ORIGINAL ARTICLE

A novel method for rapid determination of risperidone in human plasma using Reversed-Phase Liquid Chromatography with Ultra-Violet detection

Une nouvelle méthode pour la détermination rapide de la rispéridone dans le plasma humain par chromatographie liquide à haute performance en phase inverse avec détection des ultraviolets

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Abstract

Aim: A simple and sensitive High-Performance Liquid Chromatographic (HPLC) method with UV detection is described for the quantitation of risperidone in human plasma, using bisoprolol as internal standard.
Methods: After sample alkalinization with 900 µl of NaOH, the test compounds were extracted from plasma using diethylether. The tubes were centrifuged twice and an evaporation to dryness under a gently stream of nitrogen at 50°C (~10 min) was done. 50 µl of the solution was injected into a C18 Nucleosyl analytical column (5 µm, 150×4.6 mm I.D). The mobile phase consisted of phosphate buffer (10 mM, pH 4.9 and 30% acetonitrile, (70:30, v/v), and was delivered at a flowrate of 1.0 ml/min.
Results: The peaks were detected using a UV detector set at 279 nm and the total time for a chromatographic separation was about 8 min. This new method was validated for the concentration range 5–150 ng/ml. Mean recoveries were 92 % for risperidone. Intra- and inter-day relative standard deviations were less than 15% for our compound, while accuracy, expressed as percent error, ranged from 0.13 to 19 %. The limit of quantitation (LLOQ) was 3.23 ng/ml for the analyte.
Conclusion: The method shows good specificity with respect to commonly prescribed psychotropic drugs.

Key words: Risperidone- HPLC- Bisoprolol- Therapeutic drug monitoring- Pharmacokinetics

Résumé

Objectif: Une méthode simple et sensible de chromatographie liquide à haute performance (CLHP) avec détection UV est décrite pour la quantification de la rispéridone dans le plasma humain, en utilisant le bisoprolol comme étalon interne.
Méthodes: Après alcalinisation de l'échantillon avec 900 µl de NaOH, les composés à tester ont été extraits du plasma à l'aide de diéthyléther. Les tubes ont été centrifugés deux fois et une évaporation à sec sous un léger courant d'azote à 50°C (~10 min) a été effectuée. 50 µl de la solution ont été injectés dans une colonne analytique C18 Nucleosyl (5 µm, 150×4,6 mm I.D). La phase mobile était composée d'un tampon phosphate (10 mM, pH 4,9) et de 30 % d'acétonitrile, (70:30, v/v), et a été délivrée à un débit de 1,0 ml/min.
Résultats: Les pics ont été détectés à l'aide d'un détecteur UV réglé à 279 nm et le temps total pour une séparation chromatographique était d'environ 8 minutes. Cette nouvelle méthode a été validée pour la gamme de concentration 5-150 ng/ml. Les récupérations moyennes étaient de 92 % pour la rispéridone. Les écarts types relatifs intra et inter-journaliers étaient inférieurs à 15 % pour notre composé, tandis que la précision, exprimée en pourcentage d'erreur, variait de 0,13 à 19 %. La limite de quantification (LLOQ) était de 3,23 ng/ml pour l'analyte.
Conclusion: La méthode montre une bonne spécificité par rapport aux médicaments psychotropes couramment prescrits.

Mots clés: Rispéridone- CLHP- Bisoprolol- Suivi Thérapeutique Pharmacologique- Pharmacocinétique

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Introduction

Risperidone belongs to a new generation of neuroleptic drugs used in the treatment of schizophrenia, which acts mainly through selective antagonism of serotonin 5-HT2A.
Risperidone toxicity is characterized primarily by lethargy, spasm/dystonia, hypotension, tachycardia and dysrhythmia, for patients who have taken risperidone doses ranging up to 180 mg. Other cases of overdose reported drowsiness, slurred speech, altered levels of consciousness, hypertension, tachycardia, electrocardiogram abnormalities, atypical motor behavior and tremors [1]. Extrapyramidal side effects are less pronounced than observed for classical neuroleptic drugs [2]. As some of these side effects are dose dependent, an individual dose adjustment is highly recommended.
Risperidone primarily undergoes significant 9-hydroxylation within the liver by cytochrome P450 isoenzymes (CYP). N-dealkylation and 7-hydroxylation are less significant pathways. The creation of 9-hydroxyrisperidone is primarily facilitated by CYP2D6, although recent data in vitro indicate the involvement of CYP3A4. As the pharmacological effects of 9-hydroxyrisperidone closely resemble those of the parent compound, their combined plasma concentrations are commonly referred to as the 'active moiety' [2].
The normal therapeutic concentrations are between 20–60 ng/mL corresponding to the sum of risperidone and its metabolite (the drug's active moiety) [3].
The aim of this study was to develop a highly efficient chromatographic method for the determination of risperidone levels in human plasma that would be clinically useful for TDM in patients who are being treated with risperidone.

Experimental

Chemicals and Reagents

Risperidone was provided by Teriak. Bisoprolol used as the internal standards (I.S.) was provided by Saiph.
HPLC grade methanol and acetonitrile were used for the preparation of the mobile phase and the extraction. NaOH was purchased from Laboratory Chemicals. Water was deionized and purified by a Milli-Q system (Millipore). “Control” plasma was obtained in the same way from blood drawn from healthy volunteers not subjected to any pharmacological treatment.

Apparatus and chromatographic conditions

A chromatographic system (Thermo scientific) equipped with a Ultra-violet detector (UV) was used.
Separations were carried out on a C18 reversed phase column Nucleosil® (150*4.6 mm ,5 µm) using a mobile phase composed of pH 4.9, 0.01 M phosphate buffer and containing acetonitrile (70/30, v/v, final) at a flow rate of 1 mL/min.
Samples were injected through a 50 µL loop, detection and quantification were carried out at 279 nm wavelength.

Preparation of standards and quality control Samples

Stock solutions of risperidone and the I.S. (100 µg/mL and 200 µg/ml respectively) were prepared in methanol (MeOH).
These solutions were stored at −20 °C. The I.S. working solution was prepared daily after an appropriate dilution of I.S. stock solution with MeOH to achieve the final concentration of 20 µg/mL.
A series of eight standard plasma solutions of risperidone were prepared. The standard plasma solutions used for calibration were prepared at 50, 100, 200, 300, 400, 600, 1000 and 1500 ng/mL of risperidone by adding appropriate volumes of diluted standard stock solutions to analyte-free human plasma.
Additionally, three-level quality control (QC) plasma samples were prepared with the same way using another stock solution of risperidone (100 µg/mL). Each low, medium, and high concentration level QC contained 15, 75 and 120 ng/mL of risperidone.
All spiked plasma standards were stored at -20 °C.
For the estimation of extraction recovery, 100% recovery samples were prepared in a mixture of phosphate buffer – acetonitrile (70:30, v/v) at the same concentrations of risperidone as QC plasma samples.

Extraction

50 µl of I.S., (20 µg/ml) and a solution of NaOH/ diethylether (900µl/8000µl) were added to 900 µl of the plasma sample. The mixture was vortexed briefly for 10 s, and then the tube was gently rotated for 20 min on a blood mixer. After shaking, the tubes were centrifuged at 3000 g for 10 minutes. The organic phase was then evaporated to dryness under a gently stream of nitrogen at 50°C (~10 min).
The residue was dissolved in a 200 µl of the mobile phase, of which 50 µl was injected into the HPLC apparatus.

Method validation

Selectivity and specificity:
Specificity of the method was assessed by analyzing six different donors human blank plasma injected at the beginning of the validation and investigating the potential interferences at the peak region for analytes and I.S. [4].
Selectivity should be tested with QC samples by adding increasing concentrations of available drugs expected to be co-administered with risperidone. Such as diazepam, lamotrigine, levetiracetam, phenobarbital, phenytoine, carbamazepine and haloperidol.

Recovery:
The recoveries of risperidone and bisoprolol were assessed by the analyses of the low, medium and high concentrations (15, 75 and 120 ng/mL respectively). Recovery was calculated as the extracted spiked plasma peak area response compared with the response obtained after blank extraction by the liquid–liquid

extraction process. The analyte responses from the extracted samples were compared with responses of un-extracted standards.

Linearity:
The eight-point calibration curves were prepared and analyzed on three consecutive analytical days. The linearity of the standard concentrations ranging from 5 to 150 ng/ml (5, 10, 20, 30, 40, 60, 100 and 150 ng/ml) in human plasma was analyzing used the previously described HPLC method. The standard calibration curve was obtained by plotting the peak area ratio (analyte/IS) vs. the nominal concentrations using a linearly weighted 1/x2 (x = concentration) least squares regression. The coefficient of determination (r2) should be >0.99, and each calculated concentration was required to be within ± 15% of the nominal concentration other than the LLOQ, for which the allowable deviation is ± 20% [5].

Precision and accuracy:
Accuracy is the nearness of a measured value to the true or accepted value [6].
Intra- and inter-day precision (Table 1) were assessed by analyzing six samples of each level of QC (15, 75 and 120 ng/ml) for three days.
Accuracy was expressed as percent error [(measured concentration-spiked concentration)/spiked concentration] *100 (%), while precision was quantitated by calculating intra- and inter-day RSDs.

The following formulas were used in order to calculate the inter-assay precision and intra-day precision, respectively:

$$\frac{(\text{Day mean square-error mean square})/n^{1/2}}{\text{Grand Mean}} \times 100\ \%$$

$$\frac{(\text{Error mean square})^{1/2}}{\text{Grand Mean}} \times 100\ \%$$

Limit of detection and limit of quantification:
The detection limit (LLOD) and quantitation limit (LLOQ) were determined as 3 and 10 times the baseline noise.
LLOD and LLOQ were calculated from the results of the calibration curve based on the standard deviation of the intercept (SD) and the slope of the calibration curve (S) at levels approximating the LLOD according to the formulae: LOD=3.3*(SD/S) and LOQ=10*(SD/S) [6].

Results

Chromatographic conditions and method optimization

Liquid Chromatography method development was carried out focusing on achieving sufficient resolution of target drugs and endogenous interferences in matrix within a short run time, with simple buffers. The best resolution of risperidone was achieved with isocratic mobile phase consisted of a mixture of acetonitrile–10 mM phosphate buffer (30:70; v/v) with the pH adjusted to 4.9 at a flow rate of 1 mL/min on an Acquity HPLC C18 column.

These experimental conditions allowed the baseline separation of the analytes and the I.S. in a relatively short time (8 minutes).
As can be seen from Figure.1, the I.S. and risperidone are detected as neat and baseline resolved peaks, at the following retention times: 4.345 min and 7.308 min, respectively.

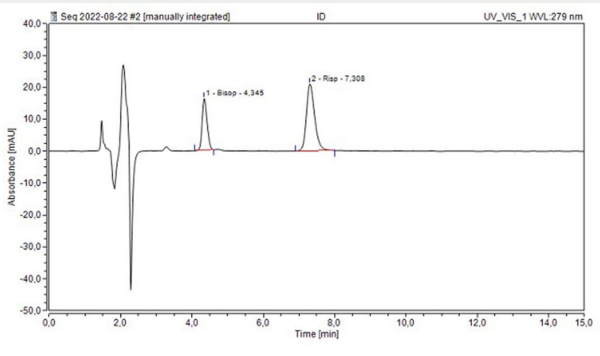

Figure 1. Plasma blank spiked with 50 µg/ml of risperidone and the internal standard (bisoprolol)

Method validation

Specificity and selectivity:
As shown in Figure. 2, no interfering peaks from endogenous compounds were observed at the retention times of analytes and I.S.
Specific selectivity tests were carried out by injecting standard solutions of other drugs, which act on the Central Nervous System (CNS) and are commonly used in Psychiatric Clinics (Diazepam, lamotrigine, levetiracetam, phenobarbital, phenytoine, carbamazepine, haloperidol and clozapine). No interfering peaks was recorded in the chromatograms.

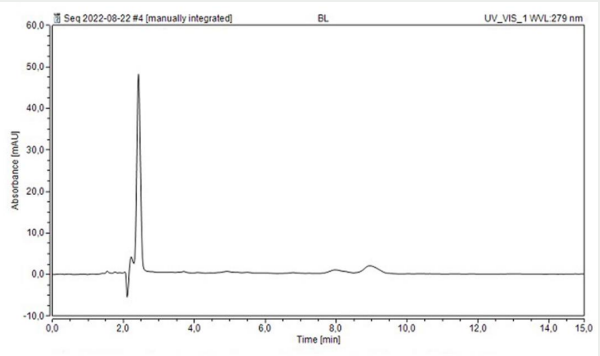

Figure 2. Chromatogram of a blank after final extraction with diéthylether

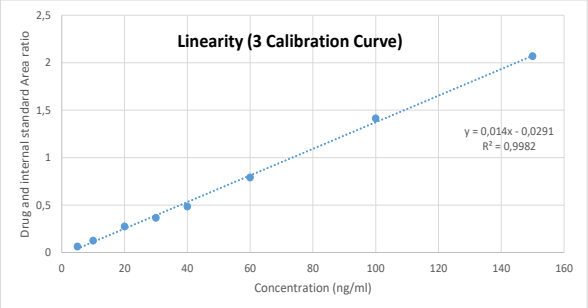

Figure 3. Risperidone standard curve (generated from 3 separate days) mean: r2=0.99; slope mean ±SD (-0.0138 ± 0.0039); intercept mean ±SD (-0.02 ± 0.0091)

Recovery:
Liquid–liquid extraction technique gave adequate recovery and cleaner samples. The results of the evaluation of neat standards vs plasma extracted standards were estimated for risperidone (15, 75 and 120 ng/mL) and the mean recoveries was 91.88 %.

Accuracy and precision:
The method was found to be reproducible as indicated by the low values obtained for the coefficients of variation, which did not exceed 15% for rispéridone (Table 1).
Accuracy, expressed as percent error, ranged from 0.13 to 19 %.

Table 1. Results of precision essays

Nominal concentration of risperidone (ng/ml)	Intra-day precision (n=6)		Inter-day precision (n=3)	
	Measured concentration (mean ± SD, ng/mL)	CV %	Measured concentration (mean ± SD, ng/mL)	CV %
CQ Low = 15	14.15 ± 0.67	4.75	13.6 ± 1.13	8.4
	13.46 ± 1.29	9.59		
	13.23 ± 1.44	10.87		
CQ Medium = 75	71 ± 8.81	12.41	77.7 ± 4.79	6.42
	78.25 ± 3.37	4.31		
	86.85 ± 2.2	2.54		
CQ High = 120	111.52 ± 5.1	4.58	122.21 ± 6.17	5.06
	124.21 ± 9.18	7.39		
	130.9 ± 4.22	3.22		

Limit of detection and quantification:
The LLOD was 1.07 for risperidone. The LLOQ expressed as the lowest concentration at which percent deviation from the nominal concentration (accuracy) and RSD (precision) are less than 20%, were determined from the linearity tests and found to be 3.23 ng/ml for our analyte.

Discussion

In the present study, we developed a very simple and fast HPLC-UV-based method for determining the concentration of risperidone in plasma. This HPLC technique offers considerable improvements compared to previously published methods.
In fact, we used bisoprolol, as the I.S., which does not interfere with our molecule. Some published methods used common psychotropic agents that had structural analogy and similar chromatographic behavior than risperidone such as clozapine [1, 2, 4, 7] or haloperidol [8]. Because psychiatric patients may frequently be treated simultaneously with several antipsychotics, we chose bisoprolol as I.S. that is rarely associated to risperidone in clinical practice. Otherwise, there is an obligation to check that the patient is not taking bisoprolol before dosing the risperidone and its metabolite.
After assessing different extraction solvents, diethyl ether allows cleaner sample with less baseline noise during the chromatographic analysis.
The average extraction yield obtained is considered close to that quoted by several authors such as [2, 4, 9-11].
Our method is sensitive; the values of LLOQ are acceptable by comparing them to those reported in the literature, which showed levels of 0.2-5 ng/ml [1, 4, 11, 12].

Conclusion

In conclusion, the assay described above has several advantages. It is fast, as the total analysis time did not exceed 10 minutes, precise and specific.
In our method, we used the bisoprolol as I.S., which comes from other therapeutic class, and it is the first published article using this I.S.
The quantitation limit obtained with the use of UV detection was found to be adequate for TDM purposes.
Nevertheless, this work must be supplemented by the pharmacogenetic study of the subjects
under treatment in order to verify the correlation between the genetic polymorphism and the blood concentration of risperidone according to the recorded clinical manifestations and the dosage of the metabolite should be completed to apply our method for the TDM in the near future.
The use of methods more sensitive than HPLC-UV such as LC-MS or LC-MS/MS is necessary to be able to detect the analytes of interest and their metabolites.

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