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Simultaneous determination of clozapine, olanzapine, risperidone and quetiapine in plasma by high-performance liquid chromatography–electrospray ionization mass spectrometry

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Abstract

Clozapine (CLZ), olanzapine (OLZ), risperidone (RIP) and quetiapine (QTP) have been widely used in the treatment of schizophrenia. However, no study (or little study) has been conducted to determine the four drugs simultaneously by the use of high-performance liquid chromatography–electrospray ionization mass spectrometry (HPLC–MS/ESI). *Objective:* To develop a sensitive method for simultaneous determination of CLZ, OLZ, RIP and QTP in human plasma by HPLC–MS/ESI. *Methods:* The analytes were extracted twice by ether after samples had been alkalinized. The HPLC separation of the analytes was performed on a MACHEREY-NAGEL C_{18} (2.0 mm × 125 mm, 3 μm, Germany) column, using water (formic acid: 2.70 mmol/l, ammonium acetate: 10 mmol/l)–acetonitrile (53:47) as mobile phase, with a flow-rate of 0.16 ml/min. The compounds were ionized in the electrospray ionization (ESI) ion source of the mass spectrometer and were detected in the selected ion recording (SIR) mode. *Results:* The calibration curves were linear in the ranges of 20–1000 ng/ml for CLZ and QTP, 1–50 ng/ml for OLZ and RIP, respectively. The average extraction recoveries for all the four analysts were at least above 80%. The methodology recoveries were higher than 91% for the analysts. The intra- and inter-day R.S.D. were less than 15%. *Conclusion:* The method is accurate, sensitive and simple for routine therapeutic drug monitoring (TDM) and for the study of the pharmacokinetics of the four drugs.

Keywords: Clozapine; Olanzapine; Risperidone; Quetiapine

1. Introduction

Clozapine, olanzapine, risperidone and quetiapine are structurally related atypical antipsychotics. They are used in the treatment of schizophrenia and other psychotic syndromes. It is reported that they are effective in the treatment of both positive and negative symptoms of schizophrenia, and that they are less likely to produce extrapyramidal side effects when compared with classical antipsychotics. The advantages of the therapeutic profile of the four drugs have led to increasing use of them in treatment of schizophrenic patients [1,2]. However, high does of these atypical antipsychotics are suspected to pose an increased risk for extrapyramidal side effects or other side effects [1,3–7]. In conditions of hepatic and renal impairment, poor metabolizers of CYP450 isoenzymes and comedication with inhibitors or inducers of these isoenzymes, routine therapeutic drug monitoring (TDM) seemed to be useful [3,4,7–15]. Furthermore, TDM is a good solution to noncompliance.

At present, determinations of some of these drugs have been established by the use of ultraviolet spectrometry [3,16–21], mass spectrometry [11,22–26] or electrochemical detection [3,27,28]. However, none of these methods makes the quick quantification and identification of these drugs in a single run. Although methods to simultaneously determine some of these drugs have been described [7–10], it appears that no simultaneous assay exists for determination of the four drugs using HPLC–MS. While some of these methods are successful in TDM and analysis of intoxication [7,9,10], it appears that for CLZ, OLZ, RIP and

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QTP are not of use since they produce too long a chromatographic run, have low sensitivity or require too expensive instruments.

The recent trend in TDM and in quick analysis of intoxication is having been developed to find methods to determine several drugs simultaneously which are expedient, quick and of low cost. Thus, we designed the method using HPLC–MS/ESI for the simultaneous determination of CLZ, OLZ, RIP and QTP in human plasma in order to explore the application of simultaneous determination of several drugs by HPLC–MS. The assay described here requires small mobile phase and sample volume, short chromatographic run and is sensitive, specific and fully validated.

2. Experiment

2.1. Equipment and reagents

A system of HPLC (Waters 2690, American)–MS with a Micromass ZQ mass spectrometer (Wythenshawe, Manchester, UK) with mass-selective detector equipped with an electrospray ionization (ESI) ion source was used. COMPAQ Deskpro Workstation and MassLynx^{TM} 3.5 software were utilized.

CLZ (>99%) and QTP (>99.6%) were kindly provided by Hunan Dongting Pharmaceutical Co. Ltd. (Changde, Hunan, China). OLZ (>99%) was kindly obtained from Eli Lilly Asia (Shanghai, PR China) and RIP (>99%) from Xian Janssen Pharmaceutical Co. Ltd. (Xi'an, China). Diazepam (I.S., >99.9%) was provided by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade reagents (for example methanol and acetonitrile) were obtained from Tedia Company Inc. (Fairfield, America). Other AR grade reagents were obtained from Chemical Reagent Factory of Hunan (Changsha, Hunan, China). Control human plasma was obtained from the Blood Center of Shanghai (Shanghai, China) or from the volunteers.

2.2. Standard solutions

The primary stock solutions of CLZ (500 μg/ml), OLZ (110 μg/ml), RIP (96 μg/ml), QTP (252 μg/ml) and diazepam (internal standard, 250 μg/ml) were prepared by dissolving appropriate amount of pure substance in methanol. Working solutions were obtained by diluting the stock solutions with methanol. All the standard solutions were stored at −20 °C.

Plasma was spiked with stock solutions of the drugs to achieve the following calibration standard concentrations: OLZ and RIP: 1, 5, 10, 15, 25, 40, and 50 ng/ml; CLZ and QTP: 20, 100, 200, 300, 500, 800, and 1000 ng/ml. Quality control samples that were run in each assay, were prepared in the same way.

2.3. Chromatographic conditions

The analytes were separated on a MACHEREY-NAGEL C_{18} (2.0 mm × 125 mm, 3 μm, Germany) column with column temperature 50 °C. The mobile phase was water (formic acid: 2.7 mmol/l, NH4AC: 10 mmol/l)–acetonitrile (53:47) and was filtered using 0.45 μm filters in a solvent filtration apparatus and was never recirculated. The flow-rate was 0.16 ml/min.

2.4. MS/ESI detection conditions

The compounds were ionized in the positive electrospray ionization ion source (ESI^+) of the mass spectrometer. Selected ion recording (SIR) mode was used for quantitation by the protonated molecular ions of each analyte.

The detection conditions were as follows: capillary voltage: 3.90 kV; cone voltage: 37 V for RIP, CLZ and QTP, 35 V for OLZ, 40 V for diazepam; extractor voltage: 1.00 V; source temperature: 130 °C; desolvation temperature: 200 °C; cone gas flow: 114 l/h; desolvation gas flow: 350 L/h.

2.5. Sample pretreatment

Fifty microliters diazepam (I.S., 250 ng/ml) and 50 μl 25% VitC were added to the sample. The sample (0.5 ml) was alkalinized by adding 0.1 ml sodium hydroxide (0.1 mol/l) then shaken for 1 min. Five milliliters of ether was added to the sample. After 5 min vortical mix, the mixture was centrifuged at 3000 *g* for 6 min at room temperature (20 °C), 4 ml of the upper layer was carefully aspirated and the remainder was extracted once again with 5 ml ether. Four milliliters of the upper layer was put together with the former, and then the ether was evaporated under a stream of nitrogen at room temperature. The residue was reconstituted in 50 μl mobile phase. Five microliters solution was injected for analysis through auto-injector.

2.6. validation

The extraction recoveries were determined by comparing peak-area ratios of the extracts of spiked plasma (three different concentration) with those obtained by direct injection of an aqueous solution of the drugs. The methodology recoveries were measured as the % difference from theoretical according to the equation:

$$\text{Methodology recovery (\%)} = \left(\frac{\text{concentration}_{\text{measured}}}{\text{concentration}_{\text{theoretical}}}\right) \times 100$$

Precision assays were carried out six times using five different concentrations (Table 2) on the same day and over 4 different days.

Calibration was performed by a least squares linear regression of the peak-area ratios of the drugs to the I.S. versus the respective standard concentration. For all drugs, a weighted regression was used with a weighting factor of $1/(\text{concentration})^2$ and with an individual run.

3. Results and discussion

3.1. HPLC–MS/ESI

The column temperature was 50 °C in order to reduce pressure of column and improve resolution. Five microliters reconstituted solution was injected for analysis in that the column (C_{18}, 2.0 mm × 125 mm, 3 μm) required small injection volume and better shape of peaks could be acquired.

The HPLC–MS/ESI in the SIR mode provided a highly selective method for the determination of CLZ, OLZ, RIP and QTP. The retention times of CLZ, OLZ, RIP and QTP were approximately 7.59, 4.80,7.45 and 5.97 min, respectively. The chromatograms of control human plasma, standard in control human plasma and patient samples are shown in Figs. 1–3 respectively. An ESI^+ mass spectrum (SIR) of standard in controlled plasma is illustrated in Fig. 4.

The protonated molecule was identified at *m/z* 327 for $[CLZ + H]^+$, 313 for $[OLZ + H]^+$, 411 for $[RIP + H]^+$, 384 for $[QTP + H]^+$ and 286 for $[\text{diazepam} + H]^+$.

3.2. Calibration curves

The concentration range was 20–1000 ng/ml for clozapine and quetiapine, 1–50 ng/ml for olanzapine and risperidone.

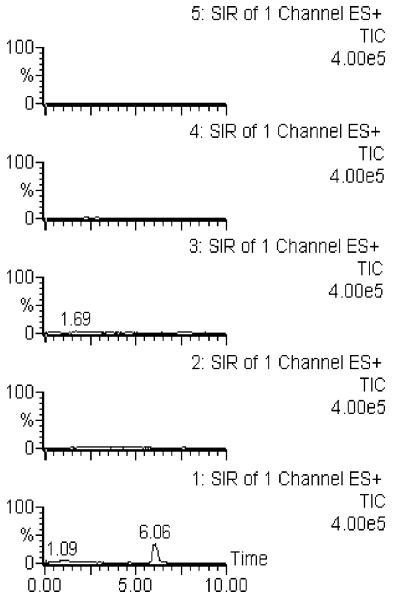

Fig. 1. Chromatograms of control human plasma. Channel 1: RIP, channel 2: OLZ, channel 3: CLZ, channel 4: QTP, channel 5: diazepam in Figs. 1–4.

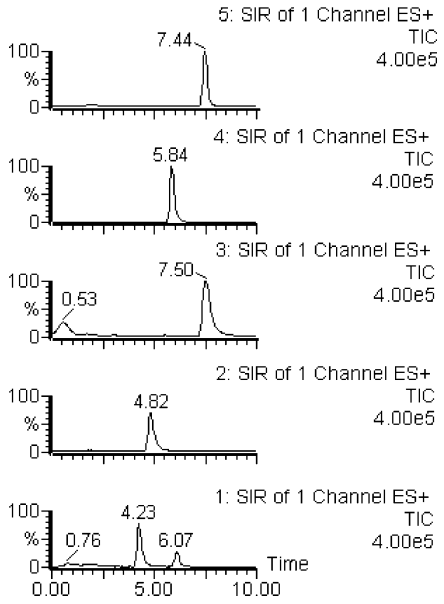

Fig. 2. Chromatograms of standard and I.S. in control human plasma. RIP: 10 ng/ml; OLZ: 15 ng/ml; CLZ: 300 ng/ml; QTP: 200 ng/ml; diazepam: 25 ng/ml.

The area ratio of each analyte to I.S. was well related to the concentration. The related coefficients (r^2) of CLZ, OLZ, RIP and QTP were 0.9903, 0.9993, 0.9979 and 0.9985, respectively.

3.3. Accuracy and precision

The mean extraction recoveries, methodology recoveries, intra- and inter-day precision for the four analyses are shown in Tables 1 and 2. The average extraction recoveries for

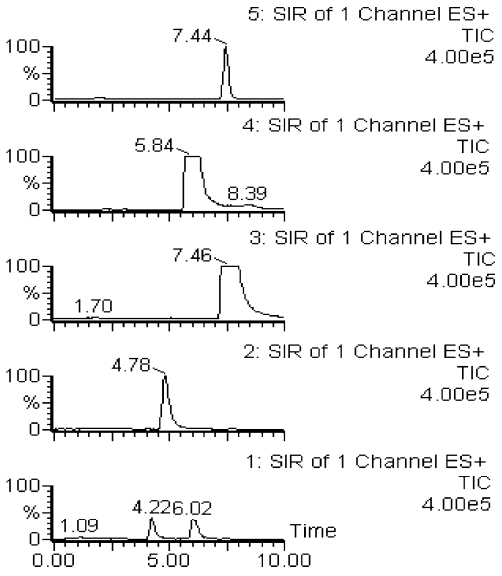

Fig. 3. Chromatograms of Patient samples. For channels 1 and 4, the represented patient is no. 11; for channel 2, the represented patient is no. 1; and for channel 3, the represented patient is no. 7.

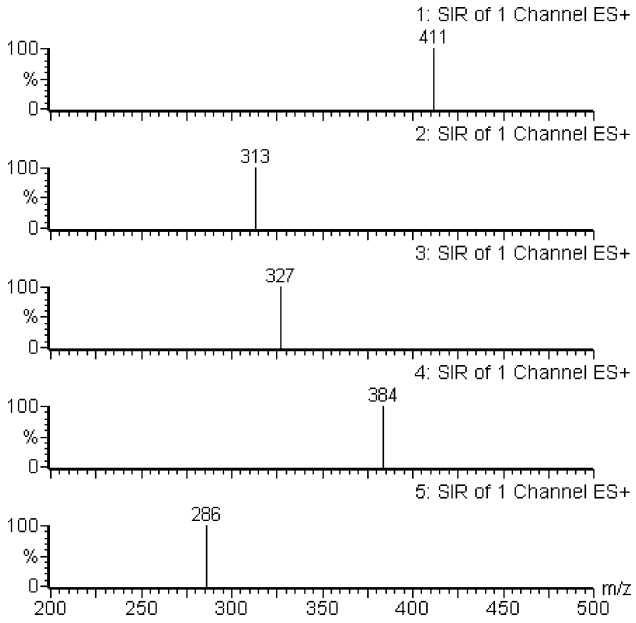

Fig. 4. ESI$^+$ mass spectra of standard in controlled plasma.

all the four analysts were at least above 80%. The average methodology recoveries were higher than 91% for the analysts. The intra- and inter-day R.S.D. are less than 15%.

Compared with CLZ, RIP and QTP, OLZ has lower twice extraction recovery, but it is still satisfactory. For CLZ, RIP and QTP, the recoveries when extracted twice showed little difference when compared to just one extraction. After the 1st extraction, the extraction recovery (mean, $n = 3$) of OLZ was 68.5, 78.1 and 73.8% for three different concentrations: 0.99, 14.8 and 49.5 ng/ml, respectively. The LOD was 1.8 ng/ml. However, after twice extraction the recovery reached about 85% and the LOD was 0.4 ng/ml. In order to increase the sensitivity of analysis in TDM of OLZ, especially in application of clinical pharmacokinetics and metabolic mechanism study of OLZ, and in order to analyze the samples in batches when undertaking TDM of the four drugs too, the double extraction is necessary.

Table 1
Ionization efficiency, mean extraction recoveries and methodology recoveries for the four compounds at the three concentrations

Drug	Added concentration (ng/ml)	Extraction recovery ($n = 3$, %)	Methodology recovery ($n = 3$, %)	Ionization efficiency ($n = 5$, %)
CLZ	20	98.7	91.4	98.1
	300	90.9	109.4	87.6
	1000	99.7	97.5	108.2
OLZ	0.99	85.3	101.2	92.5
	14.8	80.7	92.7	93.6
	49.5	91.4	93.2	84.7
QTP	20	90.7	97.2	110.2
	300	100.0	95.1	96.3
	1000	99.0	93.6	100.5
RIP	0.96	93.0	98.9	86.7
	14.4	96.6	91.7	94.6
	48	101.8	98.3	90.6

Table 2
Intra- and inter-day precision

Drug	Added concentration (ng/ml)	Precision (R.S.D.) (%)	
		Intra-day ($n = 6$)	Inter-day ($n = 4$)
CLZ	20	9.8	11.8
	150	5.9	8.1
	300	4.3	6.0
	600	8.4	3.7
	1000	7.0	5.8
OLZ	0.99	6.8	11.4
	7.4	8.9	12.5
	14.8	3.5	8.9
	29.6	2.9	6.1
	49.5	4.9	9.8
QTP	20	2.2	9.1
	150	7.2	8.6
	300	8.0	13.6
	600	6.7	6.4
	1000	5.8	7.7
RIP	0.96	11.2	8.3
	7.2	3.1	8.7
	14.4	6.4	6.6
	28.8	8.1	14.1
	48.0	3.4	6.2

3.4. Sensitivity

Five quality control plasma samples were utilized to determine the sensitivity. The limits of detection (LOD) were 1.3 ng/ml for clozapine, 0.4 ng/ml for olanzapine, 0.3 ng/ml for risperidone and 1.0 ng/ml for quetiapine, respectively (S/N = 3).

3.5. Stability

Standard solutions of CLZ (500 μg/ml), OLZ (110 μg/ml), RIP (96 μg/ml), QTP (252 μg/ml) and I.S. (250 μg/ml) in methanol were stored at −20 °C for 3 months. All analysts appeared to be stable as the publications [7,8,10,21,25,29,30] described.

Stored at −20 °C for at least 30 days and at 20 °C for 24 h, the stability of three analytes (CLZ, RIP, QTP) in samples and freeze–thaw stability of samples are good as evidenced in previous publications [3,7,8,10,16,18,19,21,22,25]. For the stability of OLZ in different conditions, however, publications [21,31,32] stated afoul. Thus, the storage stability of OLZ in sample with and without VitC was assessed. Quality control spiked samples with OLZ (0.99, 14.8, and 49.5 ng/ml; $n = 4$) were stored in the following conditions: at −20 °C for 1 month; at 20 °C for 24 h after four repeated freeze–thaw cycles. Storage at −20 °C for 1 month without VitC showed stability with C.V. less than 14% and % bias

values less than 13%, while with VitC showed stability with C.V. and % bias values less than 10%. Storage at 20 °C for 24 h without VitC showed significant degradation with accuracy bias of more than 32%, while stability of OLZ in samples added to VitC was good with accuracy bias of less than 10%. Compared with the samples added to VitC, the stability of OLZ in samples without VitC was lower after three repeated freeze–thaw cycles. The C.V. and % bias values of the former were less than 10% and the latter was more than 14%. So, it was necessary to add VitC to samples in order to enhance the recovery of OLZ.

Quality control spiked samples (added to VitC) with the four analytes (OLZ: 0.99, 14.8, and 49.5 ng/ml; QTP and CLZ: 30, 200, and 1000 ng/ml; RIP: 0.96, 14.4, and 48.0 ng/ml; $n = 4$) were pretreated according to Section 2.5. Then, the plasma extracts were determined at 0, 8, 16, 24, and 36 h at 5 °C in the HPLC unit. All analytes showed good stability with C.V. and % bias values of less than 13%.

3.6. Ionization efficiency

It was found that post-column addition of formic acid improved the signal of the analytes. Compared to methanol and H_2O, mobile phase consisted of acetonitrile and H_2O enhanced the signal of the analysts more.

The efficacy of ionization was assessed by comparing spiked cleaned-up drug-free plasma to standard mixtures prepared directly in the reconstitution solvent. The results presented in Table 1 showed that matrix suppression was minimal for the analytes with a signal response always exceeding 85%.

3.7. Analysis of patient plasma

Plasma samples were obtained from 11 schizophrenia patients ($25y \pm 4y$, female). They had been not administered these drugs simultaneously. Only some patients had been switched from one antipsychotic to another. The administered drug and concentrations determined by the method are shown in Table 3. Concentrations of the drugs in plasma have large individual differences, so TDM is necessary to acquire the best treatment effect.

Table 3
Plasma concentrations in schizophrenia patients (female)

Patients no.	Drug administered	DOSE (mg/day)	Concentration (t^a) (ng/ml)	Concentration (t^a) (ng/ml)
1	OLZ	10	22.0 (5)	8.2 (24)
2[b]	OLZ	10	18.3 (5)	4.8 (24)
	(CLZ)		25.4	No quantification
3	RIP	2	5.1 (2)	1.6 (12)
4	RIP	4	4.6 (2)	1.8 (12)
5[c]	RIP	4	21.4 (2)	11.0 (12)
	(CLZ)		125.6	70.9
6	CLZ	450	983.4 (2)	656.0 (12)
7	CLZ	300	725.1 (2)	308.6 (12)
8[d]	CLZ	300	699.5 (2)	287.9 (12)
	(RIP)		7.8	2.3
9	QTP	400	347.7 (1)	75.0 (12)
10	QTP	400	645.8 (1)	34.8 (12)
11[e]	QTP	400	751.9 (1)	147.1 (12)
	(RIP)		6.4	1.8

[a] The time to acquire blood sample after oral.
[b] The patient switched from CLZ to OLZ. The state was 2 days after switching.
[c] The patient switched from CLZ to RIP. The state was 1 day after switching.
[d] The patient switched from RIP to CLZ. The state was 1 day after switching.
[e] The patient switched from RIP to QTP. The state was 1 day after switching.

4. Conclusion

Compared with the HPLC method, HPLC–MS/ESI improved the specificity and sensitivity, shortened the analysis time and simplified the preparation of the sample. The main aim of the study was to establish a HPLC/MS method that was suitable for the determination of CLZ, OLZ, RIP and QTP in plasma of patients undergoing antipsychotic treatment. The method described here was found to be specific and accurate in application. To the best of our knowledge, this method meets the request of the present pharmacokinetic studies of the four drugs. Thus, the method is also suitable for the study of pharmacokinetic and metabolism.

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