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Short communication

Ultra-performance liquid chromatography–tandem mass spectrometry for the determination of atypical antipsychotics and some metabolites in *in vitro* samples

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Abstract

The ultra-performance™ liquid chromatography–electrospray tandem mass spectrometry (UPLC–ESI-MS/MS) method has been developed to perform the determination of quetiapine, perospirone, aripiprazole and quetiapine sulfoxide in *in vitro* samples in less than 3 min. The UPLC separation was carried out using an Acquity UPLC™ BEH C_{18} column (100 mm × 2.1 mm i.d., 1.7 μm particle size) that provided high efficiency and resolution in combination with high linear velocities. The UPLC system was coupled to a Waters Micromass Quattro Premier XE tandem quadrupole mass spectrometer. This system permits high-speed data acquisition without peak intensity degradation, and produces sharp and narrow chromatographic peaks (w_h about 2.5 s) of compounds. The determination was performed in multiple reaction monitoring (MRM) mode. The quantification parameters of the developed method were established, obtaining instrumental LODs lower than 0.005 μg/l and a repeatability at a low concentration level lower than 10% CV ($n = 10$). Finally, the method was successfully applied to the analysis of atypical antipsychotics and some metabolites in *in vitro* samples.

Keywords: UPLC–MS/MS; Quetiapine; Perospirone; Aripiprazole; Quetiapine sulfoxide

1. Introduction

Quetiapine, perospirone and aripiprazole (Fig. 1) are new atypical antipsychotic drugs used for the treatment of schizophrenia and other psychotic syndromes [1–3]. They must penetrate the blood–brain barrier (BBB) before reaching the central nerve system (CNS). So the BBB is generally believed to be an obstacle in the transportation of antipsychotic drugs. Recently, some studies have found that there are some drug transporters at the luminal membrane of the endothelial cells of brain capillaries, which limit the access of drugs to the CNS. Among the drug transporters, P-glycoprotein is a classic and very important one [4,5]. It is still not clear if P-glycoprotein influences the penetration of quetiapine, perospirone and aripiprazole through BBB and if quetiapine interacts with its metabolite, quetiapine sulfoxide, on P-glycoprotein. To help resolve these questions, a method for simultaneous determination of quetiapine, perospirone, aripiprazole and quetiapine sulfoxide in *in vitro* samples is urgently needed. But until now, only some HPLC-UV, HPLC–MS and HPLC–MS/MS methods for determination of these drugs separately have been established [6–12]. Compared with HPLC, UPLC is recently developed technology and provides a higher peak capacity, greater resolution, increased sensitivity and high speed of analysis [13,14]. In this work, a fast new UPLC–MS/MS method was developed for simultaneous determination of quetiapine, perospirone, aripiprazole and quetiapine sulfoxide in *in vitro* samples.

2. Experimental conditions

2.1. Chemicals

Quetiapine sulfoxide (purity = 74%) was donated by AstraZeneca Pharmaceuticals (London, UK). Quetiapine (purity > 99.6%) was kindly provided by Dongting Pharmaceutical Co. Ltd. (Changde, China). Aripiprazole (purity > 98.0%)

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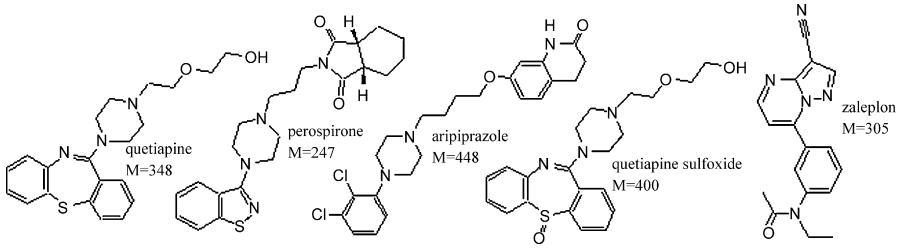

Fig. 1. Chemical structure of quetiapine, perospirone, aripiprazole, quetiapine sulfoxide and zaleplon.

was purchased from Aike Pharmaceutical Technology Co. Ltd. (Xuzhou, China). Perospirone (purity > 99.0%) was purchased from Venture Pharmaceutical Technology Co. Ltd. (Beijing, China). Zaleplon (purity > 98.0%) was purchased from Watson Fine Chemical Co. Ltd. (Changzhou, China). Working standard mixtures, containing 920 μg/l of each target compound, were prepared in acetonitrile for use as spiked solutions. The pH of PBS buffer was 7.25.

Acetonitrile and 1-chlorobuthane (HPLC grade) were obtained from Sigma Chemical Company (Steinheim, Germany). Ammonium acetate and triethylamine (analytical reagent grade) were obtained from Merck (Darmstadt, Germany). Mobile phases were filtered with a 0.22 μm nylon filter (Whatman, England).

2.2. Equipment

UPLC analyses were performed using a Waters Acquity Ultra Performance LC system (Waters, Milford, MA, USA). UPLC separation was achieved using an Acquity UPLC^{TM} BEH C_{18} column (100 mm × 2.1 mm, i.d., 1.7 μm particle size, Waters, Milford MA, US), maintained at 40 °C, with a mobile phase flow rate of 0.3 ml min^{-1}. The mobile phase contained 62% acetonitrile and 38% ammonium acetate at a final concentration of 30 mmol/l. The total run time was 3 min. The sample volume injected was 4 μl.

Determination was performed using a Waters Micromass Quattro Premier XE tandem quadrupole mass spectrometer (Waters, Manchester, UK). The instrument was operated using an electrospray source in positive mode. The ionisation source parameters were: capillary voltage 3.1 kV; source temperature 120 °C; desolvation gas temperature 400 °C at a flow rate of 900 l/h (N_2); cone gas flow rate 50 l/h. Nitrogen (99.9% purity) and argon (99.9999% purity) were used as cone and collision gases, respectively. Multiple reaction monitoring (MRM) transitions as well as the individual cone voltage and collision energy voltages applied for the analysis were summarized in Table 1. The dwell time established for each transition was 50 ms, and the interscan delay was set at 10 ms. Data acquisition was carried out by MassLynx V 4.1 software.

2.3. Sample preparation

0.5 ml *in vitro* sample (quetiapine, perospirone, aripiprazole and quetiapine sulfoxide dissolved in PBS buffer) was added to tube 1. Then 25 μl internal standard (I.S., zaleplon, 48 ng/ml in PBS buffer) was added to tube 1. The sample was dried under nitrogen in a 40 °C water bath. The residue was redissolved in 1.5 ml 1-chlorobutane–triethylamine (5:0.5) and vortex mixed for 2 min. Then the solution was transferred to tube 2. Another 1.5 ml 1-chlorobutane–triethylamine was added to tube 1 to repeat the extraction. The solution was also transferred to tube 2 and mixed with the initially extracted solution. The solution in tube 2 was centrifuged at 3000 × *g* for 5 min, and the 2.5 ml supernatant was transferred to tube 3 and dried under nitrogen in a 40 °C water bath, and the residue was redissolved in 50 μl mobile phase.

2.4. Method performance

The performance characteristics of the method were established by using acetonitrile standard solutions and samples spiked to required concentrations by PBS buffer. Linearity, matrix effects, trueness, precision and detection limits were evaluated. The linearity in the response was investigated by using calibration solutions at seven concentration levels, ranging from 0.05 to 5 μg/l (0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 μg/l). The calibration curve was required to demonstrate a correlation coefficient of ≥0.990. The peak-area ratios of each compound to I.S. of the selected quantification masses were used to construct the curves. Trueness of the method was investigated through mean recoveries obtained for the five replicates of spiked samples at

Table 1
MS–MS conditions for multiple reaction monitoring in ESI^+

Compound	Cone voltage (V)	Collision energy (eV)	Parent ion (*m*/*z*)	Daughter ion (*m*/*z*)	Dwell time (ms)
QTP	37	24	384.3	253.1	50
QTP-SF	43	30	400.0	221.1	50
ARI	47	28	448.3	285.2	50
PER	50	30	427.4	177.4	50
I.S.	31	28	306.3	236.1	50

QTP: quetiapine; QTP-SF: quetiapine sulfoxide; ARI: aripiprazole; PER: perospirone.

three different concentration levels (see Table 2). Reproducibility assays were carried out in three different concentrations for three times within the same day and over three different days. Intra-laboratory precision under repeatability conditions was expressed in terms of relative standard deviation for 10 replicates of a spiked sample at 0.5 μg/l level. Spiked sample extracts were used for evaluating limits of detection and quantification of the method. The assay acceptance criterion was 15% deviation from the nominal value except at the lower limit of quantification (LLOQ), which was set at 20% deviation. The limits of detection (LOD) were determined at the analyte concentration that gave a S/N of 3.

3. Results and discussion

3.1. Optimization of the chromatographic separation and MS/MS working conditions

In our previous work [8], a reversed-phase column packed with silica particles in 5 μm was used for quetiapine analysis by HPLC–ESI-MS. Similar mobile phases based on ammonium acetate and acetonitrile were used in the optimization of the atypical antipsychotics separation in the UPLC system. The selection of MRM transitions and associated acquisition parameters (collision energy and cone voltage) were evaluated for best response under positive mode ESI conditions by infusing a standard solution, via a syringe pump, into the mobile phase (Table 1). The sensitivity of aripiprazole and perospirone was lower than that of quetiapine and quetiapine sulfoxide in the optimal response condition.

Retention time was shown in Table 2. Peaks were very sharp and the peak widths at half height (w_h) were around 2.5 s (Fig. 2). Quetiapine sulfoxide and I.S. were not completely separated, because the total run time for completely separation of quetiapine sulfoxide and I.S. was more than 6 min. On the other hand, this coelution could be resolved since the parent ions and daughter ions (400 > 221 for quetiapine sulfoxide, 306 > 236 for I.S.) were different and MS/MS separate detection of both compounds was feasible, allowing their correct identification and quantification.

3.2. Selection of the extraction and matrix effects

To evaluate the matrix effects, acetonitrile standard solutions of the target analytes spiked, respectively by 1‰ formic acid in acetonitrile, mobile phase, acetonitrile, and PBS buffer were injected in MS/MS directly. Compared with acetonitrile, 1‰ formic acid in acetonitrile resulted in higher peaks and unretainment of the target analytes, but mobile phase resulted in no change. PBS buffer resulted in no signal of the target analytes. This meant it was necessary to test various solvent and extraction conditions. Samples were extracted under the various conditions as shown in Table 3. Zaleplon (I.S.) was added after extraction. The extraction recoveries (Table 3) were determined by comparing peak area ratios of the extraction of samples with those of acetonitrile standard solutions of the target analytes spiked by mobile phase. The condition that yielded optimal recovery

Table 2
Summary of retention time (t_R), peak widths at half height (w_h), linearity, recovery, and precision obtained by HPLC–MS/MS (MRM mode) analysis of the four compounds

Compound	t_R (min)	w_h (s)	Linearity			LOD (μg/l)	Recovery (%)[a]		Rep[b] (%)[c]	Intra-day precision (%)[d]			Inter-day precision (%)[d]		
			Slope	Intercept	r		0.1 μg/l	0.5 μg/l	2.0 μg/l		0.1 μg/l	0.5 μg/l	2.0 μg/l	0.1 μg/l	0.5 μg/l	2.0 μg/l
QTP	1.34	2.0	0.113	−0.140	0.997	0.001	91 ± 9	82 ± 7	94 ± 7	6	10	9	7	15	14	10
QTP-SF	0.92	2.5	0.796	−0.229	0.994	0.004	85 ± 13	81 ± 7	72 ± 6	8	14	11	9	14	12	10
ARI	2.71	3.0	1.213	−0.002	0.999	0.004	81 ± 11	73 ± 9	75 ± 8	7	16	12	10	17	10	9
PER	1.96	3.0	0.586	0.011	0.995	0.005	80 ± 9	77 ± 8	80 ± 7	9	10	10	9	12	13	12

[a] Mean ± standard deviation, $n = 5$.
[b] Intra-laboratory precision under repeatability conditions.
[c] Relative standard deviation for 10 replicates of a spiked sample at 0.5 μg/l.
[d] Relative standard deviation; QTP: quetiapine; QTP-SF: quetiapine sulfoxide; ARI: aripiprazole; PER: perospirone.

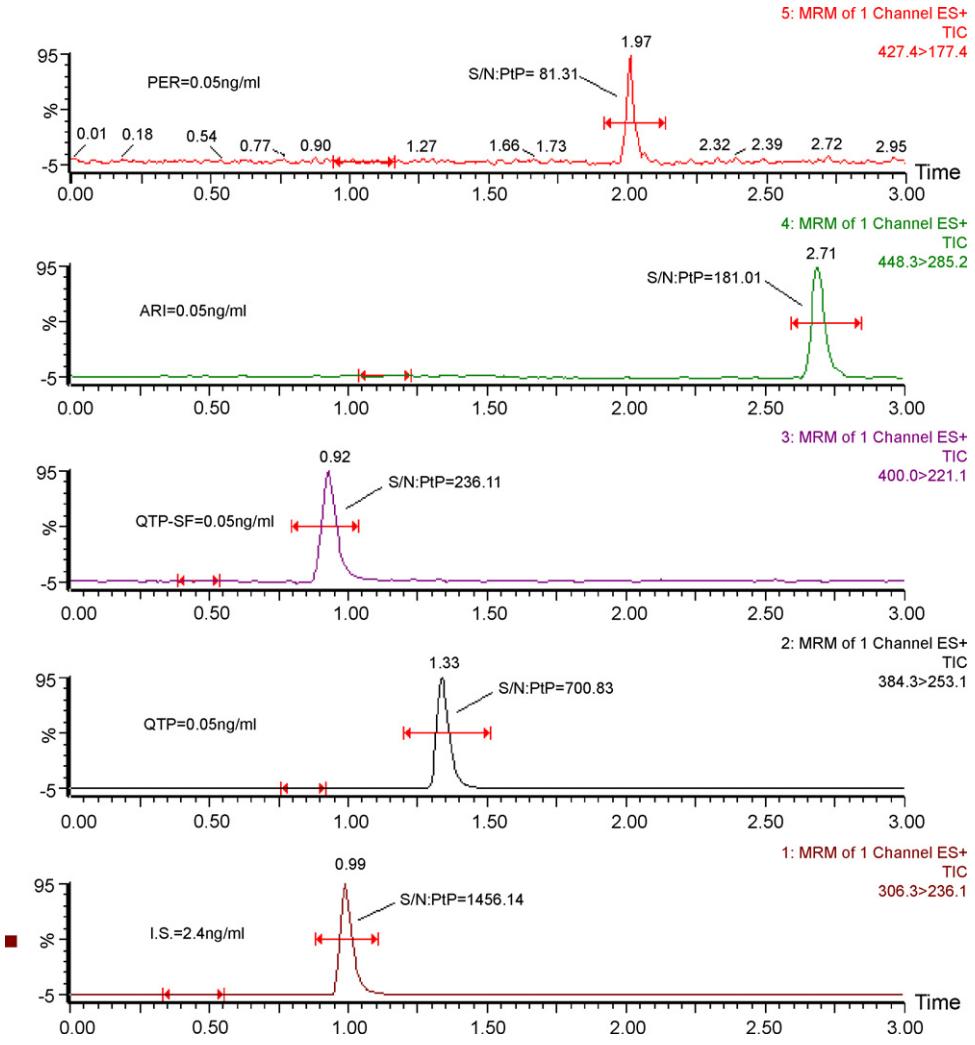

Fig. 2. Extracted MRM traces of the target compounds standard solution obtained from UPLC–MS/MS analysis. For chromatographic conditions, see Section 2.

Table 3
Recovery of four target analytes (0.5 μg/l) under varying conditions

Extraction method	*n*	Recovery (%)			
		QTP	QTP-SF	ARI	PER
Ether	5	23	15	18	10
NaOH/ether	5	47	45	21	50
NaOH/ethyl acetate	5	92	95	13	24
NaOH/hexane	5	85	5	19	33
NaOH/dichloromethane	5	67	42	50	80
NaOH/TEDIA	5	25	36	10	5
Published (ref. [7])	5	50	42	25	41
Dryness/TEA-ether[a]	5	12	19	11	10
Dryness/TEA-dichloromethane[a]	5	75	56	50	60
Dryness/TEA-chlorobutane[a]	5	81	68	62	64
Dryness/TEA-chlorobutane[b]	5	84	72	63	66
Dryness/TEA-chlorobutane[b,c]	5	82	81	73	77

Extraction time and volume: 1 × 3 ml (except the last method); NaOH: 1 mol/l × 100 μl; TEDIA: methyl *t*-butyl ether; dryness: under nitrogen; TEA: triethylamine.
[a] 0.3:3 (v/v).
[b] 0.5:5 (v/v).
[c] Extraction solvent, 2 × 1.5 ml; QTP: quetiapine; QTP-SF: quetiapine sulfoxide; ARI: aripiprazole; PER: perospirone.

of the four target analytes was dryness under nitrogen followed by 1-chlorobutane–triethylamine extraction (2 × 1.5 ml).

3.3. Other performance characteristics of the method

The efficiency of the extraction method was evaluated by using matrix spiked samples at 0.1, 0.5, 2.0 μg/l. The recoveries were similar at the levels assayed, with RSD lower than 15% (Table 2). The intra-day and inter-day precisions for the four target analytes at the three concentrations were from 7% to 17% (Table 2). The intra-laboratory precision expressed in terms of repeatability was considered satisfactory, with relative standard deviations varying from 6% to 9%. The linearity was also good for all compounds with correlation coefficients all higher than 0.990 over the studied concentration range (0.05–5 μg/l). LLOQs were 0.05 μg/l for all compounds. LODs were from 0.001 μg/l to 0.005 μg/l. Under the experimental conditions applied, quetiapine was the most sensitive compound.

3.4. Stability

Working standard mixtures, containing 92 μg/l of each compound in acetonitrile, were stored at −20 °C for 3 months and at 20 °C for 24 h. All target analytes appeared to be stable.

4. Conclusions

The application of the recently developed ultra-performance™ liquid chromatography technology combined with tandem mass spectrometry for the determination of quetiapine sulfoxide, quetiapine, aripiprazole and perospirone in PBS buffer has been established. This technique has provided enhanced characteristics regarding resolution, sensitivity and speed of analysis. Separation of the four target compounds was obtained in less than 3 min, which significantly reduced the mobile phase and time for analysis required. The very narrow chromatographic peaks generated by UPLC™, with peak widths lower than 2.5 s, result in an increase in the chromatographic efficiency and sensitivity, with LODs in the range between 0.001 μg/l and 0.005 μg/l. Other performance characteristics of the developed analytical method include good linearity, precision, selectivity and absence of ion suppression effects. Therefore, this analytical UPLC–MS/MS means can be considered as a promising technique that has obvious advantages compared with conventional HPLC–MS techniques in this field of application.

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