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Journal of Chromatography B, 744 (2000) 177–181

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Short communication

Determination of clemastine in human plasma by gas chromatography with nitrogen–phosphorus detection

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Received 16 August 1999; received in revised form 21 March 2000; accepted 23 March 2000

Abstract

A method for the quantitative determination of clemastine in human plasma has been developed and validated. The assay uses gas chromatography with nitrogen–phosphorus detection and a HP-1 capillary column (25 m×0.22 mm, film thickness 0.33 mm) coated with dimethylpolysiloxane. Clemastine (with orphenadrine as internal standard) was isolated from human plasma using liquid–liquid extraction. A linear relationship was observed between 0.1 and 12.8 ng/ml using the peak area ratio of clemastine to orphenadrine with a correlation coefficient greater than 0.99 (the detection limit for clemastine was 0.06 ng/ml). The intra- and inter-day coefficients of variation were less than 11%. The developed method was used for the analysis of plasma samples from healthy volunteers ($n=19$) to examine the pharmacokinetics of the antihistamine clemastine after single and multiple oral doses of clemastine fumarate.

Keywords: Clemastine

1. Introduction

Clemastine (Fig. 1) is an ethanolamine-derivative antihistamine used for the relief of symptoms associated with allergic rhinitis, seasonal rhinitis, urticaria, angioedema, and the common cold. Because of difficulties in measuring the low levels of clemastine in plasma, there is only limited information available in the literature on the pharmacokinetics of this drug. HPLC–MS, GC–MS, GC–ECD, GC–SID, and radioimmunoassay methods have been described for the detection of clemastine in plasma [1–5]. GC–ECD method, however, involves an oxidation step

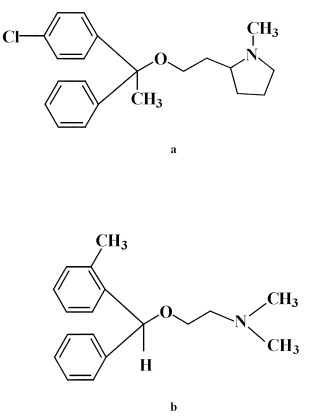

Fig. 1. Structure of clemastine (a), and orphenadrine (b).

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0378-4347/00/$ – see front matter
PII: S0378-4347(00)00216-4

and is not able to distinguishing the parent compound from metabolites [1]. The GC–MS method of evaluating clemastine does not give sufficient sensitivity to enable the study of its pharmacokinetics [2]. From the reports about LC–MS and GC–SID methods it is possible to conclude that these methods are potentially sensitive enough to detect clemastine in biological samples, but these methods were neither validated nor used for the analysis of clemastine in plasma samples from pharmacokinetic studies [3,4]. The radioimmunoassay provides adequate sensitivity to determine the clinical pharmacokinetics of clemastine at several dose levels [5].

This paper describes development and validation of a GC method with nitrogen–phosphorus detection for quantitative determination of clemastine in human plasma. The method was applied for the analysis of plasma samples from the healthy volunteers to examine the pharmacokinetics of clemastine after single and multiple oral doses of clemastine fumarate.

2. Experimental

2.1. Reagents and chemicals

Clemastine fumarate and orphenadrine were purchased from Sigma (St. Louis, MO, USA), toluene 'Optima', HPLC-grade water and sodium chloride were from Fisher Scientific (Fair Lawn, NJ, USA), and sodium bicarbonate was purchased from EM Science (Darmstadt, Germany).

2.2. Apparatus and conditions

GC analyses were carried out on a Hewlett-Packard Model 5890 gas chromatograph equipped with nitrogen–phosphorus detector (NPD). A HP-1 capillary column coated with dimethylpolysiloxane (25 m×0.20 mm, film thickness 0.33 mm) and splitless injection were used. The GC conditions were as follows: column temperature, programmed from 170 to 260°C at 6°C/min followed by isothermal operation for 3 min; injection temperature, 280°C; detector temperature, 290°C; and helium flow-rate, 1 ml/min. Aliquots of 1 μl of the samples were injected. Each cycle of automatic injection took 20 min.

2.3. Preparation of stock solutions

Standard stock solution of 10 μg/ml clemastine in HPLC-grade water was prepared. Standard stock solution of the internal standard (orphenadrine), 5 μg/ml, was prepared. Working standards from the clemastine stock solution were prepared by serial dilution to yield concentrations of 256, 128, 64, 32, 16, 8, 4 and 2 ng/ml and stored at 4°C.

2.4. Calibration standard preparation

Aliquots of 950 ml human plasma were spiked with 50 ml each of clemastine working standard to yield concentrations of 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2 and 0.1 ng/ml. A calibration curve was obtained by plotting the peak–area ratio of the clemastine to the internal standard as a function of clemastine concentration.

2.5. Sample preparation

One ml of plasma was mixed with 25 μl of internal standard (orphenadrine), 1 ml of 1 *M* $NaHCO_3$ and 0.5 ml of 2 *M* NaCl. The structure of orphenadrine is shown in Fig. 1b. The mixture was extracted three times with 2 ml of toluene by the vortex for 30 s followed by the centrifugation at 3400 *g* for 15 min. The organic layer was collected into borosilicate glass culture tubes and evaporated to dryness in Speed Vac SC110 (Savant) concentrator. The residue was dissolved in 100 μl methanol and used for the analysis.

2.6. Intra- and inter-day validation

Intra- and inter-day validation studies for precision and accuracy were performed on five low-, medium- and high-concentration quality control standards in plasma. Quality control samples with the following clemastine concentrations were prepared: 0.1, 0.5 and 5.0 ng/ml. The analyses were repeated on three separate days.

2.7. Subjects

Nineteen healthy volunteers (10 men, nine women, aged 19–56 years) took part in this study.

For the single-dose study the 2.68 mg clemastine fumarate (Tavist, Sandoz Pharmaceuticals) was administered orally and the blood samples were collected immediately before and 1, 2, 3, 4, 6, 8, 10, 14 and 24 h after administration. In the multiple-dose study, the tablets of clemastine fumarate were administered to subjects three times a day for a total of nine doses. On the morning of the last dose, blood samples were collected prior to dosing, and at 1, 2, 3, 4, 6, 8, 10, 14 and 24 h after the dose. The samples were centrifuged immediately and the plasma stored at −80°C until analysis.

3. Results and discussion

3.1. Chromatograms

In the development of this assay it was necessary to identify conditions that could enable efficient extraction of clemastine and internal standard from the human plasma with no interference of endogenous plasma components. Many impurity peaks appeared on chromatograms after isolation of clemastine from plasma by solid-phase extraction with various types of cartridges. The lowest amount of clemastine that could be accurately estimated after the solid-phase extraction was about 1 ng/ml with nitrogen–phosphorus or electron-capture detection. The liquid–liquid extraction method, as described in this paper, produced no peaks in the blank plasma that could interfere with clemastine or orphenadrine. It allowed to achieve high sensitivity for clemastine with the nitrogen–phosphorus detection. Chromatograms of typical blank human plasma, blank plasma spiked with orphenadrine and clemastine, and a chromatogram of plasma from healthy volunteers administered clemastine fumarate are shown in Fig. 2.

There are only few reports in the literature on identification of urinary metabolites of clemastine after oral administration to humans and rats [6,7], with no information found on metabolites of clemastine in human plasma. Therefore, under the present analytical conditions, the interference from possible metabolites was not taken into consideration. However, the plasma spiked with clemastine after microsomal incubation was analyzed under several temperature programs to ensure that potential metabolites would not interfere with the measurement of clemastine and orphenadrine.

3.2. Linearity

The calibration curve was created for eight different concentrations (0.1–12.8 ng/ml). A linear relationship was observed between peak–area ratio of clemastine to orphenadrine and the nominal concentration of clemastine with a correlation coefficient greater than 0.99. The detection limit was 0.06 ng/ml. The equation for the relationship of clemastine in plasma was $y=0.0033(\pm 0.0006)x+0.0008(\pm 0.0002)$, where y represents the peak–area ratio of clemastine and internal standard, and x is the analyte concentration in ng/ml.

3.3. Stability

Control plasma samples were analyzed daily and compared to freshly prepared standard samples of clemastine in plasma. Clemastine was found to be stable in human plasma for at least 4 weeks, when stored at −20°C. Clemastine and orphenadrine were found to be stable in the final reconstituted solution for the period of 10–12 h, during which samples were in the instrument, and at room temperature for at least 48 h.

3.4. Validation

The results of intra- and inter-day precision and accuracy for low, medium and high concentrations of clemastine in plasma are presented in Table 1. All coefficients of variation were less than 15%.

3.5. Human study

Fig. 3 shows the mean plasma concentration profiles after administration of single oral doses of clemastine in three healthy volunteers and multiple oral doses of clemastine fumarate in 19 healthy volunteers. The maximum plasma concentration of clemastine occurred at 3.4±1.7 h after administration. RSTRIP (MicroMath, Salt Lake City, UT) data analysis produced the α half-life of 1.9±0.9 h. The mean elimination half-life was calculated to be

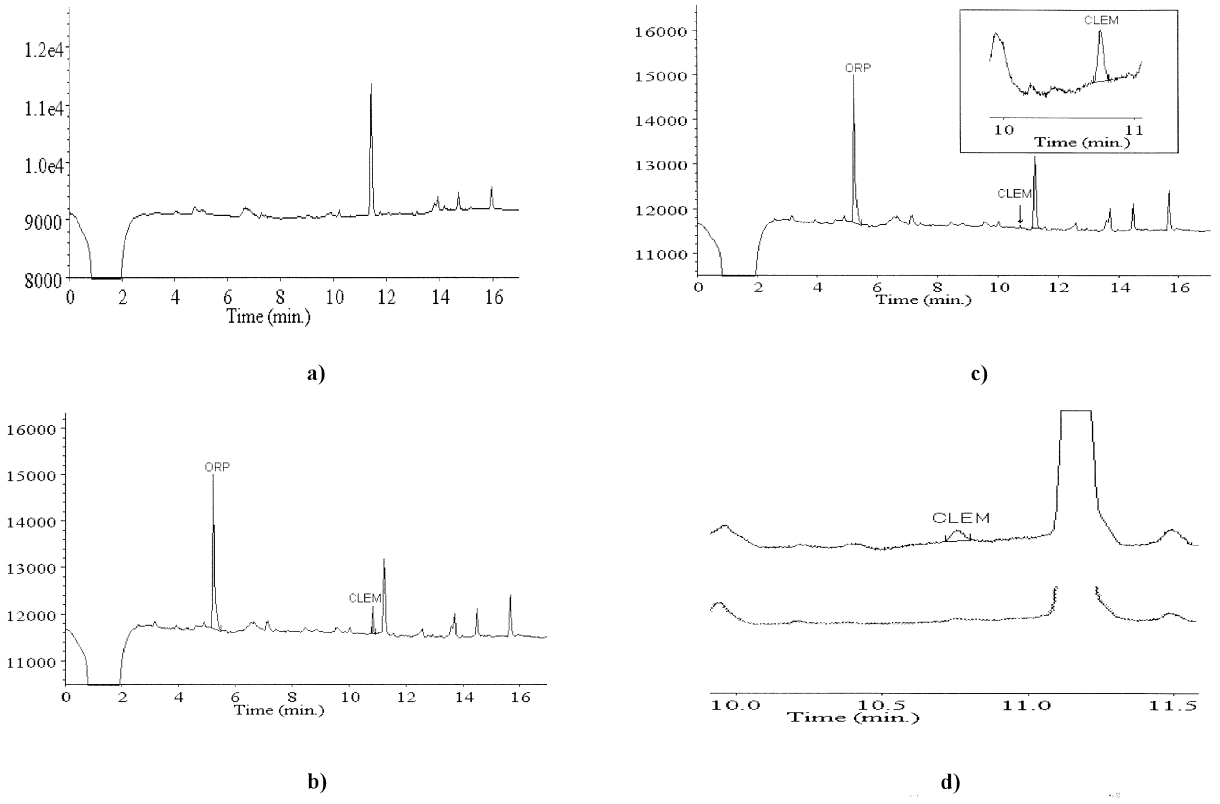

Fig. 2. Chromatograms of plasma samples. (a) Blank plasma; (b) plasma spiked with orphenadrine (ORP) and clemastine (CLEM) (concentration of clemastine 10 ng/ml); (c) plasma from healthy volunteers administered clemastine fumarate (concentration of clemastine (CLEM) 1.09 ng/ml); and (d) fragments of chromatograms of plasma with the clemastine concentration of 0.1 ng/ml and corresponding blank plasma.

Table 1
Intra- and inter-day precision and accuracy of clemastine in plasma

Clemastine	LoQC	MeQC	HiQC
Conc. (ng/ml)	0.1	0.5	5.0
Intra-day:			
n	5	5	5
mean	0.1	0.5	5.1
SD	0.0	0.1	0.3
C.V. %	11.3	9.4	5.4
Accuracy	109.3	96.0	108.4
Inter-day:			
n	13	13	15
mean	0.1	0.5	5.0
SD	0.0	0.1	0.4
C.V. %	12.7	10.2	8.2
Accuracy	110.5	106.0	99.8

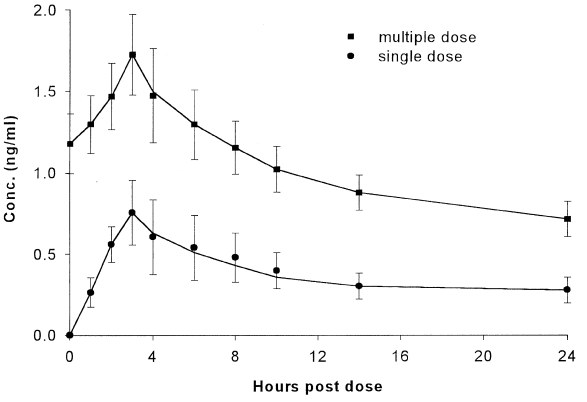

Fig. 3. Concentration of clemastine (mean±SE) after administration of single oral doses in three healthy volunteers (●), and multiple oral dose of 2.68 mg clemastine fumarate three times a day for 3 days in 19 healthy volunteers (■).

22.4±11.8 h. Values are presented as the mean±standard deviation.

4. Conclusion

The GC–NPD method developed and validated in this work was found to be accurate for estimating clemastine concentration in human plasma. The detailed pharmacokinetic and pharmacodynamic data for single and multiple oral doses of clemastine fumarate in healthy volunteers will be reported elsewhere.

Acknowledgements

The study was supported in part by NIH grants RO1 HL54590 and 1M01–RR1329701–A1.

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