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ANALYSIS

QUANTIFICATION AND PHARMACOKINETICS OF 1-METHYLPYRIDINIUM AND 1,4-DIMETHYLPYRIDINIUM IN RATS BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY. TISSUE DISTRIBUTION OF 1,4-DIMETHYLPYRIDINIUM IN RATS

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Abstract: A sensitive and specific liquid chromatography tandem mass spectrometry method for quantification of 1-methylpyridinium (1-MP) and 1,4-dimethylpyridinium (1,4-DMP) in rat plasma and tissues homogenates was developed. Chromatographic separation was performed on an Aquasil C18 analytical column with an isocratic elution of acetonitrile and water, both with an addition of formic acid (0.1%, v/v). Detection was achieved by triple quadrupole mass spectrometer TSQ Quantum Ultra equipped with a heated electrospray ionization source (HESI). The limit of quantification for both compounds was 0.05 μg/mL in plasma and 0.25 μg/g in studied tissues. The method was applied to pharmacokinetics and bioavailability of both 1-MP and 1,4-DMP with tissue distribution of 1,4-DMP in rats. Pharmacokinetic studies of 1-MP and 1,4-DMP were carried out following their intravenous or intragastric administration to male Wistar rats at the dose of 100 mg/kg. The terminal half-lives of 1-MP and 1,4-DMP after their intravenous administration were 55.3 and 70.8 min, respectively. The absolute bioavailability was 51 and 31% for 1-MP and 1,4-DMP, respectively.

Keywords: LC/MS/MS, method validation, derivatives of pyridinium compounds, pharmacokinetics

Trigonelline, a component of green coffee beans (about 1%) is a product of thermal decomposition, formed during the coffee roasting process. Evaluated compounds: 1-methylpyridinium (1-MP) and 1,4-dimethylpyridinium (1,4-DMP) are the degradation products of trigonelline and for many years they have been a subject of increased interest because of theirs potential hepatoprotective, vasoprotective and antioxidant activity (1-5). Furthermore, some pyridinium salts are known from cytotoxic activity against tumor cells and this effect is probably related to their redox properties (6, 7).

To characterize properties of 1-MP and 1,4-DMP, the structure and surface activity of these compounds were investigated using surface-enhanced Raman spectroscopy (SERS) (8). Recently, liquid chromatography–mass spectrometry method was developed to determine the concentration of 1,4-DMP in rat plasma (9), and this technique was also used for food-derived bioactive pyridines quantification, among them 1-MP and their metabolites in human plasma and urine. The method was applied to monitor the plasma appearance and the urinary excretion, and to calculate the pharmacokinetic parameters of the studied compounds (10, 11). To our knowledge there is no described method for simultaneous determination of 1-MP and 1,4-DMP in complex biological samples, like e.g., tissue homogenates.

The aim of this study was to develop and validate a selective and sensitive bioanalytical LC/MS/MS method for simultaneous quantification of 1-MP and 1,4-DMP in rat plasma and tissue homogenates according to EMA requirements, and finally to assess the pharmacokinetics and bioavail-

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ability of 1-MP and 1,4-DMP considering tissue distribution of 1,4-DMP.

EXPERIMENTAL

Reagents

The 1-methylpyridinium (1-MP) chloride, 1,4-dimethylpyridinium (1,4-DMP) chloride and their stable isotope labeled internal standards: 1-d_3-methylpyridinium 1-MP-d_3) chloride and 1-d_3-methyl-4-methylpyridinium (1,4-DMP-d_3) chloride were provided by dr. J. Adamus from the Institute of Applied Radiation Chemistry, Technical University (Poland). HPLC grade acetonitrile was purchased from J.T. Baker (Germany) and formic acid from Fluka (Germany). Sodium phosphate dibasic, potassium dihydrogen phosphate and sodium chloride were purchased from Sigma-Aldrich (USA). Deionized water was obtained from Millipore system (Direct-Q 3UV, Millipore) and used in all aqueous solutions.

Samples

Plasma and tissues were obtained from adult eight-weeks old male Wistar rats (180-220 g) (Charles River Laboratory, Germany). Rats were injected intraperitoneally with thiopental (60 mg/kg) and blood was collected into heparinized vials after decapitation. The plasma samples were separated by centrifugation (900 × g, 15 min) and stored at -20°C until used. The tissues: liver, lungs, heart, brain, small intestine and kidney were collected, rinsed with phosphate buffer saline solution (PBS, pH = 7.4) and stored at -80°C until used. A piece of thawed tissue was weighted (approximately 100 mg) and homogenized by an UltraTurrax® T10 basic homogenizer (IKA, Germany) in 500 µL of PBS solution (ratio 1 : 5, w/v). The tissue homogenates were prepared directly before the analysis.

Liquid chromatography conditions

The liquid chromatography system UltiMate 3000 (Dionex, USA) consisted of a pump (DGP 3600RS), a column compartment (TCC 3000RS), an autosampler (WPS-3000TRS) and SRD-3600 solvent rack (degasser) was used. Chromatographic separation was carried out on an Aquasil C18 analytical column (4.6 × 150 mm, 5 µm, Thermo Scientific, USA) with the oven temperature set at 30°C. Acetonitrile (A) and water (B), both with a 0.1% (v/v) of formic acid addition were used as mobile phases. Separation of analytes and IS was performed under isocratic condition (A : B; 40 : 60, v/v) at a flow rate of 0.8 mL/min. The autosampler temperature was set at 10°C and the injection volume was 10 µL. The eluent from the HPLC before being directed into the heated electrospray ionization (HESI) probe was split in the proportion of 1 to 2 (1 part to the mass spectrometer and 2 parts to waste). The whole HPLC analysis lasted 10 min.

Mass spectrometric conditions

Mass spectrometric detection was performed on TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo Scientific, USA) equipped with a HESI II probe operating in the positive ion mode. Quantification was done using selected reaction monitoring (SRM) mode to monitor precursor → product ion transitions of m/z 94 → 79 for 1-MP, m/z 97 → 79 for 1-MP-d_3, m/z 108 → 93 for 1,4-DMP and m/z 111 → 93 for 1,4-DMP-d_3. Data acquisition and processing were accomplished using Xcalibur 2.1 software (Thermo Scientific, USA).

The ion source parameters for all analytes were as follows: ion spray voltage 4000 V, vaporizer temperature 250°C, sheath gas and auxiliary gas (nitrogen) pressure 30 and 10 arbitrary units, respectively, and capillary temperature 350°C. Argon pressure in the collision cell was 1.5 mTorr. Collision energy was set at 23 V for 1-MP, 22 V for 1-MP-d_3 and 30 V for 1,4-DMP and 1,4-DMP-d_3.

Preparation of standard solutions

Stock solutions (1 mg/mL) of 1-MP chloride, 1,4-DMP chloride and its deutered analogs: 1-MP-d_3 chloride and 1,4-DMP-d_3 chloride were individually prepared in ultrapure water. The combined standard solution of 1-MP and 1,4-DMP was prepared by mixing and diluting the appropriate amounts from individual stock solutions. The final concentration of the working standard solutions was 50, 40, 35, 30, 25, 20, 10, 5, 1.5, 1 and 0.5 µg/mL. Internal standard (IS) solution consisted of 1-MP-d_3 and 1,4-DMP-d_3 at a concentration of 25 µg/mL. IS solution was prepared by mixing and diluting the appropriate amounts from individual stock solutions. All stock and working solutions were stored at 4°C until used.

Preparation of calibration and quality control samples

Calibration standards (CC) and quality control samples (QC) were prepared by spiking 10 µL of the appropriate working mixed solution of 1-MP and 1,4-DMP chlorides into 90 µL of blank tissue homogenate or plasma. The concentration of CC points were equivalent to 5, 4, 3, 2, 1, 0.5, 0.1 and 0.05 µg/mL in plasma, and 25, 20, 15, 10, 5, 2.5, 0.5, 0.25 µg/g tissue in tissue samples. Concentration of

QC samples were as follows: limit of quantification (LOQ) at 0.05 µg/mL, low QC (LQC) at 0.15 µg/mL, medium QC (MQC) at 2.5 ng/mL and high QC (HQC) at 3.5 ng/mL in plasma samples, and LOQ at 0.25 µg/g, LQC at 0.75 µg/g, MQC at 12.5 µg/g and HQC at 17.5 µg/g in tissue samples, for both analyzed compounds.

Samples preparation

All analyzed samples were prepared by the way of deproteinization with acetonitrile (12). A 100 µL aliquot of rat plasma or homogenized tissues was pipetted out into a polypropylene tube and spiked with 10 µL of the working IS solution (25 µg/mL). Then, the samples were briefly mixed and 200 µL of acidified acetonitrile (0.1%, v/v) was added. The mixture was again shaken, next, the samples were refrigerated at 4°C for 20 min, and afterwards centrifuged at 16600 × g for 15 min at 10°C. The supernatant (100 µL) was transferred into new tubes and evaporated to dryness at 37°C under a gentle stream of nitrogen gas in a TurboVap evaporator (Caliper Life Sciences, USA). The dry residue was reconstituted with 100 µL of the acetonitrile/water (50/50, v/v) mixture, and an aliquot of 10 µL was injected into the LC/MS/MS system.

Method validation

Method validation was carried out on blank matrices: plasma and brain, liver, heart, kidney, lungs, and small intestine homogenates spiked with an appropriate amounts of 1-MP, 1,4-DMP and their IS following the criteria of bioanalytical method validation (13).

Selectivity/Specificity

The specificity of the method was evaluated by analyzing blank matrices from six different rats. Each blank sample was tested for interferences using the proposed clean up procedure and chromatographic/mass spectrometric conditions.

Accuracy and precision

Precision was calculated in the terms of RSD (%) by analyzing QC samples at four concentration levels of 1-MP and 1,4-DMP (0.05, 0.15, 2.5 and 3.5 µg/mL in plasma, and 0.25, 0.75, 12.5, 17.5 µg/g in the tissues). Accuracy was evaluated as [mean found concentration / theoretical concentration] × 100. The criteria for acceptability of the data included accuracy within ± 15% deviation from the nominal values, and precision within 15% RSD except for LOQ, where it should not exceed ± 20%.

Within day precision and accuracy were executed by repeated analysis (n = 5) of the samples at different QC levels on the same day. Between days precision and accuracy were determined by repeated analysis on the following day. The concentration of each QC sample was determined using the calibration curve prepared and analyzed on the same day.

Matrix effect and extraction recovery

The relative matrix effect was estimated according to Matuszewski (14, 15) by assessing the variability of standard line slopes expressed as a coefficient of variation, RSD (%). The precision values of standard slope lines should not exceed 4% for the method to be considered reliable, and free from the relative matrix effect. For evaluation of the relative matrix effect, five different sources of rat matrices were used.

Extraction recoveries of 1-MP and 1,4-DMP from plasma were determined at LQC and HQC. They were calculated by comparing the mean peak areas obtained for deproteinized QC samples with those of blank extracts with standards added at appropriate concentration which represented the 100% recovery value.

Stability studies

Long-term, short-term, freeze and thaw stability tests were performed for plasma samples. The samples for long-term and short-term stability tests were kept at -20°C for the period of 4 months, and at the room temperature for a period that exceeded the routine sample preparation time (about 5 h), respectively. Post preparation stability test was carried out for all analyzed matrices; samples were stored in autosampler at 10°C for 24 h. A stability study was evaluated using two concentration levels (LQC and HQC). All stability samples were quantified using fresh calibration curve and compared to the nominal concentration in the sample. Samples were considered to be stable if 85-115% of the initial concentration was found.

Pharmacokinetic study in rats

Using the new LC/HESI-MS/MS method, pilot pharmacokinetic studies of 1-MP and 1,4-DMP were carried out following their intravenous or intragastric administration to male eight-weeks old Wistar rats (180-200 g). Rats were kept under conditions of constant temperature (21-25°C), and relative humidity of approximately 40-65% with a standard light/dark cycles. Animals were housed in stainless steel cages with suspended wire-mesh floors (maximum of 5 rats per cage). They were fast-

ed overnight and then weighted. Rats had free access to water throughout the experimental period. Studied compounds: 1-MP and 1,4-DMP, dissolved in 0.9% sterile isotonic saline, at the dose of 100 mg/kg body weight were administered intravenously or intragastrically. Rats were anesthetized *via i.p.* administration of thiopental (60 mg/kg) and sacrificed at the following time intervals: 5, 30, 60, 120 and 240 min after 1-MP or 1,4-DMP intravenous administration, and 10, 30, 60, 120 and 240 min after intragastric dosing. Three rats were sampled at every time point. Blood samples were collected into

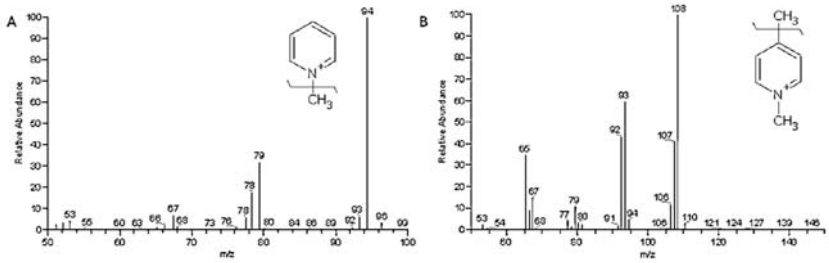

Figure 1. Fragmentation mass spectra of 1-MP (A) and 1,4-DMP (B), collision energy 30 V

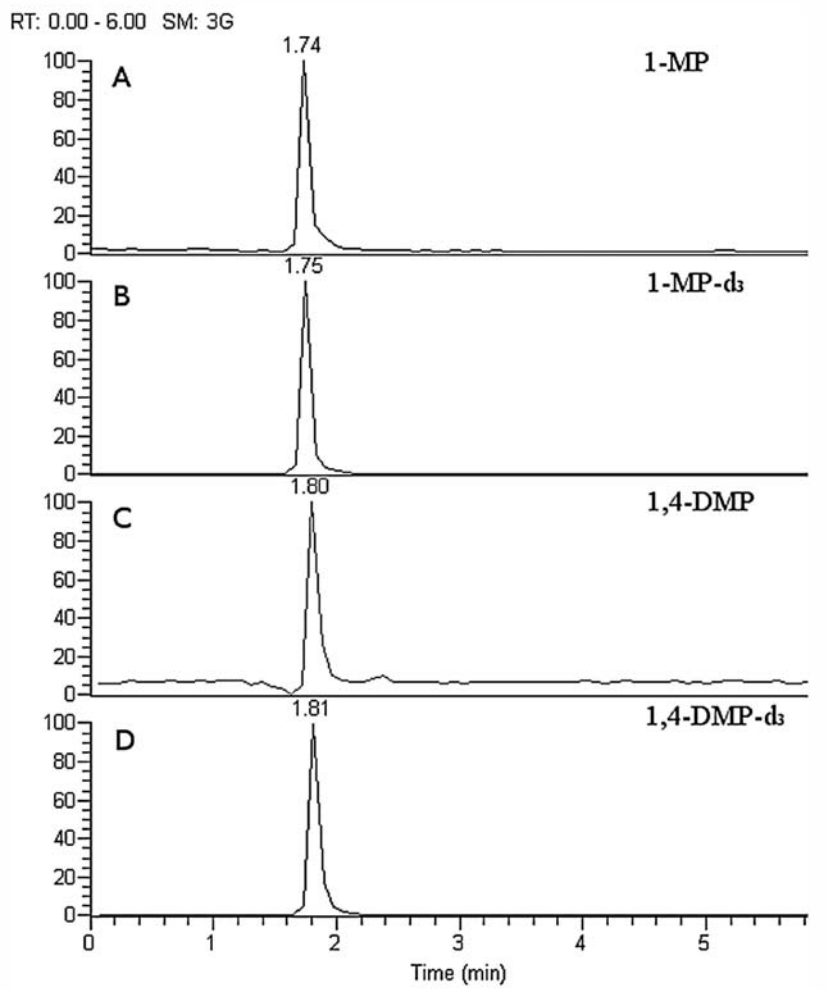

Figure 2. Extracted ion chromatograms of 1-MP (A), 1-MP-d_3 (B), 1,4-DMP (C) and 1,4-DMP-d_3 (D) in rat plasma (LLOQ sample)

microfuge tubes. Plasma and selected tissues (liver, lungs, heart, intestine, brain and kidneys) were stored at –20°C and –80°C until used, respectively.

Pharmacokinetic parameters were calculated by a non-compartmental approach from the average concentration values, using Phoenix WinNonlin software (Certara, USA). First order elimination rate constant (λ_z) was calculated by linear regression of time *versus* log concentration. Next, the area under the mean serum and tissue compound concentration *versus* time curve extrapolated to infinity ($AUC_{0\rightarrow\infty}$) was estimated using the log-linear trapezoidal rule (equation 1), where C_n is the concentration of last sampling of each compound.

Table 1. Relative matrix effect for 1-MP and 1,4-DMP.

Matrix	Lots	1-MP			1,4-DMP		
		Slope	R^2	SD [%]	Slope	R^2	SD [%]
Plasma	1	0.000350	1.0000	3.5	0.000351	0.9999	2.6
	2	0.000318	0.9973		0.000341	1.0000	
	3	0.000330	0.9998		0.000357	0.9995	
	4	0.000335	0.9971		0.000349	0.9998	
	5	0.000329	0.9992		0.000366	0.9990	
Liver	1	0.000407	0.9911	4.1	0.000461	0.9993	2.2
	2	0.000366	0.9981		0.000435	0.9989	
	3	0.000393	0.9999		0.000451	0.9997	
	4	0.000401	0.9941		0.000449	0.9997	
	5	0.000397	0.9967		0.000442	0.9979	
Heart	1	0.000464	0.9977	3.4	0.000449	0.9990	4.0
	2	0.000474	0.9997		0.000423	0.9979	
	3	0.000472	0.9998		0.000425	0.9988	
	4	0.000474	0.9980		0.000411	1.0000	
	5	0.000506	0.9991		0.000404	0.9978	
Lungs	1	0.000605	0.9999	3.3	0.000481	0.9975	2.3
	2	0.000656	0.9998		0.000507	0.9996	
	3	0.000620	0.9961		0.000497	0.9981	
	4	0.000647	0.9988		0.000505	0.9988	
	5	0.000639	0.9981		0.000511	0.9979	
Kidney	1	0.000572	0.9959	2.1	0.000484	0.9985	2.1
	2	0.000576	0.9996		0.000462	0.9999	
	3	0.000591	0.9994		0.000476	0.9985	
	4	0.000558	0.9993		0.000462	0.9998	
	5	0.000568	0.9997		0.000465	0.9996	
Brain	1	0.000581	0.9971	3.7	0.000502	0.9993	3.7
	2	0.000569	1.0000		0.000486	0.9989	
	3	0.000550	0.9984		0.000487	0.9994	
	4	0.000605	0.9966		0.000530	0.9967	
	5	0.000560	0.9996		0.000491	0.9992	
Intestine	1	0.000285	0.9989	3.6	0.000285	0.9972	3.6
	2	0.000288	1.0000		0.000288	0.9976	
	3	0.000306	0.9997		0.000306	0.9996	
	4	0.000278	0.9991		0.000278	0.9996	
	5	0.000284	0.9999		0.000284	0.9993	

Table 2. Within day accuracy (% of nominal concentration) and precision (% RSD) for 1-MP and 1,4-DMP in plasma and rat tissues (n = 5).

Matrix	QC level	1-MP			1,4-DMP		
		Mean concentration	Accuracy [%]	Precision RSD [%]	Mean concentration	Accuracy [%]	Precision RSD [%]
Plasma[a]	LLQC	0.053	106.6	7.9	0.050	100.2	1.4
	LQC	0.140	93.2	4.6	0.140	93.1	3.1
	MQC	2.539	101.6	3.2	2.434	97.4	3.8
	HQC	3.365	96.1	8.4	3.423	97.8	5.0
Liver[b]	LLQC	0.238	95.0	12.9	0.254	101.4	3.4
	LQC	0.678	90.3	3.6	0.668	89.0	0.6
	MQC	11.347	90.8	6.3	11.637	93.1	1.2
	HQC	16.497	94.3	4.7	16.658	95.2	6.0
Heart[b]	LLQC	0.225	89.8	3.8	0.260	104.0	6.9
	LQC	0.739	98.6	8.8	0.730	97.3	4.4
	MQC	12.667	101.3	3.9	12.699	101.6	1.8
	HQC	17.715	101.2	2.4	17.496	100.0	1.8
Lungs[b]	LLQC	0.242	96.7	12.3	0.238	94.9	6.0
	LQC	0.693	92.3	6.2	0.690	91.9	3.5
	MQC	11.853	94.8	6.8	11.805	94.4	2.8
	HQC	16.127	92.2	4.7	16.120	92.1	2.5
Kidney[b]	LLQC	0.288	115.0	2.9	0.237	94.8	5.4
	LQC	0.795	106.0	1.9	0.753	100.4	5.4
	MQC	13.832	110.7	1.4	13.590	108.7	1.1
	HQC	18.974	108.4	3.4	18.693	106.8	3.8
Brain[b]	LLQC	0.242	96.7	9.3	0.248	99.1	15.4
	LQC	0.750	100.0	5.4	0.779	103.8	8.9
	MQC	13.087	104.7	2.2	13.303	106.4	3.3
	HQC	17.289	98.8	1.9	17.882	102.2	3.2
Intestine[b]	LLQC	0.245	97.8	5.8	0.206	82.3	0.2
	LQC	0.679	90.6	2.4	0.700	93.3	5.7
	MQC	12.181	97.4	4.2	13.249	106.0	4.9
	HQC	16.419	93.8	3.1	17.943	102.5	7.8

[a] Mean concentration [µg/mL], [b] mean concentration [µg/g].

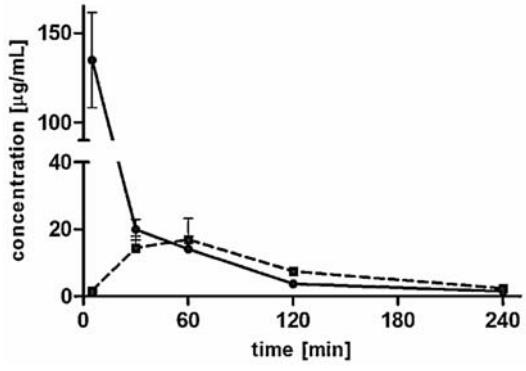

Figure 3. Plasma concentration-time profile of 1-MP after a single intravenous (●) or intragastric (■) administration to rats at the dose of 100 mg/kg

Table 3. Accuracy (% of nominal concentration) and precision (%RSD) between days for 1-MP and 1,4-DMP in plasma and rat tissues.

Matrix	QC level	1-MP			1,4-DMP		
		Mean concentration	Accuracy [%]	Precision RSD [%]	Mean concentration	Accuracy [%]	Precision RSD [%]
Plasma[a]	LLQC	0.051	102.2	8.6	0.0499	99.7	5.0
	LQC	0.148.	99.1	7.6	0.150	100.2	8.1
	MQC	2.607	104.3	4.8	2.574	103.0	7.2
	HQC	3.531	100.9	10.1	3.597	102.8	7.6
Liver[b]	LLQC	0.235	94.1	9.2	0.262	104.6	5.8
	LQC	0.720	96.0	9.6	0.675	90.0	2.8
	MQC	11.879	95.0	8.5	11.945	95.6	4.5
	HQC	16.324	93.3	5.1	17.014	97.2	4.6
Heart[b]	LLQC	0.197	94.6	11.3	0.265	105.8	6.4
	LQC	0.661	101.8	8.4	0.737	98.2	5.1
	MQC	10.202	103.7	3.8	12.902	103.2	3.0
	HQC	17.159	102.6	4.4	17.630	100.7	4.4
Lungs[b]	LLQC	0.252	100.5	14.4	0.249	99.4	6.2
	LQC	0.733	97.7	6.9	0.738	98.3	9.2
	MQC	12.442	99.5	7.3	12.799	102.4	8.8
	HQC	16.986	97.1	6.6	17.292	98.8	7.9
Kidney[b]	LLQC	51.147	102.3	13.58	0.245	97.8	7.5
	LQC	157.860	105.2	6.21	0.780	104.0	5.8
	MQC	2752.947	110.1	8.03	13.401	107.2	3.2
	HQC	3654.619	104.4	4.95	18.297	104.6	3.5
Brain[b]	LLQC	0.248	99.3	12.5	0.236	94.3	10.2
	LQC	0.751	100.1	4.9	0.745	99.3	7.9
	MQC	13.043	104.3	3.2	12.945	103.6	4.5
	HQC	17.704	101.2	5.3	17.897	102.3	4.9
Intestine[b]	LLQC	0.260	104.0	8.8	0.239	95.3	16.8
	LQC	0.721	96.1	7.4	0.743	99.0	9.0
	MQC	12.356	98.8	3.5	12.619	100.9	7.5
	HQC	16.932	96.8	4.6	17.097	97.7	8.8

[a] Mean concentration [µg/mL], [b] mean concentration [µg/g].

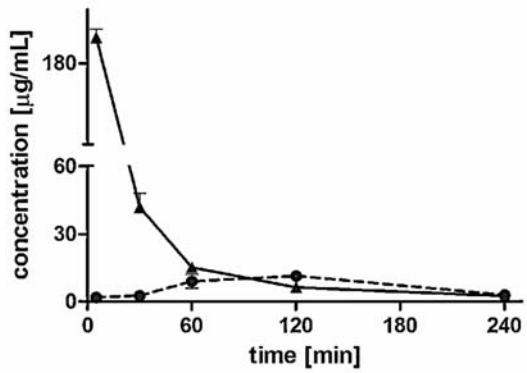

Figure 4. Plasma concentration-time profile of 1,4-DMP after a single intravenous (●) or intragastric (■) administration to rats at the dose of 100 mg/kg

Table 4. Stability for 1-MP and 1,4-DMP in rat plasma.

QC level	1-MP			1,4-DMP		
	Mean concentration (µg/mL)	Accuracy [%]	Precision RSD [%]	Mean concentration (µg/mL)	Accuracy [%]	Precision RSD [%]
Short-term stability test						
LQC	0.1463	97.5	8.7	0.151	100.6	1.5
HQC	3.135	89.6	5.7	3.141	89.7	7.5
Freeze and thaw stability test						
LQC	0.1589	105.9	4.2	0.1483	98.9	6.8
HQC	3.900	111.4	2.7	3.372	96.3	3.4
Long-term stability test						
LQC	0.1528	101.9	4.3	0.130	86.7	8.6
HQC	3.383	96.6	1.1	3.200	91.4	1.6

Table 5. Post-preparative stability for 1-MP and 1,4-DMP.

Matrix	QC level	1-MP			1,4-DMP		
		Mean concentration	Accuracy [%]	Precision RSD [%]	Mean concentration	Accuracy [%]	Precision RSD [%]
Plasma[a]	LQC	0.162	108.2	10.9	0.156	103.7	4.2
	HQC	3.557	101.6	3.3	3.647	104.2	2.8
Liver[b]	LQC	0.913	122	6.2	0.658	87.7	4.1
	HQC	16.664	95	4.7	15.629	89.3	3.6
Heart[b]	LQC	0.606	80.8	10.9	0.667	88.9	3.7
	HQC	15.731	89.9	6.0	16.081	91.9	3.2
Lungs[b]	LQC	0.789	105.2	9.1	0.919	122.5	5.0
	HQC	17.779	101.6	4.	19.362	110.6	3.4
Kidney[b]	LQC	1.012	135	3.2	0.740	98.6	6.1
	HQC	18.703	107	2.4	18.388	105.1	4.7
Brain[b]	LQC	0.793	105.7	3.9	0.727	96.9	9.0
	HQC	17.161	98.1	2.7	18.147	103.7	4.9
Intestine[b]	LQC	0.768	102.4	5.7	0.963	128.4	9.3
	HQC	17.618	100.7	3.2	18.629	106.4	8.1

[a] Mean concentration [µg/mL], [b] mean concentration [µg/g].

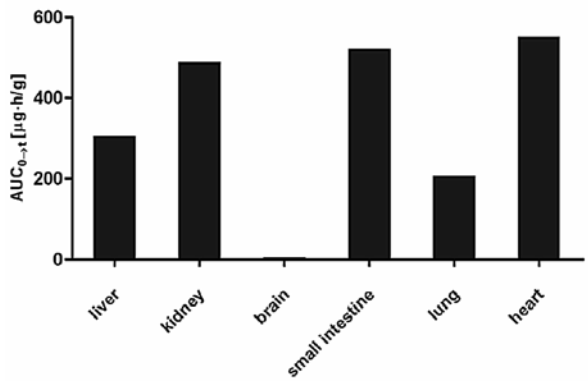

Figure 5. Tissue distribution of 1,4-DMP after a single intravenous administration of compound at the single dose of 100 mg/kg in rats

Table 6. Pharmacokinetic parameters for 1,4-DMP and 1-MP after a single intravenous and intragastric administration at the dose of 100 mg/kg in rats.

Pharmacokinetics parameters	1,4-DMP		1-MP	
	Intravenous	Intragastric	Intravenous	Intragastric
$AUC_{0\to\infty}$ [μg·min/mL]	6.284	1.944	4.245	2.159
MRT [min]	44.34	140.28	38.81	110.58
$t_{1/2}$ [min]	70.77	60.41	55.32	63.26
C_{max} [μg/mL]	-	11.38	-	16.91
V_{ss} [mL/kg]	706	-	914	-
Cl [mL/min/kg]	15.91	-	23.56	-
F [%]	-	31		51

Table 7. Pharmacokinetic parameters in rat tissues after a single intravenous administration of 1,4-DMP at the dose of 100 mg/kg.

Tissue	Pharmacokinetic parameters			
	$AUC_{0\to\infty}$ [μg·min/g]	C_{max} [μg/g]	t_{max} [min]	MRT [min]
Liver	305.03	243.80	30	115
Kidney	488.70	584.45	5	70
Brain	4.87	3.42	5	244
Intestine	530.16	232.92	5	157
Lungs	210.67	115.22	5	143
Heart	551.47	162.80	120	292

$$AUC_{0\to\infty} = \sum_{i=1}^{n}([c_i + c_{i+1}]/2) \cdot (t_{i+1} - t_i) + c_n/\lambda_z \quad (1)$$

Area under the first-moment curve ($AUMC_{0\to\infty}$) was estimated by calculation of the total area under the first-moment curve and extrapolated area using the equation 2, where t_n is the time of last sampling.

$$AUMC_{0\to\infty} = \sum_{i=1}^{n}((t_i \cdot c_i + t_{i+1} \cdot c_{i+1})/2) \cdot (t_{i+1} - t_i) + (t_n \cdot c_n)\lambda_z + c_n/\lambda_z 2 \quad (2)$$

Mean residence time (MRT) was calculated as:

$$MRT = AUMC_{0\to\infty}/AUC_{0\to\infty} \quad (3)$$

Systemic clearance (Cl) was calculated as:

$$Cl = D_{iv}/AUC_{0\to\infty} \quad (4)$$

Volume of distribution at steady state (V_{ss}) was calculated as:

$$V_{ss} = \frac{D_{iv} \cdot AUMC_{0\to\infty}}{(AUC_{0\to\infty})^2} \quad (5)$$

where D_{iv} is an intravenous dose of studied compound, AUMC is the area under the first moment curve, and AUC is the area under the zero moment curve.

The absolute bioavailability (F) after the extravascular (*e.v.*) administration compared to the intravenous (*i.v.*) route was calculated as follows:

$$F = \frac{AUC_{e.v.}}{AUC_{i.v.}} \quad (6)$$

RESULTS AND DISCUSSION

Validation data

The newly developed bioanalytical method for the simultaneous analysis of 1-MP and 1,4-DMP in rat matrices (plasma and selected tissue samples) using LC/HESI-MS/MS technique was developed and validated in the first step of the study.

In order to construct the appropriate SRM method, the most abundant parent and fragmentation ions of analyzed compounds and IS were chosen (Fig. 1). Representative chromatograms from rat plasma samples are shown in Figure 2. Retention times are around 1.7 and 1.8 min for 1-MP and 1,4-DMP, respectively. No significant interferences with other endogenous molecules in sample were observed.

The obtained limit of detection for both compounds was high and equaled 0.01 μg/mL and 0.05 μg/g in plasma and tissues, respectively. It provides the measurement of studied analogs' concentration

in the biological samples from pharmacokinetic experiments. The obtained results, in all matrices, show good linearity over the entire concentration range 0.05-5 µg/mL for plasma and 0.25-25 µg/g for tissues. Calibration curves were generated using weighted (1/x) linear regression analysis. The extraction recoveries of 1-MP and 1,4-DMP in rat plasma were 87.8 ± 8.2% and 92.9 ± 9.8%, respectively. No relative matrix effect for studied compounds (Table 1) was observed for five different tested plasma lots, what can indicate, that developed method is reliable and can be used in routine laboratory work.

Precision was evaluated as repeatability (within day precision) and reproducibility (between days precision). The accuracy and precision results for all matrices are shown in Table 2 (within day) and Table 3 (between days). The obtained results were within the acceptable limits established by EMA for bioanalytical methods (13) confirming that the method can be used for quantifying both 1-MP and 1,4-DMP compounds in the following rat tissues: liver, lungs, heart, brain, small intestine, kidneys and plasma.

The stability of analytes in rat plasma was investigated under a variety of storage and process conditions described in a previous section. Compounds showed to be stable during storage under various conditions (Table 4). Results of post-preparative stability (24 h) for all tested matrices are shown in Table 5. Moreover, results showed, that both 1-MP and 1,4-DMP were stable in all analyzed matrices, but not in liver and kidney. Since, prepared samples of liver and kidney for 1-MP, and in case of small intestine and lungs for 1,4-DMP should be analyzed within 24 h. The validated LC/HESI-MS/MS method was successfully used for quantification of 1-MP and 1,4-DMP in rat plasma following theirs intravenous or intragastric administration at a dose of 100 mg/kg. The concentration of 1,4-DMP in selected tissues were also determined with desired accuracy and precision.

Pharmacokinetic study in rats

Blood samples during experiments were collected in a regular time intervals. The mean concentration – time profiles of 1-MP and 1,4-DMP in plasma were plotted in Figures 3 and 4, respectively.

The results of the model independent pharmacokinetic data analysis obtained following intravenous or intragastric administration of 1-MP or 1,4-DMP in plasma are summarized in Table 6. Target analogs were eliminated rather slowly with the terminal half-life times for 1-MP equaled 55.3 min, and for 1,4-DMP equaled 70.8 min, after their intravenous administration. The volumes of distribution at the steady-state were in the range of 0.9 L/kg and 0.7 L/kg for 1-MP and 1,4-DMP, respectively, and might indicate theirs intracellular disposition. The absolute bioavailability estimated based on the $AUC_{0\rightarrow\infty}$ calculated from time zero to infinity yielded the values of 51% for 1-MP and 31% for 1,4-DMP, and was rather low, with the peak concentration occurring 60 min for 1-MP, and 120 min for 1,4-DMP after their intragastric administration.

1,4-DMP has significant tissue distribution which is in agreement with its high volume of distribution (0.7 L/kg). Analysis showed that the highest amount of 1,4-DMP was observed in heart, then in kidney and small intestine, and the lowest one was detected in brain (Fig. 5). Distribution was rapid and the maximal concentration occurred in the most of tissues at 5 min after administration with exception of liver (maximum at 30 min) and heart (maximum at 120 min) as seen in Table 7. These findings suggest that 1,4-DMP is mostly distributed in heart and, despite the fact that this compound is positively charged in physiological pH, it can penetrate blood-brain barrier, probably *via* a specific carrier system.

CONCLUSIONS

A rapid and simple LC/HESI-MS/MS method was developed and validated for quantification of 1-MP and 1,4-DMP in plasma and selected rat tissues. The assay showed wide linear dynamic range of 0.05–5 µg/mL for plasma, and 0.25–25 µg/g for tissues with acceptable within day and between days accuracy and precision. The method was successfully applied to assess the pharmacokinetic profiles of 1-MP and 1,4-DMP in rats after compounds intravenous and intragastric administration. The absolute bioavailability in rats was estimated at 51% for 1-MP and 31% for 1,4-DMP, respectively. The distribution of 1,4-DMP in tissues was rapid with the maximal concentration occurred at 5 min after compound administration in kidney, intestine, lungs and brain.

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