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Optimization of Bioanalytical LC Method for Simultaneous Determination of Risperidone And Its Active Metabolite 9-OH Risperidone in Human Plasma And Urine

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Abstract: *Risperidone is the most widely used first line mono therapy in schizophrenia and combined therapy in bipolar disorders. Unpredictable dose/concentration ratio, narrow therapeutic index, clinically relevant interactions, along with serious adverse reactions (ADR), raises the need for individualization of risperiodone treatment and establishing of good therapeutic regime using TDM. Optimization and validation of simple and rapid HPLC method with UV detection for simultaneous determination of risperidone and its active metabolite 9-OH risperidone in human plasma and urine samples, using liquid - liquid extraction procedure for sample preparation. Satisfactory separation was achieved on a reversed-phase C8 column, using acetonitrile and phosphate buffer as a mobile phase with gradient elution, for a total analysis time of 10 minutes. Clozapine was used as an internal standard. The validation data confirmed that bioanalytical method is selective, sensitive, linear, precise and accurate. The method was successfully applied for determination of risperidone and 9-OH risperidone in plasma and urine samples obtained from 52 hospitalized schizophrenia/bipolar disorder patients treated with risperidone as monotherapy and polytherapy. Proposed method is suitable for a reliable TDM of patients on mono or poly therapy with risperidone, with an advantage for use in pharmacokinetic studies where multiple sampling is needed.*

Key words: *risperidone, 9-OH risperidone, HPLC, liquid-liquid extraction, plasma, urine*

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I. INTRODUCTION

Risperidone is an atypical (second generation) antipsychotic drug and is the most widely used neuroleptic as first line monotherapy in schizophrenia and combined therapy in bipolar disorders. The main metabolite of risperidone is 9-OH risperidone with almost the same pharmacological antipsychotic activity as risperidone [1, 2]. The clinical response of a risperidone therapy is a result of plasma concentration of active moiety-AM (sum of risperiodne and 9-OH risperiodne). Risperiodne is highly bound (90%) to plasma proteins, including albumin and α_1–acid glycoprotein, while the active metabolite 9-OH risperiodne shows lower binding (77% bound). Since the active metabolite is found in a higher percentage in the free form in human plasma (pharmacologically active fraction) and is equipotent to the parent drug, it is assumed that 9-OH risperiodne contributes significantly to both therapeutic and adverse effects [3]. Risperidone has a narrow therapeutic index, and the relationship between dose and plasma concentrations of risperiodne may be unpredictable because of differences in genetics, age, gender, absorption, auto induction and disease state between individuals. Also, the presence of numerous clinically significant drug interactions supports the need of using therapeutic drug monitoring (TDM) of risperidone as an essential tool in designing a safe and effective therapeutic regimen for patients with psychiatric disorders [4].

Different studies are conducted for developing new analytical methods or to improve existing methods for TDM of risdperidone and its active metabolite. A number of liquid chromatography methods for simultaneous determination of risperiodne and its metabolites in plasma have been published, using different pretreatment techniques for sample preparation including liquid-liquid extraction [5, 6] and solid-phase extraction [7]. Most of the published methods for simultaneous determination of therapeutic concentration of risperidone and 9-OH risperidone in biological fluids (plasma, urine, saliva) are based on reverse phase (C8 or C18) HPLC with UV or MS detection [6-12]. However, with some of these techniques the obtained extraction yields were not satisfactory, most of them were time-consuming, some required expensive instrument, rendering them not appropriate for routine drug monitoring. Although LC-MS methods for the determination of

risperidone and its metabolites in biological fluids provide improved sensitivity and specificity compared with other analytical methods, these procedures require use of sophisticated and expensive equipment, which is not convenient for implementation in everyday clinical practice.

The aim of this study was to optimize simple and rapid liquid chromatography method with UV detection for simultaneous determination of risperidone and 9-OH risperidone in plasma and urine samples using liquid - liquid extraction procedure for sample preparation.

II. EXPERIMENTAL

2.1 Chemicals and solutions

Risperidone (reference standard) and clozapine (reference standard) were obtained from Krka, Slovenia. 9-OH risperidone (paliperidone, ≥ 98%, HPLC) were purchased from Sigma-Aldrich (St. Luis, MO, USA). Methanol (HPLC grade), acetonitrile, (HPLC grade), hexane and ethyl acetate were obtained from Merck (Darmstadt, Germany). For all analysis HPLC grade water purified with a TKA_LAB Reinstwasser system (Niederelbert, Germany) was used.

2.2 Plasma/urine samples

Plasma/urine from 6 healthy volunteers (drug - free plasma) used for the method validation, were obtained from Institute for transfusion medicine, Republic of Macedonia and stored at -20°C. Plasma/urine samples were obtained from 54 hospitalized schizophrenia/bipolar disorder patients undergoing chronic risperidone therapy (Clinic of Psychiatry, Faculty of Medicine, University "Ss Cyril and Methodius", Skopje). The participation of each subject was voluntary and could be cancelled by any individual at any time during this study (according to the Helsinki II declaration). The Ethics Committee at the Faculty of Pharmacy and the Faculty of Medicine, Ss. Cyril and Methodius University - Skopje, approved the research protocol for this study and all volunteers signed the Study Informed Consent form.

Sampling time of risperidone in relation to dose ingestion was important for the interpretation of the drug concentration. According to literature results, blood samples for TDM of risperidone should be collected after eight weeks of treatment and after a minimum of two weeks of stable dose of the drug. Blood samples were taken before the morning dose and after a minimum of 15 days [13]. Thus, blood samples were collected at 08.00h in the morning, just before the first daily drug administration, into EDTA tubes, and centrifuged at 3000 rpm for 10 min. The supernatant plasma was transferred into test tubes and frozen at - 20°C until analysis. Urine samples were collected using the identical sampling plan as the plasma samples and stored at - 20°C until analysis.

Demographic characteristic of the patients are presented in Table 1.

Table 1. Demographic characteristic of the patients

Diagnosis	Number of patients	Age	Gender (M/F)	Treatment (daily dose)		
				risperidone (2-6 mg)	risperidone (2-6 mg) biperiden (2-6 mg) lorazepam (2.5-10 mg)	risperidone (2-4 mg) carbamazepine (400 mg)
Schizophrenia	38	22-65	17/21	10	24	2
Bipolar disorder	16	22-65	10/6	8	8	0
Negative control	6	28-40	3/3	0	0	0

1.3 Apparatus and chromatographic conditions

HPLC separation was performed on Agilent 1100 LC System. ChemStation software, Version A.10.02 was used for data acquisition and instrument control. Separation was performed on a reversed-phase column (LiChrospher 60 RP B selected 125 x 4 mm; 5 μm, Merck), using acetonitrile as mobile phase A and phosphate buffer (5 mM $NaH_2PO_4xH_2O$, pH 3,0) as a mobile phase B, in a gradient elution mode: linear gradient from 20%(*v*) to 80 % (*v*) mobile phase A over 10 minutes for plasma samples and linear gradient from 18%(*v*) mobile phase A to 82 % (*v*) over 10 minutes for urine samples. The column temperature was 35°C. Flow rate was 1 mL/min. Injection volume was 30 μL. UV detection was performed at 280 nm.

2.4 Standard solutions

2.4.1 Standard stock solutions

Standard stock solutions of risperidone (1 mg/ml) and 9-OH risperidone (0.1 mg/ml) were prepared by dissolving each compound in methanol. The stock solutions were stable for at least 3 months stored at 2-8ºC.

Standard stock solution of clozapine (used as an internal standard IS) was prepared by dissolving and diluting in methanol, to obtain concentration of 1 mg/ml.

2.4.2 Standard solutions

Six standard solutions containing both risperidone and 9-OH risperidone were prepared by suitable dilution of standard stock solutions with water, to individual final concentration of 100, 200, 500, 750, 1000 and 1500 ng/ml. Standard solution of clozapine (IS) was prepared by diluting stock standard solution in water to obtain concentration of 1000 ng/ml.

2.5 Sample solutions

Plasma and urine samples were prepared by liquid/liquid extraction according to modified and adopted procedure, described by Jovanović et al., 2010 [13]. Aliquots of internal standard solution of clozapine were added to 1500 µL plasma and urine to obtain final concentration of 100 ng clozapine/ml plasma and urine. Samples were alkalized with 0.5M NaOH (150 µL in plasma samples; 600 µL in urine samples). The mixture was vortexed continuously for 30 *s*, followed by addition of equal amount of extraction solvent (hexane: ethyl acetate 30:70, *v/v*) and centrifugation for 10 minutes at 12000 rpm. Organic phase was evaporated to dryness using thermo block at a temperature of 37°C - 40°C. The dry residue was dissolved in 50 µL methanol and centrifuged for 2 minutes at 12000 rpm.

2.6 Method validation

Method validation was performed according to EMA Guideline on bioanalytical method validation [14].

2.6.1 Selectivity

Selectivity of the method was determined by analyzing and evaluation of the chromatograms obtained from blank and spiked with standard solutions (risperidone, 9-OH risperidone and clozapine) drug-free plasma and urine samples, from six different sources.

2.6.2 Calibration curves

Standard solutions for calibration curves were prepared by spiking 1500 µL drug-free plasma and urine samples with the aliquots of internal standard solution of clozapine to obtain final concentration of 100 ng/mL and different volumes of standard solutions of risperidone and 9-OH risperidone to obtain final concentrations of 10, 20, 50, 75, 100 и 150 ng/ml for both substances, followed by liquid/liquid extraction as described above (sample preparation). Standards solutions from calibration curve (50 ng/ml of risperidone and 9 OH risperidone) both in plasma and urine, were used as system suitability solutions.

2.6.3 Accuracy and precision

Accuracy and precision were assessed using QC samples prepared by spiking 1500 µL drug-free plasma and urine samples with standard solutions (containing risperidone and 9-OH risperidone) and internal standard solution to obtain final concentrations of 10 ng/ml (LLOQ sample), 20 ng/ml (low QC sample), 50 ng/ml (medium QC sample) and 100 ng/ml (high QC sample) for risperidone and 9-OH risperidone and of 100 ng/ml clozapine in all QC samples. Solutions for injections were prepared by liquid/liquid extraction (as described in sample preparation).

2.6.4 Recovery

Extraction yields were assessed at four concentration levels corresponding to the lower limit, low, medium and high point of each calibration curve.

2.6.5 Stability

Stability of the analytes in the sample matrix (plasma and urine) was evaluated using LLOQ and high QC samples which are analyzed immediately after preparation, after 24 h at room temperature (short term stability), after three freeze-thaw cycles, autosampler stability for 12 h and after 90 days on samples stored at -20 ^{0}C (long term stability). Stability tests were also performed on standard stock solutions stored for 24 h at room temperature and for 3 months at 2-8°C.

III. RESULTS AND DISCUSSION

3.1 Optimization of chromatographic conditions

The optimization of the chromatographic conditions included evaluation of different mobile phases (combinations of methanol and/or acetonitrile with water and phosphate buffers in different ratio) in order to obtain fast and efficient separation of risperidone, 9-OH risperidone and clozapine. Satisfactory separation of the three components with no interference of the components of the matrix (plasma/urine) was obtained on reversed-phase column LiChrospher 60 RP B selected (125 x 4 mm; 5 μm), using acetonitrile and phosphate buffer (5 mM $NaH_2PO_4xH_2O$, pH 3,0) with gradient elution, for a total analysis time of 10 minutes (Fig 1-6). Selection of the working pH value of mobile phase was made on the bases of the dissotiation constants (pKa) of risperidone (pKa = 8.24), 9-OH risperidone (pKa = 7.26) and internal standard clozapine (pKa = 7.6) and in accordance with the literature data for the methods (pH of the mobile phase ranges from 3 to 3.7) for simultaneous determination of risperidone and 9-OH risperidone in biological fluids [5-7].

3.2 Selection of the concentration range for risperidone and 9-OH risperidone and internal standard (IS)

The working concentration range for risperidone and 9-OH risperidone (10 - 150 ng/ml) was selected according to literature data (optimal therapeutic effect is achieved when active moiety AM (risperidone + 9-OH risperidone) concentration ranges between 20-60 ng/ml) [15]. Higher concentrations are published for patients that are on concomitant therapy with *CYP2D6* and *ABCB1* inhibitors. Clozapin and diltiazem are most commonly used as internal standards [5, 12, 13]. Since there is limited information for urine concentration of risperdione and 9-OH risperidone, the same concentration range as plasma samples (10 - 150 ng/ml) were used.

3.3. Optimization of sample preparation procedure and recovery (extraction yield)

Preparation of the samples for application on HPLC, for determination of risperidone and 9-OH risperidone concentration is usually performed using different extraction techniques, for example liquid/liquid extraction with hexane and ethyl acetate (1:1 *v/v*) as organic phase [13] or with solid-phase extraction (SPE) [7].

During the method optimization, solid phase extraction (SPE), with Oasis® HLB columns (30mg/1ml) was performed for sample preparation, but it did not give satisfactory results. Satisfactory extraction results for extraction of risperidone and 9-OH risperidone from plasma/urine were obtained using liqud/liquid extraction with mixture of hexane: ethyl acetate (30:70 *v/v*) as organic phase, alkalized with 0.5 M NaOH. The extraction yields were evaluated at four concentration levels (LLOQ, low QC, medium QC and high QC sample) of the linear concentration range for both, risperidone and 9-OH risperidone. The obtained values were in the range of 95.5% - 98.7% for risperidone and 96.1% - 97.9% for 9-OH risperidone, while the recovery value for IS was 98.8 % for the plasma samples. The gained values from urine samples were in the range of 92.4% - 98.0% for risperidone and 96.3% - 98.6% for 9-OH risperidone, while the recovery value for IS was 98.7%. These results indicate excellent recoveries for all analytes which means that the proposed liquid- liquid extraction procedure is appropriate to be applied to the determination of risperidone and its active metabolite in plasma and urine taken from patients with psychiatric disorders. When described extraction procedure was applied to plasma/urine samples of patients undergoing chronic treatment with risperidone in polytherapy, no interfering peaks due to the co-administered drugs except carbamazepine were eluted at the retention times of analytes (figure 11 and 12).

3.4 System suitability for the method used in determination of analytes in plasma samples

The results from testing the system suitability of the method calculated from the chromatograms of the system suitability solutions, using ChemStation software are given in Table 2 for plasma samples and Table 3 for urine samples. Obtained results for number of theoretical plates, as well as the results for selectivity and resolution ($\alpha > 1$, $Rs > 1.5$) indicate a good separation efficiency of the selected column and satisfactory suitability of the chromatographic system. The peak purity of the peaks corresponding to risperidone, 9-OH risperidone and clozapine, determined using ChemStation for LC 3D software for data handling indicated that the purity factors were within the calculated threshold limits.

Table 2. System suitability values for determination of analytes in plasma samples

Parametars	9-OH risperidone	Risperidone	Clozapine
Rt (min)	4,55	5,35	5,77
κ’	2, 04	2,58	2,86
As	0,84	1,39	0,98
N	5338	7369	9982
α	1,26	1,11	
Rs	2,93	1,76	

Table 3. System suitability values for determination of analytes in urine samples

Parametars	9-OH riseridone	Risperidone	Clozapine
Rt (min)	5,01	5,83	6,19
κ’	2,35	2,89	3,14
As	0,93	1,13	1,07
N	6933	9593	12189
α	1,23	1,08	
Rs	3,40	1,58	

3.5 Method validation

Chromatograms obtained from blank and spiked with standard solutions (risperidone, 9-OH risperidone and clozapine) drug-free plasma and urine samples, from six different sources showed that using the chromatographic conditions satisfactory separation of risperidone, its active metabolite 9-OH risperidone and internal standard is obtained with no interference from endogenous plasma/urine components (Figure 1-4).

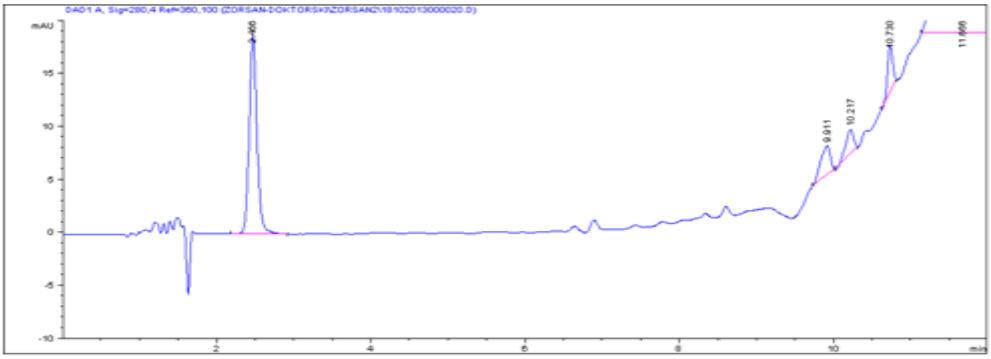

Fig. 1 Chromatograms of blank plasma

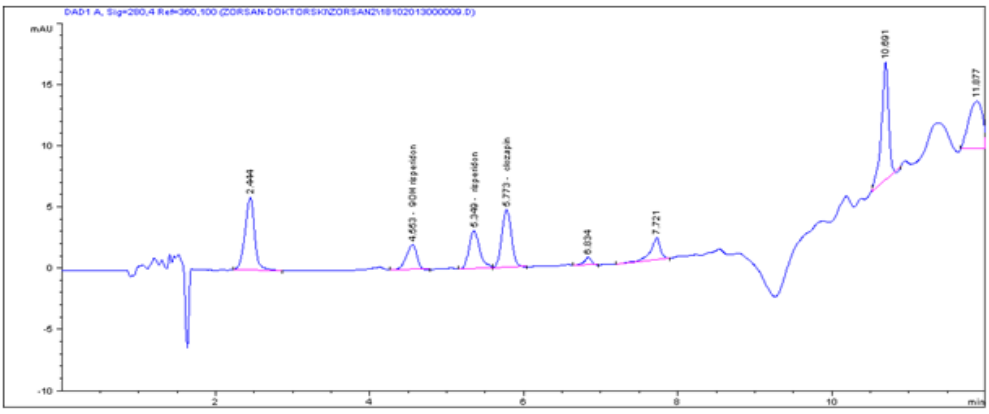

Fig. 2 Chromatograms of system suitability solution for plasma samples

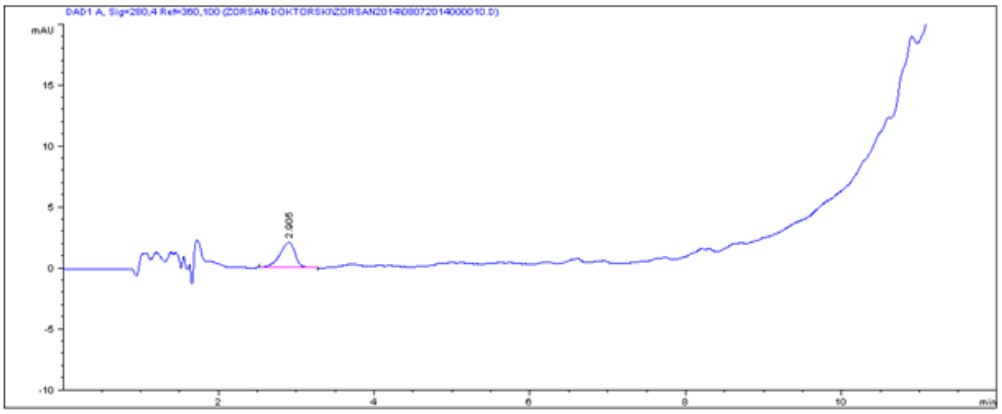

Fig. 3 Chromatograms of blank urine sample

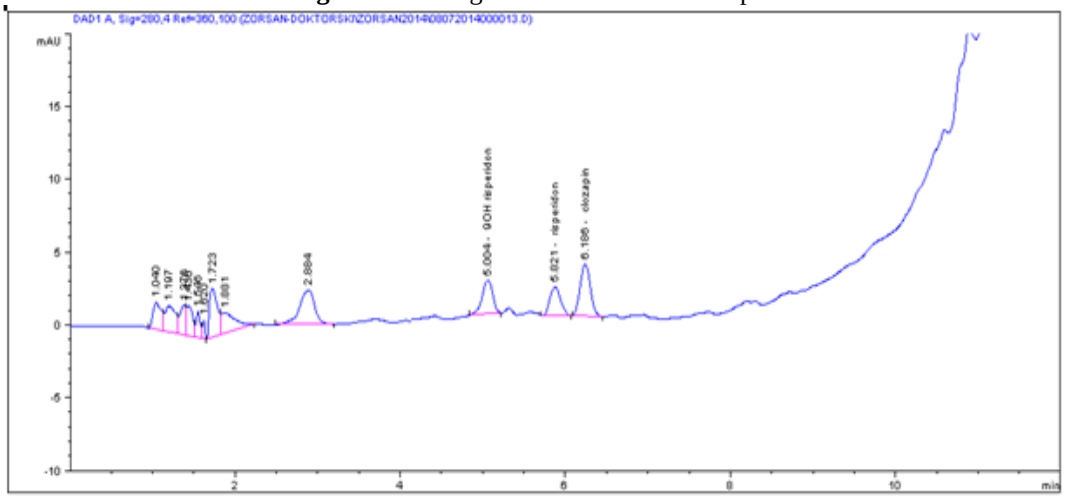

Fig. 4 Chromatograms of system suitability solution for urine samples

The lowest concentration of standard solution for the calibration (10 ng/ml risperidone and 9-OH risperidone) was determined to be the lower limit of quantification (*lower limit of quantification, LLOQ*). The obtained signal/noise ratio were: 23.9 for risperidone, 16.1 for 9-OH risperidone and 185.4 for clozapine in chromatograms from plasma samples and 13.5 for risperidone, 34.9 for 9-OH risperidone and 95.4 for clozapine in chromatograms from urine samples.

The calibration curves were linear over the defined concentration ranges 10 - 150.0 ng/ml for risperidone ($y=0.0069x + 0.0455$, $r^2 = 0.998$ for plasma samples and $y=0.0131x + 0.016$, $r^2 = 0.998$ for urine samples, and 9-OH risperidone ($y = 0.0116x +0.035$, $r^2 = 0.999$ for plasma samples and $y = 0.0126x +0.0264$, $r^2 = 0.999$ for urine samples).

The results from determination of the accuracy and precision of the method are summarized in Table 4 and 5. In plasma samples, within-run assay precision ranged from 1.7 % to 4.9 % for risperidone and from 0.5 % to 4.3 % for 9-OH risperidone, while within-run assay accuracy ranged from 94.1 % to 95.6 % and 93.8 % to 105.8 %, for risperidone and 9-OH risperidone, respectively. The between-run precision and accuracy, ranged from 2.5 % to 5.1 % and 92.6 % to 95.5 % for risperidone, while for 9-OH risperidone the ranges were 0.9 % to 4.5 % and 94.1 % to 112.6%, respectively.

In urine samples, within-run assay precision ranged from 0.8 % to 6.5 % for risperidone and from 0.4 % to 2.8 % for 9-OH risperidone, while within-run assay accuracy ranged from 98.1 % to 105.7 % and 97.9 % to 98.5 %, for risperidone and 9-OH risperidone, respectively. The between-run precision and accuracy, ranged from 3.0 % to 4.1 % and 98.7 % to 103.0 % for risperidone, while for 9-OH risperidone the ranges were 0.9 % to 5.0 % and 95.7 % to 98.6%, respectively.

All the results of the tested samples were within recommended limits. (accuracy: ± 15 % from nominal value of QC samples and ± 20 % from nominal value of LLOQ; precision: value of CV should be in range of ± 15 % for QC samples and ± 20 % for LLOQ), indicating on good accuracy and precision of the method.

Table 4. Precision and accuracy of risperidone and 9-OH risperidone in human plasma

Analyte	Nominal conc. (ng/ml)	Within-run assay (n=5)		Between-run assays (n=15)	
		accuracy (%)	precision (CV%)	accuracy (%)	precision (CV%)
ris	100.0	94.1±4.3	1.9	95.5±9.2	3.9
	50.0	94.7±2.6	1.7	92.6±5.7	2.5
	20.0	95.6±5.6	2.5	92.6±5.7	2.8
	10.0	94.2±11.6	4.9	93.5±11.7	5.1
9-OHris	100.0	96.1±4.7	2.0	94.1±6.9	2.7
	50.0	105.8±7.4	2.8	112.6±2.5	0.9
	20.0	97.0±1.3	0.5	98.5±3.0	1.2
	10.0	93.8±9.9	4.3	95.8±12.0	4.5

Confidence Level (95.0%)

Table 5. Precision and accuracy of risperidone and 9-OH risperidone in human urine

Analyte	Nominal conc. (ng/ml)	Within-run assay (n=5)		Between-run assays (n=15)	
		accuracy (%)	precision (CV%)	accuracy (%)	precision (CV%)
ris	100.0	102.4±3.3	1.3	101.1±8.4	3.4
	50.0	98.1±1.9	0.8	98.7±7.3	3.0
	20.0	98.9±2.4	1.0	98.8±5.2	3.0
	10.0	105.7±16.7	6.5	103.0±10.5	4.1
9-OHris	100.0	98.2±5.9	2.4	98.6±7.3	3.0
	50.0	98.1±6.7	2.8	96.8±9.5	4.0
	20.0	97.9±1.1	0.4	96.6±2.2	0.9
	10.0	98.5±5.8	2.4	95.7±11.9	5.0

Confidence Level (95.0%)

The obtained results from stability testing indicate that the analytes were stable under all storage conditions described above and that no stability related problems would be expected during the routine plasma/urine sample analysis (Table 6 and 7).

Table 6. Stability of risperidone and 9-OH risperidone in human plasma under various conditions (n=3)

Nominal concentration (ng/ml) RIS / 9-OH RIS	Initial concentration t = 0 min (ng/ml) RIS / 9-OH RIS	Accuracy ((%) RIS / 9-OH RIS
Short term stability (24h at room temperature)		
10 / 10	10.2 / 10.3	97.9 / 97.8
100 / 100	99.9 / 100.1	98.7 / 99.3
Autosampler stability (after 12h)		
10 / 10	10.1 / 9.9	96.3 / 97.2
100 / 100	100.0 / 100.0	101.1 / 97.7
Three freeze-thaw cycles		
10 / 10	9.9 / 10.0	98.4 / 100.9
100 / 100	100.1 / 99.6	96.7 / 95.5
Long term stability (90 days at -20^{0}C)		
10 / 10	10.0 / 9.9	97.8 / 102.0
100 / 100	99.8 / 99.7	99.0 / 102.8

Table 7. Stability of risperidone and 9-OH risperidone in human urine under various conditions (n=3)

Nominal concentration (ng/ml) RIS / 9-OH RIS	Initial concentration t = 0 min (ng/ml) RIS / 9-OH RIS	Initial concentration t = 0 min (ng/ml) RIS / 9-OH RIS
Short term stability (24h at room temperature)		
10 / 10	10.0 / 10.1	99.0 / 98.6
100 / 100	99.8 / 100.3	101.2 / 99.3
Autosampler stability (after 12h)		
10 / 10	10.1 / 10.1	97.1 / 96.0
100 / 100	100.4 / 99.9	98.8 / 97.4
Three freeze-thaw cycles		
10 / 10	10. 2 / 9.9	94.8 / 93.2
100 / 100	100.0 / 99.7	97.7 / 94.1
Long term stability (90 days at -20^{0}C)		
10 / 10	9.9 / 10.0	98.8 / 99.1
100 / 100	99.8 / 100.1	100.6 / 99.3

3.6 Determination of risperidon and 9-OH risperidone in patient plasma/urine

The proposed method was applied for simultaneous determination of risperidone and its active metabolite, 9-OH risperidone, in 54 plasma samples and 46 urine samples taken from patients with diagnosed psychiatric disorders (schizophrenia or bipolar disorder). All of the patients were under chronic risperidone treatment (2 - 6 mg/day), but 32 of the patients were on co-medication with biperiden (2-6 mg/day) and lorazepam (2.5-10 mg/day), and 2 of the patients were co-medicated with carbamazepine (400 mg/day) (Figure 5-10). Chromatograms of the plasma and urine samples from patients on risperidone treatment and co-medication with biperiden and lorazepam show that there is no interference from the peaks of biperiden and lorazepam with the peaks of risperidone, 9-OH risperidone and clozapine, which was also confirmed by the software determination of peak purity. The method is suitable for determination of risperidone and its active metabolite 9-OH risperidone in plasma and urine from patients that are on risperidone treatment and co-medication with biperiden and lorazepam.

Chromatograms of plasma and urine sample from patients on risperidone treatment and co-medication with carbamazepine show that the peak of carbamazepine co-elute with the peaks from risperidone and clozapine, indicating that the proposed method is not suitable for therapeutic drug monitoring in these patients, which were excluded from this study (Figure 9 and 10).

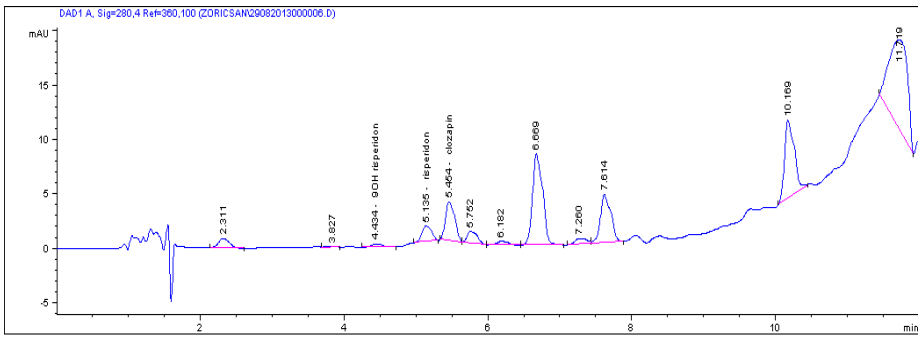

Fig. 5 Chromatograms of plasma samples from patients on risperidone 2 mg/day treatment without co-medication

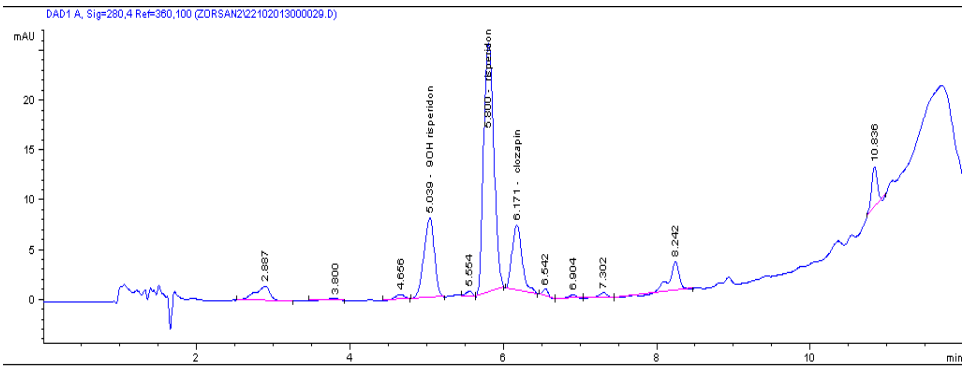

Fig. 6 Chromatograms of urine samples from patients on risperidone 2 mg/day treatment without co-medication

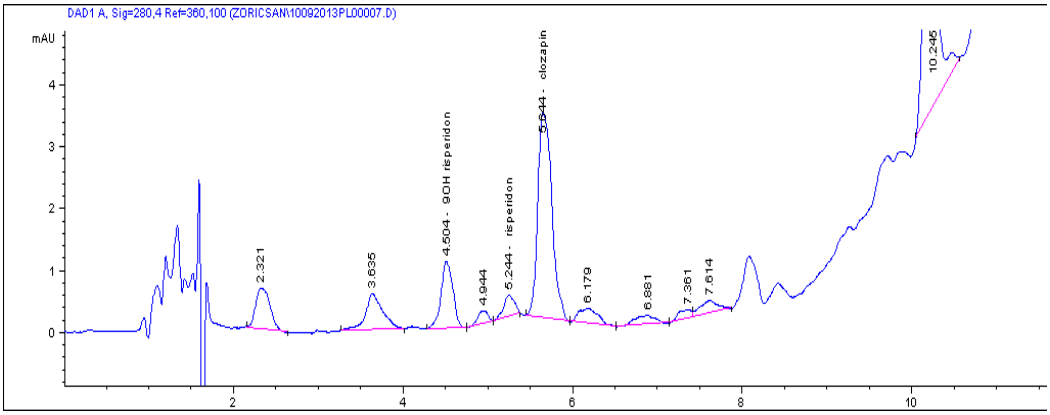

Fig. 7 Chromatograms of plasma sample from patients on risperidone 2 mg/day treatment and concomitant use of biperiden (2 mg/day) and lorazepam (2.5 mg/day)

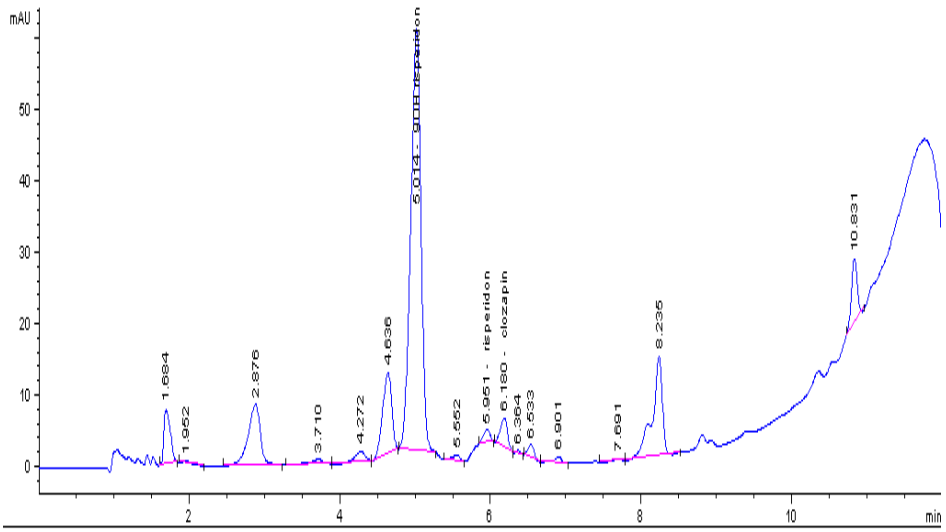

Fig. 8 Chromatograms of urine sample from patients on risperidone 2 mg/day treatment and concomitant use of biperiden (2 mg/day) and lorazepam (2.5 mg/day)

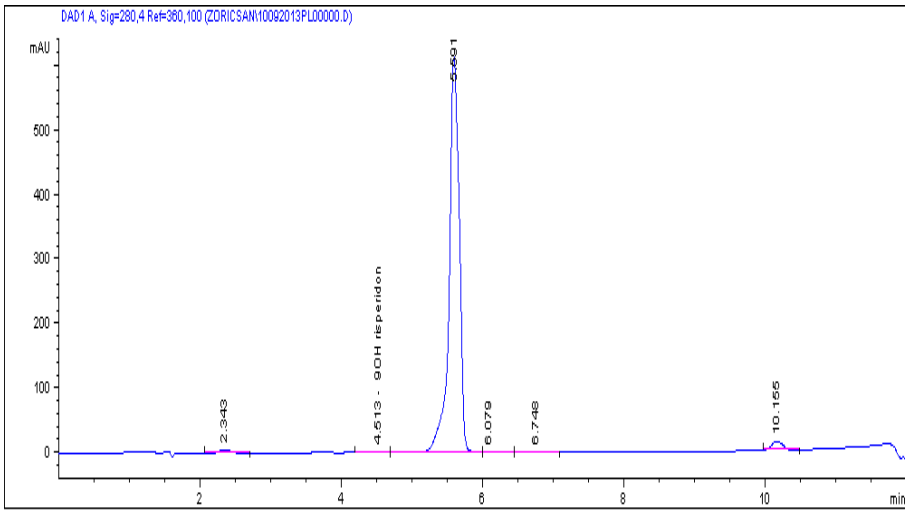

Fig. 9 Chromatograms of plasma sample from patients on risperidone 4 mg/day treatment and concomitant use carbamazepine (400 mg/day)

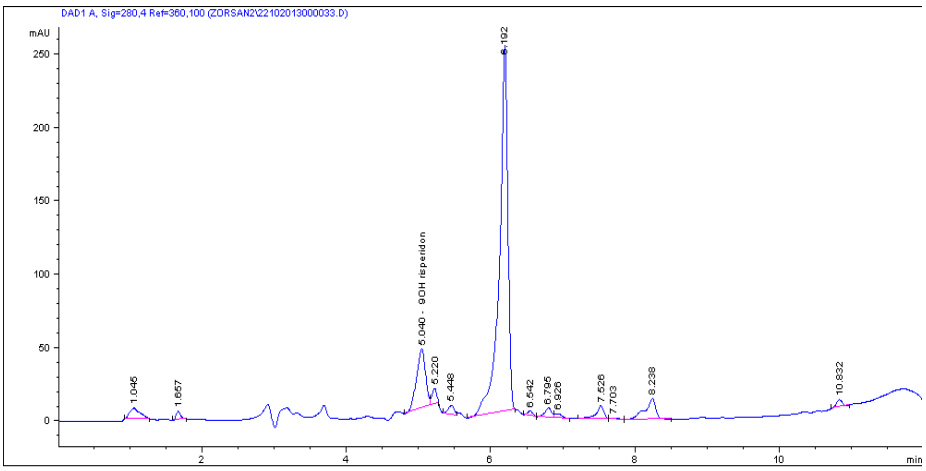

Fig. 10 Chromatograms of urine samples from patients on risperidone 4 mg/day treatment and concomitant carbamazepine (400 mg/day)

Obtained results have shown high variability of concentration of risperidon and its active metabolite 9-OH risperidone and consequently the concentration of active moiety in both plasma and urine samples, which may be a result from genetic variability of metabolic and transporter proteins in different patients (Table 8). Therapeutic plasma concentrations of risperiodone and its active metabolite are directly influenced by genetic variations in metabolic CYP450 enzymes (*CYP2D6* and *CYP3A4/5*) and transporter (*ABCB1*) enzymes and additional environmental factors [16, 17]. The obtained concentrations for risperidone that were below LLOQ probably indicate that all risperidone is metabolized in its active metabolite 9-OH risperidone as a result of specific metabolic phenotype for CYP2D6. Further studies are needed to investigate the correlation between plasma and urine concentrations of active moiety of risperidone in different metabolic types of patients.

Table 8. Mean concentration of risperidone and 9-OH risperidone from plasma/urine obtained from patient population (two patients on carbamazepine co-medication were excluded from the analysis)

	Plasma (52 patients)		Urine (46 patients)	
	9-OH ris	ris	9-OH ris	ris
Min. conc. (ng/ml)	4.01	below LLOQ	66.38	below LLOQ
Max. conc. (ng/ml)	160.61	204.01	3586.86	2076.58
Average (ng/ml)	71.46	14.01	969.68	150.03

Simple and rapid liquid chromatography method with UV detection was optimized for simultaneous determination of risperidone and 9-OH risperidone in plasma and urine samples. The described liquid - liquid extraction procedure for sample preparation gave good extraction yields with satisfactory precision for both plasma and urine samples. Validation results showed that the method is selective, sensitive, linear, precise and accurate. The proposed method was applied for determination of risperidone and 9-OH risperidone in plasma and urine obtained from patients treated with risperidone as monotherapy and in polytherapy.

IV. CONCLUSION

Proposed method has advantage for use in pharmacokinetic studies where multiple sampling is needed. It could be used as a routine method for therapeutic monitoring of risperidone and its active metabolite, but also for establishing correlations of metabolic ratio of risepridone and 9-OH risperidone in plasma and urine and studying the influence of patient's phenotype on metabolic ratio (Ris/9-OHRis) in plasma and in urine. This information could be useful for initial indication of the metabolic phenotype and for selection of patients with high risk for adverse drug reactions and establishing a good therapeutic regimen in individualization of risperidone treatment.

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