Determination of Paroxetine in Human Plasma by High-Performance Liquid Chromatography Using 7,7,8,8-Tetracyanoquinodimethane as the Derivatization Reagent

Armağan Önal and Aysel Öztunç

Abstract: A selective and sensitive reversed-phase HPLC method was developed for the determination of the antidepressant paroxetine in plasma. The method is based on the purple chromogen formed by a displacement reaction of paroxetine with 7,7,8,8-tetracyanoquinodimethane (TCNQ) in acetonitrile at 80°C for 20 minutes. For the assay, the drug was extracted from 1 mL of plasma with chloroform and, after sample alkalinization, derivatized with TCNQ; then the reaction mixture was directly injected into a C_{18} column. Desipramine was used as internal standard. The mobile phase was acetonitrile–water (70:30) at a flow-rate of 1.0 mL/min, and the derivatives were eluted at 13.1 and 15.5 minutes for paroxetine and desipramine, respectively, and detected at 567 nm. Calibration curve was found linear over the range of 20–400 ng/mL, and the detection limit was 2 ng/mL at a signal-to-noise ratio of 3/1. Recoveries determined for 3 concentrations range between 81.3% and 88.1%. Intraday and interday relative standard deviation values were found to be within 3.8%–13.5% and 8.2%–14.6%, respectively. With this developed method, a pharmacokinetic study was performed for paroxetine.

Key Words: paroxetine, TCNQ, HPLC, pharmacokinetics

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Antidepressant drugs are widely used for the treatment of a variety of depressive states and other psychiatric disorders.[1] Selective serotonin reuptake inhibitors (SSRIs) are the most widely used drugs for the treatment of depressive disorders. Paroxetine, a phenylpiperidine derivative, is the most potent inhibitor of the reuptake of serotonin (5-hydroxytryptamine, 5-HT) of all the currently available antidepressants including the class of SSRIs.[2] Paroxetine is mainly metabolized by cytochrome P450 2D6 isoform (CYP2D6). Genetic polymorphism of CYP2D6 causes wide interindividual variations of steady-state plasma paroxetine levels after the same dosage.[3–5] Consequently, the need for an analytic procedure capable of both identifying and quantifying this drug is great in both clinical and pharmacokinetic studies for effective treatments.[6]

For the determination of paroxetine in serum and plasma, various methods have been described. These methods are based on high-performance liquid chromatography with ultraviolet[7–12] or fluorescence detection,[13–18] gas chromatography,[19,20] and micellar electrokinetic capillary chromatography.[21] Paroxetine has no active metabolites.[22] Kristoffersen et al[23] developed an HPLC method to separate paroxetine and its two metabolites, BRL 36610A and BRL 36583A, in whole blood, but they have not detected these compounds in the blood sample from a forensic case.

To determine amines, chemical derivatization is often used to enhance sensitivity and selectivity and to improve separation behavior. Common derivatization reagents, fluorogenic or chromogenic, used for secondary aliphatic amines react also with primary amines, amino acids, and in some cases, such as dansyl chloride, with phenols as well.

7,7,8,8-Tetracyanoquinodimethane (TCNQ) has been widely used as the reagent for visible spectrophotometric methods of a number of n-electron donor drugs including amines.[24–29] These methods are based on the blue-colored $TCNQ\cdot^-$ radical anion formed by interaction of the drugs (as base) with the reagent in acetonitrile at room temperature.

In a previous paper,[30] we reported that if a displacement reaction[31] takes place between certain secondary aliphatic amines and TCNQ, the amines can be analyzed by TLC and HPLC through the purple derivatives formed. Using TCNQ as a derivatization reagent for TLC and HPLC for the first time, we described new TLC and HPLC methods to detect some antidepressants (fluoxetine, nortriptyline, maprotiline, paroxetine, desipramine) in plasma.

As a continuation of the earlier work, in the present paper we describe a highly selective new HPLC method for determination of the secondary aliphatic amine–bearing drug paroxetine in plasma. The developed method was applied to a pharmacokinetic study in a healthy volunteer.

MATERIALS AND METHOD

Chemicals and Reagents

Paroxetine hydrochloride (PA) was generous gift from Novartis (Basel, Switzerland). Seroxat tablets were

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From the Department of Analytical Chemistry, Istanbul University, Faculty of Pharmacy, Istanbul, Turkey.
Reprints: Prof. Dr. Aysel Öztunç, Istanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, 34116 Istanbul, Turkey (e-mail: ayseloztunc@yahoo.com).

products of Novartis (Istanbul, Turkey). The internal standard (IS), desipramine hydrochloride (DE), was from Sigma (St. Louis, MO, USA), and TCNQ from Fluka (Neu-Ulm, Germany). Chemicals and solvents were of analytic or HPLC grade. Water was purified by aquaMax^{TM}-ultra (Young Lin Instrument, Korea) water ultrapurification system.

Solutions

Stock solutions of paroxetine and desipramine were prepared by dissolving them in water at a concentration of 100 μg/mL as base. These solutions were further diluted with water to required concentrations for working solutions for PA (1 μg/mL and 0.2 μg/mL) and for DE (1 μg/mL). A freshly prepared solution of TCNQ was used at a concentration of 2 mg in 10 mL acetonitrile.

Instrumentation

The analyses were performed on a Thermo Separation Products Liquid Chromatograph (TX), which consisted of a P4000 solvent delivery system equipped with a Rheodyne injection valve with a 20 μL loop, a UV3000 detector set at 567 nm, and an SN4000 automation system software. Separations were carried out on a Phenomenex C_{18} column (250 × 4.6 mm ID, 5 μm; Thermo Separation, Texas, USA) with a guard column (4 mm × 3 mm ID, Phenomenex) packed with the same material. The mobile phase consisted of acetonitrile–water (70:30) at a flow rate of 1.0 mL/min, run time 16 minutes at ambient temperature. Before use, the mobile phase was degassed by an ultrasonic bath and filtered by a Millipore vacuum filter system equipped with a 0.45-μm HV filter.

Sample Preparation

Blood samples were collected into the tubes containing disodium EDTA as anticoagulant and immediately centrifuged at 4500 g for 15 minutes. The resultant plasma samples were separated and stored at −20°C until use.

Plasma samples (1 mL) were first spiked with working solutions to obtain the drug content ranging from 20 to 400 ng. Then 200 μL internal standard solution was added, the volume was brought to 1.6 mL with water, and the samples were alkalized with a 0.2 mL of 1 mol/L KOH solution. The free bases liberated were then extracted with 6 mL of chloroform by vortex-mixing for 5 minutes. After centrifugation (4500 g, 5 minutes), the organic layer was dried on anhydrous sodium sulfate (500 mg) and centrifuged. Then 4 mL of this solution was transferred into another tube and evaporated to dryness under nitrogen with mild heating, and 200 μL of TCNQ reagent was added to the residue. The tube was stoppered, and the mixture was heated at 80°C for 20 minutes. (For safety, evaporation and derivatization were performed in a fume cupboard.) The solvent was evaporated under nitrogen at 45°C, and the residue was dissolved in 50 μL of acetonitrile. Next, a 20-μL aliquot was directly injected into the column. The chromatograms were evaluated on the basis of the drug/IS ratios of the peak areas (n = 4).

Recovery

Extraction recovery studies were performed by analyzing plasma samples spiked with the drug at 3 different concentrations (40, 200, 300 ng/mL). For the assay, the drug was added before the extraction procedure, and the 200 μL of internal standard solution was added after extraction, and then the reaction and chromatographic procedure were carried out as described. The peak-area ratios of the drug/IS were found, and extraction recoveries were evaluated by using the calibration curve prepared with aqueous drug solutions. Recovery of IS was also studied for the concentration of 200 ng/mL.

Validation

To determine the intraday and interday precision, 1 mL of blank plasma sample was spiked with the drug at concentrations 40, 80, 160 ng/mL, and the internal standard at concentration 200 ng/mL. The samples were then analyzed (n = 4) according to the described procedure for intraday variability and at 5 different days for interday variability.

Applicability

To a 28-year-old healthy woman volunteer, a tablet containing 40 mg of paroxetine was administered on an empty stomach, and venous blood samples of 5–6 mL were drawn into the tubes containing disodium EDTA at 2.5, 3, 4, 5, 6, 8, 12, and 24 hours. Plasma samples were stored at −20°C until analysis. Analysis was performed as described.

RESULTS AND DISCUSSION

The developed HPLC method is based on the purple chromogen[30] formed by displacement reactions of PA with TCNQ in acetonitrile at 80°C for 20 minutes (Fig. 1).

The method includes extraction of the drug base from plasma (1 mL) with chloroform after alkalinization, derivatization with TCNQ, and injection of the reaction mixture directly into a reversed-phase C_{18} column. Desipramine was used as internal standard. Acetonitrile: water (70:30) was used as the mobile phase, and the derivatives were eluted at 13.1 (PA) and 15.5 (DE) minutes with detection at 567 nm. Figure 2 represents the chromatograms of (A) blank plasma and (B) a plasma sample (1 mL) spiked with paroxetine (160 ng) and internal standard (200 ng). As can be seen from the figures, chromatographic behavior was excellent for the compounds with good shapes, clear of endogenous interference and straight baseline. Figure 2C shows the chromatogram of a plasma sample obtained from a volunteer after administration of an oral dose of paroxetine (40 mg). This chromatogram did not show any interference peak either.

A calibration graph was constructed by plotting the peak area ratios of the drug/IS against the concentrations and was linear over the range of 20–400 ng/mL. Limit of quantification (LOQ) value under the described condition was 20 ng/mL, which is the lowest concentration of the

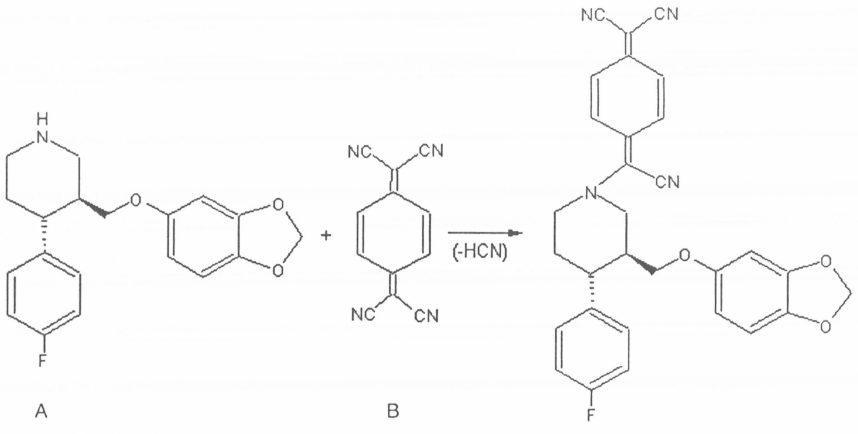

FIGURE 1. The proposed reaction between paroxetine (A) and TCNQ (B).

calibration curve. Detection limit was 2 ng/mL at a signal-to-noise ratio of 3/1. Extraction recoveries were determined at 3 concentrations using the described extraction condition and found between 81.3% and 88.1% at concentrations 40, 200, 300 ng/mL. The mean recovery of IS was determined as 86.1% for 200 ng/mL (Table 1).

Intra- and interday precision and accuracy were evaluated by assaying plasma samples spiked with PA at 3 different concentrations (40, 80, 160 ng/mL). Intra- and interday relative standard deviation values were found to be within 3.8%–13.5% and 8.2%–14.6%, respectively. Accuracy of the method expressed as relative mean error (RME) was below 3.2%, which was shown to be satisfactory. The validation data are given in Table 2.

The developed method was applied to a pharmacokinetic study for PA, and Figure 3 shows the plasma concentration–time profile after oral administration of a tablet of PA (40 mg) to a 28-year-old healthy woman volunteer. The figure demonstrates adequate sensitivity for the method to evaluate plasma concentration profile of PA in pharmacokinetic studies at the administered oral dose. The data obtained indicate that C_{max}, 60.0 ng/mL, was attained at 4 hours (t_{max}) of dosing. Plasma concentrations were measurable up to 24 hours. The preliminary data for the elimination half-life ($t_{1/2}$) was 24.8 hours. AUC_{0-24} (area under the curve) was 703 ng h/mL. Previous studies on oral pharmacokinetics in man have illustrated that a large intersubject variability is observed.[22]

The reaction between paroxetine and TCNQ is simple, and no buffer solution or any other agent is needed to proceed. The formed derivative is stable at room temperature in the dark up to 24 hours. Paroxetine is not affected by freezing, thawing, or heating processes. No significant change was observed in plasma spiked with the drug and stored at −20°C for a period of 2 months. Paroxetine is quite stable under forced degradation conditions using acid and base according to the study of Lambropoulos et al.[32] Therefore, in the developed method, the reaction procedure is not expected to cause any harm to the drug.

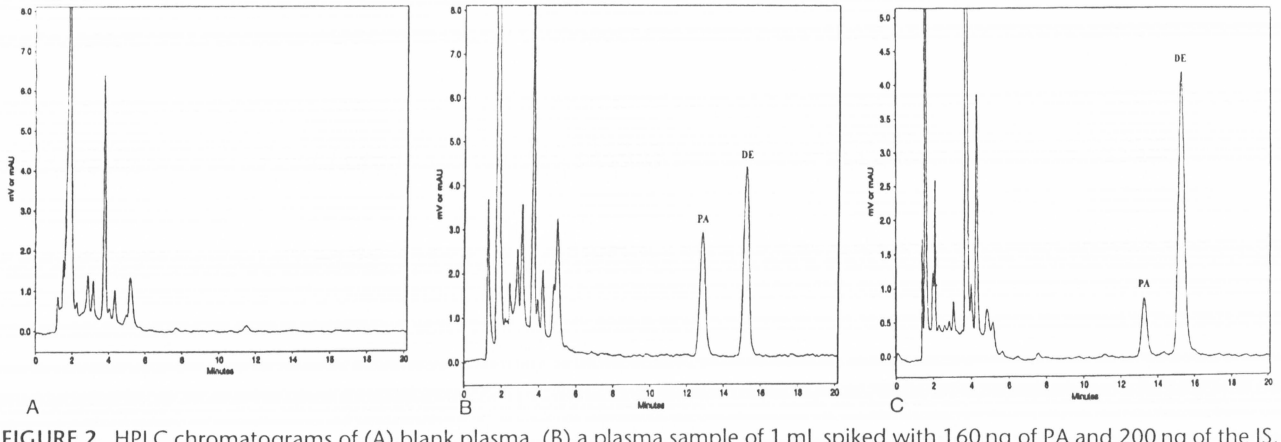

FIGURE 2. HPLC chromatograms of (A) blank plasma, (B) a plasma sample of 1 mL spiked with 160 ng of PA and 200 ng of the IS, and (C) plasma sample obtained at 4 hours after oral administration of 40 mg of PA from a healthy volunteer, IS 200 ng. For all chromatograms, 1-mL plasma samples were used.

TABLE 1. Absolute Recovery of Paroxetine and IS From Plasma (n = 3)

	Concentration (ng/mL)			
Analyte	Added	Found (mean ± SD)	Recovery (%)	RSD*(%)
Paroxetine	40	35.3 ± 4.6	88.1	12.9
	200	162.8 ± 6.0	81.4	3.7
	300	243.9 ± 7.5	81.3	3.1
Desipramine	200	172.3 ± 6.2	86.1	3.6

*RSD, relative standard deviation.

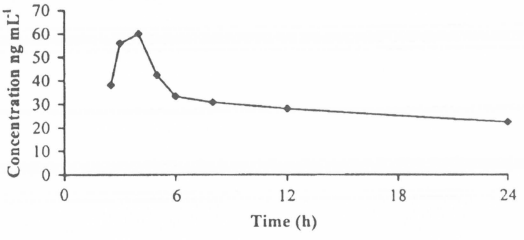

FIGURE 3. Plasma concentration–time profile of paroxetine in a healthy volunteer after a single oral administration of 40 mg paroxetine.

Previous HPLC methods reported for PA used UV or fluorescence detection. Gupta[15] and Shin[13] used the native fluorescence of PA and needed highly sensitive fluorescence detectors, which are more costly than UV-visible detectors. Brett,[16] Lacassie,[17] and Lucca[18] have also used fluorescence data obtained from the dansyl derivative of PA.

Tournel et al[11] and Zainaghi et al[10] developed HPLC methods using UV detection at 200.4 and 205 nm, respectively. At these wavelengths, the absorbances are maximum, but interference from endogenous compounds is possible. Although Tournel et al reported that no endogenous interfering substances are present, the peak shape of paroxetine alone in the serum is not very satisfactory. Our method is the first one to use absorbance measurements in the visible region.

The work by Zainaghi et al[10] did not employ an internal standard. Although the authors reported that the reproducibility of the method is satisfactory, this matter still can be considered a disadvantage for this method. Frahnert et al[12] reported an isocratic reversed-phase HPLC method with UV detection to analyze 22 psychotropic drugs, including paroxetine, and some active metabolites in serum. The method has not been applied to real serum samples. In the method, to eliminate interferences, solid-phase extraction has been used, which appears to be more expensive than liquid-liquid extraction. In the related chromatogram, the peak shape of paroxetine is not symmetrical. This method developed for extensive drugs is apparently very suitable in drug-drug interaction studies.

The proposed HPLC method has great advantage in terms of selectivity. Among numerous drugs tested, only 6 (nortriptyline, desipramine, fluoxetine, maprotiline, sertraline, barbexaclone) give purple derivative with TCNQ under the described experimental conditions. Among the above drugs, only nortriptyline and fluoxetine interfere the analysis. On the other hand, because of its high selectivity, the proposed method is mainly suitable for single-component assay of paroxetine and may not be useful for simultaneous determination of paroxetine with other drugs in drug-drug interaction studies.

TABLE 2. Intraday and Interday Precision and Accuracy of Paroxetine in Plasma (n = 4)

	Concentration (ng/mL)		
Added	Found (mean ± SD)	RSD* (%)	RME† (%)
Intraday			
40.0	41.3 ± 5.6	13.5	3.2
80.0	79.7 ± 3.8	4.7	−0.4
160.0	157.5 ± 6.0	3.8	−1.6
Interday			
40.0	39.6 ± 5.8	14.6	−1.0
80.0	80.7 ± 9.0	11.2	0.9
160.0	159.4 ± 13.0	8.2	−0.4

*RSD, relative standard deviation.
†RME, relative mean error.

CONCLUSION

A highly selective, simple, and accurate HPLC method was developed to analyze paroxetine in plasma. Satisfactory validation data were obtained for linearity, precision, and recovery.

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