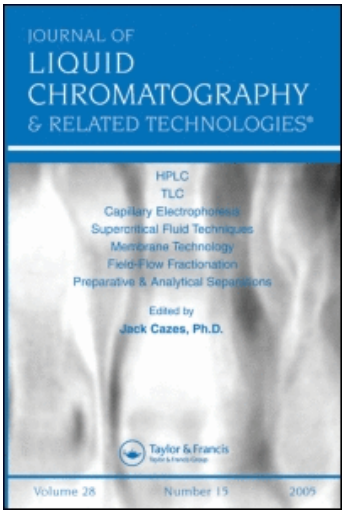

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Quantitation of Meclizine Dihydrochloride in Serum by Reversed Phase Ion Pair High Performance Liquid Chromatography

T. Imai[a]; S. Kimura[a]; Y. Imamura[a]; K. Uchiyama[b]; T. Iijima[b]; W. J. Underberg[c]; M. Otagiri[a]
[a] Faculty of Pharmaceutical Sciences Kumamoto University 5-1 Oe-honmachi, Kumamoto, Japan [b] Ueda Faculty Nisshin Chemical Co. Ltd., Nagano, Japan [c] Department of Chemical Pharmacy, Subfaculty of Pharmacy State University of Utrecht Catharijnesingel, Utrecht, The Netherlands

To cite this Article Imai, T. , Kimura, S. , Imamura, Y. , Uchiyama, K. , Iijima, T. , Underberg, W. J. and Otagiri, M.(1987) 'Quantitation of Meclizine Dihydrochloride in Serum by Reversed Phase Ion Pair High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 10: 11, 2505 — 2512
To link to this Article: DOI: 10.1080/01483918708068931
URL: http://dx.doi.org/10.1080/01483918708068931

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(11), 2505-2512 (1987)

QUANTITATION OF MECLIZINE DIHYDROCHLORIDE IN SERUM BY REVERSED PHASE ION PAIR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

T. Imai[1], S. Kimura[1], Y. Imamura[1],
K. Uchiyama[2], T. Iijima[2], W. J. Underberg[3],
and M. Otagiri[1]*

[1]*Faculty of Pharmaceutical Sciences*
Kumamoto University
5-1 Oe-honmachi
Kumamoto 862, Japan
[2]*Ueda Faculty*
Nisshin Chemical Co. Ltd.
751 Kamishiojiri Ueda
Nagano 386, Japan
[3]*Department of Chemical Pharmacy*
Subfaculty of Pharmacy
State University of Utrecht
Catharijnesingel 60
3511 GH Utrecht, The Netherlands

ABSTRACT

A specific and sensitive HPLC method has been developed for the assay of meclizine dihydrochloride in dog serum using an internal standard technique with a single step extraction. The extracts are injected into a reversed phase ion pair HPLC system using a solvent containing camphorsulfonate as paring anion. The detection limit is 5 ng/ml and the range of linearity is 5 - 250 ng/ml. The method has been used to quantitate meclizine dihydrochloride levels in bioavailability and pharmacokinetic studies in dogs.

INTRODUCTION

Meclizine (1-[(4-chlorophenyl)phenylmethyl]-4-[(3-methylphenyl)methyl]piperazine), an anti-motion-sickness drug, presently available only as dihydrochloride in oral tablets, is typically taken in 25 or 50 mg doses. An analytical method for the quantitation of meclizine in serum is desired for both pharmacokinetic and bioavailability studies. Available assays,such as fluorimetry (1) or GC with flame ionization detection (2) do not allow the detection of the low plasma levels expected after administration of therapeutical doses. A sensitive method based on the oxidation of meclizine to the corresponding benzophenone followed by GC with electron capture detection (3) was non-satisfactory due to background interferences, including those from meclizine metabolites, from the plasma samples. Although a sensitive method applying GC with selected ion monitering (4) was available for the analysis of plasma samples, this method appeared to be too complicated for routine analysis.

This paper describes a simple, rapid and sensitive high performance liquid chromatographic (HPLC) method with ultraviolet (UV) detection preceded by a single step extraction with an internal standard containing organic solvent.

EXPERIMENTAL

Chemicals and Reagents

Meclizine dihydrochloride (MZ) was kindly supplied by Nisshin Chemical Co., Ltd.(Tokyo, Japan). MZ tablets (Taizer®) were obtained from Taito Pfizer Co., Ltd.(Tokyo, Japan). All other materials were of analytical reagent grade. Water was deionized and distilled from glass.

Chromatography

The HPLC system consisted of a Model 655A-11 pump and Model 655A-21 UV detector (Hitachi,Tokyo, Japan). The column used (length 15 cm, i.d. 4 mm) contained Nucleosil $5C_{18}$ (Macherey-Nagel Gmbh & Co. Kg, Düren, West Germany).

The mobile phase was prepared by mixing methanol and 5 mM aqueous camphorsulfonate solution (7:1, v/v). The flow rate was 1 ml/min and the UV absorbance of the eluent was monitored at 232 nm.

Extraction Procedure

MZ levels in spiked blood have been shown to remain unchanged for at least 8 h when stored on ice (5). All samples were processed within 2 h. Blood obtained from dogs was centrifuged after 20 min to isolate the serum. To 2.0 ml serum 2 ml 0.1 M phosphate buffer (pH 7.0) was added in a test tube. After subsequent addition of 8.0 ml cyclohexane containing pyrene (5 ng/ml) as the internal standard, the tube was shaken mechanically for 20 min and centrifuged at 3000 rpm for 15 min. 6.0 ml of the organic phase was transferred to a new tube and the solvent was evaporated to dryness at $40^{\circ}C$ under reduced pressure. The residue was dissolved in 100 µl of methanol, and 80 µl was subjected to HPLC.

Animal Studies

Five male beagle dogs (10-13 kg, 2-5 years old) were used for a bioavailability study of MZ. The dogs were fasted for 48 h before administration of the drug. Two tablets (50 mg MZ) were administered orally. Blood samples (5 ml) were obtained from brachial veins at 0, 0.5, 1, 2, 3, 4, 6, 8 h after administration. MZ levels in the sample were measured as described above.

RESULTS AND DISCUSSION

Chromatography

MZ, being a protonated, positively charged ion, exhibits a high affinity for the stationary phase, probably due to interaction with residual free silanol groups in the column material (6). It can not be eluted with pure organic solvents such as methanol and acetonitrile. However, it can be easily eluted from the column with semi-aqueous mobile phases containing pairing anions, in this case camphorsulfonate. Fig. 1 shows typical chromatograms of blank serum and serum spiked with 250 ng/ml MZ. The retention times for MZ and the internal standard pyrene were 10 and 6 min, respectively. No interfering endogeneous peaks were observed in the chromatograms.

A calibration curve obtained from six different concentrations ranging from 5 to 250 ng/ml was constructed by plotting the ratio of the peak heights of MZ and the internal standard versus their concentrations. The standard curve shows good linearity ($r=0.9993$), the equation of the curve being $y = -0.0014 + 0.0042x$. The detection limit is 5 ng/ml using a signal-to-noise ratio of 3.

Precision and Accuracy

The inter-day precision and accuracy of the method over the entire concentration range were determined with the analysis of spiked serum samples. As shown in Table 1, the precision, expressed as the coefficient of variation, varies from 0.8 to 10.3 %. The accuracy, expressed as the relative error, varies from 0.3 to 5.3 %.

Extraction Recovery

The recovery was determined by comparing the peak height ratio obtained using 2.0 ml 0.067 M phosphate buffer (pH 7.4) instead of the serum with those obtained with serum. Spiked

TABLE 1. INTER-DAY PRECISION AND ACCURACY IN SERUM

Sample concn. (ng/ml)	Mean* (ng/ml)	S.D.	Coefficient of Variation (%)	Relative Error (%)
5	4.74	0.49	10.3	5.3
10	10.18	0.49	4.8	1.7
25	25.05	1.00	3.9	2.0
50	50.01	1.35	2.7	0.01
150	150.44	3.89	2.5	0.3
250	251.44	2.02	0.8	0.6

* : average of 5 determinations

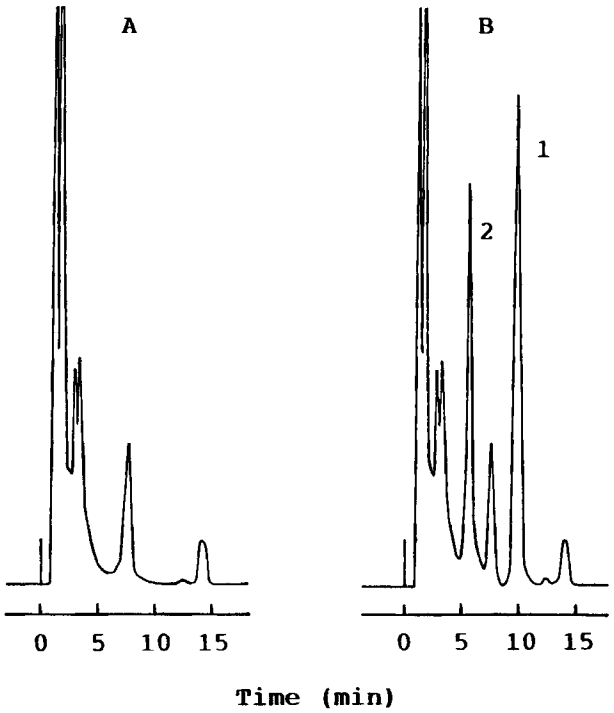

FIGURE 1. HPLC chromatograms of (A) blank serum, (B) serum spiked with 250 ng/ml of MZ.
Peak (1): MZ; peak (2): internal standard.

samples were treated as described previously except that the internal standard was not added until the evaporation step. This procedure was followed to prevent the internal standard from interaction with serum proteins.

The recoveries at six different concentrations appear in Table 2. The average recovery was 97.2 %.

Bioavailability Study

The procedure for the MZ assay has been applied successfully for a bioavailability study of the drug in dogs. Fig. 2 shows the mean serum levels of MZ following the oral administration of 50 mg MZ to each of five dogs. The measured serum levels of MZ ranged from 10 ng/ml to 390 ng/ml. The mean maximum serum level of 211 ng/ml was observed 2 h after administration. Some pharmacokinetic parameters are shown in Table 3.

The results of the study clearly indicate that the presented assay combines a simple sample preparation with a satisfactory precision. The assay is useful in pharmacokinetic and bioavailability studies as well as in the routine monitoring of MZ blood levels in hospital pharmacies and medical laboratories.

TABLE 2. EXTRACTION RECOVERY FROM SERUM

Spiked concn (ng/ml)	Recovery (%)* Mean	S.D.
10	92.9	1.8
25	97.4	1.6
50	98.5	3.1
100	97.6	4.2
150	97.6	2.6
250	99.2	3.9

* : average value of 3 determinations

TABLE 3. PHARMACOKINETIC PARAMETERS OF MZ FOLLOWING THE ORAL ADMINISTRATION

AUC (ng h/ml)	Ka (h^{-1})	Ke (h^{-1})
747.52 ± 182.11	1.28 ± 0.53	0.46 ± 0.1

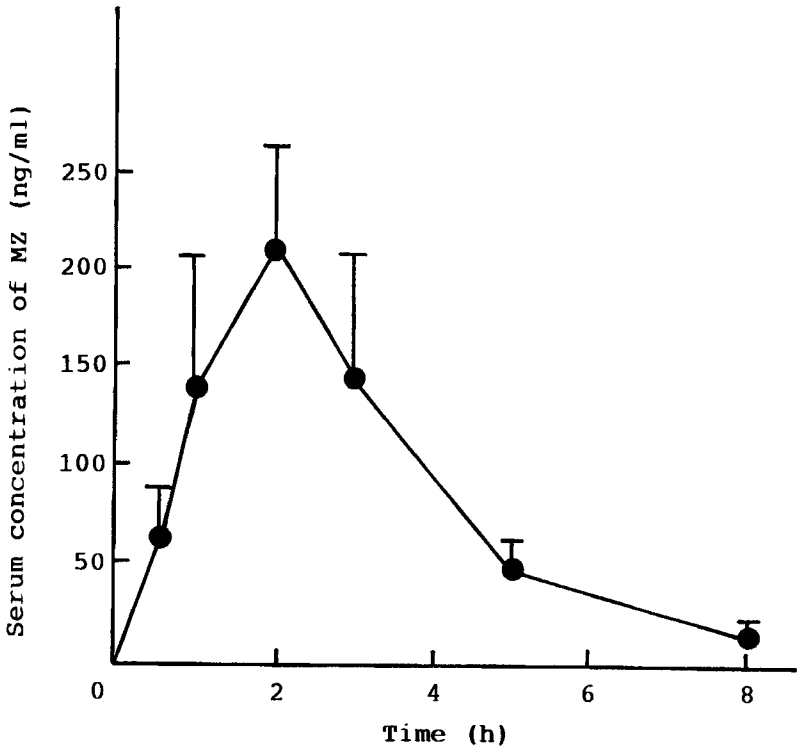

FIGURE 2. Mean serum concentration of MZ following the oral administration of 50 mg MZ.
Values represent the mean ± S.D. of 5 dogs.

REFERENCES

1) Wirz, D.R., Wilson, D.L. and Schenk, G.H. Fluorescence and Phosphoresecence of Antihistamines Having the Diphenylmethane Chromophore. Anal. Chem. 46, 896-900 (1974).
2) Wong, C.K., Urbigkit, J.R., Conca, N., Cohen, D.M. and Munnelly, K.P. GLC Determination of Meclizine Hydrochloride in Tablets Fomulation. J. Pharm. Sci., 61, 1340-1342 (1973).
3) Hartrig, P. and Handl, W. Gas Chromatography and Electron Capture Detection of Benzophenones. Acta Pharm. Suec., 12, 349-360 (1975).
4) Fouda, H.G. Falkner, F.C., Hobbs, D.C. and Luther, E.W. Selected Ion Monitoring Assay for Meclizine in Human Plasma. Biomed. Mass Spectrom.,5, 491-494 (1978).
5) Chovan, J.P., Klett, R.P. and Rakieten, N. Comparison of Meclizine Levels in Plasma of Rats and Dogs after Intranasal, Intravenous and Oral Administration. J. Pharm. Sci., 74, 1111-1113 (1985).
6) Lingeman, H., Van Munster, H.A., Beijnen, J.H., Underberg, W.J.M. and Hulshoff, A. High Performance Liquid Chromatographic Analysis of Basic Compounds on Non-modified Silica Gel and Aluminium Oxide with Aqueous Solvent Mixtures. J. Chromatogr., 352, 261-274 (1986).