Journal of Chromatography, 276 (1983) 319—328
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1740

ELECTRON-CAPTURE DETERMINATION OF METOCLOPRAMIDE IN BIOLOGICAL FLUIDS USING FUSED SILICA CAPILLARY COLUMNS

APPLICATION TO PLACENTAL TRANSPORT STUDIES IN SHEEP AND HUMANS

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(First received November 15th, 1982; revised manuscript received March 31st, 1983)

SUMMARY

An electron-capture gas—liquid chromatographic assay for metoclopramide using cross-linked fused silica capillary columns which provides improved selectivity and sensitivity is reported. A 25 m × 0.31 mm fused silica capillary column was used for all analyses. Linearity was observed in the range of 4—40 ng of metoclopramide base per 0.25—0.5 ml of plasma. This represents from ca. 0.9—9.0 pg at the detector employing a split ratio of 30:1 and an injection volume of 2 μl. Applicability of the method is demonstrated by the analysis of human and sheep plasma (maternal, fetal and neonatal) from metoclopramide placental transfer studies.

INTRODUCTION

Metoclopramide (MCP), 4-amino-5-chloro-2-methoxy-N-(2-diethyl-amino-ethyl)benzamide (I), is a procainamide analogue used clinically in the treatment of nausea and vomiting and to promote gastric emptying [1—7].

0378-4347/83/$03.00

A number of analytical methods have been reported for the measurement of MCP in biological fluids including colorimetry [8], thin-layer chromatographic photodensitometry [9, 10] and high-performance liquid chromatography (HPLC) [11—14]. Most, however, lack specificity [8—10] and require the extraction of large plasma volumes (2—5 ml) [8—14] in order to achieve adequate sensitivity in the low nanogram range for thorough pharmacokinetic studies. Several electron-capture gas—liquid chromatographic (GLC—ECD) methods have also been developed. These do provide good sensitivity following the extraction of small plasma volumes (<1.0 ml) and have been used for pharmacokinetic studies in small animals such as rats [15] and in man [16, 17]. While sensitive, these packed column GLC—ECD methods, like HPLC [13], demonstrate considerable potential for interference from endogenous substances when used at higher sensitivities as well as from other drugs in clinical studies.

The aim of the present paper is to describe a GLC—ECD procedure whereby better resolution and high sensitivity have been achieved using recently introduced nonreactive cross-linked fused silica capillary columns.

EXPERIMENTAL

Materials

Metoclopramide (MCP), 4-amino-5-chloro-2-methoxy-N-(2-diethyl-aminoethyl)benzamide monohydrochloride monohydrate ($MCP \cdot HCl \cdot H_2O$), (lot Nos. A105 and 9207) and $MCP \cdot HCl \cdot H_2O$ injectable, 5 mg/ml (Reglan® Injectable, 2 ml ampule, lot No. 80246, for sheep study) were supplied by A.H. Robins, Montreal, Canada. $MCP \cdot HCl \cdot H_2O$ injectable 5 mg/ml (Maxeran® Injectable, 2 ml ampule; Nordic Pharmaceuticals, Montreal, Canada, for human study) was supplied by Pharmacy, The Vancouver General Hospital, Vancouver, Canada. Maprotiline, N-methyl-9-10-ethanoanthracene-9(10H)propanamine (lot No. A11663096472-0) was supplied by Ciba Pharmaceuticals, Mississauga, Canada. Heptafluorobutyric anhydride (HFBA) sequanal grade and triethylamine (TEA) were purchased from Pierce, Rockford IL, U.S.A. Benzene and toluene (distilled in glass) were purchased from Caledon Laboratories, Georgetown, Canada. Solutions of 1 *N* and 5 *N* sodium hydroxide, 1 *N* hydrochloric acid and 4% ammonium hydroxide were prepared from ACS reagent-grade chemicals (American Scientific and Chemical, Seattle, WA, U.S.A.). Deionized distilled water was used in the preparation of all reagents, stock solutions and throughout analysis.

Instrumentation and chromatographic conditions

A Model 5840A Hewlett-Packard gas chromatograph equipped with a ^{63}Ni electron-capture detector (ECD) and a Model 18835B capillary inlet system was used for all analyses. A Model 18850A Hewlett-Packard integrator system was used for peak area integration and quantitation.

A 25 m × 0.31 mm I.D. cross-linked SE-54 fused silica capillary column (5% phenylmethylsilicone, Ultra No. 2, film thickness 0.15 μm, siloxane-deactivated; Hewlett-Packard, Avondale, PA, U.S.A.) was used for all plasma analyses.

The split injection mode employing a silanized, unpacked Jennings split liner (Hewlett-Packard) was used, with a 2-μl sample being injected. A fused silica insert (78 mm × 2 mm I.D.) may be used interchangeably with the Jennings split liner provided that it is packed with a tight silanized glass wool plug extending 1 cm from the base to 3 cm from the top. This is illustrated by the close agreement of the following respective calibration curve parameters obtained by injecting the same calibration curve samples using first the unpacked Jennings liner ($y = 0.0241x + 0.0086$; $r = 0.9996$) and immediately followed using a packed fused silica insert ($y = 0.0243x + 0.004$; $r = 0.9997$). A more complete evaluation of these two split liner configurations and their use in plasma MCP analysis has been made [18].

The operating conditions for routine analysis were: injection temperature, 220°C; column temperature, 235°C; detector temperature (ECD), 350°C; carrier gas (hydrogen, ultra high purity) flow-rate 1.0 ml/min ($\bar{\mu}$ = 63 cm/sec); split vent flow-rate, 30 ml/min (split ratio 30:1); inlet pressure 0.65 bar; septum purge flow-rate, 1.5 ml/min; make-up gas (argon—methane, 95:5) flow-rate, 60 ml/min.

Stock solutions

Metoclopramide hydrochloride (0.04 μg/ml, equivalent to base) and the internal standard, maprotilene hydrochloride (II) (0.4 μg/ml, equivalent to base), were prepared by dissolving these compounds in water. The solutions were stored at 4°C, following preparation, for up to three weeks.

Extraction and derivative formation

To 0.25—0.5 ml of plasma obtained from MCP treated parturient human patients or pregnant sheep were added 0.1 ml of maprotiline hydrochloride and 0.5 ml of 1 *N* sodium hydroxide in a 15-ml PTFE-lined screw-capped disposable culture tube. The final volume was adjusted to 2.1 ml (pH ≈ 14) with water. Benzene (6 ml) was added and the aqueous phase was extracted by shaking for 20 min on a rotary shaker (Labquake Tube Shaker, Model 415-110; Lab Industries, Berkeley, CA, U.S.A.). After centrifugation at 2300 *g* for 10 min, 5 ml of the organic phase were removed and back-extracted for 20 min with 2 ml of 1 *N* hydrochloric acid. The samples were centrifuged for 5 min and the organic layer aspirated and discarded. The remaining aqueous layer was washed with two 4-ml aliquots of benzene, alkalinized by adding 0.5 ml of 5 *N* sodium hydroxide (pH ≈ 14) and then re-extracted for 20 min with 6 ml of benzene. Following centrifugation at 2300 *g* for 5 min, 5 ml of the organic layer were removed and dried under a gentle stream of nitrogen in a 40°C water bath. The residue was reconstituted to a volume of 200 μl with 150 μl of toluene and 50 μl of 0.05 *M* TEA in toluene. A 20-μl volume of HFBA was added, the sample vortexed and placed in an oven at 55°C for 60 min. After cooling to room temperature, the excess derivatizing agent was removed by hydrolysis with 0.5 ml of water (vortex for 10 sec) and neutralizing with 0.5 ml of 4% ammonium hydroxide (vortex for 10 sec). Following centrifugation at 750 *g* for 1 min the derivatized organic layer was immediately transferred to a clean, dry 15-ml culture tube. Aliquots of 2 μl were used for GLC—ECD analysis.

Preparation of the calibration curve

A 0.5-ml sample of blank human or sheep plasma was spiked with serial amounts (4, 8, 16, 24, 32, or 40 ng) of the prepared metoclopramide hydrochloride stock solution and then 0.1 ml of the maprotiline hydrochloride solution (0.4 μg/ml) and 0.5 ml of 0.5 *N* sodium hydroxide were added. The aqueous phase was adjusted to a total volume of 2.1 ml (pH ≈ 14) with water and the samples extracted and derivatized as described above. Quantitative estimation of MCP in plasma was accomplished by plotting the area ratios of the heptafluorobutyryl (HFB) derivatives of MCP and maprotiline against the range of indicated MCP concentrations.

Human experiments

The placental transfer of MCP from mother to fetus was examined in normal healthy women in labour who required MCP as a pre-anaesthetic medication prior to undergoing elective Caesarian section.

MCP was administered by intravenous (i.v.) infusion over 1—2 min at the rate of 0.15 mg/kg. All patients received a standardized general anaesthetic regimen of thiopental, halothane, *d*-tubocurare and succinylcholine. One-point blood samples were drawn at delivery from a maternal vein (MV) and from a double-clamped section of the umbilical cord [umbilical vein (UV), umbilical artery (UA)]. The blood samples were immediately centrifuged, the plasma removed and stored at −20°C until analysis.

Sheep experiments

A preliminary experiment on the placental transfer of MCP was carried out on a pregnant ewe with vascular catheters chronically implanted in the mother and fetus [19]. A 10-mg i.v. dose of MCP was administered over a 3-min period via the implanted maternal jugular venous catheter. Blood samples for MCP determination were simultaneously withdrawn from implanted maternal femoral arterial and fetal venous (lateral tarsal vein) catheters at −5, 1, 5, 15, 30, 60, 90, 120, 150, and 180 min. Normally the fetal femoral arterial site is used for sampling but this catheter was not patent for this preliminary study. The blood samples were immediately centrifuged, the plasma removed and stored at −20°C until analyzed.

RESULTS

Application of the developed method to sheep plasma analysis is shown in Fig. 1. No interfering peaks from endogenous plasma components were observed in the plasma extracts (Fig. 1a). Peaks with retention times of 3.20 and 4.57 min were the HFB derivatives of MCP and maprotiline, respectively (Fig. 1b). Essentially identical chromatograms were obtained with blank and MCP-spiked human plasma.

The data for a representative calibration curve used in the quantitation of MCP in maternal and fetal sheep plasma are presented in Table I. Linearity was observed over the concentration range studied (4—40 ng/ml) with the line of best fit through the data points being described by $y = 0.0254x - 0.0023$ with a correlation coefficient of $r = 0.9993$. The precision of the assay for this

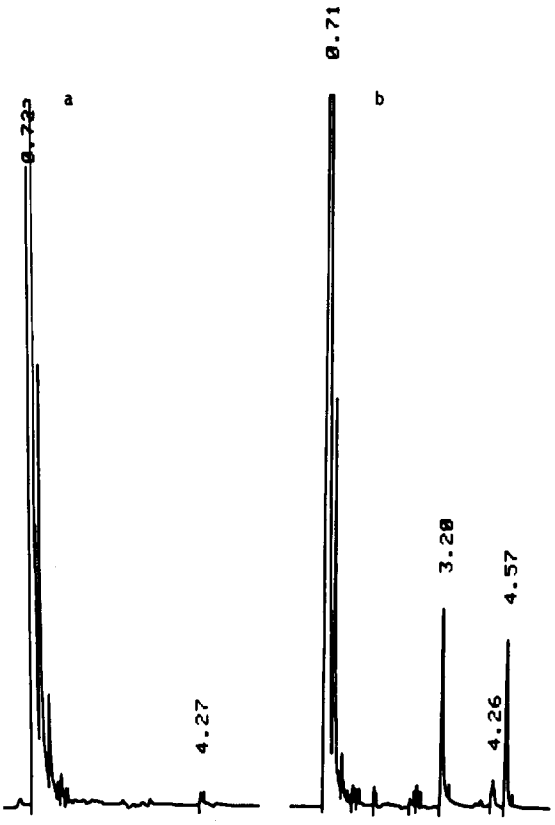

Fig. 1. Representative chromatograms obtained from blank (a) and MCP-spiked (b) plasma extracts on the 25-m SE-54 fused silica column. The spiked sample contained MCP, 139.58 pg/μl (40.20 ng/ml) and maprotiline 138.89 pg/μl (2-μl injection; split ratio, 30:1).

TABLE I

CALIBRATION CURVE DATA FOR SHEEP PLASMA

n = 2, duplicate injections.

MCP (ng/ml)	A.R.* (± S.D.)	C.V. (%)
4.02	0.1055 ± 0.0114	10.85
8.04	0.1893 ± 0.0159	8.41
16.08	0.4263 ± 0.0119	2.80
24.12	0.5939 ± 0.0235	3.95
32.16	0.8094 ± 0.0178	2.20
40.20	1.0256 ± 0.0127	1.24

*Area ratio, drug/internal standard ± 1 S.D.

calibration curve is included in Table I. In general, a range of coefficients of variation (C.V.) from 2—10% was observed for all calibration curves (human or sheep plasma) with an average of 5—6%. This is illustrated by a more extensive study of C.V. values in Table II for two extreme calibration curve MCP concentrations.

TABLE II

REPRODUCIBILITY STUDY

A fused silica insert packed with a "tight" silanized glass wool plug was used in this study.

Sample*	No. of samples**	A.R.***	C.V. (%)
8.02	6	0.3416 ± 0.0159	4.65
40.20	5	1.2152 ± 0.0815	6.71

*MCP·HCl·H_2O concentration (equivalent to MCP base), ng/ml.
**Triplicate injections.
***Mean area ratio (HFB-MCP/HFB-maprotiline) ± 1 S.D.

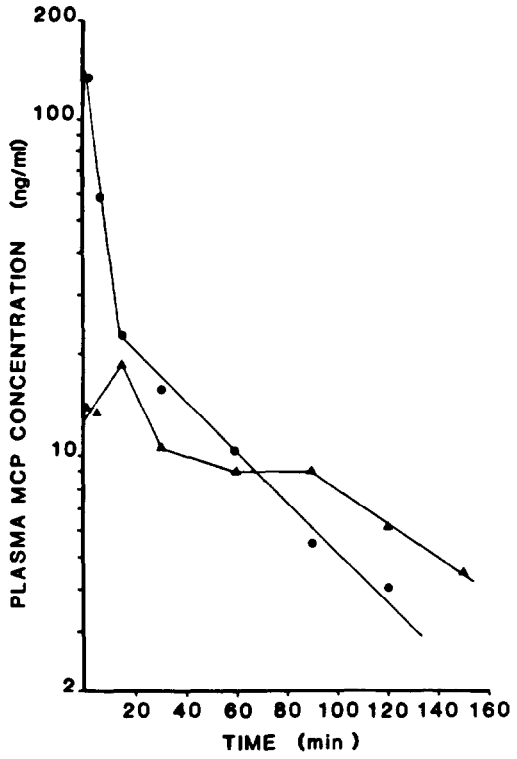

Fig. 2. A semi-logarithmic plot of the plasma profile of MCP in a pregnant ewe following a 10-mg i.v. dose (●——●) maternal plasma levels, (▲——▲) fetal plasma levels. The terminal elimination half-lives in the ewe and fetus were calculated to be 40 and 54 min, respectively.

Semilogarithmic plots of the determined MCP concentrations in maternal and fetal sheep plasma against time following administration of the 10-mg i.v. dose to the ewe are shown in Fig. 2. Maternal plasma MCP elimination was observed to follow a biexponental decay described by the following equation: $C_p = Ae^{-\alpha t} + Be^{-\beta t}$ where C_p is the plasma concentration at time t. A and B are the intercepts of the α and β phases, respectively. The parameters α and β represent the distribution and terminal elimination rate constants, respectively. The half-life of the α phase ($t_{1/2\alpha}$) was calculated to be 6 min, indicating rapid

TABLE III

HUMAN PLASMA ANALYSIS DATA

Patient code	Maternal		MCP i.v. dose (mg/kg)	MV sample*, elapsed time (min)	Umbilical samples*, elapsed time (min)	Plasma MCP concentration** ng/ml)		
	age (years)	wt. (kg)				MV	UV	UA
2S	28	68	0.15	36	29	61.16±0.22	18.82±0.13	18.28***
9S	23	80	0.15	21	30	63.01±3.12	38.75±3.27	32.78***

*Time elapsed between administration of MCP and sampling and delivery, respectively.
**Mean of two separate determinations ± 1 S.D.
***Single determination.

distribution of MCP following an i.v. injection. A maternal elimination half-life ($t_{½\beta}$) of 40 min was calculated for the terminal phase.

Transfer of MCP to the fetus was observed to be rapid with measurable concentrations at 1 min post-injection to the ewe. Plasma concentrations peaked in the fetus at 20 min and were observed to exceed maternal concentrations at 90 min. An elimination half-life ($t_{½}$) of 54 min was calculated for MCP in the fetus.

Data from preliminary studies of the placental transfer of MCP in human subjects are shown in Table III. In both instances, the MV plasma concentrations were greater than those measured in either UV or UA samples.

DISCUSSION

The high efficiency associated with glass open tubular (capillary) columns has been applied for many years to resolve complex mixtures. Analysis of environmental pollutants in air and water and use in the petroleum, food, flavor and fragrance industries is widespread. More recently this technology has been applied to the analysis of biological samples for metabolic profiling [20—22] of certain disease states, the measurement of human urine and plasma drug concentrations [23—25] and the determination of drug pharmacokinetics in man [23, 25]. The recent introduction of inert fused silica columns [20] facilitates reliable and reproducible analysis of a variety of underivatized (acidic, basic, neutral, polar) drugs [26]. Previously such procedures for underivatized substances using more reactive glass columns were often only marginally successful unless the surface was carefully deactivated.

Before a placental transfer study of MCP in hospitalized human patients undergoing multiple therapy during general or spinal anaesthesia could be undertaken, an analytical method with high separation efficiency was required, due to potential interference from structurally related compounds (e.g., local anaesthetics). In addition, a planned study of this drug's placental transfer in sheep required an assay with improved sensitivity such that the analysis of small plasma volumes (0.25—0.5 ml) was possible after serial blood sampling (1.0 ml) of the catheterized fetus.

A packed column GLC—ECD assay method developed for use in our laboratory [16] demonstrated the potential for interference from other drugs as well as from endogenous plasma components during trace drug analysis. On this basis then, the development of a capillary column GLC—ECD method was

undertaken that would provide both improved sensitivity and selectivity.

Initial tests conducted with glass capillary columns (OV-225, Silar 10-C) provided generally unsatisfactory results, with broad severely-tailing MCP peaks being obtained, possibly due to incomplete surface deactivation [20, 22].

Two Carbowax deactivated methylsilicone fluid fused silica columns were tested following experimentation with glass columns. Both provided good resolution of MCP from plasma components as well as a marked improvement in peak shape. These columns are, however, subject to phase stripping with aromatic solvents. Hexane and isooctane are recommended solvents for capillary column GLC—ECD analysis, however they provided extremely variable chromatographic results in this study. Both lack sufficient solvent strength to adequately extract and solubilize MCP and their use did not permit quantitation in the desired 4—40 ng/ml MCP concentration range.

Polysiloxane-deactivated fused silica columns with cross-linked phases (methylsilicone, phenylmethylsilicone) exhibit a high degree of inertness, excellent thermal stability (maximum temperature 325—350°C) and very low solvent extractability [20, 26]. This technological advance virtually eliminates phase stripping thus allowing the use of aromatic solvents such as benzene and toluene which possess good extractability and solubility characteristics towards MCP. The use of a cross-linked SE-54 fused silica column coupled with toluene as the derivatizing and injection solvent permitted quantitation in the range of 4—40 ng/ml MCP.

Resolution of MCP and maprotiline is excellent as illustrated in Fig. 1b; no interference from endogenous plasma (sheep) components was observed (Fig. 1a). Analysis times are short and repeat injections of plasma extracts every 5—6 min are possible without interference from late eluting plasma components. An identical chromatographic pattern was obtained with blank and clinical patient plasma; again no interference from plasma components or from the drugs used during general anaesthesia (viz., thiopental, halothane) was observed. Diazepam, a commonly prescribed therapeutic agent, and a congener, prazepam, have also been shown not to interfere. The antiarrhythmic procainamide, a close structural analogue of MCP, also shows no interference eluting well before MCP.

A series of samples containing 20 ng of MCP and 40 ng of maprotiline were incubated in the presence of the catalyst TEA, for periods ranging from 0—180 min to determine the optimum time required for reaction of both drugs with HFBA. No significant differences were observed in area ratios of MCP to internal standard between 20—180 min; a 60-min reaction time was subsequently chosen to ensure complete derivatization.

Derivatized samples have been found to be stable for at least four days when stored at −4°C with repeat injections showing no significant decline in area ratios over this time period.

The developed assay method has been found to show good linearity over the 4—40 ng/ml concentration range studied with replicate calibration curves having correlation coefficients of at least $r = 0.989$. This represents ca. 0.9—9 pg at the detector employing a 30:1 split ratio (2 μl injection) and demonstrates the sensitivity of the method.

Within-run precision (repeatability) of a representative calibration curve is shown in Table I with coefficients of variation ranging from 1.24—10.85%. Between-run precision studies (reproducibility) have shown the method to be reliable and reproducible with average coefficients of variation between 5 and 6% (Table II).

The plasma concentration data in Table III illustrate the applicability of the method to the analysis of human plasma samples in the clinical setting. The low sensitivity limit of the method (ca. 4 ng/ml) has also been found to be suitable for a preliminary pharmacokinetic assessment of plasma MCP concentration vs. time profiles in chronically catheterized maternal and fetal sheep (Fig. 2).

In summary, the developed capillary GLC—ECD method:

(1) provides a short analysis time; a desired property for a routine analytical method;

(2) is reliable, reproducible and offers improved sensitivity over the previous packed column GLC—ECD method in use in our laboratory [16];

(3) has been applied to patient samples in the clinical setting without interference from the other drugs used during general anaesthesia in this study;

(4) has been used to demonstrate placental transfer of MCP both in humans and in sheep, which has not been reported to date [5];

(5) has shown pharmacokinetic applicability to the study of MCP placental transfer in sheep.

More extensive evaluations of the pharmacokinetics of MCP placental transfer in human patients as well as in pregnant and non-pregnant sheep are currently underway in our laboratory using the method described in this paper.

ACKNOWLEDGEMENTS

This work was supported by the Medical Research Council of Canada and the British Columbia Medical Services Foundation. The authors wish to thank Ms. Tracy Lakevold for her capable assistance in the preparation of this manuscript.

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