Talanta 81 (2010) 1547–1553

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Analysis of risperidone and its metabolite in plasma and saliva by LC with coulometric detection and a novel MEPS procedure

Maria Addolorata Saracino[a], Anna de Palma[a], Giancarlo Boncompagni[b], Maria Augusta Raggi[a,*]

[a] Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, I-40126 Bologna, Italy
[b] Department of Mental Health, Malpighi Hospital, Via P. Palagi 9, I-40126 Bologna, Italy

ARTICLE INFO

Article history:
Received 28 December 2009
Received in revised form 23 February 2010
Accepted 25 February 2010
Available online 6 March 2010

Keywords:
Risperidone
9-Hydroxyrisperidone
Liquid chromatography
Microextraction by packed sorbent
Saliva
Plasma

ABSTRACT

A new analytical method, based on the use of liquid chromatography with coulometric detection, has been developed and applied to quantify risperidone and its main active metabolite 9-hydroxyrisperidone in human plasma and saliva. The analytes were separated on a reversed phase C18 column, using a mobile phase composed of acetonitrile (26%) and a pH 6.5 phosphate buffer (74%). Pipamperone was used as the internal standard. A high sensitivity coulometric detection analytical cell containing two flow-through working electrodes was used: electrode 1 was set at +0.500 V and electrode 2 at +0.700 V. The detector response was linear over a plasma and saliva concentration range of 0.5–50.0 ng mL^{-1} for risperidone and 0.5–100.0 ng mL^{-1} for 9-hydroxyrisperidone. The limit of quantitation and the limit of detection for risperidone and 9-hydroxyrisperidone were 0.5 ng mL^{-1} and 0.17 ng mL^{-1}, respectively. A novel clean-up procedure of biological samples was developed using the microextraction by packed sorbent technique, which gave good extraction yield for both the analytes, with absolute recovery values higher than 90.1%. The intra-day and the inter-day precision results, expressed by relative standard deviation values, were lower than 5.8%. Accuracy and selectivity assays were also satisfactory. The validated method has been successfully applied to the analysis of risperidone and 9-hydroxyrisperidone in plasma and saliva of psychiatric patients undergoing therapy with risperidone.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Risperidone (3-{2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl}-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-α]pyrimidine-4-one, RISP, Fig. 1a) is an atypical antipsychotic with a benzisoxazolic structure, approved for the treatment of schizophrenia and for schizoaffective and bipolar disorders [1]. Recently, the Food and Drug Administration approved RISP for the treatment of irritability in children and adolescents with autism [2].

RISP is available as oral tablets and solution (Risperdal®) and it is usually administered at doses between 4 mg day^{-1} and 16 mg day^{-1} for the treatment of schizophrenia and at 1–6 mg day^{-1} for mania. A depot intramuscular injectable formulation has become available; this formulation could be administered at 2-week intervals and seems to have comparable efficacy of the oral administration [3].

The drug effects are mainly related to the blocking of serotonine 5-HT$_{2A}$ and 5-HT$_7$ receptors and dopamine D$_2$ receptors [4], thus RISP is also very effective against negative symptoms of schizophrenia.

RISP is rapidly and completely absorbed after oral administration and it undergoes extensive hepatic metabolism mainly *via* cytochrome P450 (CYP) isoenzymes (CYP2D6 and CYP3A4 isoforms); 9-hydroxyrisperidone (RISP9-OH, Fig. 1b) is the major active metabolite [5].

RISP is extensively bound (90%) to plasma proteins, while protein binding for RISP9-OH is lower (77%) [3]. For the patients under RISP treatment it is evaluated the "*active moiety*" which is the sum of RISP and RISP9-OH concentrations. The levels of the *active moiety* are considered therapeutic in the 10–60 ng mL^{-1} range [3].

Therapeutic drug monitoring (TDM) of RISP is usually recommended to avoid severe side effects, such as extrapyramidal symptoms (at high doses), hyperprolactinemia, weight gain, headache and dizziness [1].

Numerous papers have been published on the analysis of RISP and its active metabolite in different matrices. Most

Abbreviations: RISP, risperidone; RISP9-OH, 9-hydroxyrisperidone; TDM, therapeutic drug monitoring; CNS, central nervous system; MEPS, microextraction by packed sorbent.

* Corresponding author at: Laboratory of Pharmaco-Toxicological Analysis, Department of Pharmaceutical Sciences, Via Belmeloro 6, 40126 Bologna, Italy. Tel.: +39 051 2099739; fax: +39 051 2099740.
E-mail address: mariaaugusta.raggi@unibo.it (M.A. Raggi).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.02.067

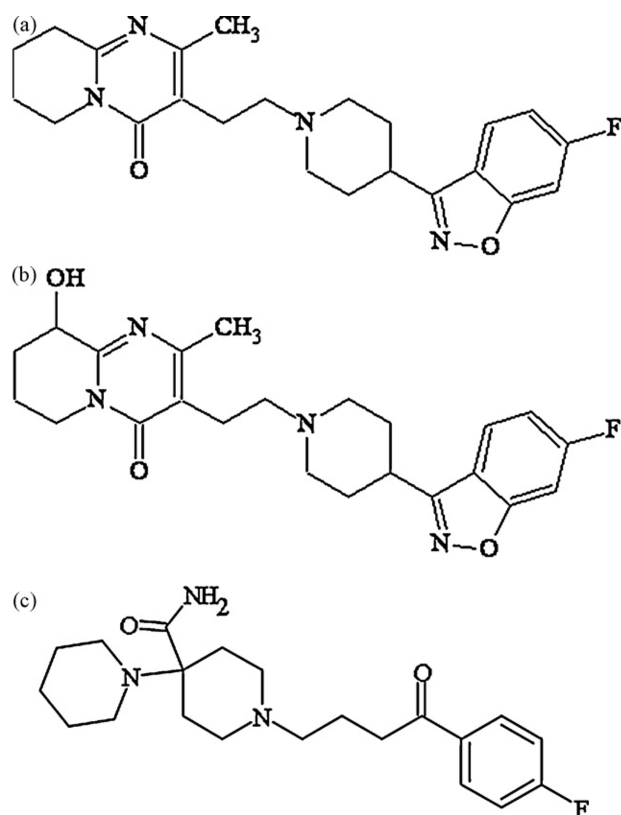

Fig. 1. Chemical structures of (a) risperidone (RISP), (b) 9-hydroxyrisperidone (RISP 9-OH), and (c) pipamperone, used as internal standard (IS).

of them concern with liquid chromatographic (LC) methods with UV or diode array detection [6–16]; other methods use electrochemical detection [17–21] or mass spectrometry (MS) [22–33]. The sample pre-treatment is carried out by means of liquid–liquid extraction (LLE) procedures with organic solvents [9,11,12,14,15,17–22,24,25,29,31,32], solid-phase extraction (SPE) [6,7,13,16,23,28] and deproteinization techniques [10,26,27,33]. Today, there is a constant need for the development of faster and more selective sample clean-up procedures. Recent developments of sample handling are directed toward miniaturization and automation. The microextraction by packed sorbent (MEPS) is a new technique for miniaturized solid-phase extraction because it reduces the sample volume and the time necessary for the analysis; thus, it is suitable for the rapid analysis of biological samples, as already reported in the literature for the determination of other drugs [34–38].

The aim of this study is the development of a sensitive, selective and fast analytical method which allows the analysis of RISP and its main active metabolite in human plasma and saliva. The proposed method is based on the use of LC with coulometric detection and a novel MEPS procedure.

2. Experimental

2.1. Chemicals and solutions

Risperidone and 9-hydroxyrisperidone were kindly provided by Janssen-Cilag (Titusville, USA); pipamperone dihydrochloride (used as internal standard, IS, Fig. 1c) was purchased from Sigma–Aldrich (St. Louis, MO, USA).

Potassium dihydrogen phosphate, 85% (w/w) phosphoric acid and 2 M sodium hydroxide, all pure for analysis, methanol and acetonitrile (HPLC grade) were from Sigma–Aldrich. Ultrapure water (18.2 MΩ cm) was obtained by means of a MilliQ apparatus by Millipore (Milford, MA, USA).

Stock solutions of RISP and RISP9-OH (1 mg mL^{-1} of each one) were prepared by dissolving 5 mg of each pure substance in 5 mL of methanol. Stock solution of the IS (1 mg mL^{-1}) was prepared by dissolving 5.9 mg of the hydrochloride salt in 5 mL of methanol. The stock solutions were stable for at least three months when stored at −20 °C, as assessed by LC assays. Standard solutions were prepared fresh every day by diluting the stock solutions with the mobile phase.

2.2. Human plasma and saliva collection

Blood and saliva samples were obtained from 6 patients (4 men, mean age = 28 years; 2 women, mean age = 35 years) subjected to oral therapy with Risperdal® at daily doses between 2 mg and 16 mg or to depot therapy at biweekly doses between 25 mg and 50 mg. Written consent form was obtained from the patients after their acceptance of the criteria for the analytical investigation. The biological samples were usually drawn early in the morning from fasting patients (12 h after the last drug administration in the oral treatment). Blood (3 mL) was stored in glass tubes containing ethylenediaminetetraacetic acid as the anticoagulant, then centrifuged (within 2 h from collection) at 4000 rpm for 15 min at 5 °C; the supernatant (plasma) was then transferred to polypropylene tubes and stored at −20 °C until LC analysis.

Unstimulated saliva samples (0.5 mL) were collected from patients half an hour after blood sampling, with a disposable plastic pipette. It was put into polypropylene tubes and stored frozen at −20 °C until the time of LC analysis, when they were centrifuged at 4000 rpm for 15 min at 5 °C; the assays were carried out on the clear supernatant.

Blood and saliva samples from healthy volunteers, used as "blank", were treated in the same way.

2.3. Apparatus and chromatographic conditions

The chromatographic apparatus for the determination of RISP and RISP9-OH was composed of a Jasco (Tokyo, Japan) PU-1580 chromatographic pump and an ESA (Milford, MA, USA) Coulochem III coulometric detector equipped with a high sensitivity analytical cell (model 5011 ESA) and a conditioning cell (model 5021 ESA). The high sensitivity analytical cell contained two flow-through working electrodes positioned serially. They were made from porous graphite with large surface area. The applied voltages for electrodes 1 and 2 were +0.500 V and +0.700 V, respectively. Electrode 1 was the screening electrode for potential interfering substances; electrode 2 was the detection electrode. The conditioning cell was set at a potential of +0.050 V and was used as an additional screening electrode for interference. Data handling was carried out using a DataApex (Prague, Czech Republic) Chromatography Station (CSW 32 v. 1.4) software.

The chromatographic separation was carried out on a Gemini C18 reversed phase column (150 × 4.6 mm I.D., 5 μm) from Phenomenex (Torrance, CA, USA) equipped with a C18 cartridge precolumn (4 × 3 mm I.D., 5 μm) and kept at room temperature. The mobile phase was composed of a mixture of acetonitrile and a 7.07 mM, pH 6.5 phosphate buffer (26:74; v/v). The mobile phase was filtered through a Phenomenex membrane filter (47 mm membrane, 0.2 μm, NY) and degassed by an ultrasonic apparatus. The flow rate was 1.5 mL min^{-1}. The samples were injected into the LC system by means of a 50 (L loop.

A Crison (Barcelona, Spain) MicropH 2000 pHmeter, a Universal 32 R centrifuge from Hettich (Tuttlingen, Germany), a Büchi (Flawill, Switzerland) RE 111 rotary evaporator, an Elma (Berlin,

Germany) Transsonic T310 ultrasonic bath and a vortex agitator were also used.

2.4. Microextraction by packed sorbent procedure

Using the MEPS procedure, the sample pre-treatment takes place on the packed bed, available in a variety of SPE sorbents and inserted inside a syringe between the barrel and the needle (Barrel Insert and Needle Assembly, BIN). The MEPS BIN is easily installed into the syringe housing and then secured by a locking nut. In the present work, MEPS was carried out using a BIN containing 4 mg of solid-phase material silica-C8, inserted into a 250 μL gas-tight syringe from SGE Analytical Science (Melbourne, VIC, Australia). The packed syringe was activated with 100 (L of methanol three times and then conditioned with 100 (L of water three times before being used. The volumes of methanol and water were picked up and then discarded every time at a flow rate of 20 (L s^{-1}.

For the analysis of biological samples from patients aliquots of 25 μL of IS working solution (20 ng mL^{-1}) and 100 μL of ultrapure water were added to 100 μL of plasma or saliva sample. For the preparation of the spiked blank samples, aliquots of 25 μL of analyte standard solutions at different concentrations (containing the IS at a constant concentration) and 100 μL of ultrapure water were added to 100 μL of plasma or saliva sample. The prepared mixtures were put into polypropylene tubes; then, they were drawn up and down through the syringe 15 times (at a flow rate of 5 μL s^{-1}) without discarding it. The solid-phase was washed once with water (100 μL) and once with a mixture of water and methanol (95:5, v/v) to remove biological interference. Then the analytes were eluted with 250 μL of methanol. The eluate was dried under vacuum (rotary evaporator), re-dissolved with 100 μL of mobile phase and injected into the LC system. All MEPS steps including activation, loading, washing and elution were carried out manually. After each extraction, cleaning of the sorbent was done with 3× 100 μL of methanol followed by 3× 100 μL of water. This step decreased memory effects, but also acted as the conditioning step for the next extraction. The same packing bed was used for about 50 extractions; then it was discarded due to low analyte extraction yields and clogging of the sorbent.

2.5. Method validation

Method validation procedures were carried out according to USP XXXII [39] and Crystal City [40] guidelines.

2.5.1. Extraction yield (absolute recovery) and precision

Standard solutions at three different concentrations of RISP and RISP9-OH were added to 100 μL of blank plasma or saliva, in order to obtain concentrations of 0.5, 25.0 and 50.0 ng mL^{-1} for RISP and 0.5, 50.0 and 100.0 ng mL^{-1} for RISP9-OH; these mixtures were subjected to the MEPS procedure and injected into the LC system.

The peak areas of analytes obtained in this way were compared to those obtained from standard solutions at the same theoretical concentration, and the percentage extraction yield was calculated.

The assays described above were repeated six times within the same day to obtain repeatability (*intra-day precision*) and six times over six different days to obtain intermediate precision (*inter-day precision*), both expressed as percentage relative standard deviation values (RSD%).

2.5.2. Calibration curves, limit of quantitation, and limit of detection

The calibration curves were constructed by adding aliquots of 25 μL of analyte standard solutions at ten different concentrations, containing the IS at a constant concentration, to 100 μL of blank plasma and saliva; the resulting mixtures were diluted with 100 μL of ultrapure water and, then subjected to MEPS procedure and injected into the LC system. This procedure was done in triplicate for each point. The analyte/IS peak area ratios (pure numbers) obtained were plotted against the corresponding concentrations of the analytes (expressed as ng mL^{-1}) in the following ranges: 0.5–50.0 ng mL^{-1} for RISP and 0.5–100.0 ng mL^{-1} for RISO9-OH. The calibration curves were constructed by means of the least-square method. One stock solution was used for each replicate; different working solutions were prepared from the stock solutions and added to the blank plasma and saliva samples to obtain the different concentrations.

The values of limit of quantification (LOQ) and limit of detection (LOD) were calculated according to official guidelines [39,40] as the analyte concentrations which give rise to peaks whose heights are 10 and 3 times the baseline noise, respectively.

2.5.3. Accuracy

Accuracy was evaluated by means of recovery assays. Aliquots of 25 μL of analyte standard solutions at three different concentrations (i.e. additions of 2.5–5.0–10.0 ng mL^{-1} for RISP and 5.0–10.0–25.0 ng mL^{-1} for RISP-9OH), containing the IS at a constant concentration, were added to 100 (L of plasma and saliva from patients treated with Risperdal®. The resulting mixtures were diluted with 100 μL of ultrapure water and, then, subjected to the MEPS procedure. Recovery values were calculated according to the following formula:

$$100 \times \frac{[\text{after spiking}] - [\text{before spiking}]}{[\text{added}]}$$

3. Results and discussion

3.1. Choice of the chromatographic and detection conditions

Preliminary assays were carried out using a cyano column as the stationary phase, while the mobile phase was a mixture of acetonitrile and a pH 6.5 phosphate buffer [21]. These chromatographic conditions were found unsuitable for a good separation of the analytes. A C18 column together with a mobile phase more hydrophilic (74% of phosphate buffer and 26% of acetonitrile) provided a better separation of the analytes in an acceptable run time (<10 min), coupled with a good selectivity and peak shape.

The next step was to find the best electrochemical conditions for improving the sensitivity of the coulometric detector, being RISP and RISP9-OH difficultly oxidizable compounds. Fig. 2 shows the hydrodynamic voltammograms of RISP and RISP9-OH, monitored at detector 2 keeping the detector 1 at 0.000 V. As one can see, for values of potentials higher than +0.700 V the sensitivity improved, but since the signal/noise ratio worse, the +0.700 V value was selected as the working potential. For detector 1, used as the screening electrode, some trials were carried out in a range from −0.200 V to +0.500 V. An oxidation potential of +0.500 V was chosen obtaining a satisfactory cut-off of biological interference without any significant oxidation on the analytes.

Pipamperone was chosen as the internal standard, having an acceptable retention time and chemical–physical properties similar to the analytes.

The chromatogram of a standard solution containing 12.5 ng mL^{-1} of RISP, 25.0 ng mL^{-1} of RISP9-OH and 5.0 ng mL^{-1} of IS is reported in Fig. 3. As can be seen, the peaks are neat and well resolved. Retention times (t_R) are: RISP, t_R = 9.1 min; RISP9-OH, t_R = 5.9 min; IS, t_R = 7.5 min.

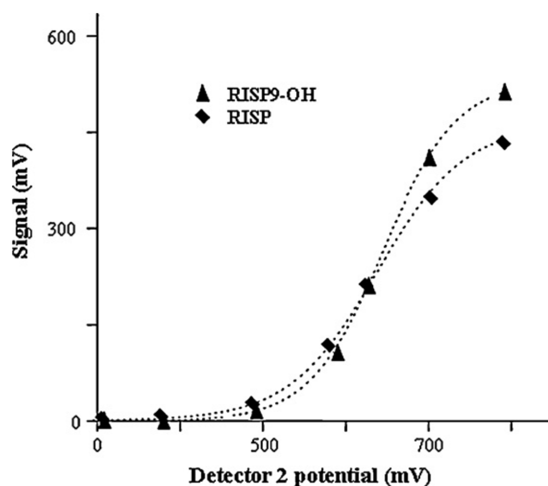

Fig. 2. Voltammograms of RISP and RISP9-OH (50.0 ng mL^{-1}) monitored at detector 2. Chromatographic conditions are as described in Section 2, except that detector 1 was set at 0.000 V.

3.2. Development of a microextraction by packed sorbent procedure

The biological sample pre-treatment is an important step of the analysis since it influences the overall method time, accuracy, sensitivity and precision. Moreover, proteins and other interference from biological matrices may result in precocious deterioration of the performance of chromatographic columns. As concerning the analysis of RISP and its metabolite, the most frequently used extraction procedures was LLE with organic solvents. In the last few years, SPE has also become widespread and it is used to extract, pre-concentrate and clean-up the analytes from the biological matrix. Today, miniaturization, short extraction times and consumption of small organic solvent volumes are useful parameters especially for TDM purposes. Furthermore, the procedure should be highly selective and reproducible, grant acceptable extraction yields and involve a minimum number of steps. MEPS is a novel development for miniaturized solid-phase extraction in the field of sample handling and preparation. In this paper, some factors affecting the performance of MEPS and the absolute recovery such as the composition of washing and elution solutions, the number of extraction cycles and the flow rate were studied using C8 as sorbent.

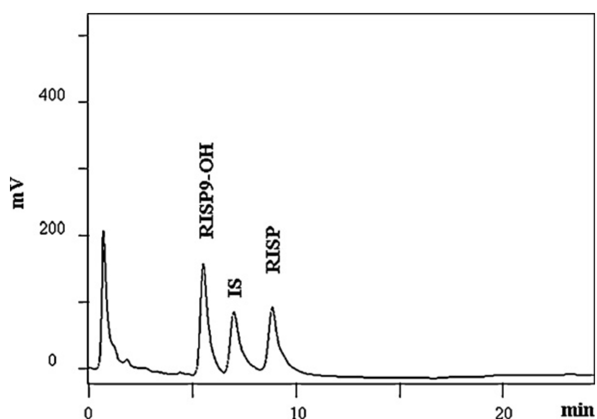

Fig. 3. Chromatogram of a standard solution containing 12.5 ng mL^{-1} of RISP, 25.0 ng mL^{-1} of RISP9-OH and 5.0 ng mL^{-1} of IS.

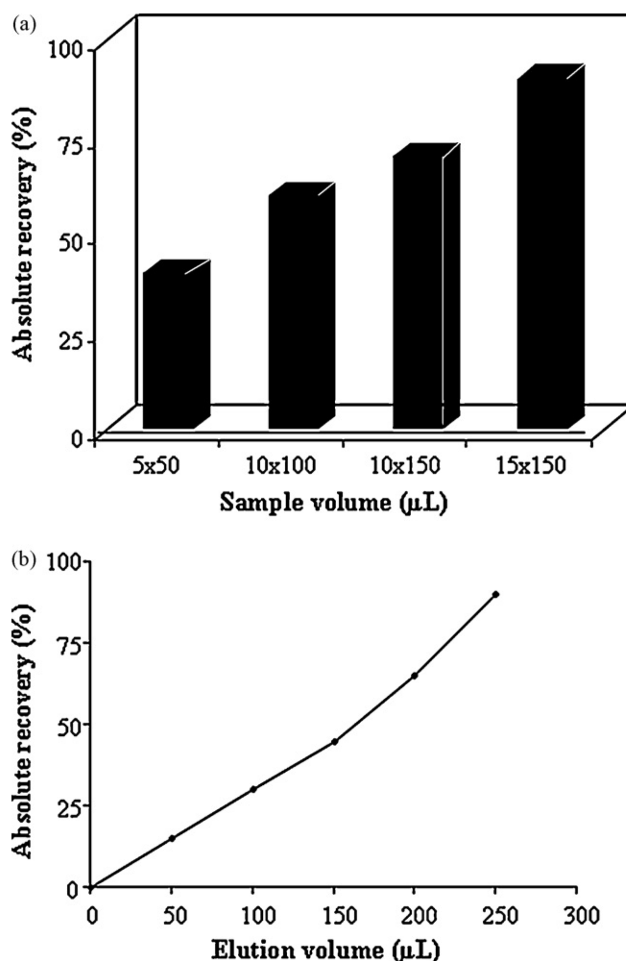

Fig. 4. Mean absolute recovery of RISP and RISP9-OH as a function of (a) applied sample volume; and (b) elution volume. MEPS conditions are as described in Section 2.

3.2.1. Effect of number of extraction cycles and flow rate on extraction efficiency

In MEPS an aliquot of the volume of the sample can be drawn up and down through the syringe, once or several times (cycles) without discarding it. Fig. 4a shows the effect of such procedure: sample response increased as applied extraction number and sample volume increased. The recovery increased from 40% to 90% using 15× 150 µL sample volume compared to 5× 50 µL using. Moreover, during method development, it is important to evaluate the effect of different sample flow rates on the efficiency of analyte adsorption and desorption. The response increased by 15% with flow rate of 5 µL s^{-1} compared to flow rate of 20 µL s^{-1} and using in both trials 15× 150 µL sample volume with C8 sorbent.

3.2.2. Nature and volume of washing solutions and elution solutions

The effect of different washing solutions on the absolute recovery of the analytes and on the cleanliness of the extracts was investigated. The only step washing with water is inadequate to remove interference from the matrices. Thus, a low amount of methanol in the washing mixture was also tested. Clean extracts were obtained using an aliquot of 50 µL of the mixture of water/methanol 95:5 (v/v). The elution efficiency was tried using acetonitrile and methanol as solvents. Acetonitrile proved to be almost ineffective (analytes extraction yield <60.0%); better results were obtained using methanol. A linear relationship was there between the analyte response and the elution volume (range of

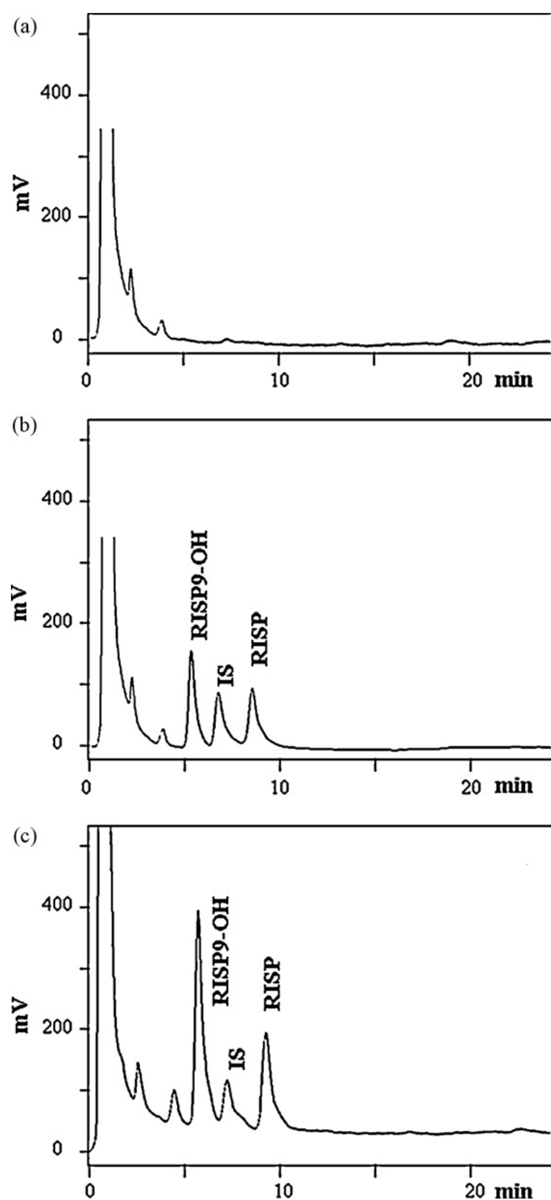

Fig. 5. Chromatograms of (a) a blank plasma sample; (b) the same blank plasma sample spiked with 12.5 ng mL^{-1} of RISP, 25.0 ng mL^{-1} of RISP9-OH and 5.0 ng mL^{-1} of IS; and (c) a plasma sample from a patient taking 9 mg day^{-1} of risperidone.

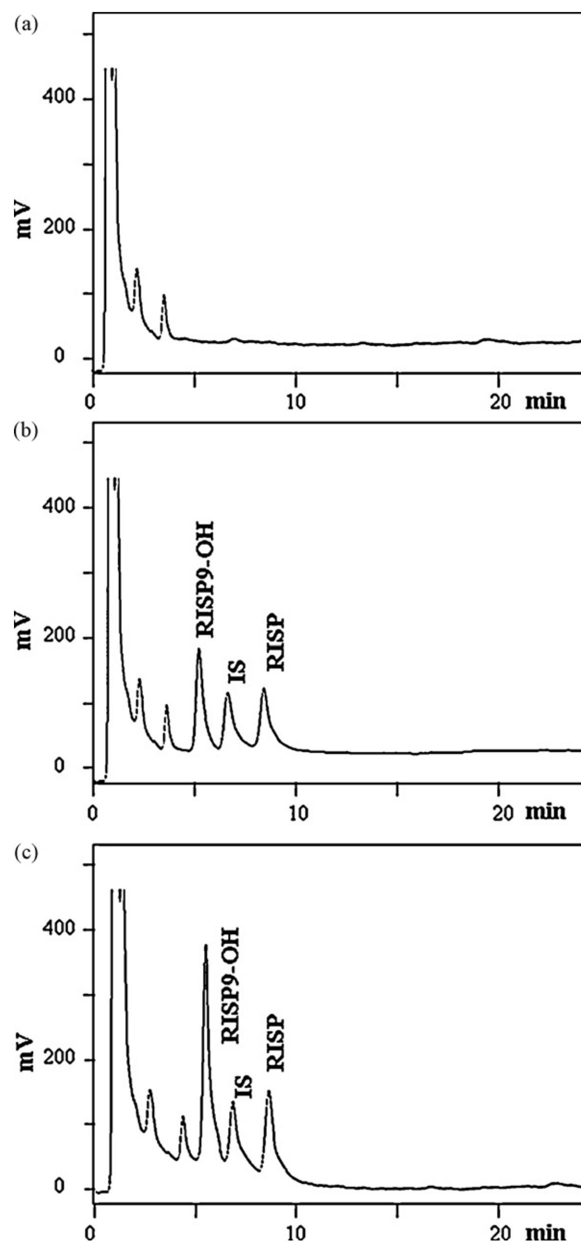

Fig. 6. Chromatograms of (a) a blank saliva sample; (b) the same blank saliva sample spiked with 12.5 ng mL^{-1} of RISP, 25.0 ng mL^{-1} of RISP9-OH and 5.0 ng mL^{-1} of IS; and (c) a saliva sample from a patient taking 9 mg day^{-1} of risperidone.

methanol between 50 and 250 μL), as reported in Fig. 4b. Thus, a volume of methanol of 250 μL was used to obtain the best elution efficiency: the organic extracts were evaporated to dryness and, then the analytes were re-dissolved with 100 μL of mobile phase.

The chromatograms from blank plasma and saliva samples are represented in Figs. 5a and 6a, respectively. Figs. 5b and 6b show blank plasma and saliva samples spiked with 12.5 ng mL^{-1} of RISP, 25.0 ng mL^{-1} of RISP9-OH and 5.0 ng mL^{-1} of IS. The peaks of all the analytes are neat and symmetric without any interference in both the biological matrices.

3.3. Method validation

Calibration curves were set up for RISP and its metabolite and good linearity (rc > 0.9990) was found in the concentration ranges studied (Table 1). The limit of quantitation (LOQ) and the limit of detection (LOD) for RISP and RISP9-OH were 0.5 ng mL^{-1} and 0.17 ng mL^{-1}, respectively.

Extraction yield and precision assays were carried out at three different concentration levels, corresponding to the lowest level, highest level and middle point of each calibration curve. The results of these assays are reported in Table 2. As one can see, the results are satisfactory, being the extraction yield values higher than 90.1%. The mean extraction yield of the IS was 89.8%. The precision was also satisfactory, with RSD values always lower than 5.8% for all analytes.

3.4. Selectivity

The method selectivity was evaluated injecting under the optimized chromatographic conditions standard solutions of several drugs usually co-administered in clinical practice. Retention times were compared with those of the analytes. The compounds tested for possible interference are reported in Table 3. As one can see,

Table 1
Linearity parameters.

Compound	Linearity range ($ng\,mL^{-1}$)	Equation coefficients, $y = ax + b$[a]		r_c	LOQ ($ng\,mL^{-1}$)	LOD ($ng\,mL^{-1}$)
		a	*b*			
RISP	0.5–50.0	0.0695	0.0035	0.9996	0.5	0.17
		0.0729	*−0.0473*	*0.9990*		
RISP9-OH	0.5–100.0	0.0719	−0.0012	0.9993	0.5	0.17
		0.0813	*−0.0158*	*0.9993*		

The values in italics are referred to linearity parameters on saliva samples.
[a] y = analyte/IS peak area ratio; x = analyte concentration ($ng\,mL^{-1}$).

Table 2
Validation parameters.

Analyte	Amount added ($ng\,mL^{-1}$)	Extraction yield (%)[a]		Repeatability (RSD%)[a]		Inter-day precision (RSD%)[a]	
		Plasma	Saliva	Plasma	Saliva	Plasma	Saliva
RISP	0.5	92.0	96.7	4.6	4.9	5.3	5.8
	12.5	91.2	95.0	4.3	4.5	4.7	4.9
	25.0	90.8	92.0	4.1	4.2	4.5	4.5
RISP9-OH	0.5	95.0	96.0	4.5	4.9	5.1	5.1
	50.0	93.0	95.8	4.3	4.4	4.6	4.9
	100.0	90.1	93.4	4.0	4.2	4.1	4.3
IS	20.0	89.5	90.0	3.5	4.0	3.7	4.0

[a] Each value is the mean of 6 independent assays.

Table 3
Compounds tested for interference.

Drug	Retention time (min)	Drug	Retention time (min)
Antipsychotics		**Sedative-hypnotics**	
Aripiprazole	n.d.[a]	Clonazepam	n.d.
Clozapine	15.6	Flurazepam	24.0
N-desmethylclozapine	12.6	Lorazepam	n.d.
Clozapine N-oxide	n.d.	**Antiepileptic agents**	
Amisulpride	1.8	Valproic acid	n.d.
Olanzapine	n.d.	Lamotrigine	n.d.
Ziprasidone	n.d.	Topiramate	n.d.
Antidepressants		Oxcarbamazepine	n.d.
Chlomipramine	n.d.	**Antiparkinson drugs**	
Fluoxetine	n.d.	Orphenadrine	n.d.
Sertraline	n.d.	Biperiden	n.d.

[a] n.d. = not detected within 30 min.

none of the tested drugs interfered with the chromatographic peaks of RISP and its metabolites.

3.5. Analysis of patient plasma and saliva samples

The method was applied to the analysis of plasma and saliva samples from some patients treated with Risperdal®. The chromatogram of a plasma sample from a patient taking 9 mg day^{-1} of RISP is reported in Fig. 5c; the chromatogram of a saliva sample from the same patient is reported in Fig. 6c. As one can see, no interference from the matrices is apparent and the analyte separation is good. The plasma analyte levels found were: 22.0 $ng\,mL^{-1}$ for RISP and 48.7 $ng\,mL^{-1}$ for RISP9-OH; the saliva levels found were: 21.0 $ng\,mL^{-1}$ for RISP and 47.9 $ng\,mL^{-1}$ for RISP9-OH.

Method accuracy was evaluated by means of recovery assays at three different concentration levels for the analytes ($n = 3$ for each level), as reported in the Section 2.5.3. Results were satisfactory: recovery values were always higher than 90.0% in both plasma and saliva samples from patients treated with Risperdal®.

Then, the method was applied to the analysis of plasma and saliva levels of RISP and its metabolite from 6 psychiatric patients. The results are reported in Table 4, where a good correlation can be seen between plasma and salivary levels of the analytes. For three patients of 6 the value of the *active moiety* (RISP plus RISP9-OH

Table 4
Plasma and saliva concentrations in patients under therapy with risperidone.

Patient	Gender	Age (years)	Risperdal® dose (mg day^{-1})	Plasma levels ($ng\,mL^{-1}$)		Salivary levels ($ng\,mL^{-1}$)	
				RISP	RISP9-OH	RISP	RISP9-OH
1	M	18	9	22.0	48.7	21.0	47.9
2	F	36	2	n.d.[a]	12.0	n.d.[a]	15.0
3	M	21	50 mg *per* depot[b]	5.1	16.0	4.0	14.0
4	M	28	12	5.5	50.0	9.0	75.0
5	F	34	25 mg *per* depot[b]	3.3	6.6	3.0	6.0
6	M	45	9 (+37.5 mg *per* depot[b])	9.0	44.0	8.2	40.0

[a] n.d. = not detected.
[b] Depot administration of Risperdal® to patients was carried out every 15 days.

concentrations) was in the therapeutic range (10–60 ng mL^{-1}) and the patients were found to be *responder* to the therapy. Patient 1 showed an *active moiety* of 70.7 ng mL^{-1} without any severe side effect; patients 2 and 5 had sub-therapeutic drug levels and were found to be *non-responder* to RISP.

4. Conclusion

It was developed an analytical method based on the use of an LC system coupled with a coulometric detector for the determination of risperidone and 9-hydroxyrisperidone in plasma and saliva samples from psychiatric patients. A novel pre-treatment of the biological samples by means of a microextraction by packed sorbent procedure has provided good extraction yields.

MEPS procedure by means of C8 sorbent if compared to conventional extraction techniques (LLE and SPE) is more selective, simple and rapid in execution, limiting the use and volumes of organic solvents and minimizing the time of one analysis. In addition, good results in terms of precision (RSD < 5.8%), sensitivity (LOQ = 0.5 ng mL^{-1} for both the analytes) and accuracy (recoveries always higher than 90.0%) were obtained.

In comparison with other analytical methods for the simultaneous analysis of risperidone and its metabolite, the present method is more advantageous for the shorter time of the sample pretreatment and the chromatographic run and for the use of a more sensitive and selective detector (i.e. coulometric *vs* UV detection). Furthermore, compared with an LC–MS method [27] that analyzes RISP and its metabolite in plasma and saliva, the method presented herein is fully validated on both the matrices and it is more sensitive, accurate and less expensive.

The proposed method is suitable for the analysis of the active moiety in plasma of patients with psychiatric disorders treated with risperidone, hence for TDM of these patients. Moreover, the use of saliva as a biological specimen for TDM of RISP seems to be a good alternative for patients difficult to treat (such as children and the elderly).

Assays are in progress to apply the present method to a numerous number of patients in order to confirm our hypothesis.

Acknowledgements

Thanks are due to Janssen-Cilag for providing the pure Risperidone and its metabolite used for the development of this method. This study was supported by grants from MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca, Italy): RFO (ex-60%) and PRIN funds.

References

[1] S.C. Sweetman, Martindale—The Complete Drug Reference, 36th edn., Pharmaceutical Press, London, 2009, pp. 1024–1027.
[2] U.S. Food and Drug Administration. Available from URL: http://www.fda.gov/CDER/DRUG/infopage/antipsychotics/default.htm [accessed 02.07.08].
[3] A. Musenga, M.A. Saracino, G. Sani, M.A. Raggi, Curr. Med. Chem. 16 (2009) 1463.
[4] A.A.H.P. Megens, F.H.L. Awouters, A. Schotte, T.F. Meert, C. Dugovic, C.J.E. Niemegeers, J.E. Leysen, Psychopharmacology 114 (1994) 9.
[5] R.L. Borison, B. Diamond, A. Pathiraja, R.C. Meibach, Psychopharmacol. Bull. 30 (1994) 193.
[6] K.M. Kirschbaum, S. Finger, F. Vogel, R. Burger, M. Gerlach, P. Riederer, C. Hiemke, Chromatographia 67 (2008) 321.
[7] L. Mercolini, M. Grillo, C. Bartoletti, G. Boncompagni, M.A. Raggi, Anal. Bioanal. Chem. 388 (2007) 235.
[8] K. Titier, S. Bouchet, F. Pehourcq, N. Moore, M. Molimard, J. Chromatogr. B 788 (2003) 179.
[9] K. Titier, E. Deridet, E. Cardone, A. Abouelfath, N. Moore, J. Chromatogr. B 772 (2002) 373.
[10] S.M. Foroutan, A. Zarghi, A. Shafaati, A. Khoddam, Iran. J. Pharmaceut. Res. 5 (2006) 37.
[11] A. LLerena, R. Berecz, P. Dorado, C. Sanz de la Garza, M.J. Norberto, M. Caceres, J.R. Gutierrez, J. Chromatogr. B 783 (2003) 213.
[12] A. Avenoso, G. Facciolà, M. Salemi, E. Spina, J. Chromatogr. B 746 (2000) 173.
[13] T. Nagasaki, T. Ohkubo, K. Sugawara, N. Yasui, H. Furukori, S. Kaneko, J. Pharmaceut. Biomed. 19 (1999) 595.
[14] O.V. Olesen, K. Linnet, J. Chromatogr. B 698 (1997) 209.
[15] Y.L. Shen, H.L. Wu, W.K. Ko, S.M. Wu, Anal. Chim. Acta 460 (2002) 201.
[16] M.A. Raggi, F. Bugamelli, C. Sabbioni, M.A. Saracino, C. Petio, J. Sep. Sci. 28 (2005) 245.
[17] A.E. Balant-Gorgia, M. Gex-Fabry, C. Genet, L.P. Balant, Ther. Drug Monit. 21 (1999) 105.
[18] M. Aravagiri, S.R. Marder, T. Van Putten, K.K. Midha, J. Pharm. Sci. 82 (1993) 447.
[19] M. Aravagiri, S.R. Marder, D. Wirshing, W.C. Wirshing, Pharmacopsychiatry 31 (1998) 102.
[20] J.P. Le Moing, S. Edouard, J.C. Levron, J. Chromatogr. B 614 (1993) 333.
[21] D.S. Schatz, A. Saria, Pharmacology 60 (2000) 51.
[22] S. Schneider, E. Sibille, M. Yegles, H. Neels, R. Wennig, A. Muehe, J. Chromatogr. B 877 (2009) 2589.
[23] M. De Meulder, B.M.M. Remmerie, R. de Vries, L.L.A. Sips, S. Boom, E.W.J. Hooijschuur, N.C. van de Merbel, P.M.M.B.L. Timmerman, J. Chromatogr. B 870 (2008) 8.
[24] M. Roman, R. Kronstrand, D. Lindstedt, M. Josefsson, J. Anal. Toxicol. 32 (2008) 147.
[25] B. Cabovska, S.L. Cox, A.A. Vinks, J. Chromatogr. B 852 (2007) 497.
[26] C. Kousoulos, Y. Dotsikas, Y.L. Loukas, Talanta 72 (2007) 360.
[27] J. Flarakos, W. Luo, M. Aman, D. Svinarov, N. Gerber, P. Vouros, J. Chromatogr. A 1026 (2004) 175.
[28] B.M.M. Remmerie, L.L.A. Sips, R. de Vries, J. de Jong, A.M. Schothuis, E.W.J. Hooijschuur, N.C. van de Merbel, J. Chromatogr. B 783 (2003) 461.
[29] M. Aravagiri, S.R. Marder, J. Mass Spectrom. 35 (2000) 718.
[30] S. McClean, E.J. O'Kane, W.F. Smyth, J. Chromatogr. B 740 (2000) 141.
[31] D.E. Moody, J.D. Laycock, W. Huang, R.L. Foltz, J. Anal. Toxicol. 28 (2004) 494.
[32] L. Zhang, Z. Jiao, Z. Yao, Y. Zhong, M. Zhong, Y. Yu, Chromatographia 61 (2005) 245.
[33] J. Bhatt, G. Subbaiah, S. Singh, Rapid Commun. Mass Spectrom. 20 (2006) 2109.
[34] M. Abdel-Rehim, J. Chromatogr. B 801 (2004) 317.
[35] M. Abdel-Rehim, P. Skansen, M. Vita, Z. Hassan, L. Blomberg, M. Hassan, Anal. Chim. Acta 539 (2005) 35.
[36] M. Vita, P. Skansen, M. Hassan, M. Abdel-Rehim, J. Chromatogr. B 817 (2005) 303.
[37] Z. Altun, M. Abdel-Rehim, Anal. Chim. Acta 630 (2008) 116.
[38] M.A. Saracino, K. Tallarico, M.A. Raggi, Anal. Chim. Acta 661 (2010) 222.
[39] United States Pharmacopeia, 32th edn., United States Pharmacopeial Convention, Rockville, MD, 2009, pp. 734–736.
[40] V.P. Shah, K.K. Midha, J.W.A. Findlay, W.A. John, H.M. Hill, J.D. Hulse, I.J. McGilveray, G. McKay, K.J. Miller, R.N. Patnaik, M.L. Powell, A. Tonelli, C.T. Viswanathan, A. Yacobi, Pharm. Res. 17 (2000) 1551.