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Short communication

Sensitive high-performance liquid chromatographic determination of chlorpheniramine in human serum using column switching

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Abstract

A sensitive method for the determination of chlorpheniramine in human serum was developed using column-switching high-performance liquid chromatography (HPLC) with ultraviolet detection at 210 nm. The analyte was extracted with diethyl ether from alkalinized serum. After evaporation of the organic layer, the reconstituted residue was analyzed by HPLC using a heart-cut technique. Good recoveries of the analyte from spiked human serum samples were obtained with a coefficient of variation below 7%. A good linear response was obtained for the concentration range 0.5–50 ng/ml, with a correlation coefficient higher than 0.999. The lower limit of quantitation for chlorpheniramine in human serum was 0.5 ng/ml. The method was satisfactorily applied to the determination of chlorpheniramine in human serum after oral administration of chlorpheniramine maleate.

1. Introduction

Chlorpheniramine (CP) maleate {2-[*p*-chloro-α-(2-dimethylaminoethyl)benzyl]pyridine maleate}, a well-known commercially available antihistaminic drug, has been widely used for symptomatic relief of common cold and allergic diseases. A previous pharmacokinetic study revealed that a low concentration of CP was found in human serum after oral administration [1]. For the development of a new pharmaceutical formulation, e.g. a sustained-release dosage form, it is essential to perform pharmacokinetic studies. For that purpose, a simple and highly sensitive method is necessary for the determination of CP in human serum after oral administration of CP maleate. Various methods for the determination of CP in human serum have been developed including gas chromatography (GC) [2–6], GC–mass spectrometry (MS) [7,8], high-performance liquid chromatography (HPLC) [9–13] and radioimmunoassay (RIA) [14]. GC and GC–MS need a large amount of serum sample (more than 2 ml) [2–8], and some of them lack sensitivity [2–4]. Further, GC–MS is not always suitable for routine analysis. CP shows a UV absorption maximum at 263 nm but much stronger absorption in the short-wavelength region (< 220 nm). HPLC with UV detection near 260 nm [9,12] requires a large amount of serum sample (1 or 2

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ml) and a tedious sample preparation procedure. Moreover the sensitivity was still insufficient for the present purpose. Shi et al. [11] reported a high sensitivity with UV absorption at 200 nm with a special combination of a silica-gel column and an aqueous mobile phase. HPLC with fluorescence detection offered high sensitivity, but required a laborious derivatization step prior to analysis [10]. Although RIA was highly sensitive, it required the preparation of antisera and showed some cross-reactivity with metabolites of CP [14].

Previously we described that a column-switching technique combined with ion-pair chromatography offered high selectivity and sensitivity for the determination of primary [15], secondary [16] or tertiary [17–20] amines, the elution of which was delayed by ion-pair formation on the first column, even with poorly selective short-wavelength UV detection. The aim of this study was to develop an HPLC method with a simple clean-up procedure for the determination of CP in human serum using this column-switching technique. The present method was applied to the determination of CP in human serum samples after oral administration of CP maleate.

2. Experimental

2.1. Reagents and materials

CP maleate was of biochemical reagent grade (Wako, Osaka, Japan). Acetonitrile and methanol were of HPLC grade (Wako). Diethyl ether was of analytical reagent grade (Wako). Sodium 1-octanesulphonate (SOS) was of ion-pair reagent grade (Tokyo Kasei, Tokyo, Japan). All other reagents were of reagent grade and used without further purification.

2.2. Instrumentation and conditions

The column-switching HPLC system consisted of two LC-10AS pumps, an SIL-10A autosampler with cooling system, an SCL-10A controller, a CTO-10AC column oven, two SPD-10A UV detectors, an FCV-12AH six-port switching valve, a C-R4AX integrator (all from Shimadzu, Kyoto, Japan) and a U-228 dual-pen recorder (Nippon Denshi Kagaku, Kyoto, Japan). Both columns (C1 and C2) used were Inertsil ODS-2 (5 μm particle size, 150 × 4.6 mm I.D.; GL Science, Tokyo, Japan). The mobile phase for C1 (MP 1) was 20 m*M* potassium dihydrogenphosphate–acetonitrile (7:3, v/v) containing 5 m*M* SOS. The mobile phase for C2 (MP 2) was 20 m*M* potassium dihydrogenphosphate–acetonitrile (7:3, v/v). The column temperature and the flow-rate were 40°C and 1 ml/min, respectively. The sample cooling system was set at 4°C. UV detection was carried out at 210 nm.

2.3. Analytical system and procedure

The analytical system and the procedure using column switching (heart-cut technique) were almost the same as those reported previously [15–20]. In brief, the retention time of CP on C1 was checked each day prior to analysis to determine the time program for the column switching. The injected sample was first separated on C1 with MP1 and the eluate fraction containing the analyte was transferred to C2 by valve operation. The eluate introduced on C2 was further separated with MP2 followed by UV detection.

2.4. Serum sample pretreatment

To 500 μl of human serum in a disposable glass culture tube (16 × 100 mm) were added 100 μl of 0.2 *M* NaOH. The mixture was extracted twice with 2 ml of diethyl ether by vortex-mixing for 30 s followed by centrifugation for 5 min at 2000 *g*. The aqueous layer was frozen in a dry ice–acetone bath. The organic layer was transferred to another tube and evaporated to dryness under a stream of nitrogen gas at room temperature. The residue was dissolved in 200 μl of MP 1 and a 150-μl aliquot of the solution was injected onto the HPLC system.

2.5. Calibration graph and quantitation

Drug-free human serum samples spiked with known amounts of CP maleate were analyzed according to the procedure described above. The peak heights of CP were plotted against the serum concentrations to give the calibration graph. A weighted (1/concentration) linear regression was used to determine slope, intercept and correlation coefficient. The percentage recoveries of CP in human serum were calculated from the peak-height ratio of spiked standard samples relative to directly injected standard solutions. Intra- and inter-day accuracy and precision of the assay procedure were assessed in the concentration range 0.5–50 ng/ml in serum. The CP concentrations in samples were calculated using calibration graphs obtained with spiked standard.

3. Results and discussion

CP, being a hydrophobic basic compound, could be readily extracted with an organic solvent from aqueous samples under alkaline conditions. Therefore, liquid–liquid extraction has been employed for the pretreatment of serum samples in the determination of CP [2–6,8,9,11–13]. CP, dissolved in methanol, was found to adsorb on the wall of a glass tube during the evaporation under a stream of nitrogen gas at room temperature; this was also reported by Athanikar et al. [9] and Fujiwara et al. [13]. However, this phenomenon was not observed for spiked serum samples. Some endogenous substances in the serum were presumed to inhibit the adsorption of CP on the glass wall. Based on the above findings, the pretreatment of serum samples was performed as described in Experimental.

CP shows strong absorption only in the short-wavelength UV region. Although with poor selectivity, only this detection method offered the required sensitivity for the determination of CP without derivatization. The shorter wavelength gave a higher sensitivity. However, in the present study, considering the base line stability, UV absorption at 210 nm was selected for detection of CP. Fig. 1 shows typical chromatograms of a drug-free human serum and a standard solution equivalent to 5 ng/ml in serum obtained only with column C2 without column switching, monitored by UV detection at 210 nm. To acquire a higher sensitivity, CP should be eluted as fast as possible. However, large interfering peaks were observed at the retention time of CP, and thus conventional HPLC analysis could hardly offer the required high sensitivity. We previously reported a sensitive HPLC method for basic compounds in biological fluids with short-wavelength UV detection using col-

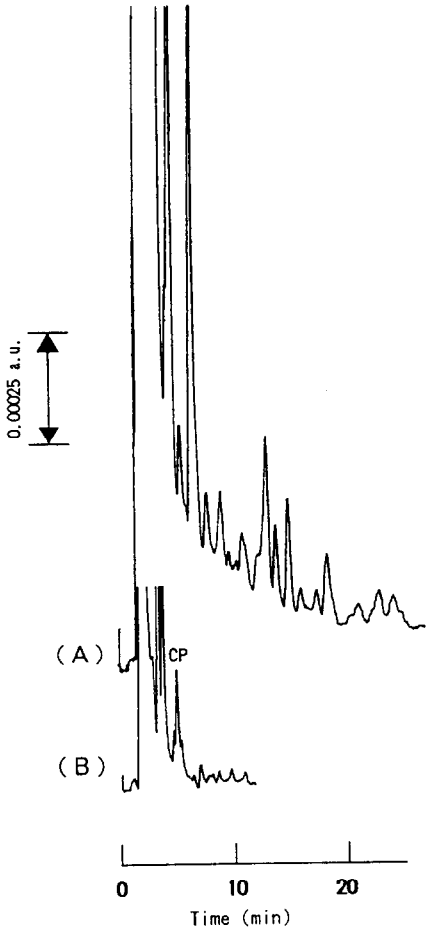

Fig. 1. Chromatograms of (A) a drug-free human serum and (B) standard solution of CP equivalent to 5 ng/ml obtained only with column C2 without column switching.

umn switching combined with ion-pair chromatography [15–20]. As the elution of CP, a tertiary amine, was predicted to be delayed by ion-pair formation, this column-switching technique was considered to be applicable. Testing of various sodium alkyl sulphonates ($C_nH_{2n+1}SO_3Na$) as the ion-pair reagent in MP 1 indicated that SOS ($n = 8$) was the most suitable for present study, as based on selectivity and total analysis time. Fig. 2 shows typical chromatograms of human serum samples after column switching. No interference was observed at the retention time of CP and the analyte could be determined with high selectivity and sensitivity. The mechanism for the separation from endogenous compounds in serum was previously described in detail [15].

A calibration graph was obtained by analyzing drug-free human serum samples spiked with CP in the concentration range 0.5–50 ng/ml. Good linearity was observed between the peak heights (y) and the corresponding serum concentrations (x). The equation for the regression line was $y = 75.3x - 15.1$ (correlation coefficient, $r = 0.99950$). The recoveries of CP from spiked serum samples were 88.9–107.4%. The accuracy and reproducibility for CP in human serum are also shown in Table 1. With this method, the limit of quantitation for CP was 0.5 ng/ml in serum, although the peak corresponding to 0.2 ng/ml was detectable at a signal-to-noise ratio of 3 (data not shown). The use of a larger amount of serum sample (e.g. 2 ml) would offer a higher sensitivity because no interference was observed at the retention time of CP (Fig. 2). The sensitivity obtained by the present method was comparable with that obtained by RIA [14]. It should be noted that this high sensitivity was obtained with a poorly selective detection method and a simple clean-up procedure prior to analysis.

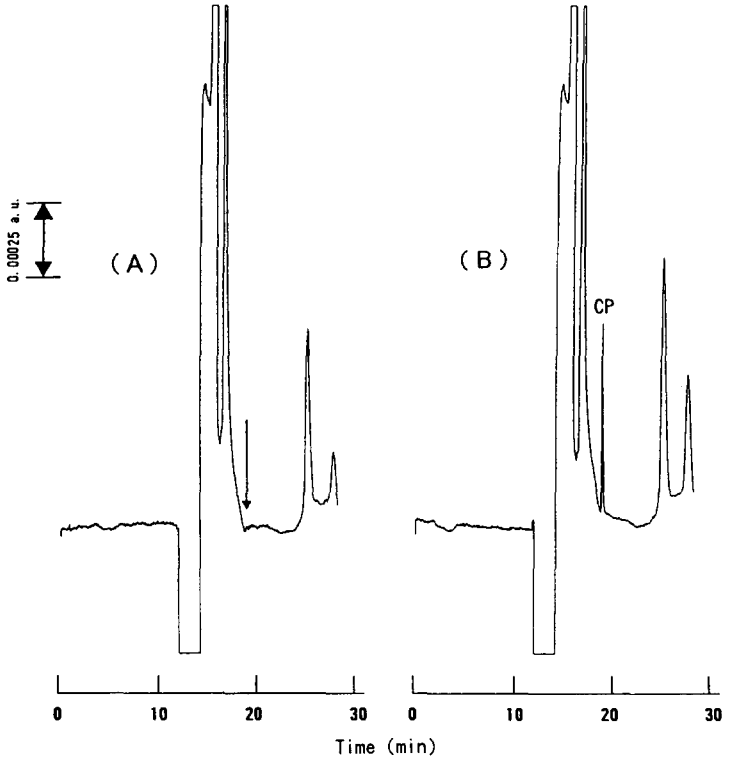

Fig. 2. Chromatograms of (A) a drug-free human serum and (B) human serum spiked with CP (10 ng/ml) obtained with column switching.

Table 1
Accuracy and reproducibility of the determination of CP added to a drug-free human serum

Concentration added (ng/ml)	Concentration found (ng/ml)		
	Day 1	Day 2	Day 3
0.500	0.556 (4.32)	0.548 (6.57)	0.565 (5.13)
5.000	4.559 (0.79)	4.897 (1.65)	4.661 (1.76)
10.000	9.623 (0.87)	9.219 (2.65)	9.223 (0.90)
25.000	24.748 (0.74)	24.296 (2.54)	24.729 (1.53)
50.000	50.958 (0.47)	51.537 (1.21)	51.318 (0.95)

Values in parentheses are coefficients of variation (%) ($n = 5$).

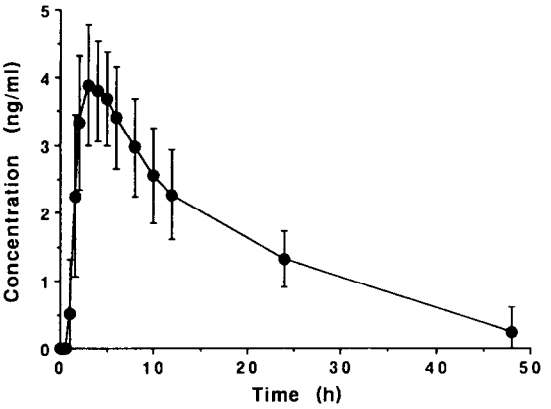

Fig. 3. Serum levels of CP in human volunteers after oral administration of a single dose of 2.67 mg of CP maleate. Each point and bar represent the mean ($n = 6$) and the standard deviation, respectively.

The proposed method was applied to the determination of CP in human serum after oral administration. The mean serum concentration–time profile of CP after oral administration of a single dose of 2.67 mg of CP maleate is shown in Fig. 3. The mean serum concentration of CP showed a peak of 3.9 ng/ml three hours after administration. These concentrations were almost the same as those reported previously [1,6,9–11].

In conclusion, a selective and sensitive method for the determination of CP in human serum was established by column-switching HPLC. This method was suitable for the determination of CP after oral administration of CP maleate.

References

[1] S.M. Huang, N.K. Athanikar, K. Sridhar, Y.C. Huang and W.L. Chiou, *Eur. J. Clin. Pharmacol.*, 22 (1982) 359.
[2] E. Townley, I. Perez and P. Kabasakalian, *Anal. Chem.*, 42 (1970) 1759.
[3] S. Hanna and A. Tang, *J. Pharm. Sci.*, 63 (1974) 1954.
[4] J.W. Barnhart and J.D. Johnson, *Anal. Chem.*, 49 (1977) 1085.
[5] H.T. Smith, J.T. Jacob and R.G. Achari, *J. Chromatogr. Sci.*, 16 (1978) 561.
[6] K. Masumoto, Y. Tashiro, K. Matsumoto, A. Yoshida, M. Hirayama and S. Hayashi, *J. Chromatogr.*, 381 (1986) 323.
[7] H. Miyazaki and H. Abuki, *Chem. Pharm. Bull.*, 24 (1976) 2572.
[8] J.A. Thompson and F.H. Leffert, *J. Pharm. Sci.*, 69 (1980) 707.
[9] N.K. Athanikar, G.W. Peng, R.L. Nation, S. Huang and W.L. Chiou, *J. Chromatogr.*, 162 (1979) 367.
[10] Y. Miyamoto, *J. Chromatogr.*, 420 (1987) 63.
[11] R.J. Shi, W.L. Gee and E.T. Lin, *Liq. Chromatogr.*, 10 (1987) 3101.
[12] S.A. Sande, T. Waaler and P.T. Nielsen, *Acta Pharm. Technol.*, 35 (1989) 22.
[13] K. Fujiwara, K. Iwamoto, S. Kawai and T. Sakamoto, *Yakugakuzasshi*, 109 (1989) 59.
[14] K.K. Midha, G. Rauw, G. Mckay, J.K. Cooper and J. Mcvittie, *J. Pharm. Sci.*, 73 (1984) 1144.
[15] K. Yamashita, M. Motohashi and T. Yashiki, *J. Chromatogr.*, 527 (1990) 103.
[16] K. Yamashita, M. Motohashi and T. Yashiki, *J. Chromatogr.*, 527 (1990) 196.
[17] K. Yamashita, M. Motohashi and T. Yashiki, *J. Chromatogr.*, 487 (1989) 357.
[18] T. Miyabayashi, K. Yamashita, I. Aoki, M. Motohashi, T. Yashiki and K. Yatani, *J. Chromatogr.*, 494 (1989) 209.
[19] M. Yamaguchi, K. Yamashita, I. Aoki, T. Tabata S. Hirai and T. Yashiki, *J. Chromatogr.*, 575 (1992) 123.
[20] K. Yamashita, M. Motohashi and T. Yashiki, *J. Chromatogr.*, 616 (1993) 144.