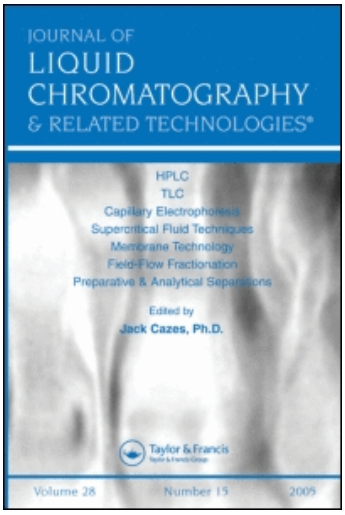

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Shields, Brenda J. and Mackichan, Janis J.(1990) 'High-Performance Liquid Chromatographic Method for the Determination of Metoclopramide in Plasma', Journal of Liquid Chromatography & Related Technologies, 13: 13, 2643 — 2659
To link to this Article: DOI: 10.1080/01483919008049060
URL: http://dx.doi.org/10.1080/01483919008049060

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(13), 2643-2659 (1990)

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF METOCLOPRAMIDE IN PLASMA

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ABSTRACT

A reversed-phase high performance liquid chromatographic method for the determination of metoclopramide in plasma has been developed. Metoclopramide was extracted from alkalinized plasma (0.25 - 0.5 ml) into methyl-t-butyl ether, injected onto an alkylnitrile column and eluted with a mobile phase of methanol and tetrahydrofuran in acetate buffer, pH 4.3. The eluate was monitored with a variable wavelength UV detector at 309 nm. Extraction efficiencies for metoclopramide and the internal standard (a dipropyl analog of procainamide) ranged from 89 to 93 %. The limit of sensitivity for the method was 2.5 ng/ml when 0.5 ml of plasma was extracted. Application of the method to single dose pharmacokinetic studies was demonstrated by the analysis of plasma samples following the administration of a single 10 mg oral dose of metoclopramide hydrochloride to two healthy volunteers.

INTRODUCTION

Metoclopramide (4-amino-5-chloro-2-methoxy-N-(2-diethyl aminoethyl)benzamide) is a dopamine-receptor antagonist, an antiemetic and a stimulant of upper gastrointestinal motility (1). It is used for the management of gastrointestinal motility disorders and gastrointestinal reflux and for the prevention of cancer chemotherapy-induced emesis at much higher doses (1).

Previously reported methods for quantitating metoclopramide in biological fluids include thin-layer chromatography (2,3), spectrophotometry (4), electron-capture gas chromatography (5-7), and both normal- (8-11) and reversed-phase (12-19) high-performance liquid chromatography. Most of these methods require large sample volumes (1-5 ml) and lengthy sample preparation procedures. Some require elevated temperatures (18), large sample injection volumes (11), slurry-packed columns (13) or suffer from a lack of sensitivity (2,3,4,8,19). One method (12) requires only 150 µl of sample and no sample pretreatment, but uses 3 pumps and 2 columns in the analysis. The advantages of the present reversed-phase HPLC method include ease in sample preparation and a small sample volume requirement. The application of this method to a single-dose pharmacokinetic study is demonstrated.

EXPERIMENTAL

Reagents

Metoclopramide monohydrochloride was purchased from Sigma Chemical Company, St. Louis, MO. The internal standard, p-amino-

N-2-dipropylaminoethyl benzamide hydrochloride, was obtained from E.R. Squibb and Sons, Inc., Princeton, NJ. Methanol, methyl-t-butyl ether and tetrahydrofuran, HPLC grade, were purchased from Baxter Healthcare Corporation, Burdick and Jackson Division, Muskegon, MI. Glacial acetic acid, analytical grade, was purchased from Mallinckrodt, Paris, KY and sodium hydroxide, analytical grade, was purchased from VWR Scientific, San Francisco, CA. All water used in the preparation of solutions was demineralized and double-distilled. The Red Cross, Columbus, OH, provided outdated blood bank plasma for use in the preparation of standards and quality controls.

Instrumentation and Chromatographic Conditions

A Model 222B isocratic chromatographic pump equipped with a Model 02-0249 injector port and a 50 µl sample loop (Scientific Systems Inc., State College, PA) was used. Separations were performed on a Microsorb CN column (25 cm, 5 µm particle size) from Rainin Instrument Company, Woburn, MA. The effluent was monitored with a Model 2050 variable wavelength UV detector (Varian Associates, Walnut Creek, CA) at 309 nm and a range of 0.005 absorbance units full scale (a.u.f.s.) (range = 0.0025 when 0.25 ml of sample is extracted). Detector output was recorded at 1 mV with a strip chart recorder (Beckman Instruments, Fullerton, CA) at a chart speed of 0.25 cm/min.

The mobile phase was composed of acetate buffer:methanol:tetrahydrofuran (65:35:1, v/v). The acetate buffer was prepared by bringing 10 ml of glacial acetic acid to a final volume of 1 L with distilled water and adjusting to a pH of 4.3 with 5 N NaOH. After degassing the mobile phase by sonication under a vacuum, it was pumped through the column at a flow rate of 0.9 ml/min.

Calibration Standards and Quality Controls

Stock solutions (1 mg/ml) of metoclopramide and the internal standard base were prepared in 50% methanol in water. Working solutions of metoclopramide (10 ng/µl) and the internal standard (1 and 2 ng/µl) were prepared in 50% methanol in water. All stock and working solutions were stored in polypropylene tubes at 4° C.

Plasma standards were prepared with pooled drug-free plasma by serial dilution of the 10 ng/µl metoclopramide solution to give final concentrations of 200, 150, 100, 50, 25, 12.5, 6.25 and 3.125 ng/ml. Low (20 ng/ml) and high (150 ng/ml) metoclopramide quality controls were likewise prepared in pooled plasma. All plasma samples were stored in polypropylene tubes at -20° C.

Extraction and Quantitation

Extractions were performed using 8 ml polypropylene tubes. To 0.5 ml of human plasma were added 50 µl of 5 N NaOH and 40 µl

of working internal standard (2 ng/µl). (If 0.25 ml of plasma was used, 40 µl of internal standard (1 ng/µl) were added). After briefly vortexing, 4 ml of methyl-t-butyl ether were added to each tube, the tubes were capped and gently shaken for 10 min. Following centrifugation for 10 min at 1200 g, the ether (top) layer was transferred to a new tube and evaporated to dryness at 50° C under a gentle stream of air. The sides of each tube were rinsed with 0.5 ml methyl-t-butyl ether and evaporated to dryness. Extracts were reconstituted with 80 µl of mobile phase, vortexed for 30 sec and 25-40 µl volumes were injected onto the column.

Peak heights were used to quantitate detector response. Peak height ratios were calculated by dividing the peak height of metoclopramide by the peak height of the internal standard. Calibration curves were constructed by plotting peak height ratios as a function of metoclopramide concentration. Least squares linear regression analysis was used to determine sample concentrations of metoclopramide from peak height ratio data.

Analytical Variables

Limit of Sensitivity. The limit of sensitivity was determined by injecting amounts of metoclopramide onto the column ranging from 1 ng to 10 ng at a range of 0.0025 a.u.f.s. Peak heights as a function of amount injected were plotted. Using the

criterion of a signal-to-noise ratio of 3, linear regression analysis was used to determine the minimum detectable amount of metoclopramide.

Specificity. One to two micrograms of potentially interfering drugs were directly injected onto the column. Those drugs that interfered with either the metoclopramide or internal standard peak were then extracted and injected onto the column to determine whether they were removed by the extraction procedure.

The potential of interference by metoclopramide metabolites was also evaluated. Three metoclopramide plasma standards and one plasma sample obtained from a healthy volunteer following a single 10 mg oral dose of metoclopramide hydrochloride were extracted and injected onto the column at three different wavelengths (280, 309 and 320 nm). The wavelengths were selected to include the wavelength used in the assay (309 nm) which is close to the λ_{max} for metoclopramide (308 nm), as well as a wavelength 20 nm above and a wavelength 20 nm below the one used in the assay. Since metoclopramide was barely detectable at a wavelength of 330 nm, the upper wavelength of 320 nm was used. The resulting standard curves (based on the peak height of metoclopramide) were used to determine the sample concentration of metoclopramide at each of the three wavelengths.

Precision. Within-day precision was determined by analyzing 10 low (20 ng/ml) and 10 high (150 ng/ml) quality controls in one day. Coefficients of variation were determined for the

corresponding mean peak height ratios. Day-to-day precision was evaluated by analyzing each quality control on different days 13 times over a 2 month period. Coefficients of variation were determined for the corresponding mean concentrations.

Extraction efficiency. The extraction efficiencies of metoclopramide (20 and 160 ng/ml) and the internal standard (160 ng/ml) from human plasma were determined by extracting 0.5 ml of spiked plasma samples according to the above procedure with the exception that no additional internal standard was added. Reference solutions were prepared in 50% methanol in water and directly injected onto the column. Quantitative transfers and injections were made throughout. Absolute recovery was calculated by correcting the peak heights from the extracts for losses in transfer and comparing them with those obtained from direct injections of the reference solutions.

Application

Two healthy female volunteers, 50 and 60 kg, fasted for 12 h, received oral 10 mg doses of metoclopramide HCl. The 50 kg subject received a tablet manufactured by Geneva Generics, Broomfield, CO, while the 60 kg subject received a tablet manufactured by Travenol Laboratories, Ascot Pharm. Div., Deerfield, IL. Blood samples were collected in polypropylene tubes containing 5% EDTA (100 µl EDTA per 5 ml blood) at time 0

and 0.5, 1, 1.5, 2, 3, 5, 7, 9, 12, 15 and 24 h post-dose. Separated plasma samples were stored at -20° C in polypropylene tubes until analysis.

The elimination half-life was calculated by least squares regression analysis using time points between 5 and 24 h after dosing. The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal rule from time = 0 to time = 24 and extrapolated to infinity using the elimination rate constant (20).

RESULTS

Fig. 1. shows typical chromatograms for drug-free human plasma (A) and plasma containing 100 ng/ml metoclopramide and 160 ng/ml internal standard (B). There was a small interference peak in the drug-free plasma that eluted just prior to the internal standard, but it did not pose a problem with peak height measurements. If necessary, interfering plasma components may be resolved from the metoclopramide or internal standard peak by varying the percentage of tetrahydrofuran in the mobile phase. Retention times for the internal standard and metoclopramide were 8.4 and 13.0 min, respectively. The limit of sensitivity for both the internal standard and metoclopramide was 0.5 ng, using the criterion of a signal-to-noise ratio of 3. Based on this information, along with a mean recovery of 90.1% and a sample volume of 0.5 ml, it can be estimated that the limit of sensitivity for metoclopramide is 2.5 ng/ml in plasma.

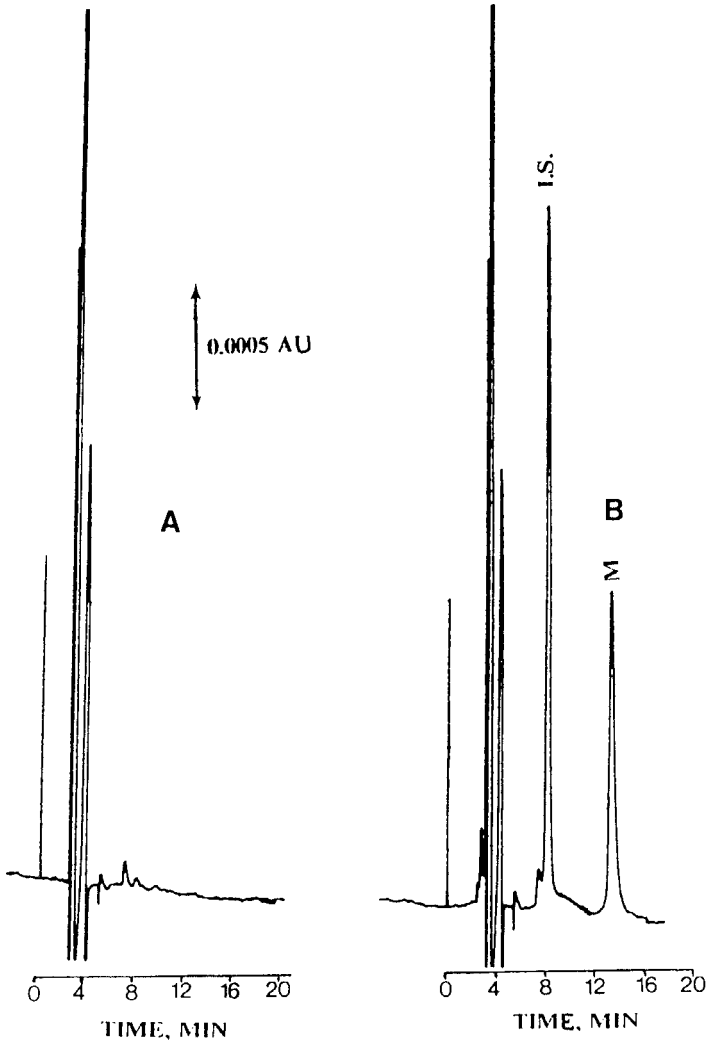

FIGURE 1. Typical chromatograms of drug-free plasma (A) and plasma containing 100 ng/ml of metoclopramide and 160 ng/ml of internal standard (B). Peaks: M = metoclopramide; I.S. = internal standard.

TABLE (I)

Retention Times for Selected Drugs. ND = not detected.

DRUG	RETENTION TIME (MIN)
Caffeine	ND
Cimetidine	ND
Clonidine	ND
Digoxin	ND
Disopyramide	ND
Furosemide	ND
Hydrochlorothiazide	ND
Ibuprofen	ND
Prednisone	ND
Verapamil	ND
Carbamazepine	4.3
Nadolol	4.7
Diazepam	4.9
Alprenolol	5.8
Propranolol	6.6
Nifedipine	6.9
Prochlorperazine	6.9
Spironolactone	7.1
Procainamide	7.2
Internal Standard	8.4
Captopril	8.5
Diltiazem	12.5
Prazosin	12.5
Metoclopramide	13.0
Dipyridamole	20.0

Table (I) lists the drugs tested for possible interference with the method and their respective retention times. Of the drugs tested by direct injection, captopril co-eluted with the internal standard and both diltiazem and prazosin co-eluted with the metoclopramide. The extraction procedure was not successful in eliminating these interfering drugs. The calculated sample

concentrations of metoclopramide at each of the three wavelengths were similar and helped to confirm the specificity of the method with regard to the metabolites.

Within-day precision, evaluated on the basis of variation in the peak height ratio, showed coefficients of variation (C.V.) of 3.1 and 2.5 % at metoclopramide concentrations of 20 and 150 ng/ml, respectively. Day-to-day precision, evaluated on the basis of measured plasma concentrations of the quality controls, showed mean (± S.D.) plasma concentrations of 21.3 ± 1.4 ng/ml and 148.5 ± 6.8 ng/ml for the 20 and 150 ng/ml quality controls, respectively. Day-to-day precision was also evaluated from variation in the slope of the standard curve over the 2 month period (C.V. = 3.7 %).

Metoclopramide extraction efficiencies from plasma (mean ± S.D.) were
89.1 % ± 3.1, at 20 ng/ml and 91.1 % ± 1.5 at 160 ng/ml. The mean ± S.D. extraction efficiency of the internal standard at 160 ng/ml was 93.1 % ± 1.6.

Fig. 2 shows plasma concentration-time curves for two normal subjects following single 10 mg oral doses of metoclopramide hydrochloride. The subject who received the Geneva Generics tablet had a peak plasma concentration of 78.1 ng/ml that occurred at 2 h following administration of the dose, an AUC of 880 (µg/L)h, an elimination half-life of 9.5 h and a 24 h plasma concentration of 11.5 ng/ml. The subject who received the

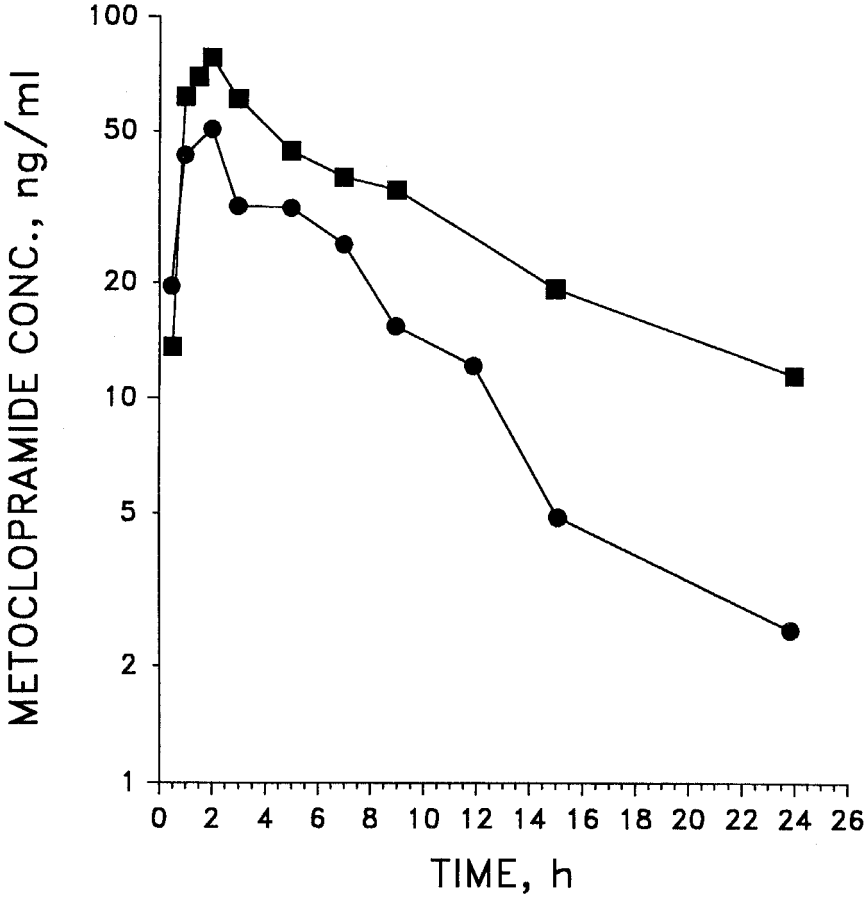

FIGURE 2. Plasma concentrations of metoclopramide at various times following the oral administration of a 10 mg tablet of metoclopramide hydrochloride to two healthy volunteers. Key: (■) 50 kg subject and (●) 60 kg subject.

Travenol Laboratories tablet had a peak plasma concentration of 50.6 ng/ml at 2 h post-dose, an AUC of 388 (μg/L)h, an elimination half-life of 5.1 h and a 24 h plasma concentration of 2.9 ng/ml. The assay of aqueous solutions of these tablets

showed that the difference in AUC was not due to differences in tablet contents.

DISCUSSION

The method presented here offers specificity and sufficient sensitivity for single-dose pharmacokinetic studies using as little as 0.25 to 0.5 ml of plasma. This is in contrast with previously published HPLC methods, which require 1 to 5 ml of sample for the same level of sensitivity (8-19). Greater sensitivity is achieved by the use of gas chromatographic methods (5-7), however these methods involve time-consuming derivatization and extraction procedures. A unique automated trace-enrichment method was developed by Fairhead et al. (12) that allows direct injection of the plasma samples onto the HPLC column, but requires three pumps and two columns. Our sample preparation procedure is simple, and the equipment used is likely to be available in most clinical laboratories.

A big difference in elimination half-lives and AUC's was observed between the two subjects in the present study. The half-life and AUC for the subject who received the tablet manufactured by Travenol Laboratories were in agreement with published values (21 and 22), while those for the subject who received the Geneva Generics tablet were much larger than the published values. These differences were not due to differences in tablet content or

nonspecificity with respect to metabolites. Differences between these two normal subjects in bioavailability and/or clearance of metoclopramide must therefore be considered. The long half-life in one subject suggests that a lower clearance or higher volume of distribution may be involved.

The oral bioavailability of metoclopramide is reported to range from 32 to 100% among normal subjects (14,16,21,22 and 23), and intersubject variations in peak plasma concentration following oral doses of metoclopramide as great as ten-fold have been reported (16 and 23). It has been suggested that this variablility may be due to differences among subjects in first-pass metabolism (16). Bateman et al. (21) suggested that wide differences in bioavailability of metoclopramide in man may contribute to the unpredictable occurrence of side effects. Our data support these findings. Our 60 kg subject experienced no side effects following the 10 mg oral dose of metoclopramide hydrochloride, however our 50 kg subject experienced facial numbness at 2 h post-dose. The considerably higher peak plasma concentration for the 50 kg subject may be the cause of the side effect and lend support for the hypothesis of Bateman et al. (21).

In conclusion, the present reversed-phase HPLC method requires small sample volumes and is sensitive enough for use in single-dose pharmacokinetic studies.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. James Visconti for financial support in developing this method.

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