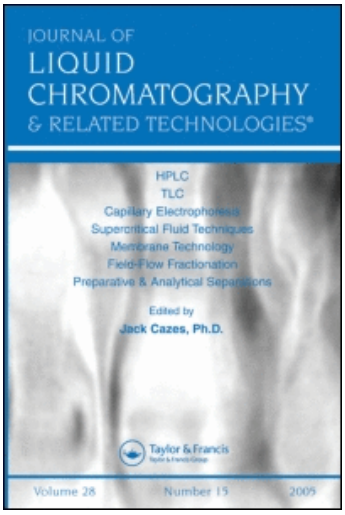

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High-Performance Liquid Chromatographic Determination of Meclofenamate Sodium in Plasma

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF MECLOFENAMATE SODIUM IN PLASMA

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ABSTRACT

A reversed phase high-performance liquid chromatographic method for the analysis of meclofenamate sodium in plasma was developed. Diclofenac was used as the internal standard. The chromatography was performed using a resolve C_{18} column; the mobile phase consisted of 60:40 % methanol to water and adjusted to pH 3.0 using acetic acid; a flow rate of 1.5 ml/min; and UV detection at 270 nm. Retention times were 3.6 and 5.9 min for diclofenac and meclofenamate, respectively. The mean (±SD) absolute and relative recovery of meclofenamate were found to be 96.49±0.59 and 100.48±0.73, respectively. The minimum detectable concentration of meclofenamate by this method was 150 ng/ml sample. The sensitivity obtained should enable the use of the method in future bioequivalency and/or pharmacokinetic studies.

INTRODUCTION

Meclofenamate sodium is a nonsteroidal anti-inflammatory drug (NSAID) administered orally and is used for the treatment of acute and chronic rheumatoid arthritis and osteoarthritis (1-5). The recommended adult dose is 200 to 400 mg/day in 3 to 4 divided doses. The drug is also used for monorrhagia in doses of 100 mg three times daily (6).

A number of analytical procedures were developed for the determination of meclofenamate sodium. These include a fluorometric assay (7), gas-liquid chromatography and a thin layer chromatography (8). Recently an HPLC method was developed for meclofenamate sodium in both urine and plasma (9). The GLC method requires highly sophisticated equipment and is not technically feasible for routine use in bioequivalency study. The HPLC method previously reported is time consuming.

This study describes a simple, sensitive, and reproducible high-performance liquid chromatographic method for the quantitative determination of meclofenamate sodium in plasma using diclofenac sodium as internal standard. A single extraction step is followed by reversed-phase chromatography, eliminating the tedious and time-consuming procedures required by the previously reported method (9). Meclofenamate sodium

and diclofenac sodium can be internal standards for each other during either assay.

MATERIALS

Meclofenamate sodium and the internal standard diclofenac (Sigma Chem. Co., St. Louis, MO, U.S.A.), glacial acetic acid and hydrochloric acid (BDH Chemicals Ltd., Poole, U.K.) were analytical grade and used without further purification. Methanol and chloroform (BDH Chemicals Ltd., Poole, U.K.) were HPLC grade.

METHODS

Instruments

The following instruments were utilized:

A Model LC-10AD solvent delivery pump, a Model SPD-10AV UV-Vis detector, a Model CTO-10A column oven, and a Model C-R4A chromatopac computing integrator (Shimadzu Corporation, Koyato, Japan), a Model 7010 Rheodyne injector (Rheodyne Inc., Catati, CA, U.S.A.), stainless steel column (Resolve C_{18}, 150 mm length x 3.9 mm i.d., 5 μm particles, Waters Associates, Milford, MA, U.S.A.), and a Model CFC-301 Gallenkamp centrifuge (Gallenkamp, Louchborough, England).

Standard Stock Solution

An accurately weighed sample of 10 mg meclofenamate and 10 mg diclofenac were dissolved in methanol in two separate 100 ml volumetric flasks to give standard stock solution of 100 μg/ml.

Chromatographic Conditions

The mobile phase was a mixture of 60% methanol and 40% HPLC water adjusted to pH 3.0 by using acetic acid. It was degassed daily by passing it through 0.45 μm membrane filter (Millipore, Bedford, MA, U.S.A.).

The mobile phase was pumped at flow rate of 1.5 ml/min, which produced a backup pressure of about 219 kgf/Cm^2. The column oven temperature was adjusted at 35°C. The effluent was monitored at 270 nm and attenuation at 0.002 AUFS. The chart speed was 1.5 mm/min.

Analytical Procedure

Drug-free blank plasma (0.5 ml) were spiked with known amounts of meclofenamate in 15 ml glass stoppered test tube. To each tube 7.5 ml chloroform, 0.2 ml 6N HCl and 50 μl of diclofenac (I.S.) were added. The samples were shaken for 2 minutes and the chloroform layer, containing the drug, was transferred to 10 ml quick fit test tube and evaporated to dryness under vacuum at 45°C. The residue was dissolved in 0.5 ml of

the mobile phase, vortexed for one minute and then an aliquot of 20 µl was directly injected into the loop.

RESULTS AND DISCUSSION

The mobile phase used for the assay provided good separation of meclofenamate and diclofenac (I.S.) and sharp peaks with no interference from plasma substances. Figure 1 represents a typical chromatogram of: blank plasma (A), plasma containing the drug (B), and plasma containing meclofenamate and the internal standard (C). Using the developed assay procedure the retention time for the internal standard and meclofenamate were 3.6 and 5.9 minutes, respectively. Heating the column to 35°C gave sharp peaks.

Quantification

Peak-height ratios of the drug to the internal standard were used in constructing four different standard calibration lines in plasma and mobile phase by spiking 0.5 ml drug-free plasma and 0.5 ml mobile phase samples with the drug standard to produce a final concentration of 0.5, 1.0, 2.0, 4.0 and 10.0 µg/ml meclofenamate. The standard curves were prepared in a period of three weeks. Least squares linear regression analysis of the mean standard calibration plots for mobile

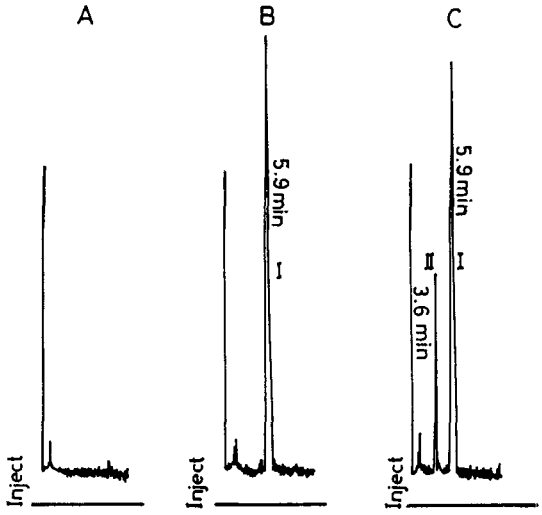

FIGURE 1: Chromatograms of blank plasma (A), plasma containing meclofenamate (B), and plasma containing meclofenamate and internal standard (C).

Key: I; Meclofenamate, II; Internal standard

phase and plasma samples resulted in the following equations:

$$Y = 0.234\ x - 0.01525,\ r = 0.999 \text{ (Plasma)}$$

and $$Y = 0.260\ x + 0.002,\ r = 0.999 \text{ (Mobile phase)}$$

Analysis of variance of the slopes, intercepts and correlation coefficients of the four standard plots from plasma indicated non-significant difference ($F=2.83$, $P>0.05$). These results confirm the linearity of the standard curves and the excellent reproducibility of the assay method.

Recovery

The absolute recovery of meclofenamate and the internal standard (diclofenac) were determined by comparing the peak-height of the drug obtained from plasma with the peak-height obtained by the direct injection of pure aqueous drug standard at three different concentrations (0.75, 3.0 & 8.0 μg/ml). The relative recovery of the drug was calculated by comparing the concentration obtained from the drug-supplemented plasma to the actual added concentrations. The results of the recovery studies are shown in Table 1. The average absolute and relative recovery of meclofenamate were found to be 96.49+0.59 and 100.48+0.73, respectively.

Precision

The intraday precision was evaluated by replicate analysis of plasma samples containing meclofenamate at three different concentrations (low, medium and high). The intraday precision showed coefficient of variations (CV) of 2.00 to 3.95 (Table 2). The interday precision was similarly evaluated over 3-weeks period. The interday CVs ranged from 1.65 to 2.97 (Table 2).

Sensitivity

The limit of quantification for this method was 150 ng/ml plasma. If additional sensitivity is needed,

Table 1 : Absolute and Relative Recovery of Meclofenamate from Plasma *.

Conc. (µg/ml)	Mean Peak Heights (cm) Aqueous	Plasma	Absolute Recovery %	Relative Recovery % Mean ± SD
0.75	1.05 ± 0.08	1.02 ± 0.07	97.14	99.67 ± 1.85
3	3.83 ± 0.07	3.69 ± 0.04	96.34	100.71 ± 1.03
8	9.99 ± 0.49	9.59 ± 0.37	95.99	101.07 ± 2.41
Diclofenac (I.S.) 1	4.63 ± 0.08	4.60 ± 0.1	99.35	

* Eight replicate analyses of each concentration.

injection volume could be increased or reconstitution in small volume of the mobile phase could be done.

Application

A typical mean plasma concentration-time profile for meclofenamate in beagle dogs (n=5) following intravenous administration of 40 mg dose is shown in

Table 2 : Intraday and Interday Precision of Meclofenamate in Plasma.

Intraday*			Interday**		
Added Conc. (μg/ml)	Measured Conc. (μg/ml)	Bias %	Added Conc. (μg/ml)	Measured Conc. (μg/ml)	Bias***
0.75			0.75		
Mean	0.76	1.33	Mean	0.74	-1.33
S.D.	0.03		S.D.	0.02	
CV %	3.95		CV%	2.7	
3			100		
Mean	3	0	Mean	3.03	1
S.D.	0.06		S.D.	0.05	
CV%	2		CV%	1.65	
8			8		
Mean	7.98	-0.25	Mean	8.09	1.13
S.D.	0.22		S.D.	0.24	
CV%	2.76		CV%	2.97	

* Mean values represent eight different plasma samples for each concentration.

** Interday precision was determined from 23 different runs over 3-weeks period at the three concentrations.

*** Bias = 100x (measured concentration-added concentration) / added concentration.

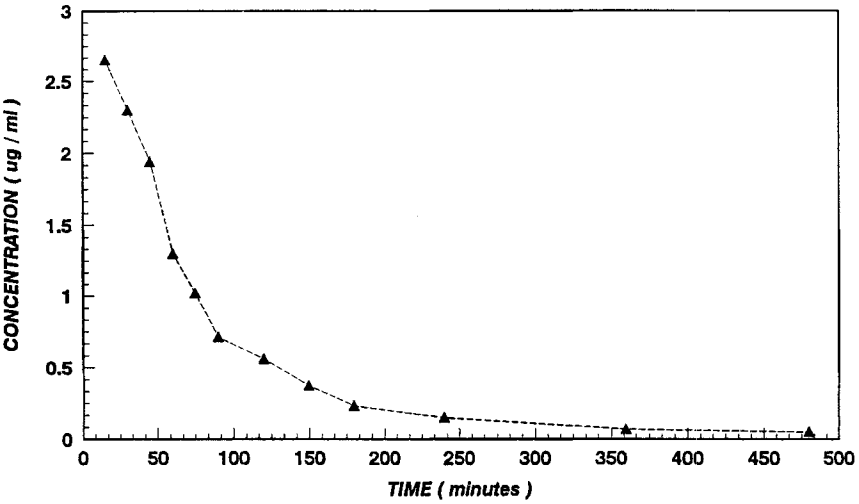

FIGURE 2: Average plasma concentration versus time profile for meclofenamate in five dogs after intravenous administration of 40 mg dose.

Fig. 2. The method was sufficiently sensitive to enable meclofenamate quantification from plasma upto 8 hours post dose.

Conclusion

The HPLC method developed in this study has the sensitivity, simplicity, reproducibility and rapidity which makes it versatile and valuable in many applications, specifically in drug level monitoring, drug-drug interactions, pharmacokinetic and bioequivalence studies.

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