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Simultaneous determination of risperidone and 9-hydroxyrisperidone enantiomers in human blood plasma by liquid chromatography with electrochemical detection

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ABSTRACT

Risperidone is a commonly prescribed antipsychotic drug. An enantioselective HPLC method with electrochemical detection was developed and validated for simultaneous determination of plasma concentrations of risperidone and its active metabolites, 9-hydroxyrisperidone enantiomers. Following solid phase extraction of 1.0 mL blood plasma, a baseline separation of the analytes was achieved on an AGP (α_1 acid glycoprotein) column using isocratic mobile phase consisting of methanol–phosphate buffer (pH 6.2; 0.1 M) (15:85, v/v). Total analysis run time was 11 min. For the detection of the analytes analytical cell potentials were set at 500, 650, 950, and 950 mV. The method linearity was attained in the range from 1.0 to 100 ng/mL for risperidone and both 9-hydroxyrisperidone enantiomers. The limit of detection was 0.5 ng/mL for all three analytes. The method was precise and accurate and was successfully applied in a clinical study investigating the stereoselectivity of risperidone 9-hydroxylation.

1. Introduction

Risperidone (RISP) is an atypical antipsychotic agent used in the treatment of schizophrenia and acute bipolar mania [1,2]. In humans, it undergoes extensive alicyclic hydroxylation at position 9 (Fig. 1), leading to two active 9-hydroxyrisperidone (9-OH-RISP) enantiomers [3,4]. It was demonstrated that the 9-hydroxylation of risperidone is catalyzed by two cytochrome P450 (CYP) enzymes: CYP2D6 and CYP3A4 [5]. Moreover, it was suggested that the formation of 9-OH-RISP might be stereoselective, with CYP2D6 having a predominant role in the formation of major (+)-9-OH-RISP metabolite, while CYP3A4 is involved in the formation of minor (−)-9-OH-RISP metabolite [6]. However, in a later study the stereoselective role of CYP2D6 and CYP3A4 was questioned [7]. Other RISP and 9-OH-RISP metabolic pathways include N-dealkylation and hydroxylation at position 7. These reactions occur to a much lesser extent, leading to the formation of inactive metabolites [3,4]. Additionally, the conjugation of risperidone and its metabolites with glucuronic acid was found to be a negligible metabolic pathway [4]. In studies with ^{14}C-labelled risperidone it was shown that RISP and 9-OH-RISP were the main plasma circulating substances with plasma half-life ranging from 3 to 24 h for RISP and 19–25 h for 9-OH-RISP [3,4]. Large interindividual variability in RISP steady-state plasma concentrations was reported [3]. Due to equal pharmacological activities the sum of RISP and 9-OH-RISP plasma concentration referred as "active moiety" was used in establishing relationships between plasma concentration and clinical effects [8,9]. Recently a racemic mixture of 9-OH-RISP (paliperidone) has been introduced in the treatment of schizophrenia [10,11].

Numerous HPLC analytical methods have been developed to quantify RISP and 9-OH-RISP plasma concentrations. These methods have included achiral separation of the analytes and included ultraviolet (UV) [12–17], electrochemical (EC) [18–22] or mass spectrometry (MS) [23–26] detection. The lowest limits of quantification among the methods utilizing UV detection were 1 and 2 ng/mL for RISP and 9-OH-RISP, respectively [14,15]. On the other hand, with EC methods the lowest quantification limit was 0.25 ng/mL for RISP and 9-OH-RISP [18], while the MS methods reached the 0.1 ng/mL level for both compounds. Stereoselective analytical methods for determination of 9-OH-RISP enantiomers are less abundant. Yasui-Furukori et al. [6] described an HPLC–UV method in which RISP and total 9-OH-RISP were determined using achiral separation, while 9-OH-RISP enantiomers were separated using chiral α_1 acid glycoprotein (AGP) stationary phase. The lower limit of quantification (LLOQ) for the 9-OH-RISP enantiomers was 25 nM, equivalent to 10 ng/mL, which is much higher than the LLOQ of the previously developed achiral HPLC–UV methods, and probably not sensitive enough to determine steady-state plasma concentrations in all patients. Another enantioselective HPLC–UV method using AGP

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column was developed and validated for pure standard solutions in order to assess the enantiomeric purity of semipreparative enantioseparation of 9-OH-RISP [27], however, this method has not been applied to plasma samples.

Recently two LC–MS/MS methods for simultaneous quantification of RISP and 9-OH-RISP enantiomers using Chiralcel OJ chiral column were published [28,29]. Both of these methods used a low sample amount (200 μL), were rapid, and sensitive (LLOQ for all analytes was 0.2 ng/mL). Additionally, interference from the minor metabolite 7-hydroxyrisperidone, which cannot be discriminated from 9-hydroxyrisperidone by mass spectrometry, was chromatographically resolved. However, these methods operated in normal phase mode and required expensive LC–MS equipment and were thus too complex for our application.

The purpose of this study was to develop and validate a simple HPLC method for simultaneous determination of plasma concentration of the three analytes of interest; (−)-9-OH-RISP, (+)-9-OH-RISP, and RISP. The main idea was to combine reverse phase chiral separation with a relatively simple EC detection method with sufficient sensitivity to investigate the stereoselectivity of risperidone 9-hydroxylation in a clinical study with therapeutic doses of risperidone. So far no chiral method utilizing EC detection has been described in the literature.

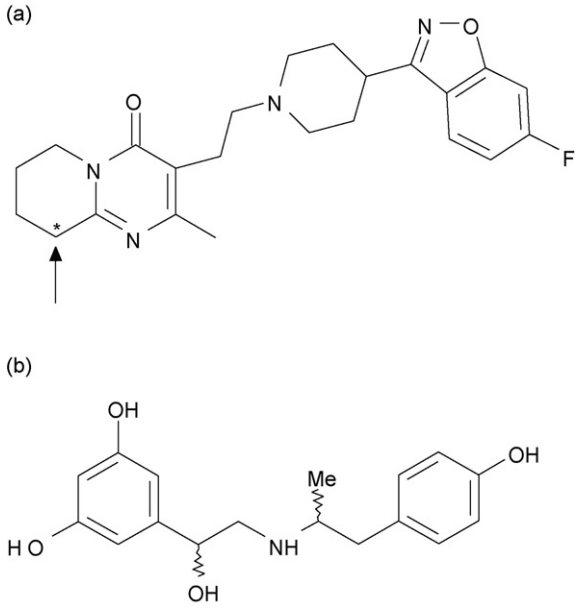

Fig. 1. Chemical formulae of risperidone (a) and I.S. fenoterol (b). Arrow on risperidone structure denotes formation of chiral carbon by hydroxylation at position 9.

2. Experimental

2.1. Chemicals

Risperidone and 9-hydroxyrisperidone were purchased from Sequoia Research Products (Pangbourne, UK) and RDI division of Fitzgerald Industries Intl. (Concord, MA, USA), respectively. Fenoterol hydrobromide (I.S.) was supplied from Sigma–Aldrich (Steinheim, Germany). Extra pure formic acid, potassium dihydrogen phosphate, anhydrous disodium hydrogen phosphate, and anhydrous sodium acetate were obtained from Merck (Darmstadt, Germany). HPLC grade acetonitrile and methanol were from Sigma–Aldrich (Steinheim, Germany). In all assay procedures bidistilled water was used.

2.2. Chromatographic system

The Agilent 1100/1200 Series HPLC system (Agilent Techologies Inc., Santa Clara, CA, USA) consisted of model 1100 vacuum degasser and quaternary pump, model 1200 thermostated autosampler, model 1200 thermostated column compartment with valve switching device, and model 1100 variable wavelength detector. The HPLC system was controlled by Agilent ChemStation software version A.09. Additional isocratic pump (HPLC pump 64, Knauer, Berlin, Germany) and model 5600 CoulArray EC detector equipped with model 6210 four sensor analytical cell (ESA Inc., Chelmsford, MA, USA) were coupled to the HPLC system using the valve switching device. Model 5020 guard cells from ESA Inc. were connected to each of the two pumps. The EC detector was controlled by ESA CoulArray software version 1.04.

Chiral separation of 9-hydroxyrisperidone enantiomers was achieved on Chiral-AGP analytical column (5 μm, 100 mm × 4.0 mm I.D.) from Chromtech (Congleton, UK) equipped with Chiral-AGP guard column (5 μm, 10 mm × 4.0 mm I.D.). The column temperature was maintained at 25 °C. The isocratic mobile phase consisted of methanol–phosphate buffer (pH 6.2; 0.1 M) (15:85, v/v). The mobile phase flow rate was maintained at 0.8 mL/min during the first 4 min of the analysis, and then it was linearly increased up to 0.9 mL/min in the period between 4.0 and 11.0 min. The total analysis run time was 11 min. After the analysis, flow rate was instantaneously set back to 0.8 mL/min. The UV wavelength was set at 276 nm. The autosampler temperature was kept at 5 °C. The analytical cell of the EC detector was set at 500, 650, 950, and 950 mV, for electrodes 1–4, respectively, while 1100 mV was used for the two guard cells. The column was connected to the EC detector, except for the time period between 0.5 and 2 min after the sample injection, when the column outlet was connected to waste and the mobile phase was delivered to the EC detector by the second pump, bypassing the column. EC chromatograms were recorded from 2.5 minutes after the sample injection.

2.3. Study design

Plasma samples from 50 patients on risperidone therapy (2–6 mg per day) were assayed. Two steady-state blood samples were taken from each patient on day 8 after the initiation of risperidone therapy. The samples were taken immediately before dosing (trough concentration) and 2 h after dosing (approximately peak steady-state concentration). Using EDTA as anticoagulant, blood samples were centrifuged and plasma samples were stored at −20 °C until analyzed. An informed consent was obtained from all the patients. The study was approved by the Slovenian Ethics Committee for Research in Medicine.

2.4. Standard solutions

The standard stock solutions of racemic 9-OH-RISP (0.1 mg/mL), RISP (0.1 mg/mL) and I.S. (0.5 mg/mL), were prepared by dissolving the appropriate amounts of the compounds in methanol. These solutions were stored at −20 °C. The I.S. working solution was prepared on a daily basis by appropriate dilution of I.S. standard stock solution with water to achieve the final concentration of 110 ng/mL. A series of 6 standard plasma solutions of mixture of racemic 9-OH-RISP and RISP were prepared. The standard plasma solutions used for calibration were prepared at 2, 5, 15, 50, 100, and 200 ng/mL of racemic 9-OH-RISP; and 1, 2, 5, 15, 50, and 100 ng/mL of RISP by adding appropriate volumes of diluted standard stock solutions to analyte-free human plasma. Concentrations of each 9-OH-RISP enantiomer in standard solutions were one half of the concentrations of racemic 9-OH-RISP. Additionally, three-level quality

control (QC) plasma samples were prepared. Each low, medium, and high concentration level QC contained 2.72, 13.6 and 136 ng/mL of racemic 9-OH-RISP and 2.04, 10.2, and 102 ng/mL of RISP, respectively. All spiked plasma standards were stored at −20 °C.

For the estimation of extraction recovery, 100% recovery samples were prepared in a mixture of formic acid–methanol–acetonitrile (2:49:49, v/v/v) at the same concentrations of 9-OH-RISP and RISP as QC plasma samples.

2.5. Hydrodynamic voltamogram

50 μL of an aqueous solution of 130, 180, and 90 ng/mL of RISP, 9-OH-RISP, and I.S., respectively, was injected into the HPLC system. The potential of electrode 4 was set at 950 mV, while the potential of electrode 3 was varied between 500 and 950 mV with 50 mV increments. No potential was applied to electrodes 1 and 2. Each measurement was made in triplicate.

2.6. Solid phase extraction (SPE) procedure

The analytes were extracted from plasma using SPE cartridges with weak cation exchange sorbent: Strata-X-CW 60 mg (Phenomenex, Torrance, CA, USA). The cartridges were placed on a vacuum manifold connected to a water aspirator. The mobile phase flow through the cartridges was regulated manually by adjusting the vacuum inside the basin. Sorbent was conditioned with 2 mL of methanol and equilibrated with 2 mL of water; then 1.0 mL of plasma sample, 100 μL of I.S. working solution and 2 mL of sodium acetate buffer (pH 4.5; 0.1 M) was loaded into the cartridge. The cartridge was washed with 2 mL of sodium acetate buffer (pH 4.5; 0.1 M,) followed by 2 mL of methanol. After drying the sorbent by increasing the vacuum inside the basin, the analytes elution was achieved with 2 mL of mixture containing formic acid-methanol-acetonitrile (2:49:49, v/v/v). The eluate was evaporated to dryness at 40 °C under a gentle stream of nitrogen in a Turbovap apparatus (Zymark, MA, USA). The dried samples were reconstituted in 200 μL of the mobile phase and 50 μL was injected into the HPLC system.

2.7. Method validation

Selectivity, linear range, intraday and interday precision, accuracy, and extraction recovery were determined according to FDA guidance on bioanalytical method validation [30]. Additionally, 24 h post-preparative (autosampler) stability, I.S. stock solution stability at −20 °C, and I.S. working solution stability at +4 °C and at room temperature were evaluated.

To establish selectivity of the method, six independent sources of human blood plasma were investigated for potential matrix interferences. The limit of detection (LOD) was defined as the lowest detectable concentration, taking into consideration a signal-to-noise ratio of 3. LLOQ was determined as the lowest identifiable concentration with a precision of 20% and accuracy of 80–120%. The calibration line consisted of six standard plasma solutions. The I.S. response was calculated as the sum of peak heights obtained from electrodes 2, 3 and 4. The response of the 9-OH-RISP enantiomers and RISP was calculated as the sum of peak heights and areas, respectively. A non-weighted linear regression was applied to calculate slopes and intercepts of the calibration lines constructed as the ratio of analyte to I.S. response versus analyte concentration. To determine intraday precision, the three-level QC samples were injected in three replicates per day for three consecutive days. Intraday precision was calculated for each day and each QC as CV of the three results. The interday precision was determined as CV of the mean values computed for three consecutive days. The accuracy was determined as percent ratio of the analyte concentration calculated from the calibration line versus nominal analyte concentration. For the estimation of extraction recoveries 100 μL of I.S. working solution was added to the 1 mL of the 100% recovery samples, then evaporated to dryness and reconstituted in 200 μL of the mobile phase. Extraction recoveries of the analytes from plasma were calculated as percent ratio of the response of extracted QC samples versus 100% recovery samples.

3. Results and discussion

3.1. Method development

The aim of this study was to develop a reverse phase enantioselective method for simultaneous determination of RISP and 9-OH-RISP enantiomers. Method development was started with the previously published enantioselective HPLC–UV method [6]. Using the same AGP column, injection of 30 μL of 9-OH-RISP solution (0.02 mg/L) resulted in two baseline separated peaks at 5.5 and 7.0 min with identical UV spectra and similar peak areas (50.1% vs. 49.9%). The retention times of these two peaks were close to the retention times reported by Yasui-Furukori et al. [6]: 5.2 min for the (−)-9-OH-RISP and 6.5 min for the (+)-9-OH-RISP. The two peak fractions were collected and used for identification of 9-OH-RISP enantiomers in subsequent method modifications.

When using the same method with EC detector, baseline separation of the enantiomers was not achieved due to increased peak tailing. We tried to improve the enantioselectivity by decreasing pH of the mobile phase and increasing the buffer concentration as suggested by Hermansson and Grahn [31]. The optimal mobile phase was 100 mM phosphate buffer with pH 6.2. Separation of the enantiomers was additionally improved by decreasing the mobile phase flow rate to 0.8 mL/min; however, this change resulted in a very wide RISP peak, which was inadequate for the required sensitivity. To solve this problem, a moderate linear gradient of mobile phase flow rate was applied. Increasing the flow rate during the detection phase of the analytes did not result in deterioration of the baseline drift. With optimized chromatographic conditions baseline resolution of the analytes was achieved (R_S for the enantiomers was 1.45). As shown in Fig. 2, the analytes eluted in the following order: I.S. (3.3 min), (−)-9-OH-RISP (5.0 min), (+)-9-OH-RISP (6.6 min), and RISP (9.1 min). To diminish the long-lasting baseline drift at higher potentials (950 mV) the solvent peak was sent to waste using a switch valve.

In previously published HPLC-EC methods for determination of 9-OH-RISP and RISP in plasma samples, remoxipride [18,19,22] or methylrisperidone (R68808) [20,21] were used as internal standards. The retention times of these two I.S. were longer compared to the compounds of interest. In our case, however, using such I.S. might considerably increase the analysis run time. We found that fenoterol is a suitable cationic I.S. with short retention time and favourable electrochemical properties. Under the conditions of the present method, fenoterol enantiomers did not separate and a single well-shaped peak was observed at 3.3 min (Fig. 2).

In previous methods RISP and 9-OH-RISP were detected by setting the electrode potential at 800 mV [20–22], 920 mV [18] or 960 mV [19]. Hydrodynamic voltamograms were constructed in order to select the optimal electrode potentials (Fig. 3). As expected, the voltamograms of 9-OH-RISP enantiomers overlaid, when constructed from the analytes peak areas. None of the analytes reached the plateau at the potentials below 950 mV, which was considered the maximum potential with optimal signal to noise ratio and acceptable baseline drift. Potential of 950 mV was applied to two detector electrodes (electrodes 3 and 4) resulting in the increased sensitivity of the method, as the responses of both of the electrodes were summed. Electrodes 1 and 2 were mainly used as screening electrodes.

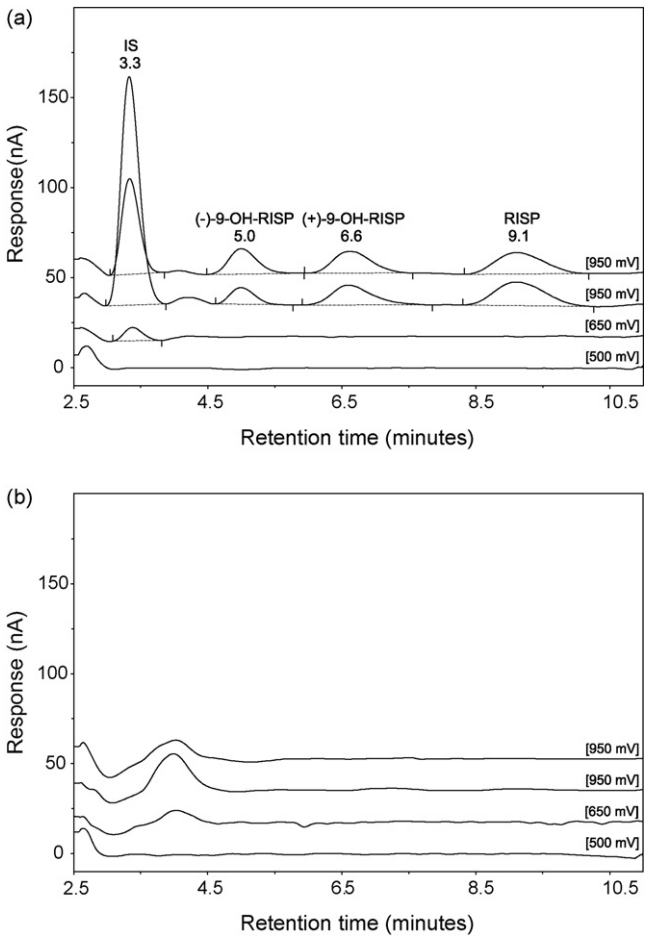

Fig. 2. Chromatograms of the extracted spiked (a) and blank plasma sample (b). The spiked concentrations were 6.8 ng/mL for both 9-hyxdroxyrisperidone enantiomers and 10.2 ng/mL for risperidone.

3.2. SPE extraction recovery

In most methods, one- or multi-step liquid/liquid extraction was used for sample preparation. SPE was performed using CN bonded cartridges [14] or Bond Elut Certify columns with strong cation exchanger [22,26,29]. We applied SPE for sample preparation as this procedure allows full automation. SPE cartridges with strong cation exchanger were used as the starting point (Strata-X-C), however, the extraction recovery of I.S. was low due to its basic properties (pKa ~10). The optimal extraction was achieved on weak cation exchange sorbent (Strata-X-CW). Extraction recoveries of the analytes ranged between 77.9 and 100.9% (Table 1), while for the I.S. it was 99% with a CV of 1.5%.

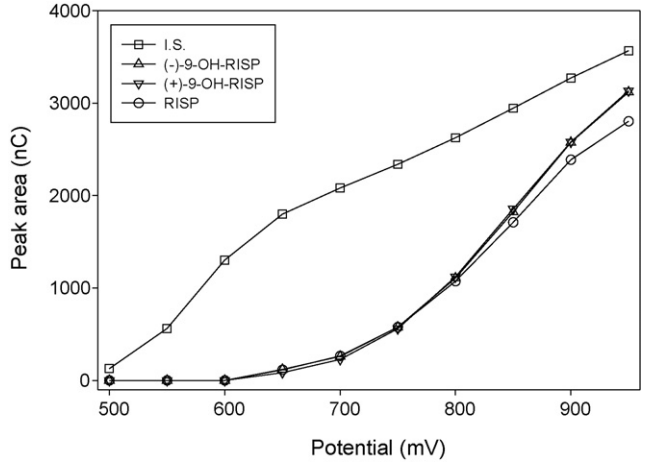

Fig. 3. Voltamograms of the I.S. (fenoterol), (−)-9-hydroxyrisperidone, (+)-9-hydroxyrisperidone, and risperidone normalised to equal concentration (100 ng/mL).

Table 1
Extraction recoveries (%) of 9-hydroxyrisperidone enantiomers and risperidone.

Quality control sample	(−)-9-OH-RISP	(+)-9-OH-RISP	RISP
Low	77.9 (5.7)	82.2 (6.7)	100.9 (4.7)
Medium	87.5 (2.0)	93.2 (4.7)	96.8 (0.6)
High	92.1 (1.1)	97.4 (1.2)	89.9 (1.3)
Mean	85.8 (7.2)	90.9 (7.8)	95.9 (5.6)

Mean (CV) of three replicates. Low, medium, and high concentration levels contained 1.36, 6.80 and 68.0 ng/mL of 9-OH-RISP enantiomers and 2.04, 10.2, and 102 ng/mL of RISP, respectively.

3.3. Method validation

When analyzing six different sources of analyte-free human blood plasma, one peak was found to elute adjacent to the analytes of interest. With the retention time of 4.4 min it eluted between the I.S. and (−)-9-OH-RISP (Fig. 2). However, this endogenous peak did not interfere with their quantification and the method was found adequately selective. No other noticeable peaks were found at working electrodes 3 and 4 in any of the tested lot of plasma. The validation parameters are summarized in Table 2. The lower level of linear range was approximately 10 times lower compared to previous HPLC–UV method [6]. On the other hand, the published enantioselective LC–MS methods are five times more sensitive and require five times smaller sample volume [28,29].

Previous stability studies demonstrate that RISP and racemic 9-OH-RISP plasma samples pass the freeze and thaw stability, long-term storage and short-term temperature stability tests [25,26,29]. Plasma samples were found stable for up to 11 months at −20 °C and at room temperature for up to 72 h, and methanolic stock solutions can be stored for 6 months at −20 °C or for 1 month at +4 °C. Additionally, no racemization was observed for (−)-9-OH-RISP and (+)-9-OH-RISP in plasma samples kept at −20 °C for 6 months [28]. Since stability of the analytes was already confirmed, we tested only post-preparative stability of the processed plasma samples and stability of stock solution and working solution of I.S. (Table 2). The processed plasma samples were found stable for 24 h, when stored in the autosampler at +5 °C. Stability of methanolic I.S. stock solution at −20 °C after 1 week was 99.2%, while the stability of aqueous working solution at +4 °C was 97.7%. Working solution of I.S. was prepared on a daily basis from methanolic stock solution.

3.4. Analytical interferences

Possible analytical interferences with concomitantly prescribed drugs were tested, by injecting standard solutions of some of these drugs into the HPLC system (Table 3). In the majority of the patients lorazepam was the only concomitantly prescribed drug. It was eluted at 9.3 min after injection and was not detected by the EC detector; however, in most of the patients receiving lorazepam, an additional peak appeared at 2.7 min and was seen by the EC detector. The height of this peak varied between the different samples from patients receiving lorazepam. All things considered it seems plausible that this peak originates from lorazepam metabolite(s). Nevertheless, this peak did not interfere with the determination of RISP and 9-OH-RISP enantiomers.

Other co-administered drugs include biperiden ($n = 5$), midazolam ($n = 5$), and zolpidem ($n = 5$). Considering patients' chro-

Table 2
Method validation parameters and mean calibration curves for the determination of 9-hydroxyrisperidone enantiomers and risperidone.

Validation parameter	(−)-9-OH-RISP	(+)-9-OH-RISP	RISP
Intraday precision[a] (%)			
Low	4.8–6.4	4.6–7.9	3.1–13.9
Medium	4.9–7.7	4.8–8.9	2.4–4.5
High	2.6–6.4	2.9–5.7	3.7–9.5
Interday precision (%)			
LLOQ	7.3	16.8	15.7
Low	2.8	7.0	7.2
Medium	6.6	0.5	6.5
High	3.2	0.2	5.2
Accuracy[b] (%)			
LLOQ	103.3	101.7	89.3
Low	103.7	104.5	106.2
Medium	95.1	98.1	97.1
High	96.5	99.0	104.6
Post-preparative stability[b] (%)			
Low	98.4	101.6	105.2
Medium	94.2	98.3	101.6
High	92.5	94.8	101.1
Detection limit (ng/mL)	0.50	0.50	0.50
Linear range (ng/mL)	1.0–100	1.0–100	1.0–100
y-Intercept[b] ($\times 10^3$ (S.D., $n=3$))	−21.0 (1.1)	−6.26 (1.0)	−621 (89)
Slope[b] ($\times 10^3$ (S.D., $n=3$))	26.5 (1.6)	20.4 (3.3)	711 (101)
Correlation coefficient[a]	0.9982–0.9994	0.9979–0.9993	0.9988–0.9999

Low, medium, and high concentration levels contained 1.36, 6.80 and 68.0 ng/mL of 9-OH-RISP enantiomers and 2.04, 10.2, and 102 ng/mL of RISP, respectively.
[a] Results are presented as range of the parameter values.
[b] Results are presented as mean of the parameter values ($n=3$).

matograms no analytical interference was observed for these drugs. Among all drugs tested only norfloxacin was EC detected and impeded determination of the I.S. and (−)-9-OH-RISP.

Additionally, fenoterol used as I.S. is a pharmacologically active drug substance, used mainly as a tocolytic agent for treatment of premature labour. Besides very rare use, pharmacologically effective plasma concentrations are below 2 ng/mL. Therefore, only limited analytical interferences are expected and fenoterol appears as a good choice for I.S. for analysis of samples from clinical studies.

3.5. Relevance of the analytical method to clinical studies

In total 100 blood plasma samples from 50 patients on risperidone therapy were analyzed. Peak and trough steady-state plasma concentrations were determined. A typical chromatogram from a patient plasma sample is presented in Fig. 4. Peak steady-state plasma concentrations of (−)-9-OH-RISP and (+)-9-OH-RISP ranged from 2.78 to 37.6 ng/mL and 4.28–57.2 ng/mL, respectively. Peak steady-state RISP concentration was quantified in 48 patients and ranged from 2.26 to 98.2 ng/mL. In two patients peak RISP concentration was outside the linear range of the method. Semi-quantitative estimates of these two concentrations obtained by extrapolation of the calibration line were 0.90 and 133 ng/mL. Of the 50 trough plasma samples analyzed, RISP concentrations were below LOD (0.50 ng/mL) in 4 patients and in another 4 patients they were below linear range but above LOD (between 0.50 and 1.0 ng/mL). Using the previously published LC-MS/MS methods [28,29] with five times lower LLOQ, RISP concentrations could probably be determined in all samples.

Mean (range) trough plasma concentrations of (−)-9-OH-RISP and (+)-9-OH-RISP were 8.7 (2.46–27.1) ng/mL and 12.0 (3.12–50.4) ng/mL, respectively. Trough plasma concentrations of 9-OH-RISP enantiomers found in our study are lower compared to those reported by Yasui-Furukori et al. [7]. This difference can be attributed to the difference in the dosing regimen and race of the patients. The daily dose of RISP in the present study was 2–6 mg compared to 6 mg in previous study in Japanese patients with schizophrenia [7].

The limitation of the presented method is the potential interference from 7-hydroxyrisperidone, the minor metabolite of risperidone. Because analytical standards of 7-hydroxyrisperidone enantiomers were not available, this interference was not

Table 3
Retention times of some co-administered drugs (number of patients) and their possible analytical interference.

Co-administered drug	UV detection	EC detection	Interference (EC)
Sertraline ($n=2$)	Not detected	Not detected	None
Enalaprilat ($n=3$)	Not detected	Not detected	None
Lorazepam ($n=40$)	9.3 min	Not detected	None
Diazepam ($n=4$)	5.3 min	Not detected	None
Nitrazepam ($n=2$)	9.3 min	Not detected	None
Carbamazepine ($n=1$)	19 min	Not detected	None
Norfloxacin ($n=1$)	3.8 min	3.8 min	I.S. and (−)-9-OH-RISP

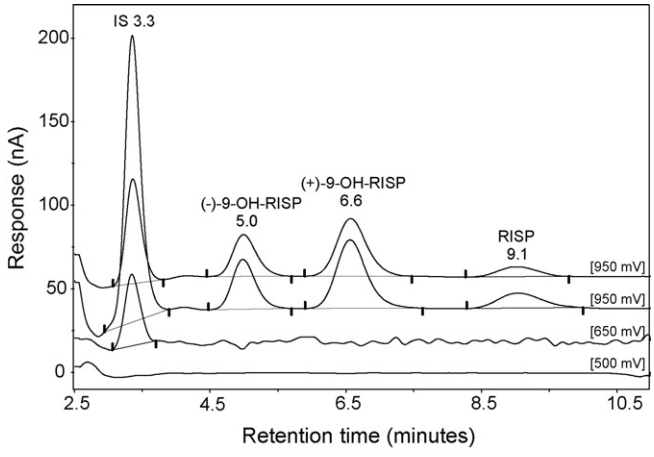

Fig. 4. Typical chromatogram of a patient plasma sample drawn at 2 h postdose (2 mg twice daily). Concentration of (−)-9OH-RISP, (+)-9OH-RISP, and RISP was estimated at 12.0, 18.1, and 6.97 ng/mL, respectively.

investigated. It is likely that 7-hydroxyrisperidone has similar electrochemical properties as 9-OH-RISP. Although the concentrations of 7-hydroxyrisperidone enantiomers are low compared to 9-OH-RISP enantiomers, accounting up to 6%, they should not be neglected, since they can lead to overestimation of 9-OH-RISP concentration, if not separated [26,28,29].

The published enantioselective LC–MS methods [28,29] are superior in terms of sensitivity and selectivity, however, they rely on chiral separation in normal phase mode and more expensive equipment. The present method is the only alternative method reported so far, which can be applied to therapeutic drug monitoring in routine patient care and in clinical studies investigating the influence of various factors on interindividual variability in pharmacokinetics of risperidone and paliperidone.

4. Conclusion

A simple HPLC method with EC detection and SPE extraction procedure was developed and validated for simultaneous determination of (−)-9-OH-RISP, (+)-9-OH-RISP, and RISP in plasma samples from patients on risperidone therapy. The analytes were baseline separated with total analysis run time of 11 min. Present validation data demonstrate that the method is consistent and reliable with low values of CV and bias. The detection limits of the analytes of interest were low enough to measure steady-state plasma concentrations in patients on risperidone therapy. The method may also be applied to therapeutic drug monitoring of risperidone and paliperidone.

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