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Simultaneous analysis of 2-methoxyphenylmetyrapone and its seven potential metabolites by high-performance liquid chromatography

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Abstract

A sensitive and specific high-performance liquid chromatographic (HPLC) assay has been developed for the quantification of 2-methoxyphenylmetyrapone (2-MPMP) and its seven potential metabolites in rat urine and whole blood. 2-MPMP, 2-hydroxyphenylmetyrapone and their *N*-oxides, together with 2-methoxyphenylmetyrapol, 2-hydroxyphenylmetyrapol and their *N*-oxides were separated on an Isco Spherisorb ODS-2 reversed-phase column (250×4.6 mm, I.D., 5 μm), with an Isco Spherisorb ODS-2 guard cartridge (10×4.6 mm I.D.). A gradient elution was employed using solvent system A (acetonitrile–water–triethylamine–acetic acid, 27.3:69.1:0.9:2.7%, v/v) and solvent system B (methanol), the gradient program being as follows: initial 0–4 min A:B=74:26; 4–10 min linear change to A:B=50:50; 10–16 min maintain A:B=50:50; 16 min return to initial conditions (A:B=74:26). Flow-rate was maintained at 1.25 ml/min, and the eluent monitored using a diode array multiple wavelength UV detector set at 260 nm. Most of the analytes were baseline resolved, and analysis of samples recovered from blood or urine (pH 12, 3×5 ml of dichloromethane, recovery ~20–95%) revealed no interference from any co-extracted endogenous compounds in the biological matrices, except for 2-hydroxyphenylmetyrapol *N*-oxide (2-OHPMPOL-NO) at low concentrations. The calibrations ($n=6$) were linear ($r\geq0.996$) for all analytes (~0.5–100 μg/ml), with acceptable inter- and intra-day variability. Subsequent validation of the assay revealed acceptable precision, as measured by coefficient of variation (C.V.) at the low (0.5 mg/ml), medium (50 μg/ml) and high (100 μg/ml) concentrations. The limits of detection for 2-MPMP and their available potential metabolites, except 2-OHPMPOL-NO, in rat urine and blood were both 0.5 μg/ml, respectively.

Keywords: 2-Methoxyphenylmetyrapone

1. Introduction

In order to achieve an effective adrenal scintigraphy with positron emission tomography (PET) and single photon emission computed tomography (SPECT), an ideal radioligand should be selective and have an elimination half-time within hours. The current available radioligands for functional diagnosis of adrenal cortex, such as 6β-[^{131}I]-iodomethyl-19-norcholesterol, have long biological half-lives (days), hence radioactivity resides in the body for a longer time than desirable [1,2]. There-

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fore, the need for the development of new radioligands with shorter biological half-lives has been well recognized.

Metyrapone (MP, Fig. 1), a potent competitive inhibitor of adrenal mitochondrial cytochrome P-450 11β-hydrolase (P-450$_{11\beta}$) [3,4] and of other steroid hydrolases [5], and recently its 2-substituted phenylmetyrapone derivatives such as 2-methoxyphenyl-metyrapone [2-MPMP, 1-(2-methoxyphenyl)-2-methyl-2-(3-pyridyl)-1-propanone, Fig. 1] have been shown to be selectively extracted into the adrenal cortex [6,7]. Hence, radiolabeled 2-MPMP may be considered as a suitable agent for further development as an imaging agent for the functional diagnosis of adrenal pathology. However, the metabolic fate of 2-MPMP must be determined prior to its clinical use. The pharmicokinetic studies of 2-MPMP, with a terminal biological half-life of 23.1 min, has been conducted in rats [8]. Only two metabolites, 2-hydroxyoxyphenylmetyrapone (2-OHPMP) and 2-methoxyphenylmetyrapone *N*-oxide (2-MPMP-NO) were identified as the major blood metabolites [8], whereas three Phase I metabolites, namely 2-MPMP-NO, 2-OHPMP-NO (2-hydroxyoxyphenylmetyrapone *N*-oxide) and 2-OHPMP, have been identified in the rat urine dosed with 2-MPMP. In addition, the conjugates of 2-OHPMP and 2-OHPMP-NO have been determined as the major urinary metabolites in rats by using indirect enzymatic hydrolysis [9]. The analysis of 2-MPMP and its metabolites in the above studies were conducted utilizing the presently developed HPLC method. Currently, no other analytical method has been reported for 2-MPMP and its metabolites. The present study was carried out to develop an HPLC assay for the simultaneous analysis of 2-MPMP and its seven potential Phase I metabolites in rat urine and blood, details of which are reported in this paper.

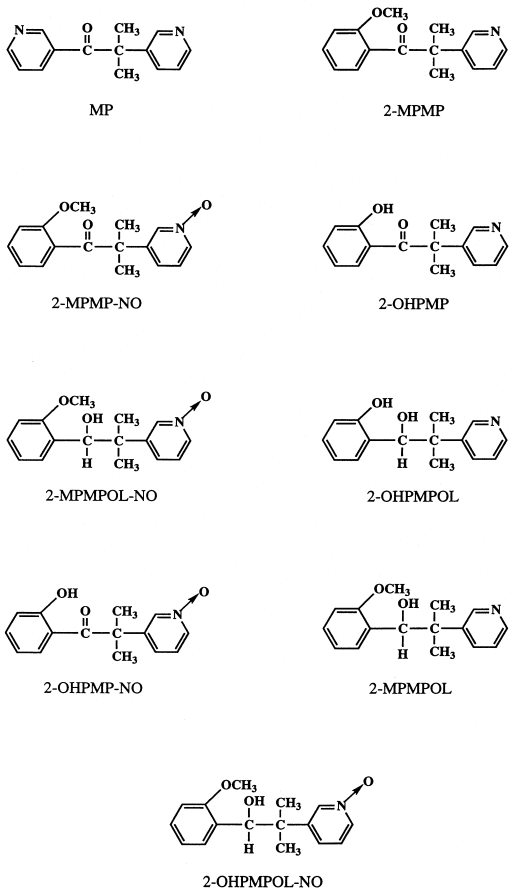

Fig. 1. Chemical structures of metyrapone (MP), 2-methoxyphenylmetyrapone (2-MPMP) and its potential metabolites. Where 2-MPMPOL=2-methoxyphenylmetyrapol, 2-OHPMP=2-hydroxyphenylmetyrapone, 2-OHPMPOL=2-hydroxyphenylmetyrapol, 2-MPMP-NO=2-methoxyphenylmetyrapone *N*-oxide, 2-OHPMP-NO=2-hydroxyphenylmetyrapone *N*-oxide, 2-MPMPOL-NO=2-methoxyphenylmetyrapol *N*-oxide and 2-OHPMPOL-NO=2-hydroxyphenylmetyrapol *N*-oxide.

2. Experimental

2.1. Chemicals

2-MPMP, 2-OHPMP and 2-bromophenylmetyrapone (2-BrPMP), which was used as an internal standard for the quantitative HPLC analyses, were synthesized by Dr. Zolle and co-workers in Vienna, Austria. The other six putative metabolites of 2-MPMP were synthesized by our laboratories. 2-Methoxyphenylmetyrapol (2-MPMPOL) and 2-hydroxyphenylmetyrapol (2-OHPMPOL) were synthesized by sodium borohydride reduction as previously described [10]. 2-MPMP-NO and 2-OHPMP-NO were synthesized by *m*-chloroperoxybenzoic acid oxidation of the corresponding parent compounds [10], while 2-methoxyphenylmetyrapol *N*-oxide (2-MPMPOL-NO) and 2-hydroxyphenylmetyrapol *N*-oxide (2-OHPMPOL-NO) were produced by selective borohydride keto-reduction of 2-MPMP-NO and 2-OHPMP-NO, respectively [11]. The purity and

structural identities of each synthesized compound were characterized and confirmed by TLC, UV, IR, NMR and mass spectral analyses [12]. HPLC-grade methanol, triethylamine and acetonitrile were purchased from BDH Laboratory Supplies (Poole, UK). All other chemicals were of commercially analytical grade obtained from Aldrich Chemical (Milwaukee, USA).

2.2. Animals

Male Sprague–Dawley rats (body weight 250–300 g) were supplied by the animal facility unit of The Chinese University of Hong Kong. Two groups of conscious cannulated rats ($n=6$) were intravenously administered with 2-MPMP as the HCl salt in normal saline (25 mg/kg). Serial arterial blood samples (0.25 ml) were taken at selected 10-min intervals for up to 1 h for one group of rats. Rats in the other group were placed in individual metabolic cages, food and water were allowed ad libitum. Urine samples (0–24 h) were collected from the individual dosed rat of this second group. The third group of rats ($n=5$) were treated with normal saline only. Blank urine samples (0–48 h) were collected, and subsequently the animals were sacrificed and blank whole blood was obtained by cardiac puncture. Heparinized normal saline (50%, v/v, 2 ml) was added to the combined blood samples to prevent clotting. Both pooled blank urine (~100 ml) and blood samples (~50 ml) were stored at 20°C and 4°C, respectively, until required.

2.3. Apparatus

The chromatographic system consisted of a Hewlett–Packard (HP) 1050 chromatography and HP-autosampler. The column configuration consisted of a 10×4.6 mm I.D. Isco Spherisorb ODS-2 guard cartridge and 250×4.6 mm, I.D., 5 μm, Isco Spherisorb ODS-2 reversed-phase column (Nebraska, USA). The UV absorbance was measured by a diode array multiple wavelength UV detector (HP 1050) set at 260 nm for routine analysis, or continuously monitored between 200 and 400 nm for validation studies. The UV spectra of the synthetic authentic compounds were stored in a spectral library, and those of the metabolic extracts were compared to these standards in terms of retention times and spectral characteristics. The peak purities were calculated automatically by the data acquisition system.

2.4. Chromatographic conditions

A gradient elution was employed using solvent system A (acetonitrile–water–triethylamine–acetic acid, 27.3:69.1:0.9:2.7%, v/v) and solvent system B (methanol) at ambient temperature. The gradient program used was as follows: initial 0–4 min, A:B=74:26; 4–10 min linear change to A:B=50:50 and maintain for 10–16 mm; and at 16 min return to initial conditions (A:B=74:26). Flow-rate was kept constant at 1.25 ml/min.

2.5. Calibration curves

Two stock solutions of concentrations of 10 mg/ml and 100 mg/ml, which contained the following compounds: 2-MPMP, 2-MPMPOL, 2-OHPMP, 2-OHPMPOL, 2-MPMP-NO, 2-MPMPOL-NO, 2-OHPMP-NO and 2-OHPMPOL-NO, were prepared. Serial dilution of the stock solutions (0.5–6 μg/ml and 5–100 μg/ml, $n=6$) were prepared. This range of concentrations was used for the construction of two separate calibration curves in rat blank urine and whole blood such as one in the low range (0.5–6 μg/ml) and the other in the high range (5–100 μg/ml). Methanolic solution of 2-BrPMP was used as an internal standard (2.5 μg for low level and 40 μg for the high level calibrations). Calibration curves, plotting concentrations of the analytes in urine or whole blood as a function of the analyte/internal standard peak area ratios, were derived for 2-MPMP and its seven potential metabolites.

2.6. Sample preparation

2.6.1. Urine

Aliquots (100 μl) of the prepared standard solutions of appropriate concentrations of analytes were added into rat blank urine (1 ml), after alkalinization (0. 1 ml, 3.5 *M* NaOH) and mixed with water (0.5 ml), followed by the addition of 2-BrPMP (2.5 μg or 40 μg) as internal standard. The mixture was extracted using dichloromethane (3×5 ml). The organic extracts were combined and evaporated to dryness using Centrivap concentrator (Labconco,

Missouri, USA). The residues were reconstituted into solvent system A (100 μl) and aliquots (20 μl) were analysed using HPLC. Urine samples (1.0 ml) collected from rat dosed with 2-MPMP were also treated in an identical manner to that for the standards.

2.6.2. Whole blood

Aliquots (100 μl) of the prepared standard solutions of appropriate concentrations were added into control rat blood (0.25 ml) alkalinized with sodium hydroxide (0.1 ml, 3.5 *M* NaOH), followed by addition of internal standard 2-BrPMP (2.5 μg or 40 μg). The final volume was made up to 1.7 ml by addition of distilled water, and samples processed for HPLC analysis as described Section 2.6.1 for urine samples. The blood samples (0.25 ml) collected from 2-MPMP-dosed rat were also treated in an identical manner to that for the standards.

2.7. Validation studies

2.7.1. Recovery

The recovery or extractability of the standards in urine and blood was determined using three different concentrations of 0.5, 50 and 100 μg/ml with the inclusion of internal standard (200 μg). The peak area ratios of the extracted samples were compared with those of unextracted samples to derive percentage recovery.

2.7.2. Accuracy and precision

The three concentrations (i.e. 0.5, 50 and 100 μg/ml) were used to test intra-day and inter-day variability. Five samples at each given concentration were extracted and analysed for each of four consecutive days. Blinded unknowns which were prepared by a different analyst were also carried out to test the accuracy of the method.

2.7.3. Limits of detection

The standard concentrations ranging from 0.5 to 6 μg/ml in both urine and whole blood were extracted

Table 1
Chromatographic parameters for 2-MPMP and its seven potential metabolites

Compound	Capacity factor, k'	Retention time (min)
2-MPMP	5.57	12.3
2-OHPMP	6.59	14.5
2-MPMPOL	4.45	9.8
2-OHPMPOL	2.41	5.3
2-MPMP-NO	3.41	7.5
2-OHPMP-NO	3.75	8.3
2-MPMPOL-NO	3.07	6.7
2-OHPMPOL-NO	2.00	4.4
2-BrPMP	7.27	16.0

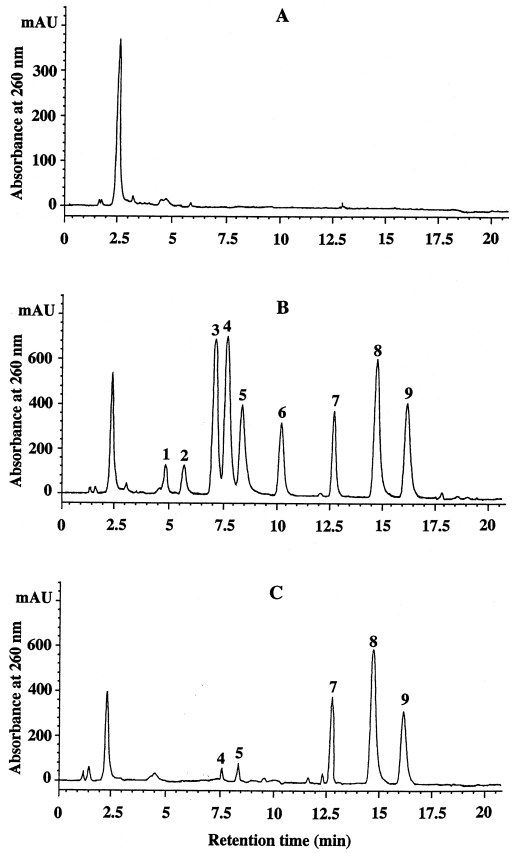

Fig. 2. Representative HPLC chromatograms of the extracts of (A) blank rat urine, (B) urinary standards (5.0 μg/ml), and (C) 0–24 h urine samples of rat dosed with 2-MPMP (25 mg/kg, i.v.) on Spherisorb ODS-2 reversed-phase column. Peaks: 1=2-hydroxyphenylmetyrapol *N*-oxide (2-OHPMPOL-NO), 2=2-hydroxyphenylmetyrapol (2-OHPMPOL), 3=2-methoxyphenylmetyrapol *N*-oxide (2-MPMPOL-NO), 4=2-methoxyphenylmetyrapone *N*-oxide (2-MPMP-NO), 5=2-hydroxyphenylmetyrapone *N*-oxide (2-OHPMP-NO), 6=2-methoxyphenylmetyrapol (2-MPMPOL), 7=2-methoxyphenylmetyrapone (2-MPMP), 8=2-hydroxyphenylmetyrapone (2-OHPMP) and 9=2-bromophenylmetyrapone (2-BrPMP).

and analysed using the previously described procedures for the sample preparation. The signal-to-noise ratios for all analytes were determined.

3. Results and discussion

3.1. Chromatographic separation of 2-MPMP and its metabolites

Under the chromatographic conditions used in this study, the capacity factors (expressed as k') and the retention times for 2-MPMP and its metabolites are shown in the Table 1 and a typical chromatogram depicted in Fig. 2, showing that most of the components are base-line resolved. The elution order of these compounds, using Spherisorb ODS-2 reversed-phase column, were 2-OHPMPOL-NO, 2-OHPMPOL, 2-MPMPOL-NO, 2-MPMP-NO, 2-OHPMP-NO, 2-MPMPOL, 2-MPMP, and 2-OHPMP. This order indicates that the *N*-oxide of 2-MPMP is more hydrophilic than the *N*-oxide of 2-OHPMP. It is also noteworthy that 2-OHPMP, the major primary metabolite of 2-MPMP, has a longer elution time than 2-MPMP itself. In addition, 2-OHPMP also had a R_F value larger than that for 2-MPMP when developed with normal-phase TLC [12]. From NMR studies, the proton signals generated for the 2-hydroxyl group of 2-OHPMP was much downfield with respect to that of 2-MPMP [12]. This could be explained by the formation of an intra-molecular hydrogen bond between 2-hydroxyl group on the phenyl ring with the carboxyl oxygen, rendering the 2-hydroxyl compound more lipophilic in nature than 2-MPMP. The same explanation can also be applied to 2-OHPMP-NO [12]. The internal standard (2-BrPMP) eluted at much longer retention time than the other studied compounds, and was therefore a suitable internal standard.

3.2. Assay of urine samples

Typical HPLC chromatograms resulting from the analysis of the extracts of blank rat urine, urinary standards and test urine samples are represented in Fig. 2. HPLC analysis of control urine extracts (Fig. 2, Panel A) demonstrated that endogenous components did not interfere with the assay of the last eluted eight compounds of interest, except for the earliest eluting compound, namely 2-OHPMPOL-

Table 2
Standard curves derived for the eight urinary standards for the low concentration ranges (0.5–6 μg/ml) and the high concentration ranges (5–100 μg/ml)

Compound	$y=mx+c$		
	Slope (m)	Intercept (c)	Correlation coefficient (r)
Low range (0.5–6 μg/ml)			
2-MPMP	0.155	8.617×10^{-3}	0.996
2–OHPMP	0.408	3.240×10^{-3}	0.999
2-MPMPOL	0.154	7.836×10^{-3}	0.998
2-OHPMPOL	0.032	0.130	0.999
2-MPMP-NO	0.427	0.041	0.998
2-OHPMP-NO	1.246	−0.035	0.999
2-MPMPOL-NO	0.361	0.064	0.998
High range (5–100 μg/ml)			
2-MPMP	8.041×10^{-3}	1.463×10^{-3}	0.999
2-OHPMP	0.019	−0.022	0.999
2-MPMPOL	8.753×10^{-3}	1.829×10^{-3}	0.999
2-OHPMPOL	2.974×10^{-3}	-1.987×10^{-3}	0.998
2-MPMP-NO	0.022	7.317×10^{-4}	0.999
2-OHPMP-NO	0.013	−0.0241	0.999
2-MPMPOL-NO	0.018	1.951×10^{-3}	0.999
2-OHPMPOL-NO	1.183×10^{-3}	9.878×10^{-3}	0.996

NO. HPLC analysis of the extracts of the urinary standards (Fig. 2, Panel B), and the extracts of test urine samples (Fig. 2, Panel C, rat dosed with 2-MPMP, 25 mg/kg, i.v.), with diode array UV detector revealed that drug and metabolite peaks were 'pure', by a standard peak-matching technique. Interestingly, the components detected in 24 h rat urine samples were 2-MPMP, 2-OHPMP, and their respective *N*-oxides. In fact, 2-OHPMP and 2-OHPMP-NO have been previously identified to be present predominantly as their *O*-glucuronide conjugates in urine [9].

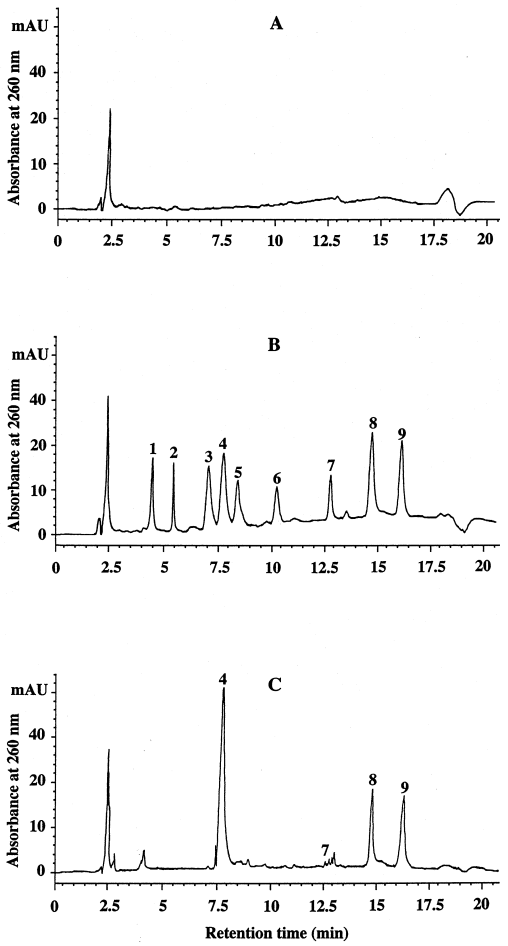

Fig. 3. Representative HPLC chromatograms of the extracts of (A) blank rat whole blood, (B) blood standards (0.5 μg/ml), and (C) 10-min blood sample of rat dosed with 2-MPMP (25 mg/kg, i.v.) on Spherisorb ODS-2 reversed-phase column. Peaks: 1=2-hydroxyphenylmetyrapol *N*-oxide (2-OHPMPOL-NO), 2=2-hydroxyphenylmetyrapol (2-OHPMPOL), 3=2-methoxyphenylmetyrapol *N*-oxide (2-MPMPOL-NO), 4=2-methoxyphenylmetyrapone *N*-oxide (2-MPMP-NO), 5=2-hydroxyphenylmetyrapone *N*-oxide (2-OHPMP-NO), 6=2-methoxyphenylmetyrapol (2-MPMPOL), 7=2-methoxyphenylmetyrapone (2-MPMP), 8=2-hydroxyphenylmetyrapone (2-OHPMP) and 9=2-bromophenylmetyrapone (2-BrPMP).

The standard curves, for the low range (0.5–6 μg/ml) and high range (5–100 μg/ml) urine standards, were constructed. Standard curves derived for each of the seven compounds with the regression equations and correlation coefficients (*r*) for the low range concentrations (0.5–6 μg/ml) are shown in Table 2. Analyses of 2-OHPMPOL-NO at the low concentration range, using the present HPLC system, were complicated by the presence of polar endogenous substances in the urine. Hence, no standard curve for 2-OHPMPOL-NO at low concentration range was obtained. For the high concentrations range (5–100 μg/ml) of urinary standards. The regression equations and correlation coefficients are also shown in Table 2.

3.3. Assay of whole blood samples

Typical HPLC chromatograms resulting from the analysis of the extracts of blank rat whole blood, blood standards and test rat blood samples are represented in Fig. 3. As for urine analysis, endogenous components did not interfere with the HPLC analysis of the last eluting eight compounds of interest except 2-OHPMPOL-NO. The compounds detected in the blood of rats dosed with 2-MPMP (25 mg/kg, i.v.) were 2-OHPMP, 2-MPMP and its respective *N*-oxide.

Standard curves for the blood standards at the low concentration range (0.5–6 μg/ml) are shown in Table 3. As in the case of urine, no standard curve for 2-OHPMPOL-NO in rat blood at low concentration range was obtained due to early eluting polar endogenous components which interfered with its analysis. For the high blood concentration range (5–100 μg/ml), the regression equations and correlation coefficients of the standards are also shown in Table 3.

Table 3
Standard curves derived for the eight blood standards for the low concentration ranges (0.5–6 μg/ml) and the high concentration ranges (5–100 μg/ml)

Compound	$y=mx+c$		
	Slope (m)	Intercept (c)	Correlation coefficient (r)
Low range (0.5–6 μg/ml)			
2-MPMP	0.212	0.056	0.997
2-OHPMP	0.453	0.049	0.999
2-MPMPOL	0.314	0.022	0.999
2-OHPMPOL	0.032	-2.908×10^{-3}	0.998
2-MPMP-NO	0.501	0.047	0.999
2-OHPMP-NO	0.280	0.074	0.997
2-MPMPOL-NO	0.388	0.050	0.999
High range (5–100 μg/ml)			
2-MPMP	7.290×10^{-3}	7.146×10^{-3}	0.998
2-OHPMP	0.019	0.011	0.999
2-MPMPOL	7.863×10^{-3}	6.473×10^{-3}	0.998
2-OHPMPOL	1.625×10^{-3}	1.834×10^{-3}	0.999
2-MPMP-NO	0.020	0.051	0.999
2-OHPMP-NO	0.016	8.629×10^{-3}	0.999
2-MPMPOL-NO	0.017	0.013	0.999
2-OHPMPOL-NO	1.016×10^{-3}	0.010	0.998

3.4. Recovery

The recoveries (%) for the eight urinary standards averaged over three concentrations previously mentioned in Section 2.7 are shown in Table 4. The results indicated that 2-OHPMPOL-NO (<20%) was the only compound which was not well-extracted by dichloromethane. All the remaining compounds had an average extraction recovery of greater than 60% from the urine. In this study, the concentration of internal standard used was 200 μg instead of 2.5 or 40 μg for high and low level calibrations. Previous studies showing an inclusion of a higher concentration of 2-BrPMP (200 μg) in urine and blood did not affect the extraction of the standards using dichloromethane. The recoveries (%) for the eight standards in whole blood averaged over three concentrations are also shown in Table 4. The results showed that the blood recoveries of all the standards tested, except 2-OHPMPOL-NO, were similar to those from urine (>60%).

Table 4
Recovery of analytes from rat urine and whole blood averaged over three concentrations (0.5, 50 and 100 μg/ml)

Compounds	Recovery (mean±S.D.) (%)	
	Urine ($n=3$)	Whole blood ($n=3$)
2-MPMP	72.2±4.3	85.7±2.1
2-OHPMP	75.7±6.3	79.6±5.6
2-MPMPOL	82.7±3.5	89.5±4.2
2-OHPMPOL	89.0±7.5	85.9±10.9
2-MPMP-NO	90.9±2.5	83.8±8.4
2-OHPMP-NO	60.7±4.4	73.8±6.7
2-MPMPOL-NO	91.7±2.1	89.5±3.6
2-OHPMPOL-NO	10.9±1.3	16.8±2.5

3.5. Accuracy and precision

Two blinded urinary samples containing unknown concentrations of all eight standards produced accuracy of 85–97%. The intra- and inter-day variabilities of the assay for urine samples are shown in Table 5, indicating that the precision of the assay, as

Table 5
Intra- and inter-day variabilities for the assay of 2-MPMP and its potential metabolites

Concentration (μg/ml)	Coefficient of variation (%)			
	Urine		Whole blood	
	Single day (n=5)	Four days (n=20)	Single day (n=5)	Four days (n=20)
2-OHPMPOL-NO				
5.0[a]	14.0	13.5	17.0	15.5
50.0	14.0	12.9	11.5	10.9
100.0	5.5	5.1	10.9	11.2
2-OHPMPOL				
0.5	9.9	7.5	8.6	9.4
50.0	8.9	8.6	6.1	5.7
100.0	1.5	1.3	1.7	1.5
2-MPMPOL-NO				
0.5	5.6	6.3	10.6	11.9
10.0	2.3	3.1	15.0	15.4
100.0	2.4	2.1	7.2	6.9
2-MPMP-NO				
0.5	4.5	4.8	5.7	5.9
10.0	2.1	1.9	4.8	4.5
100.0	2.6	2.9	4.9	5.3
2-OHPMP-NO				
0.5	4.5	3.2	16.5	16.3
10.0	3.8	5.1	19.6	18.9
100.0	2.4	3.2	6.7	6.1
2-MPMPOL				
0.5	12.5	13.4	18.4	17.6
10.0	10.9	9.9	15.2	18.4
100.0	5.5	4.9	8.9	7.5
2-MPMP				
0.5	6.4	5.9	13.7	14.2
10.0	3.7	3.9	8.8	9.7
100.0	1.4	1.9	4.3	5.7
2-OHPMP				
0.5	4.2	4.8	12.7	11.5
10.0	4.7	6.2	10.2	9.8
100.0	2.4	3.5	5.4	5.0

[a] Interference from endogenous materials for concentrations lower than 5 μg/ml.

measured by coefficient of variation (C.V.) was acceptable, being 10–19% for the lower concentrations (0.5–5 μg/ml) and 2–<10% for higher concentrations. Two blinded samples containing unknown concentrations of all eight compounds in the whole blood produced accuracies of 83–95%. The intra- or inter-day variabilities in whole blood are also presented in Table 5. The results showed that there are no large intra- or inter-day variabilities in analysis of 2-MPMP and its metabolites in rat urine or blood.

3.6. Limit of detection

The limits of detection for 2-MPMP and the authentic standards of its potential metabolites in rat urine were 0.5 μg/ml with a signal-to-noise ratio of 3 to 1. Whereas in the case of blood samples, the

limits of detection for all analytes were 0.5 μg/ml with a signal-to-noise ratio of 4 to 1.

In conclusion, a simple and sensitive HPLC assay for the simultaneous analysis of 2-MPMP and its seven potential metabolites has been developed and validated. With an application of this developed HPLC method for analysis of urine and blood samples obtained from the rats dosed with 2-MPMP, three metabolites such as 2-MPMP-NO, 2-OHPMP-NO and 2-OHPMP, and two metabolites namely 2-MPMP-NO and 2-OHPMP were detected in urine and blood, respectively. These results are in a good agreement with previous performed pharmacokinetic and metabolic studies [8,9]. Species and sex differences in metabolism of metyrapone have been previously reported [13]. It is, therefore, very likely that these two intrinsic factors may also affect the metabolism of 2-MPMP. Although other putative metabolites of 2-MPMP could not be detected in the urine and blood of the rats dosed with 2-MPMP, the presently developed HPLC assay should be useful for the investigation of the metabolic fate of 2-MPMP in other species including man, that may form some of the other Phase I metabolites which are not generated by rats, and also for the study of factors affecting the pharmacokinetic and metabolism of 2-MPMP.

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