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Short communication

Simultaneous high-performance liquid chromatographic determination of carboplatin, epirubicin hydrochloride and mitomycin C in a Lipiodol emulsion

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Abstract

A novel HPLC method for the simultaneous determination of carboplatin (CBDCA), epirubicin hydrochloride (EPI) and mitomycin C (MMC) was developed in order to determine these drugs in a Lipiodol emulsion for intra-arterial injection and in the medium of a drug-release test of the emulsion. The HPLC conditions included a reversed-phase column and a gradient programme of the mobile phase using 0.01 *M* phosphate buffer (pH 3.0)–acetonitrile. The accuracies of analysis for each drug added to the emulsion and the drug-release medium were in the ranges 96–104% and 98–108%, respectively. The R.S.D.s in the repeatability of the determination of each drug in the emulsion and in the drug-release medium were <8% and <2%, respectively. The method is simple and reliable enough to be utilized for the investigation of drug contents and drug release of the Lipiodol emulsion containing CBDCA, EPI and MMC.

Keywords: Lipiodol emulsion; Carboplatin; Epirubicin hydrochloride; Mitomycin C

1. Introduction

The lipid lymphographic agent Lipiodol Ultra-Fluid (LPD) has selective distribution and retention in liver tumours [1–3]. Therefore, LPD has been used as a carrier of anticancer agents for the chemotherapy of liver tumours. For this chemotherapy, lipid preparations containing one or two anticancer agents have commonly been used clinically [4–7]. We prepared an LPD emulsion for intra-arterial injection composed of carboplatin (CBDCA), epirubicin hydrochloride (EPI) and mitomycin C (MMC). Before clinical application of the emulsion, the chemical stability of these drugs in the emulsion and the drug-release profile from the emulsion should be examined. For this purpose, it is desirable to determine CBDCA, EPI and MMC simply and simultaneously. However, the investigation of the simultaneous determination of these agents has not been reported.

The methods commonly used for the determination of CBDCA are normal- or reversed-phase HPLC systems with a number of detection techniques including UV detection [8–12]. Reversed-phase HPLC systems have frequently been used

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for the assay of EPI [13,14] and MMC [15,16]. Therefore, for the simultaneous determination of CBDCA, EPI and MMC, we attempted to use an HPLC method with a reversed-phase column and UV detection. It was difficult to retain highly polar CBDCA on reversed-phase columns such as the octadecylsilanol type with a high proportion of organic component in the mobile phase. On the other hand, it was necessary to use such a higher organic content in the mobile phase in order to elute EPI and MMC rapidly under the reversed-phase conditions. Therefore, we attempted to employ a gradient programme using a mixture of phosphate buffer and acetonitrile as the mobile phase.

This paper describes the simultaneous HPLC determination of CBDCA, EPI and MMC in an LPD emulsion and in the medium of a drug-release test of the emulsion.

2. Experimental

2.1. Chemicals

Authentic CBDCA, EPI and MMC were kindly supplied by Bristol-Myers Squibb (Tokyo, Japan), Pharmacia (Tokyo, Japan) and Kyowa Hakko Kogyo (Tokyo, Japan), respectively. LPD, an ethyl ester of the fatty acid of poppyseed oil, was purchased from Kodama (Tokyo, Japan). Iomeprol (IOM; Iomeron), an aqueous contrast medium, was kindly supplied by Eizai (Tokyo, Japan). Polyoxyethylene sorbitan monooleate (Tween 80) was of chemical reagent grade and acetonitrile and methanol were of liquid chromatographic grade.

2.2. Preparation of emulsion

We prepared an anticancer agents–LPD emulsion using injection preparations of CBDCA (Paraplatin, 150 mg/vial; Bristol-Myers Squibb), EPI (Farmorubicin, 10 mg/vial; Pharmacia) and MMC (Mitomycin Kyowa S, 2 mg/vial; Kyowa Hakko Kogyo). First, 150 mg of CBDCA were suspended into 4.5 ml of LPD with an ultrasonicator (Sonifier; Branson, CT, USA); 40 mg of EPI and 8 mg of MMC were dissolved in 1.5 ml of IOM; these solutions were then mixed by manual pumping with syringes for homogeneous emulsification.

2.3. Analytical procedure

The drug concentrations in the emulsion and in the drug-release medium were determined by the following procedures. For analysis of drugs in the emulsion, to a portion (0.5 ml) of the emulsion, 10 ml of methanol containing 0.4 mg/ml of hydroquinone (as an internal standard, I.S.) and 40 ml of methanol containing 0.1% Tween 80 were mixed and shaken so far as to be dissolved clearly. An aliquot (10 μl) of the mixture was directly applied onto HPLC system.

For analysis of drugs in the drug-release medium, to a portion (1 ml) of the drug-release medium, 0.2 ml of methanol containing 0.1 mg/ml of hydroquinone (I.S.) was added. An aliquot (50 μl) of the mixture was directly applied onto HPLC system.

2.4. Apparatus and HPLC conditions

The HPLC system was composed of a Model 600E system controller (Waters, Milford, MA, USA) equipped with a WISP Model 712 sample processor (Waters) and a Model 490 programmable multi-wavelength detector (Waters) operating at a wavelength of 220 nm. A reversed-phase Cosmosil 10 C_{18} column (25 cm $\times$ 4.6 mm I.D., 10 μm) (Nacalai Tesque, Kyoto, Japan) was used. The mobile phase consisted of a linear gradient programme with a mixture of 0.01 *M* phosphate buffer and acetonitrile: from 0 to 1 min, 2% acetonitrile (constant); from 1 to 6.5 min, gradient from 2 to 45% acetonitrile; from 6.5 to 8.5 min, 45% acetonitrile (constant); and from 8.5 to 9.5 min, gradient from 45 to 2% acetonitrile. The flow-rate was 2.0 ml/min, and separation was performed at 20 $\pm$ 3°C. The chromatographic data were calculated using a Maxima 820 data module (Waters).

2.5. Effect of mobile phase on the determination of EPI

The chromatographic profiles of EPI were compared under three mobile phase conditions. EPI was chromatographed with the gradient programme described above using mobile phases of distilled water–acetonitrile, 0.01 *M* phosphate buffer (pH 6.0)–acetonitrile and 0.01 *M* phosphate buffer (pH 3.0)–acetonitrile.

2.6. Samples for calibration

The samples for calibration for the determination of CBDCA, EPI and MMC in the emulsion and in the drug-release medium were prepared by dissolving each authentic drug in methanol and in distilled water.

2.7. Preliminary studies on chemical stability and drug release of the emulsion

Periodically, an aliquot of the emulsion stored at 20 ± 3°C was treated according to the analytical procedure and the residual content of each drug compared with the initial level was determined. The drug release from the emulsion was investigated by the rotating dialysis cell method [17], the cell containing the emulsion and an equivalent volume of isotonic saline solution being agitated in isotonic saline solution at 37°C.

3. Results and discussion

The effect of the mobile phase on the determination of EPI was investigated. Distilled water, and 0.01 *M* phosphate buffers of pH 6.0 and 3.0 as aqueous components in the mobile phase were examined. EPI gave different chromatograms with the three mobile phases conditions as shown in Fig. 1. EPI exhibited a very sharp peak using the buffer of pH 3.0 in the mobile phase, but did not appear when using distilled water and exhibited a broad peak when using the buffer of pH 6.0. It is well known that a basic compound is likely to be adsorbed by the residual silanol groups of the gel and is likely to show a broad peak. In this study, EPI, a basic drug, could have interacted with the residual silanol groups; EPI might be adsorbed on the gel support. CBDCA and MMC were affected only slightly on changing the pH of the mobile phase, because CBDCA is a neutral compound and MMC has an acidic quinone skeleton with amino groups ($pK_a = 3.2$). Typical chromatograms of CBDCA, EPI and MMC in the emulsion and in the drug-release medium obtained using the buffer of pH 3.0 in the mobile phase are shown in Fig. 2. There was no interference in their blank. Mobile phase conditions with a linear gradient programme using 0.01 *M* phosphate buffer (pH 3.0)–acetonitrile achieved a satisfactory separation of CBDCA, I.S., IOM, MMC and EPI, and allowed CBDCA to be retained and EPI to be eluted at about 11 min with a flow-rate of 2.0 ml/min. However, an additional period (6–8 min) was required until the next analysis because of the need to equilibrate to the initial condition with a lower acetonitrile content.

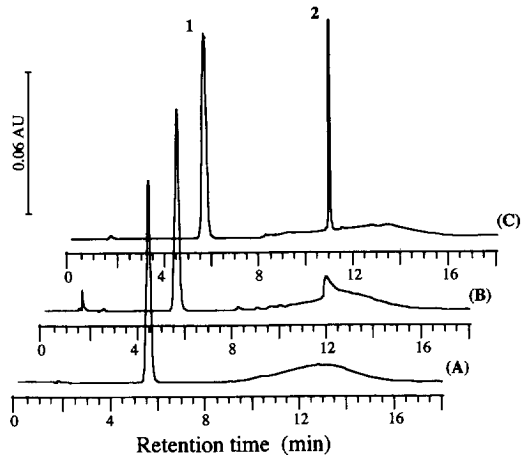

Fig. 1. Chromatograms obtained with three different conditions of the aqueous component of the mobile phase. (A) Distilled water; (B) pH 6.0; (C) pH 3.0. Peaks: 1 = I.S. (hydroquinone); 2 = EPI. Other HPLC conditions as described under Experimental.

Tables 1 and 2 show the analytical characteristics of the method. The calibration graph for EPI had a higher quantification limit and showed a narrow range of linearity because of non-linearity at lower concentrations (Table 2). This phe-

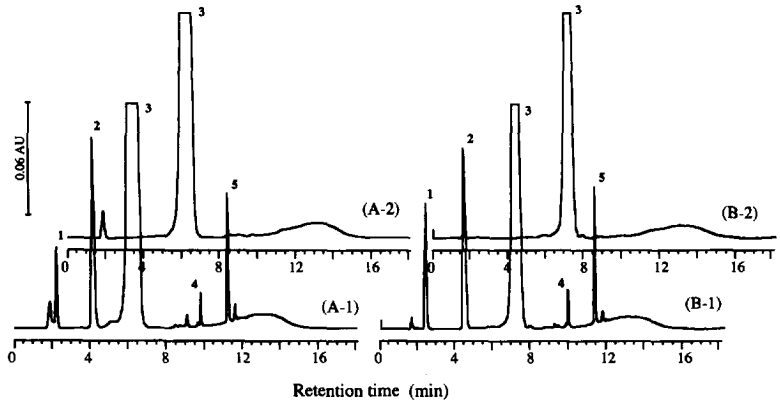

Fig. 2. Typical chromatograms obtained using a 0.01 *M* phosphate buffer (pH 3.0)–acetonitrile gradient programme. (A-1) and (A-2) emulsion and its blank; (B-1) and (B-2) drug-release medium and its blank. Peaks: 1 = CBDCA; 2 = I.S. (hydroquinone); 3 = IOM; 4 = MMC; 5 = EPI. Other HPLC conditions as described under Experimental.

Table 1
Analytical characteristics of the method in application to the emulsion

Drug	Detection limit (mg/ml)	Linearity (*r*)	Linear range (mg/ml)	Accuracy[a]		Repeatability[b]	
				Added (mg/ml)	Measured (%)[c]	Concentration (mg/ml)	R.S.D. (%)
CBDCA	0.2	0.9995	1.2–38	2.4	104	9.3	7.4
				24	100	18.7	4.8
EPI	0.1	0.9994	0.3– 8.9	0.7	101	3.1	6.8
				6.5	102	6.1	4.6
MMC	0.02	0.9995	0.1– 2.4	0.1	102	0.3	6.9
				1.3	96	0.7	4.4

[a] Five replicates.
[b] Six replicates.
[c] (Measured amounts/added amounts) × 100.

Table 2
Analytical characteristics of the method in application to the drug-release medium

Drug	Detection limit (μg/ml)	Linearity (*r*)	Linear range (μg/ml)	Accuracy[a]		Repeatability[a]	
				Added (μg/ml)	Measured (%)[b]	Concentration (μg/ml)	R.S.D. (%)
CBDCA	0.5	0.9996	6–125	6	104	26.4	0.6
				60	103	53.4	1.9
EPI	1.0	0.9986	6–29	16.3	108	8.5	1.0
						22.5	1.7
MMC	0.1	0.9994	0.3–8	0.3	98	0.9	1.1
				3.4	102	2.1	1.5

[a] Six replicates.
[b] (Measured amounts/added amounts) × 100.

nomenon might result from adsorption of EPI on the gel support at lower concentrations as described above. The accuracies of analysis for each drug added to the emulsion and the drug-release medium were in the ranges 96–104% and 98–108%, respectively. The repeatability of analysis for each drug in the emulsion and in the drug-release medium was <8% and <2% (R.S.D.), respectively. Both the accuracy and repeatability are considered satisfactory.

The method was then utilized in preliminary studies on chemical stability and drug release of the emulsion. From the results of the stability test, EPI in the emulsion retained more than 95% of the initial content after 72 h, whereas the contents of CBDCA and MMC decreased below 90%. From the results of the drug-release test, about 80% of CBDCA and EPI were released from the emulsion in 300 min. However, the release of MMC was about 50% in 300 min. These results indicate differences in the chemical stabilities and release profiles of the three drugs in the emulsion.

4. Conclusions

An HPLC method for the simultaneous determination of CBDCA, EPI and MMC was developed in order to determine these drugs in a Lipiodol emulsion and in the medium of a drug-release test of the emulsion. The results indicated differences in the chemical stabilities and release profiles of the three drugs. The proposed method is simple and reliable enough to be utilized for the investigation of drug contents and drug release in a Lipiodol emulsion.

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