaku (Seto, Aichi, Japan). The mobile phase used was a mixture of 10 m*M* ammonium formate (pH 3.0) and acetonitrile at a ratio of 75:25 (v/v). The flow-rate was 0.2 $ml\ min^{-1}$ and all the effluent was directed to the mass spectrometer. Nitrogen was used as the nebulizing gas (25 $l\ h^{-1}$). The capillary (spray) voltage was 3.1 kV. The ion source temperature was maintained at 150°C. The cone voltage was set at 20 V and the skimmer lens offset was 5 V. For quantitative analysis multiple reaction monitoring (MRM) was used, monitoring the following transitions: m/z 358→331 for I; m/z 331→259 for II; m/z 295→195 for I.S. The Q1 and Q3 resolutions were both adjusted to 150 at m/z 358. The I.S. was selected from various synthetic compounds for its appropriate retention time and molecular mass in the LC–ESI-MS–MS analysis, because no suitable analogue of DX-52-1 or quinocarmycin was available. Linearity of peak area ratios (y) of I and II to I.S were assessed by means of a weighted least square fit of $1/y^2$ within 0.25–50 $ng\ ml^{-1}$ and 5–100 $ng\ ml^{-1}$ respectively. The precision and accuracy were determined by the replicate analyses ($n=5$) of plasma samples spiked with I at concentrations of 0.25, 5 and 40 $ng\ ml^{-1}$, and II at concentrations of 5, 20 and 80 $ng\ ml^{-1}$.

3. Results and discussion

The positive product ion mass spectra of the molecular ions of I (m/z 358), II (m/z 331) and the I.S. (m/z 295) shown in Fig. 2 gave the most intense fragment ions at m/z 331, 259 and 195, respectively. Thus the parent→product ion combinations of m/z of 358→331, 331→259 and 295→195 were selected for monitoring I, II and the I.S. respectively.

Simple protein precipitation pretreatment using acetonitrile or trichloroacetate did not give sufficient recovery of I added in human blank plasma (less than 50%). Therefore use of solid-phase extraction using an octadecylsilane coated cartridge was evaluated. Extraction at pH 2.0 was optimal to reduce the degradation of I to II. Nitrogen blowing of the eluent caused the degradation of I. Thus, the sample was concentrated by freeze drying. The freeze-dried sample was reconstituted with the mobile phase of the LC–ESI-MS–MS and passed through a filter to avoid the increase of column pressure observed when injected directly into the apparatus. Adsorption of the compounds to the filter used was found to be negligible. Fig. 3 shows the LC–ESI-MS–MS chromatograms of blank human plasma and those spiked with I, II and the I.S. In the chromatogram of the blank plasma no interfering peak was observed at the retention time of I (c.a. 3.2min). Good linearity was observed over the concentration range 0.25–50 $ng\ ml^{-1}$ of I; $y=0.02399x+0.00125$ ($r=0.99445$). The recovery of I at 5 $ng\ ml^{-1}$ was about 80%. In all the chromatograms of blank plasma samples (from five volunteers) monitoring the product ion of II (m/z 331→259), a contaminant peak eluting just before the retention time of II (c.a. 2.5 min) was observed. The peak area was not variable among the plasma of the five volunteers and was approximately equivalent to that of 0.5 $ng\ ml^{-1}$ of II in blank plasma. Thus the tentative lower limit of quantitation (LLOQ) was set at 5 $ng\ ml^{-1}$ which is ten times as high as the equivalent concentration of the contaminating peak. Good linearity was also obtained over the concentration range 5–50 $ng\ ml^{-1}$ of II; $y=0.01097x+0.02329$ ($r=0.99856$). The recovery of II at 10 $ng\ ml^{-1}$ was about 50%. Intra-day precision and accuracy data are summarised in Table 1. The coefficient of variation (C.V.) for each concentration

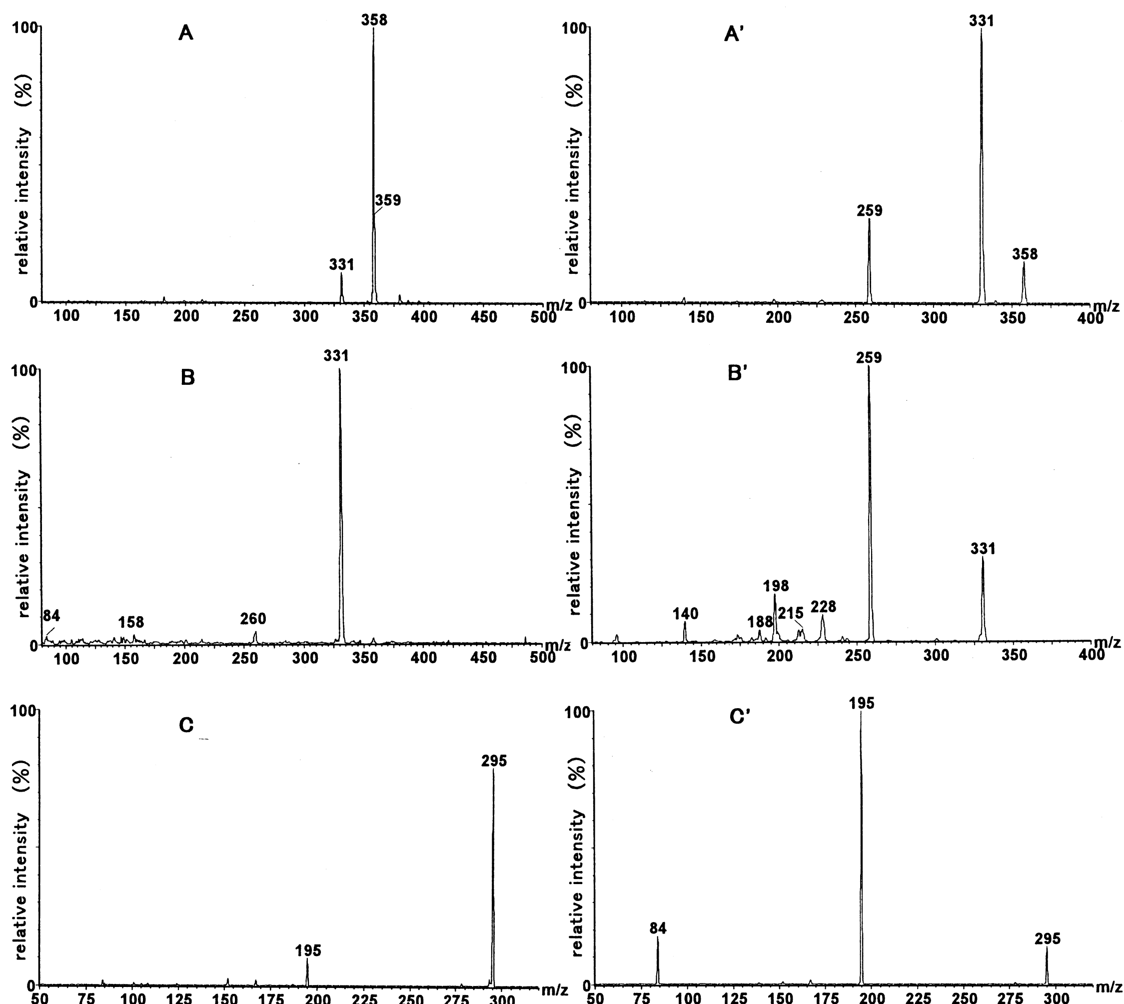

Fig. 2. Q1 spectra of (A) I, (B) II and (C) I.S and product ion spectra of (A′) I, (B′) II and (C′) I.S.

of DX-52-1 was less than 20% except that at 5 ng ml^{-1} (20.5%). The C.V. at the LLOQ was 8.7%. The accuracy [expressed as mean (observed concentration−added concentration)/added concentration] for each concentration of I was within 11%. The C.V. and the accuracy for each concentration of II was less than 16% and within 11% respectively. These values suggested that the method is applicable to the simultaneous monitoring of the plasma concentrations of I and II in the clinical trials [the plasma concentration of I at a tentatively expected starting dose (infusion at 4 mg m^{-2} $(6\ h)^{-1}$) to human is estimated to be approximately 50 ng ml^{-1} during the infusion and 1 ng ml^{-1} after four times the half life from the termination of the infusion].

LC–ESI-MS–MS was confirmed to be a very powerful technique for the determination of compounds with low UV absorption such as I. In addition, its high selectivity allows the simultaneous determination of I and II which is an advantage since it is expected that both compounds will be present in plasma, II being formed from I by metabolism and/

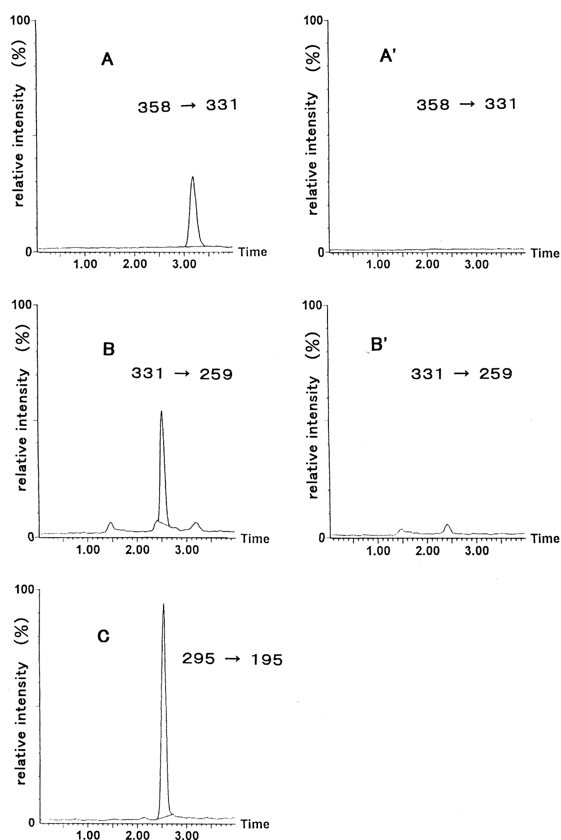

Fig. 3. Typical MRM chromatograms of human plasma spiked with (A) I, (B) II and (C) I.S. and (A′,B′) those of blank plasma.

or degradation. The very short run time for each assay, less than 5 min provides very high throughput essential for the analysis of clinical samples.

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Table 1
Intra-day assay precision and accuracy for the LC–ESI-MS–MS determination of I and II in human plasma

Compound	Added concentration (ng ml^{-1})	Observed concentration (mean±S.D., n=5, ng ml^{-1})	C.V. (%)	Accuracy (R.E.%)
I	0.25[a]	0.223±0.0195	8.7	−10.8
	5	5.21±1.07	20.5	4.2
	40	42.8±2.30	5.4	7.0
II	5[a]	4.51±0.704	15.6	−9.8
	20	22.8±1.16	5.1	14.0
	80	92.4±3.47	3.8	15.5

[a] Concentration of the lower limit of quantitation (LLOQ).
C.V.: Coefficient of variation.
Accuracy: (observed concentration−added concentration)/added concentration.