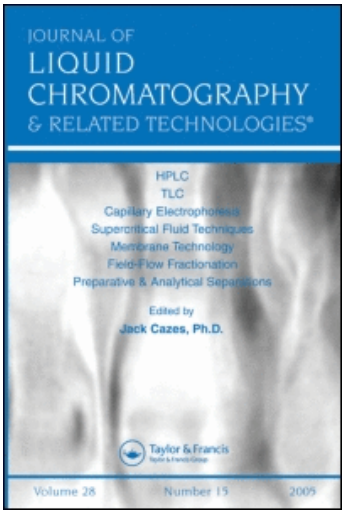

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Jang, W. C. and Lee, H. S.(1994) 'On-Line Trace Enrichment of Mifentidine in Plasma Using Column-Switching High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 17: 6, 1375 — 1384
To link to this Article: DOI: 10.1080/10826079408013770
URL: http://dx.doi.org/10.1080/10826079408013770

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(6), 1375-1384 (1994)

ON-LINE TRACE ENRICHMENT OF MIFENTIDINE IN PLASMA USING COLUMN-SWITCHING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The purpose of this study was to develop for the determination of mifentidine, a new long-acting histamine H_2 receptor antagonist, in plasma using a column-switching HPLC with a ultraviolet (UV) detection. Changing the pH influenced the species equilibria of mifentidine and these were well monitored and quantitated. This method showed a excellent precision with good sensitivity and speed, and a detection limit of 10 ng/mL. The total analysis time per sample was less than 20 min and mean coefficients of variation for intra- and inter- assay were both less than 3 %. The method has been successfully applied to plasma samples from rats receiving oral administration of mifentidine.

INTRODUCTION

Mifentidine, a new (imidazolylphenyl) formamidine derivative and a novel class of H_2-receptor antagonists that exhibit strong gastric antisecretory activity in different animal models (1), has been recently investigated. Few high performance liquid chromatographic (HPLC) methods have been reported for the determination of mifentidine in biological samples (2,3). In analyzing pharmaceutical formulations and biological samples, typical sample preparations such as liquid-liquid, liquid-solid extractions, solvent evaporations, and sample reconstitutions with suitable solvents are quite time-consuming and often give rise to loss of sample from incomplete extraction. In order to avoid these problems and accommodate the large number of samples commonly encountered in many pharmaceutical laboratories, a column-switching technique (4 -10) which allows on-line trace enrichment using precolumn without an extraction procedure is the best candidate for the determination of general drugs. This study describes an automated HPLC with the direct injection of plasma for the determination of mifentidine in plasma using the column-switching technique.

MATERIALS AND METHODS

Reagents

Pure sample of mifentidine (imidazole phenyl formamidine) was provided by Il-yang Pharm. Co., Ltd. (Suwon, Korea). Acetonitrile and methanol were HPLC grade (E. Merck, Darmstardt, Germany) and the water was deionized with NANOpure II (Barnstead, Iowa, USA). All other reagents were of analytical grade including phosphoric acid and potassium phosphate.

Standard Solutions and Plasma Samples

A stock solution was prepared by dissolving mifentidine in methanol and diluted to the appropriate concentrations with a phosphate buffer (pH 2.5). Spiked plasma samples containing mifentidine in the range of 10 - 500 ng/mL were prepared by adding aliquots of mifentidine stock solution to plasma.

Animal Treatment

Male Fisher F344 rats (KRICT animal laboratory, Taejon, Korea) weighing 140 - 160 g were used. After overnight fasting, the left femoral artery was cannulated and 10 mg/kg of mifentidine was orally administered to the conscious rats. The blood was collected at intervals of 0.25,0.5,1,2,4,8,12,18 and 24 h, heparinized, and centrifuged for 10 min at 3,000 x g to obtain plasma.

Chromatographic System

The HPLC system consisted of Waters 501 pump (Milford, MA, USA), Spectra-Physics Model SP 8800 pump (Santaclala, CA, USA), a Rheodyne 7125 injector (Cotati, CA, USA), a ten-port multifunction valve (Valco, Houston, TX, USA) and Spectra-Physics 8450 uv/vis detector. Data handling was performed by a Spectra-Physics 4270 computing integrator. The instrument arrangement for ten-port column-switching system is shown in Figure 1.

The precolumn (20 x 3.9 mm i.d.) was tap-filled with LiChrosorb RP-8 (25 - 40 μm, Merck) and changed after injection of 100 samples. A guard column was GuarPak Nova-Pak C8 (Waters Assoc.) and the analytical column was Ultracarb 5 ODS 20 (250 x 4.6 mm i.d., Phenomenex, Torrance, CA, USA).

The washing solvent was 0.05 M phosphate buffer (pH 2.5) at a flow rate of 0.5 mL/min. The mobile phase was

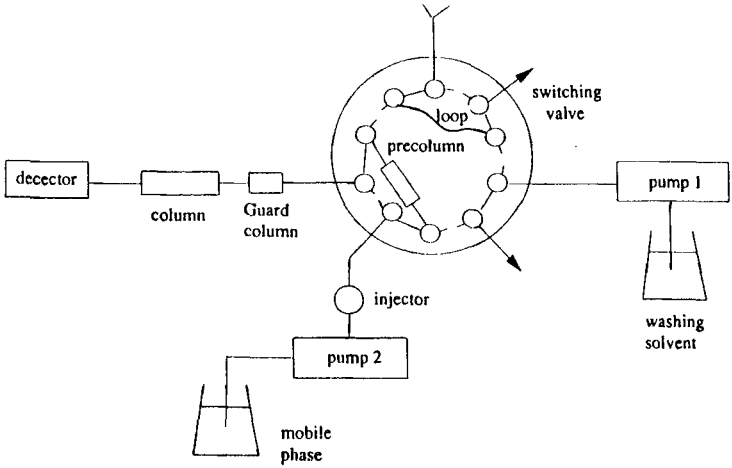


FIGURE 1: Schematic diagram of ten-port switching system (———— inject - - - - load).

methanol - 0.05 M phosphate buffer (pH 2.5) (1:9, v/v). The column temperature was ambient and the wavelength of detection was at 266 nm.

Analytical Procedure

A 100 μL of the spiked plasma or plasma samples was injected. The sequence of the sample analysis included the following three steps and required about 20 min.

Step I (0 - 5 min) : The plasma sample was injected onto the precolumn. Possible polar interfering plasma components were washed out to waste. Guard column and analytical column were equilibrated with the mobile phase.

Step II (6 - 15 min) : Washing solvent passed through to waste. The retained components were eluted from the precolumn to guard column/analytical column in back-flush mode by the mobile phase.

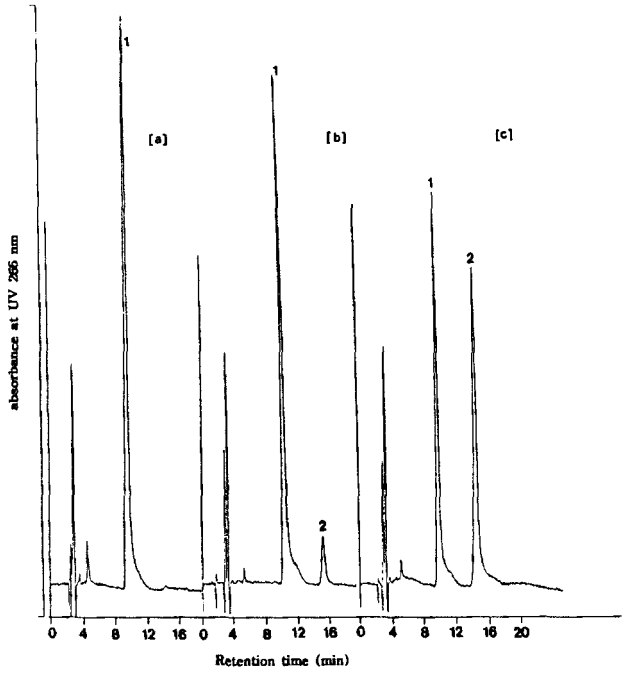


FIGURE 2: Chromatograms of 500 ng/mL mifentidine in the various pH buffers (a) pH 2.5, (b) pH 7.5 and (c) pH 9.0. Peaks: 1 = neutral form; 2 = protonated form.

Step III (16 - 20 min) : The eluted drugs were separated in the analytical column. Meanwhile precolumn was re-equilibrated with the washing solvent for the next injection.

RESULTS AND DISCUSSION

Chromatography

Chromatograms of mifentidine standard solution in the various pH values of phosphate buffer are shown in Figure 2. Figure 2 shows the effect of pH on the species

equilibria of mifentidine. According to Haaksma et al.(11) it was of interest to consider that protonation on the imino nitrogen of amidine group results in the resonance-stabilized amidinium cation, where the positive charge is delocalized over a plane of sp^2-hybridized atoms. In this study, these species of equilibria are shown. Chromatogram (a) shows a dominant neutral species of mifentidine at pH 2.5. Chromatogram (b) and (c) indicate protonated mifentidine at pH 7.5 which has pKa value of 8.82, due to the loss of proton from the amidine, and diprotonated mifentidine at pH 9.0 which has pKa value of 5.64, due to the loss of a proton from the protonated imidazole ring.

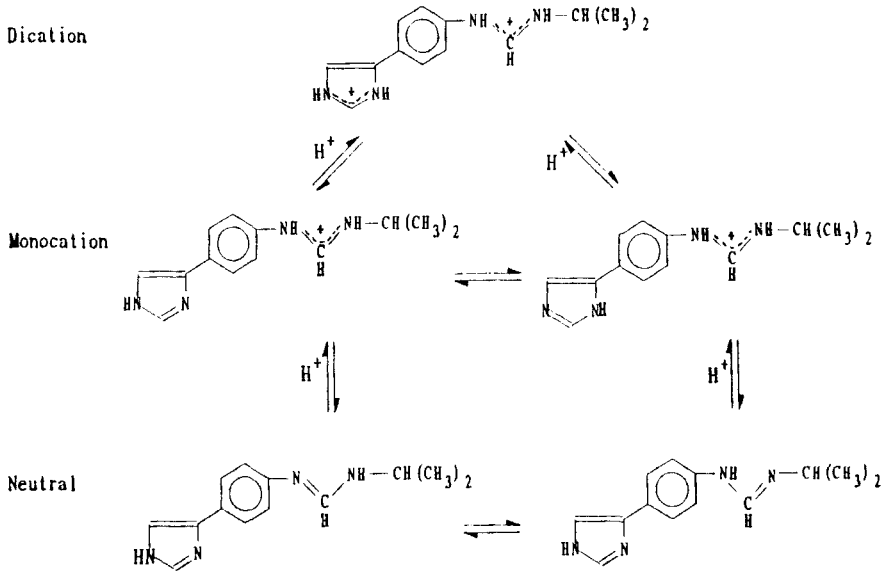
It indicated that mifentidine may exist above physiological pH 7.4 as a mixture of protonated and neutral species in their possible tautomeric forms (Scheme 1).

Therefore, to obtain good resolution of the neutral species of mifentidine in plasma, pH 2.5 phosphate buffer was chosen for mobile phase.

Chromatogram of blank plasma, spiked plasma, and plasma from a rat after oral administration of 10 mg/kg mifentidine are shown in Figure 3. As shown in Figure 3a, there was no interfering peaks at the retention times of mifentidine and its protonated form. Figure 3b and c show distinct, well-resolved peaks.

Column-Switching Procedure

In column-switching technique, it was necessary to choose the precolumn packing material, washing solvent and washing time in such a way that mifentidine and its protonated form would be completely adsorbed while the interfering endogenous components in plasma would be washed out from the precolumn to the waste port.



SCHEME 1: Ionic and tautomeric species of mifentidine.

LiChrosorb RP-8 (25 - 40 μm), a nonpolar octylsilane bonded phase adsorbent, was chosen for precolumn packing because of its strong adsorptivity for mifentidine and its protonated form at acidic pH, stability at pH 1 - 7 and easy availability.

To obtain high percent recovery of mifentidine as the neutral species and to clean the plasma components from the precolumn, 0.05 M phosphate buffer (pH 2.5) was chosen for washing solvent.

Washing the precolumn with the buffer at a flow rate of 0.5 mL/min for 5 min was good enough to get good peaks and clean chromatograms.

Recovery and Reproducibility

A series of five plasma samples spiked with mifentidine in the range of 10 - 500 ng/mL was assayed.

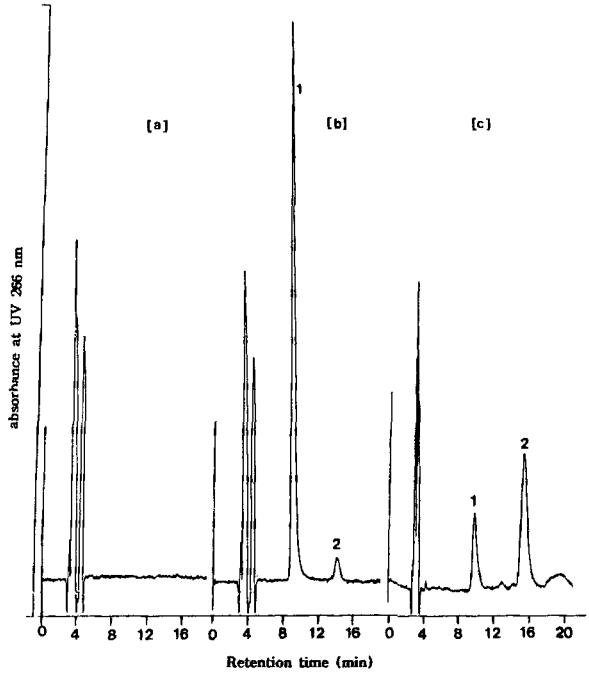


FIGURE 3: Chromatograms of (a) blank plasma, (b) blank plasma spiked with mifentidine (400 ng/mL) and (c) plasma of a rat 30 min after 10 mg/kg oral administration of mifentidine. Peaks: 1 = neutral form ; 2 = protonated form.

TABLE 1

Intra- and Inter-Assay Reproducibility of Mifentidine in Plasma Samples (n = 5)

Amount Added (ng/mL)	Amount Found (ng/mL)		Coefficient of Variation (%)	
	Intra	Inter	Intra	Inter
10	9.50	9.54	2.7	2.8
50	45.80	46.40	2.0	3.7
100	96.30	95.60	3.0	3.2
400	373.50	373.80	0.8	1.3
500	457.00	462.50	1.5	2.7

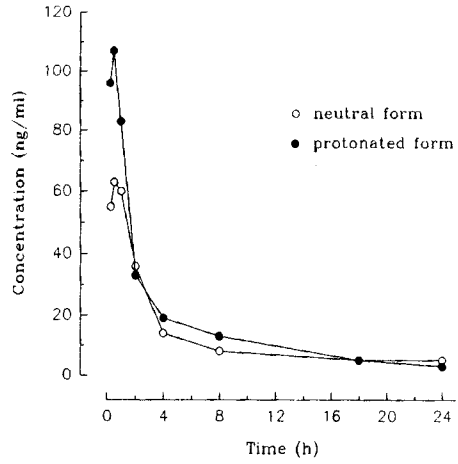


FIGURE 4: Plasma concentrations versus time curve of a rat after a 10 mg/kg oral administration of mifentidine.

Recovery of mifentidine in plasma was quantitative with a mean value of 93.8 ± 2.7 %.

Precision (defined as the coefficient of variation of replicate analysis) of the assay for mifentidine was evaluated over the concentration range studied (Table 1). The coefficient of variation for intra- and inter- assay were less than 3 %.

Application of the Method to Biological Samples

The present method was ideally suitable for mifentidine quantitation in plasma samples. Figure 4 shows the plasma concentration versus time plot of mifentidine and its protonated form after single oral administration of 10 mg/kg mifentidine to rats. Plasma concentrations of mifentidine and its protonated form are maximum 0.5 h and almost below the detection limit 18 h after oral administration.

In conclusion, the present work provided that column-switching technique was an ideal method for mifentidine quantitation from microvolumes of plasma samples without laborious sample manipulations because of its excellent precision, sensitivity and speed.

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Received: July 26, 1993
Accepted: October 8, 1993