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Original Article

Rapid High Performance Liquid Chromatographic Determination of Risperidone in Human Plasma

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Abstract

A simple, rapid and sensitive high-performance liquid chromatographic (HPLC) method for the determination of risperidone in human plasma was developed. An HPLC system based on a Nucleosil C_8 column (150×4 mm) and a UV detector (λ= 280 nm) were used. A mixture of sodium dihydrogen phosphate buffer-acetonitrile (55:45, *v/v*) adjusted to pH 6.0 at a flow rate of 1.5 ml min^{-1} was used as mobile phase. The proteins were precipitated with an acetonitrile solution containing diltiazem as internal standard and the average recovery was 93.9±3.4%. The detection limit for risperidone in plasma was 0.5 $ngml^{-1}$. The calibration curve was linear over the concentration range 2-50 $ngml^{-1}$. The inter-day and intra-day assay coefficients of variation were found to be less than 5%. The present validated method was successfully used for pharmacokinetic studies of risperidone in human subjects.

Keywords: Risperidone; Plasma; HPLC.

Introduction

Risperidone is a synthetic benzisoxazole derivative with both dopamine (D_2) and serotonin (5-HT_2) receptor antagonist activities. Risperidone is rapidly absorbed from the gastrointestinal tract with 70% bioavailability, peak levels in 1-2 hours and an elimination half-life of 3 hours (1-3). As therapeutic doses of resperidone recommended in patients are low (4 to 8 mg daily) and these doses produce very low therapeutic concentrations in plasma a sensitive method is required in order to determine risperidone concentrations in samples from clinical studies. Several liquid chromatographic methods have been developed for the quantitation of this drug in biological fluids including liquid chromatography–mass spectrometry (LC-MS) (4, 5) and high-performance liquid chromatography (HPLC) (6-10). The LC-MS technique is excellent with respect to selectivity and sensitivity, but it cannot be used for clinical routine analysis because of their speciality requirement and cost. HPLC methods differ with respect to the mode of detection (ultraviolet or electrochemical) and sample preparation (solvent extraction and solid-phase extraction). Martin and Douglas (6) reported an HPLC method with columetric detection for the measurement of risperidone in serum using solid phase extraction. However, the method requires expensive tools and the applicability to the real samples from patients

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or volunteers have not been reported. Nagasaki et al (8) reported an HPLC method with UV-detection for the determination of risperidone and its metabolite using a CN bonded-solid phase cartridge. Although this method is sensitive but the run time for a chromatographic separation is about 20 min. On the other hand, the other two reported methods (9,10) are time-consuming because of tedious liquid-liquid extraction procedure. Moreover these methods require large volume plasma sample for drug analysis. The problem of sample preparation, in particular, has attracted much attention in recent years, as this procedure has often been rate-limiting step in HPLC analyses of biological fluids. Most of the reported methods require tedious liquid-liquid extraction or solid-phase extraction and therefore sample preparation is time-consuming, complex or both. Therefore, a highly sensitive and simple assay method is needed to determine resperidone for pharmacokinetic studies and clinical monitoring of risperidone levels in patients. This paper describes a rapid and sensitive HPLC method, which enables the determination of risperidone with good accuracy at low drug concentrations in plasma using simple extraction procedure. We also demonstrate the applicability of this method for pharmacokinetic studies in humans.

Experimental

Instrumentation conditions

A Knauer HPLC system (Germany) employed Consisting of Wellchrom K-1001 pump, Rheodyne 7125 injector and K 2600 UV detector connected to Eurochrom 2000 integrator. The separation was performed on an analytical 150×4.0 mm i.d. Nucleosil-100 C_8 (5 µm, particle size) column. The wavelength was set at 280 nm. The mobile phase was 45% acetonitrile, 0.01 M sodium dihydrogen phosphate in distilled water to 100%, adjusted to pH 6.0 at a flow rate of 1.5 $mlmin^{-1}$. The mobile phase was prepared daily and degassed by ultrasonication before use.

Chemicals

Risperidone and diltiazem were supplied by Rooz-Daru Pharmaceuticals (Tehran, Iran). Risperidone is available as oral tablets containing 4 mg of risperidone and the other inactive ingredients. HPLC-grade acetonitrile and all other chemicals were obtained from Merck (Darmstadt, Germany). Water was obtained by double distillation and purified additionally with a Milli-Q system.

Solutions

Stock solutions (1 $mgml^{-1}$) and appropriate dilutions of risperidone were prepared in methanol and stored at +4ºC. The internal standard stocks solution of diltiazem was prepared at a concentration of 1 $mgml^{-1}$ in acetonitrile and appropriate dilutions were made to obtain working solution of 1 μgml^{-1}.

Sample preparation

Blood samples (3-4 ml) were collected into heparinized tubes. Following centrifugation at 4000 rpm for 10 min, resultant plasma was separated and then frozen immediately at -20ºC until assayed.

To 500 µl of plasma in a glass-stoppered 15 ml centrifuge tube were added.

Five hundred µl of acetonitrile containing diltiazem (50 $ngml^{-1}$) and 200 µl NaCl saturated solution and after mixing (30 s), the mixture was centrifuged for 10 min at 4000 rpm. The acetonitrile phase was separated and evaporated to dryness below 40ºC under a gentle stream of nitrogen. The residue was dissolved in 50 µl of mobile phase and then 40 µl of solution was injected into liquid chromatograph.

Biological samples

Risperidone was administered in a single dose of 4 mg to healthy volunteers after over night fasting. Plasma samples were collected at several intervals after dosing and then frozen immediately at –20ºC until assayed.

Stability

The stability of risperidone was assessed during all the storage steps and during all steps of the analytical method. No change in stability over the period of one month was observed.

Plasma standard curve

Blank plasma was prepared from heparinized whole-blood samples collected from healthy

Table 1. Extraction recovery for the assay of risperidone in plasma (n=5)

Drug	Nominal concentration (ng ml^{-1})	Measured concentration (mean±SD) (ng ml^{-1})	RSD (%)	Recovery (%)
Risperidone	4	3.74±0.16	4.27	93.60
	15	14.12±0.35	2.47	94.12
	35	32.90±1.34	4.07	94.10

volunteers and stored at –20ºC. After thawing, stock solution of risperidone was added to yield final concentrations ranging from 2-50 ngml^{-1}. Internal standard solution (50 ngml^{-1}) was added to each of these samples. The samples were then prepared for analysis as described above.

Results and discussion

Under the chromatographic conditions described, risperidone and the internal standard peaks were well resolved. Endogenous plasma components did not give any interfering peaks. Figure 1 shows typical chromatograms of blank plasma in comparison to spiked samples analyzed for a pharmacokinetic study. The average retention times of risperidone and diltiazem were 4.6 and 6.2 min, respectively. The chromatographic run time was less than 10 min. The calibration curve for the determination of risperidone in plasma was linear over the range 2-50 ngml^{-1} (Y = 0.0794X + 0.1468). The linearity of this method was statistically confirmed. For each calibration curve, the intercept was not statistically different from zero. The correlation coefficients (r) for calibration curves were equal to or better than 0.995. The relative analytical recovery for plasma at three different concentrations of risperidone was determined (Table 1). Known amounts of risperidone were added to drug-free plasma in concentrations ranging from 4-35 ng ml^{-1}. The internal standard was added and the relative recovery of risperidone was calculated by comparing the peak areas for extracted risperidone from spiked plasma and a standard solution of risperidone in methanol containing internal standard with the same initial concentration. The average recovery was 93.9±3.4% (n = 5). The limit of detection was defined as the risperidone concentration that produced a signal-to-noise ratio greater than 3. The limit of detection in plasma was 0.5 ngml^{-1}. This is sensitive enough for drug monitoring and other purposes such as pharmacokinetic studies. We assessed the precision of the method by repeated analysis of plasma specimens

Table 2. Reproducibility of the analysis of risperidone in plasma (n=5)

Nominal concentration (ng ml^{-1})	Measured concentration (mean±SD)(ng ml^{-1})	RSD (%)
Intra-day		
5	4.70±0.20	4.26
15	15.40±0.72	4.68
35	35.30±1.44	4.08
Inter-day		
5	4.70±0.22	4.68
15	15.66±0.66	4.21
35	35.18±1.22	3.47

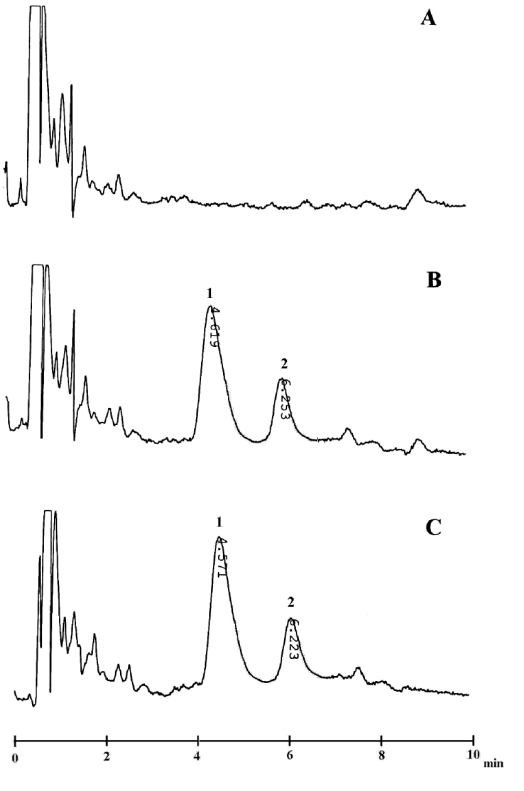

Fig. 1. Chromatograms of (A) blank plasma. (B) blank plasma spiked (1) risperidone (35 ng ml^{-1}) and (2) diltiazem (internal standard). (C) plasma sample from a healthy volunteer 1h after oral administration 4 mg of risperidone.

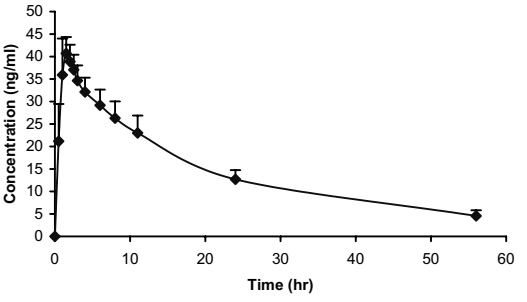

Figure 2. Mean plasma concentration-time profile of risperidone in healthy volunteers (n=12) after a single oral administration of 4 mg risperidone.

containing known concentrations of risperidone. As shown in Table 2, coefficients of variation were less than 5%, which is acceptable for the routine measurement of risperidone. Hence, the sensitivity and reproducibility of our method are comparable with the most sensitive reported methods (6,8). Also, the use of a smaller sample volume provides an advantage as compared with some previous methods (9,10) that require large volume of plasma samples for drug analysis. In addition, sample preparation in our method is considerably faster and easier than those in previous methods. The extraction procedure is simple and no other clean up steps are required in our method. The aim of our study was to develop a rapid and sensitive method for the routine analysis of biological samples in pharmacokinetic study of risperidone. This method is well suited for routine application in the clinical laboratory because of the simple extraction procedure and good sensitivity. Over 350 plasma samples were analyzed by this method without problems, thus proving its suitability. In this study plasma concentrations were determined in twelve healthy volunteers, who received 4 mg of risperidone each. Plasma concentrations were calculated from the regression equations of the calibration curve. Figure 2 shows the mean plasma concentration-time curve of risperidone: plasma concentration reached a maximum 1.25±0.26 hr after dosing with a level of 41.40±3.64 $ngml^{-1}$. These results are agreement with previous report (11).

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