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Analytical method development for the simultaneous quantitation of dexmedetomidine and three potential metabolites in plasma

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Abstract

Dexmedetomidine, a novel α_2-adrenergic receptor agonist, is being developed as an anesthetic adjunct for perioperative use. An assay method has been developed for the sensitive quantitation of dexmedetomidine and three metabolites in plasma: MPV-1305, MPV-1306 and MPV-1709. The method involves solid-phase extraction (C_{18} cartridge) of dexmedetomidine and metabolites followed by a two-step derivatization. The first step utilized BF_3–MeOH to simultaneously mask a primary alcohol in MPV-1305 and a carboxylic acid in MPV-1306. The second step applied PFB-Cl to derivatize the imidazole ring for sensitive detection of these compounds by GC–negative chemical ionization MS at pg/ml concentrations. MPV-1709 was not derivatized in the process and was detected by GC–positive chemical ionization MS. Optimization of extraction and derivatization is discussed. The method is suitable for quantitation of dexmedetomidine, MPV-1305, MPV-1306 and MPV-1709 over concentration ranges of 0.1–40, 0.5–100, 0.5–100 and 1.0–500 ng/ml, respectively. The method showed excellent specificity, linearity and sensitivity and is useful for profiling the pharmacokinetic disposition of these compounds.

Keywords: Derivatization, GC; Sample preparation; Dexmedetomidine; Medetomidine

1. Introduction

Dexmedetomidine represents a novel class of agents in the anesthetic setting. A number of clinical studies over two decades have indicated that it possesses potent sympatholytic effects with ensuing sedation, hemodynamic stabilization and analgesic effects in patients undergoing surgery. The pharmacodynamic characteristics of dexmedetomidine have been reviewed [1]. Another review demonstrated the correlation between dexmedetomidine pharmacokinetics and pharmacodynamics [2]. An analytical method for the determination of medetomidine, the racemic mixture of the dex and levo forms, in the low pg/ml (limit of quantitation ~50 pg/ml) concentration range has been developed by Veorilehto et al. [3]. This method utilized extractive derivatization of medetomidine with 2,3,4,5,6-pentafluorobenzoyl chloride (PFB-Cl) followed by sensitive GC–negative chemical ionization (NCI) MS detection. A radioreceptor assay for dexmedetomidine was also developed for large numbers of samples which required no special laboratory equipment [4]. The latter method has a detection limit of 50 pg/ml but has potential problems related to specificity, as a radioreceptor assay will detect any ligand that binds to the receptor (parent drug and metabolites).

Medetomidine is eliminated principally by biotransformation in liver [5,6]. Hydroxylation of a methyl group is the primary metabolic event reported for medetomidine in rats, which provided 3′-hydroxymedetomidine and medetomidine 3′-carboxylic acid as the major metabolites [6]. In vitro studies

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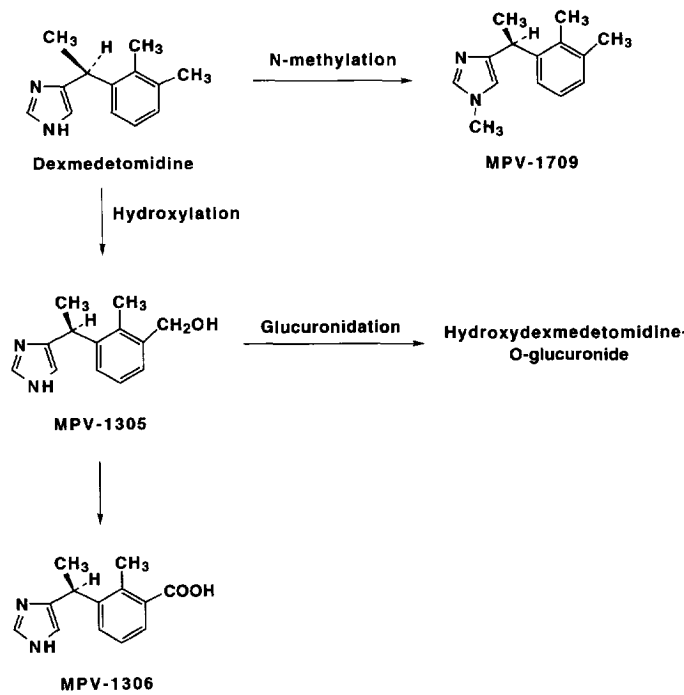

Fig. 1. Proposed metabolic scheme for dexmedetomidine.

with dexmedetomidine, the bioactive isomer of medetomidine, in rat liver microsomes produced 3′-hydroxydexmedetomidine and N-methyldexmedetomidine [7]. The proposed metabolic scheme for dexmedetomidine is shown in Fig. 1. It is important to take the metabolites into account when evaluating the kinetics and response of dexmedetomidine. Currently an assay does not exist for the simultaneous determination of dexmedetomidine and metabolites in a biological matrix. The purpose of this investigation was to develop a sensitive analytical method for the simultaneous quantitation of dexmedetomidine and three metabolites (MPV-1305, MPV-1306 and MPV-1709) in plasma.

2. Experimental

2.1. Materials

The derivatization reagent PFB-Cl (pentafluorobenzoyl chloride, 99%) was purchased from Aldrich (Milwaukee, WI, USA). Boron trifluoride–methanol (BF_3–MeOH, 50:50) was purchased from Sigma (St. Louis, MO, USA). Bakerbond SPE octadecyl (C_{18}) cartridges were purchased from J.T. Baker (Phillipsburg, NJ, USA). Sodium carbonate (Na_2CO_3, anhydrous, granular) was purchased from EM Sciences (Gibbstown, NJ, USA). All solvents, such as hexane and toluene (EM Sciences), were GC–MS grade. Dexmedetomidine·HCl, MPV-1305, MPV-1306, MPV-1709 and the internal standard (I.S.), *d*-MPV-872·HCl, were provided by Orion-Farmos, Finland. Abbott-51984, used as the internal standard for MPV-1709, was obtained from the Abbott Drug Sample Room.

2.2. GC–MS apparatus and condition

Chromatographic separation was achieved with an HP 5890 Series II GC (Hewlett-Packard) directly connected to the HP 5989B MS Engine. The system was controlled by MS Chemstation (Windows system). Resolution of the analyte peaks was achieved using a 30 m×0.25 mm I.D., 0.25 μm DB-1701 capillary column (J and W Scientific, Folsom, CA,

USA). A retention gap was used for larger volume injections (5 m×0.25 mm I.D. retention gap, Alltech Associates, Deerfield, IL, USA). Temperature programming consisted of an initial oven temperature (100°C) isothermal for 0.5 min, a 30°C/min ramp to 250°C, then isothermal at 250°C for 12.5 min. Normal red septum, a 4 mm single tapered liner and a 10 μl 23-gauge straight needle syringe were used. Injection was in the splitless mode with the injector purge on for 1 min following the 5 μl injection of the samples in toluene. Injector temperature was 250°C; transfer line temperature was 275°C. Helium was used as a carrier gas at a flow of 1.3 ml/min on the capillary column. A pressure program, employed to maintain constant helium flow, started with initial pressure pulse of 45 p.s.i. for 1 min (1 p.s.i.= 6894.76 Pa); the pressure was then decreased at a rate of 99 p.s.i./min to 15 p.s.i. with constant flow.

Dexmedetomidine, MPV-1305, MPV-1306 and the internal standard (*d*-MPV-872) derivatives were ionized in the NCI mode. Ion source temperature was 250°C and vacuum chamber temperature was 100°C. Detection was in the selected ion monitoring (SIM) mode. Ions of *m/z* 394.3, 424.3, 438.3 and 380.1 were monitored for dexmedetomidine, MPV-1305, MPV-1306 and the internal standard, respectively. Ions were slightly under resolved to improve sensitivity. The reagent gas used for NCI was ultra high purity methane (99.999%; AGA Gas, Maumee, OH, USA). The electron multiplier voltage (EMV) was set about 200 units higher than the tune voltage. Parameter values were optimized for maximum sensitivity prior to each assay batch.

MPV-1709 and its internal standard (Abbott-51981) were ionized in the positive chemical ionization mode (PCI). Ion source temperature was 250°C and MS temperature was 100°C. Detection was in the SIM mode. Ions of *m/z* 215.30 and 207.10 were monitored for MPV-1709 and the internal standard, respectively. The reagent gas was anhydrous ammonia (99.999%; AGA Gas).

For data acquisition, the dwell time was set at 85 ms. This permitted about 15 scans across the chromatographic peak which was generally 3.0 s wide. Prior to each analysis run, instrument performance was determined by injection of a standard hexachlorobenzene sample (obtained from Hewlett-Packard) at an approximate concentration of 0.04 ng/ml. An ion count of over 30 000 for the ion *m/z* 284.0 indicated adequate performance for the system. Another reference sample which contained approximately 600 pg/ml of dexmedetomidine and I.S. derivatives, which had been stored at −20°C, was injected using the identical conditions as standards and samples. An ion count of 6000 or more for dexmedetomidine indicated an adequate performance.

2.3. Optimization studies

2.3.1. Optimization of the solid-phase extraction (SPE) procedure

Evaluation of different SPE columns

The recovery of dexmedetomidine, MPV-1305 and MPV-1306 from plasma was evaluated on C_{18}, C_8, C_2, CN and phenyl SPE cartridges under identical conditions. HPLC followed by UV detection was used to estimate recovery.

Optimization of recovery from C18 SPE

The recovery of parent drug and metabolites using water and buffer containing various fractions of methanol (0, 30, 50 or 100%, v/v) was evaluated. HPLC followed by UV detection was again used to determine the recovery.

2.3.2. Optimization of the derivatization procedures

Effect of temperature and time when reacting with BF_3–MeOH

To evaluate the effect of reaction temperature and time on the formation of the methylation product of BF_3–MeOH with MPV-1305 and MPV-1306, a series of plasma samples containing dexmedetomidine, MPV-1305 and MPV-1306 was extracted using identical SPE sample cleanup procedures. The samples were then reacted with BF_3–MeOH at temperatures of 60°C, 65°C, 70°C, 75°C and 80°C for 0.17, 0.5, 1, 2, 3 and 15 h.

Effect of pH when reacting with PFB-Cl

Different bases such as sodium carbonate (1 *M*) and sodium hydroxide (6 *M*) were investigated to

basify the reaction mixture following derivatization with BF_3–MeOH.

Effect of PFB-Cl concentration

The effect of reagent concentration on the yield of dexmedetomidine, MPV-1305 and MPV-1306 derivatives was investigated. Concentrations of PFB-Cl ranging from 0.5% to 5% were investigated.

2.3.3. Optimization of the detection mode for MPV-1709

MPV-1709 is not derivatized so it has poor sensitivity in NCI. Other ionization methods for MPV-1709 and its internal standard (Abbott-51984) were investigated with samples extracted from plasma. Ionization methods investigated included electron impact (EI), positive chemical ionization (PCI) with reagent gas such as methane, isobutane and ammonia and NCI with reagent gas methane.

2.4. Optimized extraction and reaction procedure

The parent compound, dexmedetomidine, and three metabolites MPV-1305, MPV-1306 and MPV-1709, were separated from the plasma matrix utilizing a SPE (C_{18} cartridge) procedure followed by two-step derivatization before analysis with GC–MS. The first derivatization step utilized BF_3–MeOH to simultaneously mask the primary alcohol in MPV-1305 and the carboxylic acid in MPV-1306. The second derivatization step applied PFB-Cl to derivatize the imidazole ring for sensitive detection by GC–NCI–MS at pg/ml level (see Fig. 2). MPV-1709 was not derivatized in the process and was detected by GC–PCI-MS. The conditions for the optimized procedure for quantitation in plasma are described below.

Plasma samples (1 ml) containing both internal standards (*d*-MPV-872 for dexmedetomidine, MPV-

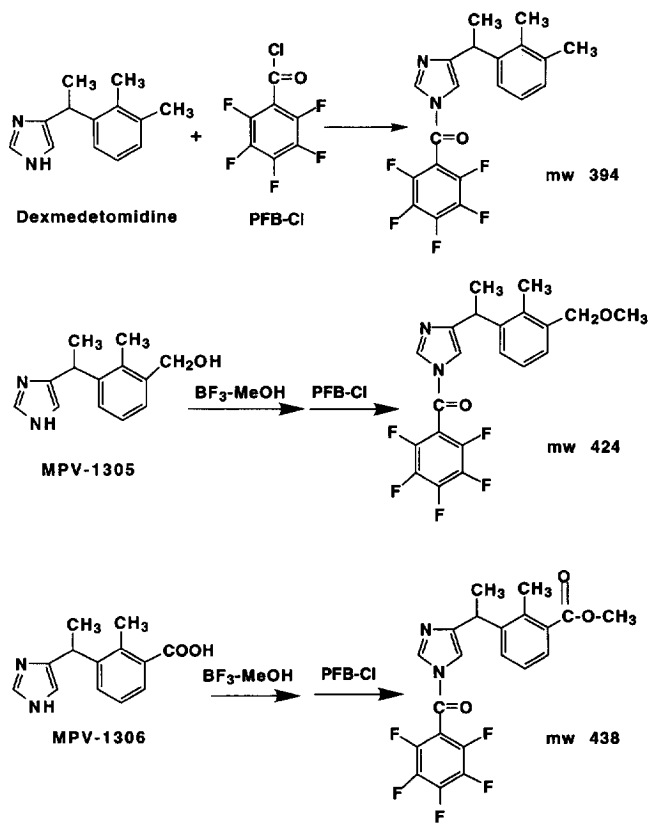

Fig. 2. Proposed reaction of dexmedetomidine, MPV-1305 and MPV-1306 with BF_3–MeOH and PFB-Cl.

1305 and MPV-1306 and Abbott-51984 for MPV-1709) were applied to a Bakerbond SPE octadecyl (C_{18}) cartridge which has been preconditioned with 6.0 ml of methanol and 3.0 ml of 0.9% NaCl/0.05 *M* phosphate buffer (pH 7.4, PBS). The columns were washed with 6 ml of PBS, 300 μl of MeOH–PBS (3:7, v/v) and 300 μl of MeOH–PBS (1:1, v/v). Dexmedetomidine, MPV-1305, MPV-1306, MPV-1709 and the internal standards were then eluted with 4.0 ml of methanol and separated into two equal volumes before evaporating to dryness with a gentle stream of dry air at 35°C. The residue of one half of extract from SPE was used to derivatize dexmedetomidine, MPV-1305 and MPV-1306 as follows. The residue was dissolved in 200 μl of BF_3–MeOH (50:50) and the mixture shaken (about 200 oscillations/min) for 2 h at 65°C. The pH of the acidic reaction mixture was adjusted through the sequential addition of 500 μl 1 *M* sodium carbonate and 1.0 ml 6 *M* sodium hydroxide (final pH>9). A volume of hexane (7.0 ml) was added to the basified solution. The PFB-Cl derivatization reagent (50 μl, 5% in hexane) was added and shaken rapidly (~300 oscillations/min) for 1 min followed by more gentle shaking (~170 oscillations/min) for 10 min (Eberbach shaker) at room temperature. The derivatized compounds partition into the hexane phase during the agitation. After centrifuging (about 2600 *g*) at 4°C for 10 min, the organic layer was transferred into a conical centrifuge tube and evaporated to dryness with a gentle stream of dry air at room temperature. The samples were reconstituted in 100 μl of toluene; 5 μl was injected into GC–MS system. The residue of the other half of the extract from the solid-phase extraction was reconstituted in 100 μl of toluene for PCI detection (5 μl injection) of MPV-1709 and its internal standard without additional sample handling.

2.5. Preparation of standards

Dexmedetomidine, MPV-1305, MPV-1306 and MPV-1709 were dried in an oven at 105°C for 30 min before use. Stock solutions of dexmedetomidine, MPV-1306 and MPV-1709 (as hydrochloride salts, provided by Orion-Farmos) were prepared by dissolving the compounds in HPLC-grade water at concentrations of about 150 μg/ml. MPV-1305, as the free base, was dissolved in ethanol at a similar concentration. Stock solutions were refrigerated when not in use. Calibration solutions were prepared by spiking 1.0 ml of drug-free plasma with 100 μl of the intermediate stock solutions to achieve a final concentration of about 0.1, 0.5, 1, 2, 5, 20, and 40 ng/ml for dexmedetomidine, about 0.5, 1, 2, 5, 10, 20, 50, 100 and 300 ng/ml for MPV-1305 and MPV-1306 and 1, 2, 5, 10, 20, 50, 100, 300 and 500 ng/ml for MPV-1709. An internal standard working solution of *d*-MPV-872 (100 ng/ml) and Abbott-51981 (5 μg/ml) was prepared by appropriate dilution of the stock standard solution with HPLC-grade water.

2.6. Validation studies

The intra-day precision and accuracy of the method were evaluated by analysis of duplicate spiked plasma standards at each of three separate concentrations. Assay precision was reported as the relative standard deviation (R.S.D.) of replicate analysis. The indication of accuracy (bias) was estimated from the relative error of the observation with respect to the theoretical, i.e., $[(F-T)/T]*100$, where F=the found concentration and T=the theoretical concentration. Inter-day precision was assessed from the results of analysis of replicate standards on multiple days.

2.7. Quantitation procedure

Peak areas of the compounds of interest were determined using the HP-Windows Chemstation software (version 5). Calibration curves were derived from the peak area ratio (analyte/internal standard) using least squares linear regression of the area ratio versus the theoretical concentration of the standards. A weighting of $1/x$ (where x is the concentration of a given standard level) was used if this was found to provide the optimum fit of the concentration/response data. Deviations from the regression line were calculated using the regression equation to back calculate the expected concentration at each standard level.

3. Results and discussion

The requirement of an assay detection limit in the pg/ml range limits the methods that might be applied to this class of compounds. HPLC followed by UV detection and LC–MS were eliminated from consideration because the sensitivity for dexmedetomidine was not sufficient for the characterization of pharmacokinetic behavior (data not shown). The GC–MS with NCI method for quantitation of dexmedetomidine provided the greatest sensitivity for the parent compound. An extension of the method to include quantitation of metabolites was explored. We faced several major challenges due to the diversity in the chemical properties of dexmedetomidine and the three metabolites. The first challenge was to extract all four compounds from plasma in a single step. The second challenge was to mask $-CH_2OH$ in MPV-1305 and –COOH in MPV-1306 for better GC behavior while derivatizing the imidazole for GC–NCI-MS detection. The third challenge was to detect MPV-1709 sensitively with GC–MS, since the imidazole functionality is no longer available for derivatization by PFB-Cl in this metabolite. The purpose of the optimization studies was to achieve the best compromise between the formation of the reaction products and degradation of products during sample preparation. The proposed scheme for the derivatization of dexmedetomidine, MPV-1305 and MPV-1306 is shown in Fig. 2.

Dexmedetomidine, MPV-1305, MPV-1306 and MPV-1709, as shown in Fig. 1, have different chemical properties. In order to simultaneously extract all four compounds from plasma, several methods were explored. MPV-1306 is unique in that it contains a 3′-carboxylic acid functional group. Three ion-pairing reagents including tetraoctylammonium bromide, tetrabutylammonium hydrogen sulfate and tetramethylammonium hydroxide were investigated in order to extract MPV-1306 into an organic solvent. MPV-1306 did not partition into organic solvent [such as ethyl acetate–hexane (9:1, 1:1 or 1:9, v/v), 10% ethanol in methylene chloride or *tert.*-butylmethyl ether] from plasma adjusted to pH 6.9, 8.0 or >11 as evaluated by HPLC with UV detection when any of these ion-pairing reagents were employed. Extraction of MPV-1306 from plasma was also investigated by forming zwitterions (pH 5.01, 5.26 and 5.52) as estimated from pK_a values of 3.5 to 4.0 for the carboxylic acid and 6–7 for an imidazole amine; the recovery was less than 1%. Solid-phase ion-exchange extraction was also attempted for MPV-1306. No peaks were detected by either HPLC-UV or GC–MS after derivatization. Protein precipitation with acetonitrile followed by a one phase derivatization was tried for MPV-1306. No peaks were detected by GC–MS.

In the optimization of SPE procedure, the recovery of dexmedetomidine, MPV-1305 and MPV-1306 was evaluated on C_{18}, C_8, C_2, CN and phenyl solid-phase extraction columns. C_{18} SPE columns provided the best recovery for all three compounds based on peak areas from HPLC followed by UV detection at a relatively high concentration of 2 μg/ml. MPV-1306 was easily removed from the SPE during sample cleanup. Water washes of SPE columns after the sample loading step removed over 90% of this compound; similar response with weakly acidic solutions was observed. SPE columns could be rinsed with PBS (pH 7.4) with minimal loss of MPV-1306 (<20%). Small volumes (0.3 ml) of methanol–PBS mixtures (3:7 or 1:1, v/v) could also be used to wash the C_{18} SPE column without significant loss. These results indicated that the ionic strength of washing solvent was crucial for the retention of MPV-1306 on C_{18} SPE cartridges. All compounds were eluted from the SPE columns with methanol. The recoveries for dexmedetomidine, MPV-1305, MPV-1306 and MPV-1709 were greater than 90% using this cleanup procedure.

Derivatization of dexmedetomidine and three metabolites was investigated with different reagents. The initial assumption was that PFB-Cl would be used for the sensitive derivatization of the imidazole functionality [3]. However, this derivatization must be done as the second step, as the PFB-Cl-imidazole derivative is not stable at elevated temperatures and/or extremes in pH. Two reagents were evaluated for blocking the carboxylic acid group in MPV-1306. These two reagents were *p*-Bromophenacyl-8 ($Br–C_6H_4–CO–CH_2Br$) and Phenacyl-8 ($C_6H_5–CO–CH_2Br$). No response was obtained for MPV-1306 through GC–MS following extractive derivatization with these two reagents. Several reagents were investigated for masking the primary alcohol in MPV-1305. These reagents included acylation re-

agent PFAA (pentafluoropropionic acid anhydride) and TFAI (N-trifluoroacetylimidazole) and silylation reagent Sylon TP (N-trimethylsilylimidazole-pyridine, 1:4). Sylon TP and TFAI followed by PFB-Cl derivatization provided derivatives with GC–MS response at m/z 482 in the NCI mode for MPV-1305 in aqueous solution; little or no response was obtained with plasma samples, however. PFAA followed by PFB-Cl derivatization provided a response for MPV-1305 at m/z 556 using NCI, but there were a number of contaminants which raised the baseline noise significantly. The alkylation reagent PFB-Br (pentafluorobenzyl bromide, $C_6F_5CH_2Br$) was evaluated to mask both the $-CH_2OH$ and $-COOH$ functional groups at pH 6.9, 8 and >11 without any success. A single peak was observed at m/z 438 for MPV-1306 and m/z 424 for MPV-1305 in NCI mode after a two-step derivatization with MeOH–HCl followed by PFB-Cl; concentrated HCl was too corrosive however. Milder reaction conditions for methylation of MPV-1305 and

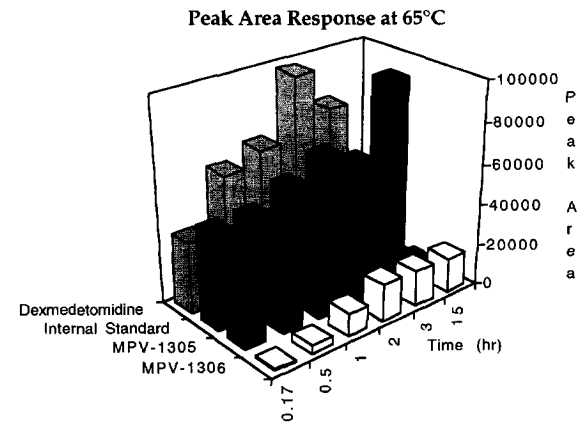

Fig. 4. Comparison of peak area response of dexmedetomidine, MPV-1305, MPV-1306 and internal standard derivatized with BF_3–MeOH at 65°C.

MPV-1306 with BF_3–MeOH proved to be more successful.

The BF_3–MeOH reaction was optimized by investigating the effect of temperature (60, 65, 70, 75 and

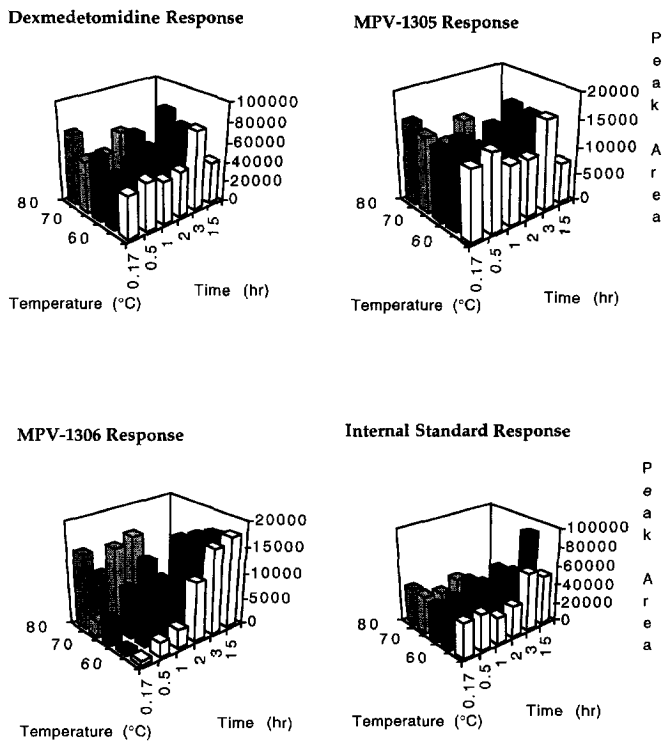

Fig. 3. Effect of temperature and time on the yield from the reaction of dexmedetomidine, MPV-1305, MPV-1306 and internal standard with 50% BF_3 in MeOH.

Table 1
Effect of ionization method on the GC–MS response of MPV-1709 and Abbott-51984 (I.S.)

Ionization method	Reagent gas	MPV-1709		A-51984	
		Ion (m/z)[a]	Peak area	Ion (m/z)[a]	Peak area
NCI	CH_4	213.1	63 150	207.0	72 411
PCI	CH_4	215.3	82 458	207.1	65 593
PCI	NH_3	215.3	288 446	207.1	276 471
PCI	i-C_4H_{10}	215.3	195 194	207.1	113 887
EI	–	214.4	229 728	206.2	285 872

NCI, negative chemical ionization; PCI, positive chemical ionization.
EI, electron impact.
[a] Ion with largest peak area.

80°C) and reaction time (0.17, 0.5, 1, 2, 3 and 15 h) on the yield of dexmedetomidine, MPV-1305 and MPV-1306 derivatives (fixed reaction conditions with PFB-Cl). Both the temperature and reaction time were found to contribute to the yield of the final derivatized product (see Fig. 3). The best response for all three analytes was achieved at 65°C for 2 h. As these studies started with similar concentrations of dexmedetomidine, MPV-1305, MPV-1306 and internal standard, the higher recovery of dexmedetomidine and internal standard compared to MPV-1305 and MPV-1306 is apparent (see Fig. 4).

The second derivatization step with PFB-Cl reaction requires a pH of greater than 9.0 [3]. The effect of reaction mixture pH was investigated with different bases such as sodium carbonate and sodium hydroxide. It was found that a mixture of 0.5 ml of 1 M Na_2CO_3 plus 1.0 ml of 6 M NaOH (sequential addition) was sufficient to bring the reaction mixture to the desired pH.

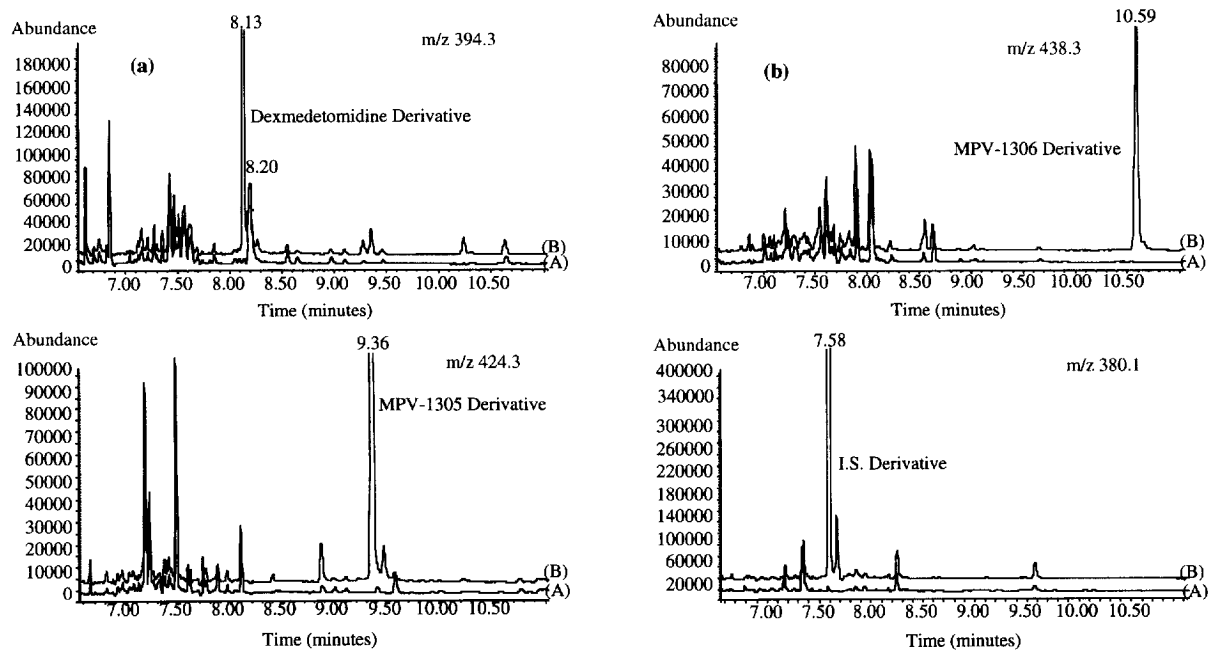

Fig. 5. Representative chromatograms of (A) blank human plasma and (B) spiked human plasma with dexmedetomidine (t_R=8.13 min, m/z 394.3), MPV-1305 (t_R=9.36 min, m/z 424.3), MPV-1306 (t_R=10.59 min, m/z 438.3) and internal standard (d-MPV-872, t_R=7.58 min, m/z 380.1).

More concentrated PFB-Cl reagent was needed for the derivatization of dexmedetomidine and its metabolites compared to derivatization of dexmedetomidine alone. 5% PFB-Cl (50 μl) in hexane provided a better response for dexmedetomidine, MPV-1305 and MPV-1306 than 50 μl of 0.5% PFB-Cl. A reagent concentration of 5% PFB-Cl in hexane (50 μl), therefore, was chosen in the validation study of dexmedetomidine and metabolites.

Dexmedetomidine, MPV-1305 and MPV-1306 contain a imidazole ring which can be derivatized by PFB-Cl to obtain a good electron-capture derivative for sensitive detection with negative chemical ionization. The amine in the imidazole ring of MPV-1709 was blocked by an N-methyl group, which prohibits this derivatization strategy. Sensitive detection of MPV-1709 required a different route. Various ionization methods were investigated (see Table 1). Positive chemical ionization with ammonia gas gave the best sensitivity for both MPV-1709 and its internal standard among the ionization methods tested (methane NCI, methane PCI, ammonia PCI, isobutane PCI and EI, see Table 1). Excellent recovery (>90%) of MPV-1709 was obtained using the solid-phase extraction procedure described above. However, subjecting the extract to the BF_3–MeOH derivatization step resulted in a loss of 65% of the MPV-1709. For optimum sensitivity, detection of MPV-1709 was separated out from the mixture of the parent compound and the other two metabolites following SPE sample preparation. A second internal standard (Abbott-51984) was added to plasma prior to SPE and MPV-1709 was analyzed by ammonia PCI following SPE extraction without additional derivatization.

The selectivity of the method was excellent as shown in Fig. 5 (NCI) and Fig. 6 (PCI). No endogenous contaminants and reaction by-products interfered with the detection of the targeted derivatives in plasma samples. There is a small peak at 8.20 min at the side of dexmedetomidine derivative peak which elutes at 8.13 min. It was well separated from the dexmedetomidine derivative peak at low concentrations and did not affect the quantitation of dexmedetomidine. MS scans of dexmedetomidine, MPV-1305, MPV-1306 and the internal standard derivatives produced essentially only the $[M+e]^-$ electron-capture ion with no fragmentation. The

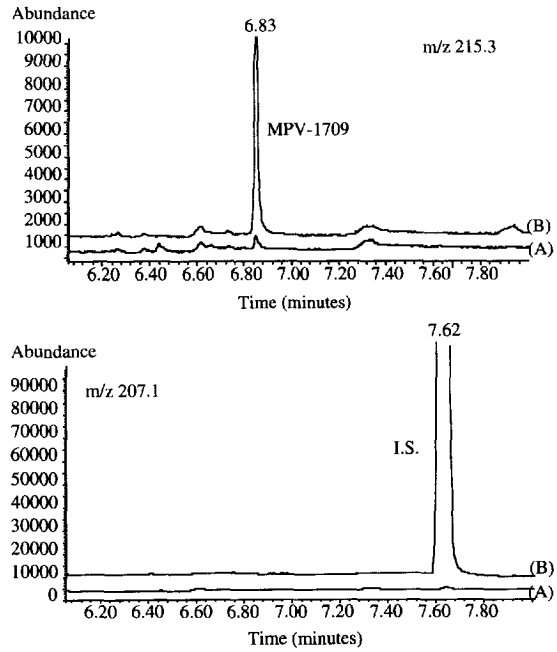

Fig. 6. Representative chromatograms of (A) blank human plasma and (B) spiked human plasma with MPV-1709 (t_R=6.83 min, m/z 215.3) and internal standard (Abbott-51984, t_R=7.62 min, m/z 207.1).

mass spectrum of the dexmedetomidine derivative is shown in Fig. 7 as representative for this class. The selected ion channels were m/z 394.3 for the dexmedetomidine derivative, m/z 424.3 for the MPV-1305 derivative, m/z 438.3 for the MPV-1306 derivative and m/z 380.1 for the internal standard derivative.

Validation studies with human plasma spiked with dexmedetomidine and three metabolites showed that

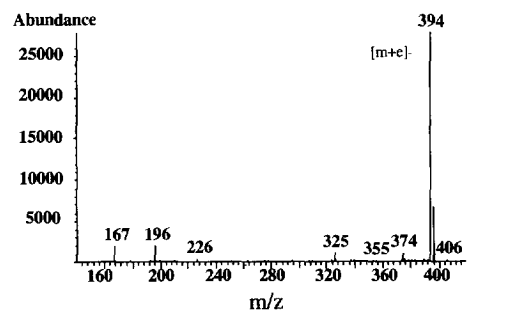

Fig. 7. Mass spectrum of dexmedetomidine-PFB derivative using methane NCI.

the method was linear for all compounds. The correlation coefficients were >0.98, >0.99, >0.97 and >0.98 over the concentration ranges of 0.1–40, 0.5–100, 0.5–100 and 1–500 ng/ml for dexmedetomidine, MPV-1305, MVP-1306 and MPV-1709, respectively, for three separate days of analysis. (MPV-1709 was only analyzed on two different days). The R.S.D.s for the analysis of duplicate samples at three concentrations on the same day were between 1.3 and 16.7% with relative errors ranging from −16.2 to +12.4% of theory (see Table 2). The mean inter-day precision, as evaluated from duplicate analysis of spiked standards on three separate days, were between 0.7–13.0% (see Table 2). The standard deviations for the slopes of the calibration curves for the three different days of assay were 14.5, 3.2, 14.2 and 9.8 for dexmedetomidine, MPV-1305, MPV-1306 and MPV-

Table 2
Reproducibility and accuracy of the method for the quantitation of dexmedetomidine, MPV-1305, MPV-1306 and MPV-1709 in plasma

Compound	Intra-day variability and accuracy					Inter-day variability and accuracy			
	Concentration					Day	Mean[b]	Grand	R.S.D.
	Spiked (ng/ml)	Found (ng/ml)	S.D. (ng/ml)	Precision (% S.D.)	Accuracy % Difference[a]		conc. (ng/ml)	mean (ng/ml)	(%)
Dex[c]	10.07	9.75			−3.2	1	9.22		
		10.15	0.29	2.9	0.8	2	9.47	9.55	3.9
						3	9.95		
	2.00	1.89			−5.7	1	1.92		
		2.02	0.09	4.6	0.7	2	1.98	1.95	1.7
						3	1.95		
	0.50	0.52			3.8	1	0.54		
		0.46	0.04	8.5	−7.9	2	0.49	0.51	4.7
						3	0.49		
MPV-1305	100.02	95.57			−4.5	1	100.50		
		93.81	1.25	1.3	−6.2	2	103.93	99.70	4.7
						3	94.69		
	10.34	10.02			−3.1	1	10.30		
		10.65	0.45	4.3	3.0	2	8.88	9.84	8.4
						3	10.33		
	1.02	1.14			12.4	1	1.10		
		0.90	0.17	16.7	−11.3	2	1.23	1.12	9.4
						3	1.02		
MPV-1306	100.32	108.32			8.0	1	97.22		
		98.49	6.95	6.7	−1.8	2	107.94	102.85	5.2
						3	103.40		
	10.15	11.04			8.8	1	8.94		
		8.99	1.45	14.5	−11.5	2	11.12	10.03	10.9
						3	10.02		
	1.02	0.85			−16.2	1	1.15		
		1.00	0.10	11.1	−1.9	2	0.93	1.00	13.0
						3	0.92		
MPV-1709	100.08	100.34			0.3	1	101.30		
		104.37	2.85	2.8	4.3	2[d]	102.35	101.83	0.7
	20.27			20.44		0.8	1	23.72	
		19.64	0.57	2.8	−3.1	2[d]	20.04	21.88	11.9
	2.00	2.39			19.5	1	1.03		
		1.79	0.42	20.3	−10.5	2[d]	1.01	1.02	0.8

[a] % Difference=[(found conc.−spiked conc.)/spiked conc.]×100.
[b] Mean of duplicate determinations on each day.
[c] Dex-dexmedetomidine.
[d] Validation for MPV-1709 was not conducted on Day 3.

Table 3
Standard curve summary-validation in human plasma

Compound	Day	Slope	r^2	Inter-assay	
				Mean slope	% S.D.
Dexmedetomidine	1	$4.04 \cdot 10^{-2}$	0.982		
	2	$4.59 \cdot 10^{-2}$	0.998	$4.67 \cdot 10^{-2}$	14.5
	3	$5.39 \cdot 10^{-2}$	0.994		
MPV-1305	1	$1.41 \cdot 10^{-2}$	0.995		
	2	$1.37 \cdot 10^{-2}$	0.998	$1.41 \cdot 10^{-2}$	3.2
	3	$1.46 \cdot 10^{-2}$	0.993		
MPV-1306	1	$2.31 \cdot 10^{-3}$	0.966		
	2	$1.75 \cdot 10^{-3}$	0.995	$2.09 \cdot 10^{-3}$	14.2
	3	$2.20 \cdot 10^{-3}$	0.984		
MPV-1709	1	$1.34 \cdot 10^{-3}$	0.975		
	2	$1.54 \cdot 10^{-3}$	0.998	$1.44 \cdot 10^{-3}$	9.8

1709, respectively (see Table 3). The lowest standards were 100 pg/ml, 1 ng/ml, 1 ng/ml and 2 ng/ml for dexmedetomidine, MPV-1305, MPV-1306 and MPV-1709, respectively. The R.S.D. for these levels were 14.5%, 11.0%, 11.1% and 16.1% indicating they could be used as the lower limits of quantitation from a 1 ml plasma sample.

4. Conclusion

A highly sensitive, specific and reproducible GC–MS assay following solid-phase extraction and two step derivatization has been developed to quantitate dexmedetomidine and three metabolites in a biological matrix. The method was found to be reliable and robust. The lack of interference from blank plasma extracts suggested that the detection mode was highly specific for the analytes of interest. The linear dynamic range of the method is satisfactory for pharmacokinetic studies in animals. Analysis of pooled plasma samples derived from a toxicology study identified concentrations of parent drug, MPV-1306 and MPV-1709 following intravenous administration of dexmedetomidine in dog. Validation of the method in human plasma showed that the method has excellent precision, accuracy and both intra- and inter-day reproducibility. The high selectivity and sensitivity of the described procedure for the quantitation of dexmedetomidine and three metabolites in biological samples makes this analytical method sufficient for pharmacokinetic evaluation of these compounds in human and animal studies.

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