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Determination of metoclopramide and two of its metabolites using a sensitive and selective gas chromatographic–mass spectrometric assay

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Abstract

A modified gas chromatographic–mass spectrometric (GC–MS) assay has been developed to quantitate metoclopramide (MCP) and two of its metabolites [monodeethylated-MCP (mdMCP), dideethylated-MCP (ddMCP)] in the plasma, bile and urine of sheep. The heptafluorobutyryl derivatives of the compounds were formed and quantitated using electron-impact ionization in the selected-ion monitoring mode (MCP, *m/z* 86, 380; mdMCP, *m/z* 380 and ddMCP, *m/z* 380). No interference was observed from endogenous compounds following the extraction of various biological fluids obtained from non-pregnant sheep. Sample preparation has been simplified and the method is more selective and sensitive (2 fold) than our previous assay using electron-capture detection. The limit of quantitation for MCP, mdMCP and ddMCP was 1 ng/ml in plasma, urine and bile, requiring 0.5 ml of sample. This represents 2.5 pg of the analytes at the detector. The standard curves were linear over a working range of 1–40 ng/ml. Absolute recoveries in plasma ranged from 76.5–94.7%, 79.2–96.8%, 80.3–102.2% for MCP, mdMCP and ddMCP, respectively. In urine, recoveries ranged from 56.5–87.8%, 61.5–87.5%, 62.6–90.2% for MCP, mdMCP and ddMCP, respectively. Recoveries in bile ranged from 83.5–100.9%, 78.5–90.5%, 66.9–79.2% for MCP, mdMCP and ddMCP, respectively. Overall intra-day precision ranged from 2.9% for MCP in plasma to 12.6% for mdMCP in bile. Overall inter-day precision ranged from 5.9% for MCP in urine to 14.9% for ddMCP in bile. Bias was the greatest at the 1 ng/ml concentration in all biological fluids ranging from a low of 2.4% for

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mdMCP in plasma to a high of 11.9% for ddMCP in urine. Applicability of the assay for pharmacokinetic studies of MCP, mdMCP and ddMCP in the plasma and urine of a non-pregnant ewe is demonstrated.

1. Introduction

Metoclopramide (MCP) is a dopamine receptor antagonist which has the properties of increasing lower esophageal sphincter pressure, preventing nausea and vomiting and promoting gastrointestinal motility. It has several clinical indications and diagnostic uses, which along with its proposed mechanisms of action and side effects have been extensively reviewed [1–11].

MCP concentrations in pharmaceutical dosage forms and various human and animal biological fluids have been determined using several assay methods including: thin-layer chromatography [12,13], gas chromatography (GC) with nitrogen–phosphorus detection [14] and GC with mass spectrometric [15] and electron-capture detection [16–22], high-performance liquid chromatography (HPLC) with fluorescence [23,24] and ultraviolet detection [25–32], potentiometry [33] and radioimmunoassay [34]. Techniques employing thin-layer chromatography lack both sensitivity and specificity limiting their use. The potentiometric method is sensitive, but can be used only for the determination of MCP in pharmaceutical preparations and in pure solutions. Radioimmunoassay also provides excellent sensitivity, however, the distinct possibility of cross-reactivity with MCP metabolites limits its application. The HPLC methods in general are convenient as minimal sample preparation is required, however, to obtain sensitivity in the low nanogram range (i.e. < 5 ng/ml) plasma volumes of 1 ml and injection of large sample volumes into the chromatograph are necessary. Because of this, these methods are not useful in studies involving extensive serial blood sampling in neonates or small animal models such as fetal sheep. Of the remaining methods, GC coupled with electron-capture detection (ECD) and capillary columns would appear to provide optimal sensitivity and selectivity in situations where small biological fluid sample volumes, in particular plasma (viz. ≤ 0.5 ml), are available.

We have been examining the pharmacokinetics of MCP in biological fluids from humans and animals extensively for several years using a GC-ECD assay employing fused-silica capillary columns and the split [21] and splitless [22] modes of sample introduction. Although these methods have provided good sensitivity and selectivity, extensive sample preparation was required to remove interfering endogenous compounds. In the present report we describe a GC–MS assay using selected-ion monitoring (SIM) with simplified sample preparation and improved sensitivity and selectivity. Applicability of the assay is illustrated for the measurement of MCP and two of its metabolites (monodeethylated-MCP [mdMCP]; dideethylated-MCP [ddMCP]) in plasma, urine and bile obtained from sheep. While the monodeethylated metabolite has been reported to be active, although less so than the parent compound [35], the aim of developing an analytical method for mdMCP and the dideethylated metabolite was to account for the dose of MCP administered on a mass balance basis.

2. Experimental

2.1. Materials

Metoclopramide monohydrochloride monohydrate (MCP · HCl), dideethylated metoclopramide monohydrochloride (ddMCP · HCl) and metoclopramide monohydrochloride monohydrate injectable 5 mg/ml (Reglan Injectable) were obtained from A.H. Robins Research Laboratories (Richmond, VA, USA). Monodeethylated metoclopramide free base and monohydrochloride (mdMCP · HCl) were synthesized in our laboratory. The internal standard, 4-amino-2-(2-butanon-3-yl)oxy-5-chloro-N-[(2-diethylamino)ethyl] benzamide monohydrochloride (BMY · HCl) was supplied by Bristol-Myers Squibb (Wallingford, CT, USA). The silica gel

(Silica Gel 60, 230–400 mesh) was obtained from BDH (Toronto, Ont., Canada). Sodium hydroxide (ACS Certified) was obtained from Fisher Scientific (Nepean, Ont., Canada), dichloromethane and toluene (distilled in glass) were purchased from Caledon Laboratories (Georgetown, Ont., Canada). Triethylamine (TEA) (Sequanal Grade) and heptafluorobutyric acid anhydride (HFBA) were purchased from Pierce (Rockford, IL, USA). HPLC grade water used in the preparation of stock solutions and during sample extraction was obtained from a Milli-Q Water System (Millipore, Bedford, MA, USA).

2.2. Synthesis of mdMCP

The synthesis of mdMCP was based on the procedure used for the synthesis of metoclopramide [36] substituting mono-N-ethyl-ethylenediamine as the starting material. A crystalline product was obtained that was composed of two compounds. In order to purify the mdMCP, the crystalline product was dissolved in chloroform and applied to a silica column which was prepared in our laboratory (Silica Gel 60, 230–400 mesh, 20 cm × 2.5 cm I.D.). It was eluted gradually with chloroform, ethyl acetate, and 10% triethylamine in ethyl acetate and finally with methanol–triethylamine–ethyl acetate (10:10:80, v/v). The product eluted from the column with the final solvent mixture. The recovered product was re-chromatographed if found to be still contaminated with the N,N′-diacylated ethylenediamine (M.W. 454). The structure of mdMCP was confirmed with thermospray mass spectrometry showing an intense M^+ ion at m/z 272.

2.3. Gas chromatography–mass spectrometry

A Hewlett-Packard 5890 Series II gas chromatograph equipped with a Hewlett-Packard 7673 automatic sampler (Hewlett-Packard, Avondale, PA, USA) was used for the analyses. Samples were introduced through a Thermogreen LB-2 11 mm septum (Supelco, Bellefonte, PA, USA) into a split/splitless capillary injection port system using an 80 × 2 mm I.D. quartz glass split/splitless injection port liner with a 3–4 mm silanized glass wool plug in its center. Sample injections onto the GC were made in the splitless mode. A crosslinked 5% phenylmethyl silicone fused-silica capillary column (25 m × 0.2 mm I.D., 0.33 μm film thickness; Hewlett-Packard HP Ultra 2) was used for analysis. Operating conditions for routine use were: injection port temperature, 260°C; initial oven temperature, 100°C for 0.8 min, increased to 270°C at a rate of 40°C/min and held for 5.5 min, then increased to a final temperature of 290°C at 70°C/min and held for 2 min. Helium (Ultra High Purity, Matheson Gas Products, Edmonton, Alta., Canada) was used as carrier gas with a total inlet flow of 30 ml/min and septum purge 0.8 ml/min. The column head pressure was 70 kPa which provided a 1 ml/min column flow. For detection, a Hewlett-Packard 5971A mass selective detector (MSD) was used in the electron-impact (EI) ionization mode with 70 eV ionization energy and 300 μA emission current. The transfer line temperature was kept at 300°C. To increase sensitivity, the MSD was manually tuned to the ions of m/z 100, 264 and 414 using perfluoro-tri-*n*-butylamine. Chromatograms were generated both in full-scale mass scanning (SCAN) and selected-ion monitoring (SIM) modes. MCP and its metabolites were quantitated in the SIM mode by detecting the total ion current of m/z 380 for mdMCP and ddMCP (group 1), m/z 86 and 380 for MCP and m/z 86 and 366 for BMY (group 2). The dwell-time was set at 700 ms. per group (low resolution off) which provided 1.37 scan cycles/s for group 1 and 0.697 scan cycles/s for group 2.

2.4. Sheep experiments

Seven non-pregnant sheep of Suffolk or Dorset breeds were used in the experiments. The surgical procedure used in the catheterization of the sheep is described elsewhere [37]. Metoclopramide (Reglan Injectable) was administered to the ewe as a 40-mg intravenous bolus over 1 min via the implanted femoral venous catheter. Blood samples for MCP determination were

collected from a femoral arterial catheter at −5, 5, 10, 15, 30, 45, 60, 75 min, 1.75, 2.5, 3.5, 4.5, 5.5, 7, 9, 11, 24, 36 and 48 h. Cumulative urine samples were obtained at −30–0, 0–30, 60–90 min, 1.5–2, 2–3, 3–4, 4–5, 5–6, 6–8, 8–10, 10–12, 12–24, 24–36, 36–48 and 48–72 h. Cumulative samples of bile were obtained from the catheterized bile duct over various time intervals in two of the animals only. Plasma, urine and bile samples were stored at −20°C until analysis.

2.5. Sample preparation

The extraction procedure is based on our previously published method [19] but with substantial modifications. Plasma, urine or bile samples (0.05–0.5 ml) were pipetted into clean 15-ml borosilicate Kimax culture tubes and 0.3 ml of BMY internal standard solution (100 ng/ml) and 0.5 ml of a 1 *M* NaOH solution were added and the mixture adjusted to a final volume of 2.2 ml with HPLC grade water. Then 6.0 ml of dichloromethane was added, the tubes closed with screw caps lined with polytetrafluoroethylene (PTFE) and mixed on a Labquake rotary shaker (Labindustries, Berkeley, CA, USA) for 20 min. Any emulsion formed during mixing was broken by freezing the samples for 30 min at −10°C followed by centrifugation for 10 min at 3000 *g* with an IEC HN-SII centrifuge (Damon/IEC Division, Needham Heights, MA, USA). Following centrifugation the upper aqueous layer was aspirated and discarded. The remaining organic phase was transferred to a clean 15-ml tube and evaporated to dryness using a Savant AS 290 Automatic Speedvac sample concentrator (Savant Instruments, Farmingdale, NY, USA). The dried residues were reconstituted with 0.8 ml of 0.0125 *M* TEA in toluene and 20 μl of HFBA was added. After incubating the samples for 60 min at 54°C in an Isotemp 500 Series Model 526G oven (Fisher Sci., Nepean, Ont., Canada), they were cooled to room temperature and the excess HFBA removed by mixing first with 0.5 ml of HPLC grade water for 20 s followed by 0.5 ml of 4% NH_4OH for 20 s using a Maxi Mix Model M-16715 test-tube shaker (Sybron Corporation, Dubuque, IA, USA). The tubes were centrifuged for 2 min at 3000 *g* and the upper toluene layer transferred to 0.2-ml borosilicate glass autosampler vial inserts and 2 μl were injected onto the gas chromatograph.

2.6. Calibration curve

Aqueous stock solutions of MCP·HCl, mdMCP·HCl, ddMCP·HCl and BMY·HCl were prepared in HPLC grade water. The weights were normalized to free base resulting in a final concentration of 50 ng/ml for MCP, mdMCP, ddMCP and 100 ng/ml for BMY. A 0.3-ml aliquot of blank plasma, urine or bile was pipetted into duplicate sets of 15-ml culture tubes and spiked with 1, 2, 4, 8, 16, 24, 32, or 40 ng/ml MCP, mdMCP and ddMCP by adding appropriate amounts of the prepared stock solutions. A 0.3-ml volume of the internal standard solution was then added and the samples were processed as described above. The quantitation of MCP, mdMCP and ddMCP was accomplished, using weighted linear regression analysis (weighting factor = $1/Y$), by plotting the area ratios of the heptafluorobutyryl derivatives of MCP, mdMCP, ddMCP to BMY versus added drug concentrations.

2.7. Recovery studies

Using the aqueous stock solutions of MCP·HCL, mdMCP·HCl and ddMCP·HCl, aliquot amounts were pipetted into clean sets of 15-ml culture tubes in the concentrations of the calibration curve. No internal standard was added. The tubes were made up to the final volume of 1 ml with HPLC grade water and dried down in the sample concentrator. The residues were reconstituted with 0.8 ml of 0.0125 *M* TEA in toluene and 20 μl of HFBA was added. Following this step, they were processed according to the regular procedure. Into another series of 15-ml tubes, 0.3 ml of blank plasma, urine or bile was pipetted and spiked with the same amounts of MCP, mdMCP and ddMCP as the previous sets of tubes. No internal standard was

added and the samples underwent a regular extraction procedure. The extraction recoveries were determined by comparing the peak-area counts of the heptafluorobutyryl derivatives of MCP, mdMCP and ddMCP of the extracted samples to the peak area counts of the un-extracted ones.

2.8. Precision and accuracy

Intra-day precision

On the same day, five calibration curves for MCP, mdMCP and ddMCP were prepared in plasma, urine and bile and assayed with the method described above. Weighted linear regression analyses were performed in each case and the slopes were compared by determining the coefficient of variation (C.V.).

Inter-day precision

One calibration curve for MCP, mdMCP and ddMCP was prepared in plasma, urine and bile every day for five consecutive days and assayed on the day of preparation with the method detailed above. A weighted linear regression analysis was performed in each case and the slopes were compared by determining the coefficient of variation (C.V.).

Accuracy

Ten calibration curves for MCP, mdMCP and ddMCP were prepared in plasma, urine and bile, and assayed with the above method. Weighted linear regression analyses were carried out in each case and the accuracy was estimated by determining the percentage difference (bias) of the mean measured concentrations from the mean added concentrations throughout the calibration curve.

3. Results and discussion

3.1. Extraction procedure/GC-MSD

The objective of the current study was to modify our existing GC-ECD method [21,22] and apply it to the quantitation of MCP and two of its metabolites, mdMCP and ddMCP, in biological fluids from sheep. In our previous method a two-step liquid–liquid extraction with benzene was used to extract MCP from biological fluids. Samples underwent an initial alkaline extraction with benzene followed by an acidic back-extraction in order to remove interfering endogenous compounds. While this technique resulted in good chromatography and sensitivity it was both labour intensive and time consuming. In the present assay, MCP, mdMCP and ddMCP are analyzed simultaneously using a simplified procedure involving a single liquid–liquid extraction with dichloromethane followed by derivatization with HFBA and mass-selective detection. Dichloromethane replaces the potentially carcinogenic solvent benzene and also results in faster and better controlled drying in the sample concentrator. Although endogenous compounds from the biological fluids of sheep may not be removed as effectively as with the previously used double-extraction method, a specific selection of ions for detection improved specificity (selected-ion monitoring). The use of MSD, in fact, provides cleaner chromatograms and more stable baselines than obtained with our GC-ECD method. In addition, the internal standard maprotiline used in the GC-ECD assay was not chemically related to MCP and resulted in different extraction and chromatographic behaviour. Maprotiline has been replaced by the structurally related benzamide analogue BMY which has proven to be a much more satisfactory reference compound.

The chromatographic response of MCP was also improved by modifications to the injection port liner and oven temperature program. Placement of a silanized glass wool plug in the injection port liner improves volatilization of the compound resulting in an approximate two-fold larger peak response than when the glass wool plug is not used. Similar changes in chromatographic response were also obtained for the two metabolites. A further modification involves a change from an initial oven temperature of 204°C [22] to cold trap the analytes, to a temperature of 100°C, to utilize a solvent effect to concentrate the analytes at the head of the column. This results in sharp chromatographic

peaks with more consistent integration as well as improved resolution between MCP and its monodeethylated metabolite, mdMCP. The analytes were derivatized with HFBA to increase their volatility and thereby significantly reduce their retention times, and to obtain specific ions for mass-selective detection.

A representative total-ion chromatogram of MCP, mdMCP, ddMCP and BMY spiked in 0.3 ml blank plasma is shown in Fig. 1. The peaks are sharp and well resolved with no sign of interference from endogenous components in plasma. Retention times of the heptafluorobutyryl derivatives of ddMCP, mdMCP, MCP and BMY are 8.52 min, 9.25 min, 9.46 min and 11.32 min, respectively. Very similar chromatograms were obtained with urine and bile. Again, there was no interference from endogenous compounds. The mass spectrum of each compound obtained using the SCAN mode of detection, which is similar to that previously reported by Tam and Axelson [16], is presented in Fig. 2. The mass spectra of ddMCP and mdMCP (Fig. 2A,B) show extensive fragmentation with a base peak of m/z 380 and molecular ions (M^+) of m/z 635 and m/z 467, respectively. Due to its high abundance, it was possible to selectively and individually monitor the ion m/z 380 for both ddMCP and mdMCP. This ion provided very specific signal detection with a signal-to-noise ratio of 16 (ddMCP) and 9.7 (mdMCP) at 1 ng/ml concentration in plasma, urine and bile samples of sheep (Fig. 3.). The mass spectra of MCP and BMY (Fig. 2C,D) also show extensive fragmentation resulting in a base peak of m/z 86 for both compounds and molecular ions (M^+) of m/z 495 and m/z 551 for MCP and BMY, respectively. Although, the ion m/z 86 was abundant, its low mass weight renders it a non-specific fragment. While it is possible that endogenous compounds carried over from the extraction step and components from column bleed could yield fragments in this range, this was not observed. To enhance the selectivity of detection for MCP and BMY, an additional, but much less abundant ion was also monitored. MCP and BMY were quantitated then, by monitoring ions m/z 86 and 380 and m/z 86 and 366, respectively. With these ion combinations, MCP and BMY were selectively detected in the biological fluids

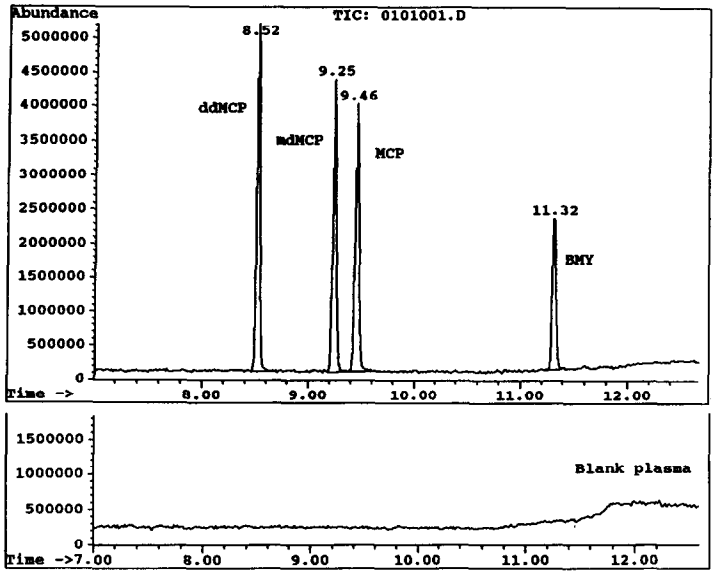

Fig. 1. Representative superimposed total-ion chromatograms (SCAN mode) of blank plasma and plasma (0.3 ml) spiked with 7 μg/ml of ddMCP and mdMCP, and 10 μg/ml of MCP and BMY.

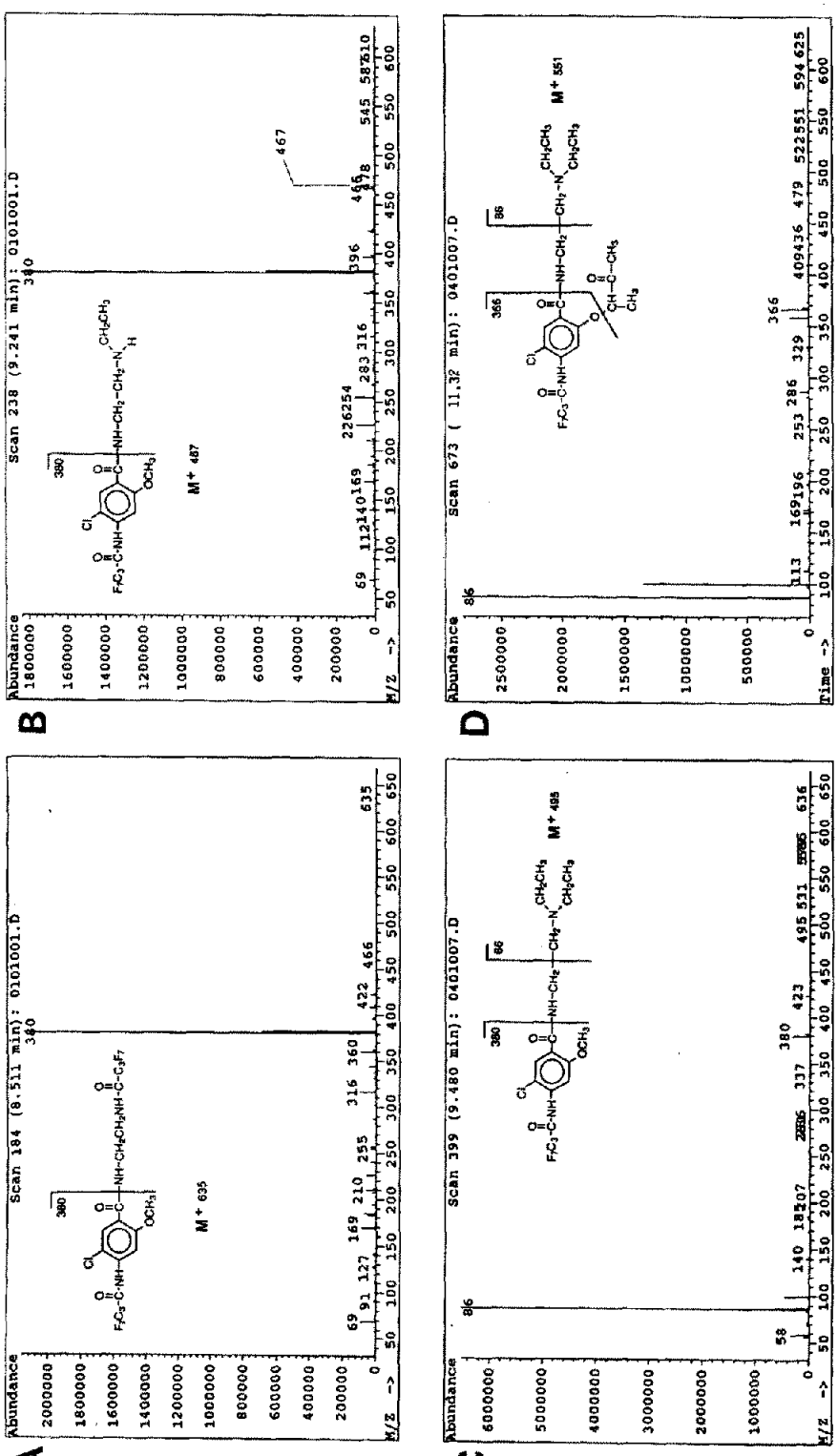

Fig. 2. Mass spectra of the heptafluorobutyryl derivatives of ddMCP (A), mdMCP (B), MCP (C) and BMY(D), obtained in SCAN mode, showing the molecular ions (M^+) and the ions used for quantitation.

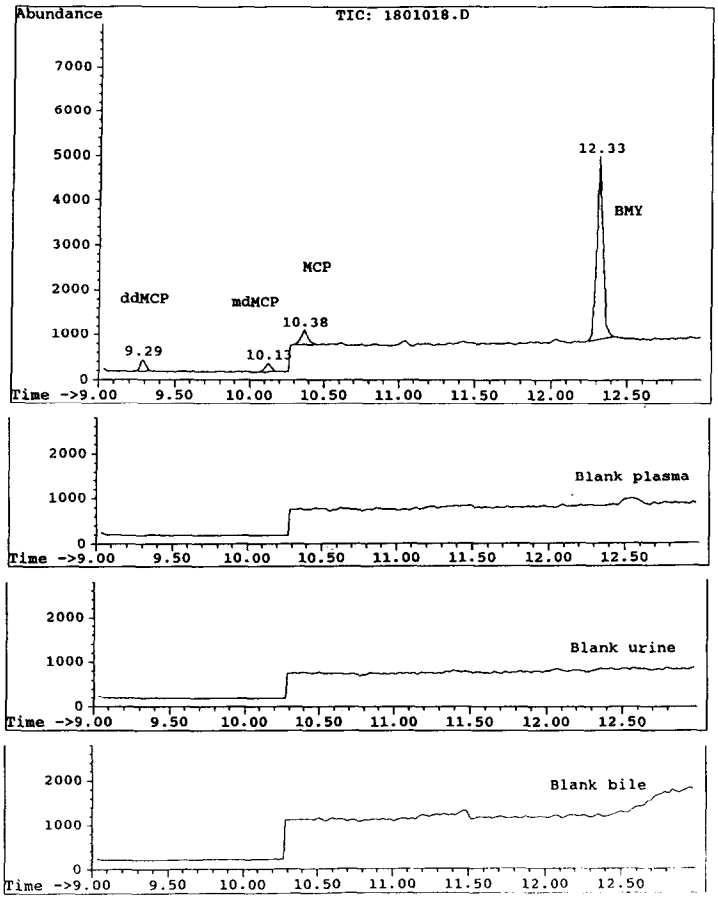

Fig. 3. Representative total-ion chromatogram (SIM mode) of the heptafluorobutyryl derivatives of ddMCP, mdMCP, MCP (1 ng/ml) and BMY (33.3 ng/ml) spiked in 0.3 ml of blank plasma. The superimposed chromatograms of blank plasma, urine and bile indicate no interference from endogenous components.

of sheep with no interference at the retention times of the peaks. The signal-to-noise ratio for MCP was 4.9 at a concentration of 1 ng/ml

As illustrated in Fig. 3., a stable baseline is obtained. The step-like shift in the baseline of the chromatograms at 10.25 min occurs as a result of switching the mass-groups to be detected in the MSD. That is, group 1 (m/z 380) is selected from 9.0–10.25 min at which time there is a switch to group 2 (m/z 86, 380, 366) for the rest of the chromatographic run. Monitoring the ions in group 2 increases the background signal resulting in an elevated but stable baseline. Use of a 700-ms dwell-time for each ion group minimizes baseline noise while providing sufficient cycles per second to achieve consistent peaks, even at a concentration of 1 ng/ml (Fig. 3.)

3.2. Calibration curve and linearity

The calibration curve parameters of MCP, mdMCP and ddMCP obtained from plasma of sheep are presented in Table 1. In urine, the slopes and intercepts of the calibration curve for MCP ($n = 11$, $r^2 = 0.999 \pm 0.001$), mdMCP ($n = 10$, $r^2 = 0.999 \pm 0.001$) and ddMCP ($n = 10$, $r^2 = 0.998 \pm 0.003$) were 0.079 ± 0.008, -0.017 ± 0.011; 0.027 ± 0.002, -0.007 ± 0.006 and 0.033 ± 0.004, -0.010 ± 0.009, respectively. The calibra-

Table 1
Weighted calibration curve data for the heptafluorobutyryl derivative of MCP, mdMCP and ddMCP to BMY versus added drug concentration in sheep plasma

Parameter	MCP (mean ± S.D.)	mdMCP (mean ± S.D.)	ddMCP (mean ± S.D.)
m	0.113 ± 0.011	0.046 ± 0.006	0.063 ± 0.007
b	−0.007 ± 0.018	0.002 ± 0.009	−0.008 ± 0.010
r^2	0.999 ± 0.006	0.999 ± 0.001	0.999 ± 0.000
n	10	9	10

$Y = mx + b$; weighting factor: $1/Y$.

tion curve data for the compounds in bile were very similar to the values obtained in urine. When unweighted regression analyses were used, the curves were linear but increasingly inaccurate at drug concentrations < 4 ng/ml. This problem was overcome by using weighted linear regression analyses with a weighting factor of $1/Y$. As a result, the calibration curves showed improved linearity throughout the working range of 1–40 ng/ml with coefficients of determination ranging from 0.998 to 0.999. The coefficients of variation (C.V.) of the slopes of the calibration curves for MCP, mdMCP and ddMCP, obtained from the same biological fluid, did not exceed 10.81%. The limit of quantitation was 1 ng/ml in all three biological fluids.

3.3. Recovery and method validation

Free base forms of MCP, mdMCP and ddMCP were not available, so extraction recoveries of the compounds from plasma, urine and bile were studied using their hydrochloric acid salts (as the aqueous stock solutions). The aqueous stock solutions were dried down directly yielding hydrochloric acid salt residues in the culture tubes. While insoluble in the non-polar solvent toluene, the presence of 0.0125 *M* TEA in the reconstituting solvent provided sufficient alkalinity for conversion to the free base. These standards were used then as the unextracted series to which samples undergoing the full extraction procedure were compared. On this basis, absolute recoveries in plasma were in the range 76.5–94.7%, 79.2–96.8%, 80.3–102.2% for MCP, mdMCP and ddMCP, respectively. In urine, the values were 56.5–87.8%, 61.5–87.5%, 62.6–90.2% for MCP, mdMCP and ddMCP, respectively, while in bile 83.5–100.9%, 78.5–90.5%, 66.9–79.2% was found for MCP, mdMCP and ddMCP, respectively.

Precision of the assay was determined from the coefficient of variation of the slopes of five weighted calibration curves for MCP, mdMCP and ddMCP prepared either on the same day (intra-day) or on consecutive days (inter-day). Overall intra-day precision was in plasma 2.87% for MCP, 7.03% for mdMCP, 9.80% for ddMCP; in urine 7.66% for MCP, 6.12% for mdMCP, 5.62% for mdMCP; in bile 3.94% for MCP, 12.61% for mdMCP, 10.74% for ddMCP. Overall inter-day precision in plasma was 8.63% for MCP, 9.16% for mdMCP, 9.97% for ddMCP; in urine 5.99% for MCP, 6.43% for mdMCP, 7.43% for ddMCP; in bile 8.39% for MCP, 12.30% for mdMCP, 14.89% for ddMCP.

Accuracy of the assay for MCP, mdMCP and ddMCP in plasma, urine and bile was calculated as [(amount measured/amount added)·100] −100 (bias, %). In plasma, accuracies for MCP, mdMCP and ddMCP at 1, 16 and 40 ng were 8.23, 2.37, 6.30; −0.48, 2.5, 2.32 and 0.40, −3.38 and −0.91%, respectively. For urine, respective values were 9.63, 9.21, 11.97; −1.01, −2.76, −2.94 and 3.44, 2.46 and 4.61%. Values obtained for bile were very similar to those for urine. Without the use of weighted linear regression (weighting factor: $1/Y$) there was a general overestimation at both low and high sample concentrations.

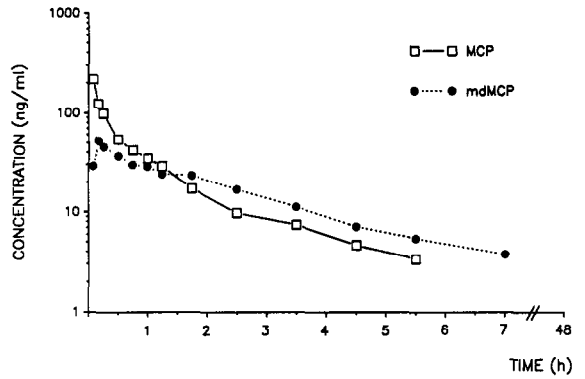

Fig. 4. Concentration–time profiles of MCP and mdMCP in plasma obtained from a non-pregnant ewe following the administration of a 40-mg intravenous bolus dose of MCP.

3.4. Determination of MCP, mdMCP and ddMCP in plasma and urine

Plasma and urine samples collected from a non-pregnant ewe following administration of a 40-mg intravenous bolus dose were analyzed. The plasma concentration versus time profiles obtained for MCP and mdMCP are shown in Fig. 4. Elimination of MCP from plasma was best described by a biexponential process with a rapid initial distribution phase. An apparent terminal elimination half-life of 1.54 h was calculated for MCP in plasma using nonlinear regression analysis [38] and agrees well with values obtained in previous studies [21,22]. Detectable plasma concentrations of the monodeethylated metabolite, mdMCP were obtained at 5 min and persisted beyond that of the parent drug. The dideethylated metabolite, ddMCP was not detected in plasma.

A representative urinary excretion rate plot for MCP, mdMCP and ddMCP is presented in Fig 5. All three compounds appeared rapidly in urine and persisted in excess of 30 h.

A detailed pharmacokinetic analysis of MCP, mdMCP and ddMCP in plasma, urine and bile will be presented elsewhere.

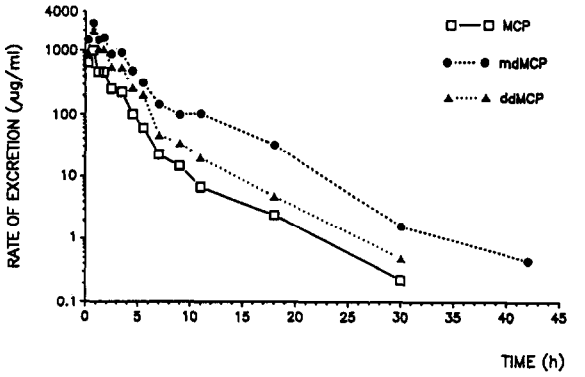

Fig. 5. Urinary excretion rate plots obtained for MCP mdMCP and ddMCP following the administration of a 40-mg intravenous bolus dose of MCP to a non-pregnant sheep.

4. Conclusions

A modified assay employing mass-selective detection has been developed for the simultaneous measurement of MCP and its mono- and dideethylated metabolites in plasma, urine and bile obtained from sheep. Sample preparation has been simplified and the method is more selective and sensitive ($\approx$2 fold) than our previous GC-ECD technique [21,22]. The lowest limit of detection using $\leq$0.5 ml of biological fluid is 1 ng/ml which represents 2.5 pg at the detector. The assay has been found to be reliable and reproducible and is being used for further studies of MCP, mdMCP and ddMCP in fluid samples obtained from non-pregnant and pregnant sheep (ewe and fetus).

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References

[1] R.M. Pinder, R.N. Brogden, P.R. Sawyer, T.M. Speight and G.S. Avery, *Drugs*, 12 (1976) 81.
[2] M.A. Smith and F.J. Salter, *Drug Intell. Clin. Pharm.*, 14 (1980) 169.

[3] C.D. Ponte and J.M. Nappi, *Am. J. Hosp. Pharm.*, 38 (1981) 829.
[4] K. Schulze-Delrieu, *N. Eng. J. Med.*, 305 (1981) 28.
[5] R. Albibi and R.W. McCallum, *Ann. Intern. Med.*, 98 (1983) 86.
[6] R.A. Harrington, C.W. Hamilton, R.N. Brogden, J.A. Linkewich, J.A. Romankiewicz and R.C. Heel, *Drugs*, 25 (1983) 451.
[7] A.F. Shaughnessy, *Drug Intell. Clin. Pharm.*, 19 (1985) 723.
[8] P.V. Desmond and K.J.R. Watson, *Med. J. Aust.*, 144 (1986) 366.
[9] J.R. DiParma, *Am. Fam. Physician*, 41 (1990) 919.
[10] R.W. McCallum, *Gastroenterol. Clin. North Am.*, 19 (1990) 551.
[11] P.J. Hesketh, *Semin. Oncol.*, 19 (1992) 33.
[12] O.M. Bakke and J. Segura, *J. Pharm. Pharmacol.*, 28 (1976) 32.
[13] G. Huizing, A.H. Beckett and J. Segura, *J. Chromatogr.*, 172 (1979) 227.
[14] R. Saller, D. Hellenbrecht, L. Briemann, A. Hellstern, H. Hess, P. Mitrou, M. Hodgson, G. Achtert, P. Brockmann and H.J. Hausleiter, *Clin. Pharmacol. Ther.*, 37 (1985) 43.
[15] D.H. Bateman, C. Kahn, K. Mashiter and D.S. Davies, *Br. J. Clin. Pharmacol.*, 6 (1978) 401.
[16] Y.K. Tam and J.E. Axelson, *J. Chromatorgr.*, 170 (1979) 157.
[17] L.M. Ross-Lee, M.J. Eadie, F. Bochner, W.D. Hooper and J.H. Tyrer, *J. Chromatorgr.*, 183 (1980) 175.
[18] Y.K. Tam and J.E. Axelson, *J. Pharm. Sci.*, 67 (1978) 1073.
[19] Y.K. Tam, J.E. Axelson and R. Ongley, *J. Pharm. Sci.*, 68 (1979) 1254.
[20] L.M. Ross-Lee, M.J. Eadie, W.D. Hooper and F. Bochner, *Eur. J. Clin. Pharmacol.*, 20 (1981) 465.
[21] K.W. Riggs, J.E. Axelson, D.W. Rurak, D.A. Hasman, B.A. McErlane, M. Bylsma-Howell, G.H. McMorland, R. Ongley and J.D.E. Price, *J. Chromatogr.*, 276 (1983) 319.
[22] K.W. Riggs, J.E. Axelson, N.C. Gruber, B.A. McErlane, G.H. McMorland and D.W. Rurak, *J. Pharm. Sci.*, 77 (1988) 373.
[23] F. Albani, R. Riva, M. Contin and A. Baruzzi, *Biomed. Chromatogr.*, 2 (1987) 135.
[24] H. Vegin, R. Krammer, V. Nitsche, M. Miczka, K. Strobel and H. Schimmel, *Arzneim. Forsch.*, 40 (1990) 679.
[25] C. Graffner, P. Lagerstrom and P. Lundborg, *Br. J. Clin. Pharmacol.*, 8 (1979) 469.
[26] G.B. Bishop-Freudling and H. Vergin, *J. Chromatogr.*, 273 (1983) 453.
[27] W.B. Taylor, S.J. Proctor and D.N. Bateman, *Br. J. Clin. Pharmacol.*, 18 (1984) 679.
[28] H. Takahashi, H. Ogata, H. Echizen and T. Ishizaki, *J. Chromatogr.*, 419 (1987) 243.
[29] A.P. DeJong, A.J. Wittebrood, W.M. Duchantinier and J. Bron, *J. Chromatogr.*, 419 (1987) 233.
[30] D.C. Buss, A.D. Hutchings, S. Scott and P.A. Routledge, *Ther. Drug Monit.*, 12 (1990) 293.
[31] R.D. Jones and J.M. Bowen, *Vet. Hum. Toxicol.*, 33 (1991) 551.
[32] J.C. Huhn, G.D. Koritz, D.R. Nelson and S.A. Brown, *J. Vet. Pharmacol. Ther.*, 15 (1992) 19.
[33] S.S. Badawy, A.F. Shoukry and Y.M. Issa, *Analyst*, 111 (1986) 1363.
[34] M. De Villiers, D. Parkin, P. Van Jaarsveld and B. Van der Walt, *J. Immunol. Methods*, 103 (1987) 33.
[35] F. Capasso, G. De Ruggiero, M. Di Rossa and L. Sorrentino, *Boll. Chim. Farm.*, 115 (1976) 649.
[36] M. Murakami, N. Inukai, A. Koda and K. Nakano, *Chem. Pharm. Bull.*, 19 (1971) 1696.
[37] D.W. Rurak, S.D. Yoo, E. Kwan, S.M. Taylor, K.W. Riggs and J.E. Axelson, *J. Pharmacol. Exp. Ther.*, 247 (1988) 271.
[38] PCNONLIN Version 3.0 SCI Software, Statistical Consultants Inc., Lexington, KY, USA.