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A rapid and simple RP-HPLC method for quantification of desipramine in human plasma

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Abstract

A high-performance liquid chromatographic (HPLC) method for the determination of antidepressant drug desipramine as active metabolite of imipramine in plasma is reported. The method is based on the purple chromogen formed by displacement reaction of desipramine with 7,7,8,8-tetracyanoquinodimethane in acetonitrile at 80°C for 20 min. The assay is based on liquid-liquid extraction with chloroform and reversed-phase chromatography with UV-visible detection. Liquid chromatography was performed on a Luna C_{18} column (250 mm×4.6 mm, 5 μm) with a mobile phase acetonitrile/water (70:30) at a flow rate of 1.0 ml/min. Paroxetine was used as internal standard. The retention time was 15.5 and 13.1 min for desipramine and paroxetine. The detector was operated at 567 nm. The method was linear in the range of 15–400 ng/ml with a correlation coefficient (r) of 0.9973. The limit of detection was 1 ng/ml at a signal-to-noise ratio of 3:1. Recoveries determined for three concentrations range between 86.14% and 88.98.1%. Intra-day and inter-day relative standard deviation values were found to be within 6.43–11.35% and 9.82–13.05%, respectively. Using the developed method, a pharmacokinetic study was performed for desipramine (as active metabolite of imipramine).

Keywords: desipramine; HPLC; imipramine; pharmacokinetic study; TCNQ.

Introduction

Imipramine and desipramine (DE) are psychiatric drugs widely used in the treatment of depressive disorders (Hardman et al. 1996). These compounds are secondary and tertiary amines with a common structure formed by two aromatic rings fused with a seven-atom cycle. Because of their structure, this class of compounds is generally named tricyclic antidepressants. The tri- or tetracyclic antidepressants are the most commonly prescribed drugs for the treatment of psychiatric patients suffering from depression. The need for an analytical procedure capable of both identifying and quantitating these drugs is broadened in both clinical and pharmacokinetic studies for effective treatments (Gupta 1992).

DE is the major metabolite of imipramine and lofepramine and it is determined in plasma simultaneously with these drugs. Many methods are available in plasma to determine DE including HPLC with UV (Kenney et al. 1989, Elm and Hansen 1995, Queiroz et al. 1995, Yoo et al. 1995, Chen et al. 1997, Theurillat and Thormann 1998, Bakkali et al. 1999, Frahnert et al. 2003), electrochemical (Virgili and Henry 1989, Bouquet et al. 1992) or chemiluminescence detection (Yoshida et al. 2000), GC (Way et al. 1998) or capillary electrophoresis (Veraart et al. 2000).

7,7,8,8-Tetracyanoquinodimethane (TCNQ) has been widely used as the reagent for visible-spectrophotometric methods of several n-electron donor drugs including amines (Kovar et al. 1981, Kovar and Abdel-Hamid 1984, Hussein et al. 1989, Abdellatef 1998, Abdel-Gawad et al. 1998, Saleh 1998). These methods are based on the blue colored $TCNQ^{-}$ radical anion formed by interaction of the drugs (as base) with the reagent in acetonitrile at room temperature. We recently published two papers regarding the determination of antidepressant drugs in plasma using TCNQ as a derivatization reagent (Öztunç et al. 2002, Önal and Öztunç 2006). Here, we describe the HPLC method which is highly selective, simple and sensitive for the determination of DE as active metabolite of imipramine in plasma as the TCNQ derivative. (Imipramine has a tertiary amino group and does not react with TCNQ reagent.) Because any pharmaceutical preparation of DE is not available in Turkey, the developed method for DE was used to follow DE in plasma as a major active metabolite of imipramine.

Materials and methods

Chemicals and reagents

Desipramine hydrochloride (DE) was from Sigma (St. Louis, MO, USA). Tofranil medication is a product from Novartis (Istanbul, Turkey). The internal standard (IS), paroxetine hydrochloride (PA), was a generous gift from Novartis (Basel, Switzerland) and TCNQ was from Fluka (Neu-Ulm, Germany). Chemicals and solvents were of analytical or HPLC grade. Water was purified by an aquaMax™-ultra, Young lin instrument (Anyang, Korea) ultrawater purification system.

Solutions

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

Instrumentation and chromatography

The analyses were performed on a Thermo Separation Products Liquid Chromatograph (Thermo Separation, TX, USA) 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, retention time of 16 min 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 tubes containing disodium EDTA as anticoagulant and immediately centrifuged at 4500 *g* for 15 min. The 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 15 to 400 ng. Then, 200 µl IS solution was added and the samples were alkalized with a 0.2-ml of 1 M KOH solution. The free bases liberated were then extracted with 6 ml of chloroform by vortex-mixing for 5 min. After centrifugation (4500 *g*, 5 min), 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. Then, 200 µl of TCNQ reagent was added to the residue, the tube was stoppered and the mixture was heated at 80°C for 20 min. (For health and safety reasons, 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. Then, 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 three different concentrations (35, 200 and 400 ng/ml). For the assay, the drug was added before the extraction procedure and the 200 µl IS solution was added, after extraction and then the reaction and chromatographic procedure were carried out as described above. 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.

The intra-day and inter-day precision

To determine the variability of the intra-day and inter-day precision, 1 ml of blank plasma samples were spiked with the drug at concentrations of 40, 80 and 160 ng/ml, and the IS at concentration of 200 ng/ml. The samples were then analyzed (n=4) according to the described procedure for intra-day variability and at five different days for inter-day variability.

Applicability

To a 28-year-old healthy woman volunteer, 40 mg of imipramine medication was administered on an empty stomach and venous blood samples of 5–6 ml were drawn into tubes containing disodium EDTA at 1.5, 2, 2.5, 5, 7, 12 and 24 h. The blood was processed to plasma and samples were stored at -20°C until analysis. Analyses were performed as described above. Volunteer gave written informed consent and the Ethics Committee of Istanbul University, Cerrahpasa Medicine Faculty approved the clinical protocol.

Results

The developed HPLC method is based on the purple chromogen (Önal and Öztunç 2006) formed by displacement

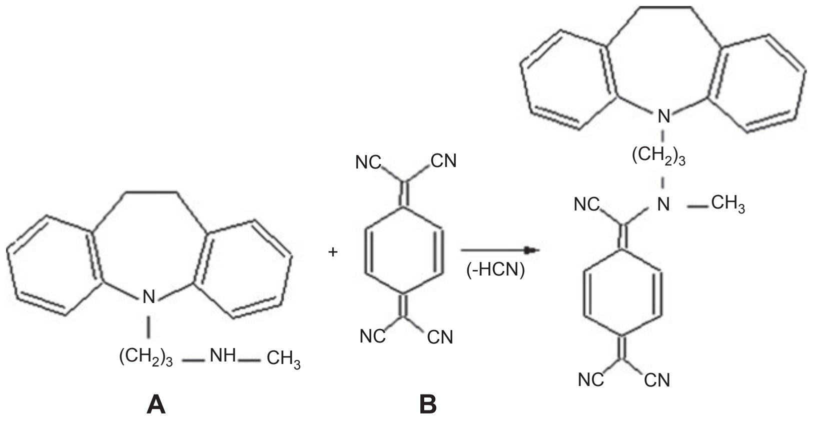

Figure 1 The proposed reaction between desipramine (A) and TCNQ (B).

reactions of DE with TCNQ in acetonitrile at 80°C for 20 min (Figure 1).

This method includes extraction of the drug base from plasma with chloroform after alkalinization, derivatization with TCNQ and injection of the reaction mixture directly into a reversed phase C_{18} column with UV-visible detection at 567 nm. PA was used as IS. Acetonitrile/water (70:30) was used as the mobile phase under the chromatographic conditions described, DE and the IS peaks were well resolved. Endogenous plasma components did not yield any interfering peaks. Figure 2A and B represents the chromatograms of blank plasma and a plasma sample spiked with DE (160 ng) and IS (200 ng). Figure 2C shows the chromatogram of a plasma sample obtained from a volunteer after administration of an oral dose of imipramine (75 mg). The average retention times of DE and IS were 15.5 and 13.1 min, respectively.

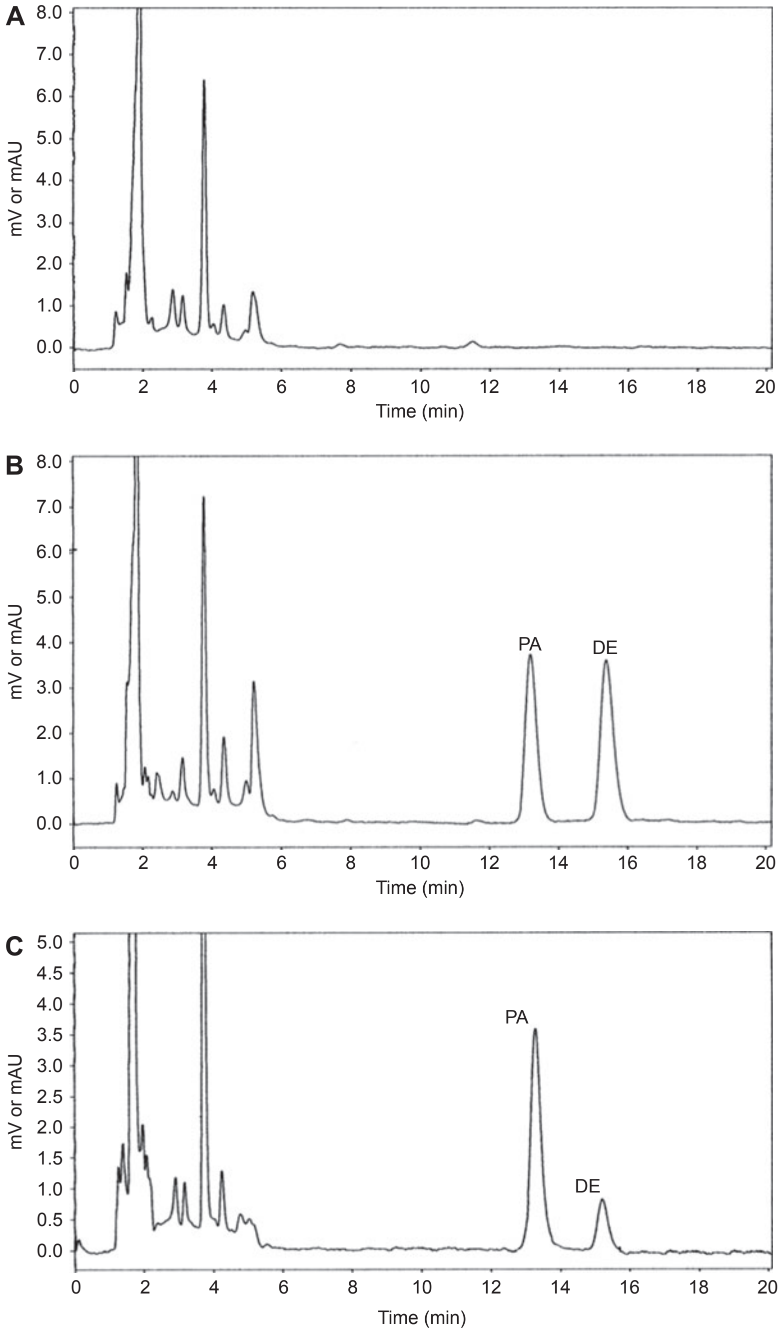

Figure 2 HPLC chromatograms of (A) a blank plasma, (B) a plasma spiked with 160 ng of DE and (C) 200 ng of the IS plasma sample obtained at 2 h after oral administration of 75 mg of imipramine from a healthy volunteer, IS 200 ng. (For all chromatograms, 1 ml plasma samples were used.)

Method validation

Linearity range Calibration graph was constructed by plotting the peak area ratios of the drug/IS against the concentrations and was linear over the range of 15–400 ng/ml. Regression equations of the calibration curve were calculated as $A_{DE/}A_{IS}$=/0.0067 C+0.08 (r=0.9988) (where C is the concentration of the drug in ng/ml, A is peak area ratios of DE versus IS).

LOD and LOQ Limit of quantification (LOQ) value under the described condition was 15 ng/ml, which is the lowest concentration of the calibration curve. Detection limit (LOD) was 1 ng/ml at a signal-to-noise ratio of 3:1. The obtained results are summarized in Table 1.

Recovery Extraction recoveries were determined at three concentrations using the described extraction condition and were found to be between 86.14% and 88.98% at concentrations of 35, 200 and 400 ng/ml. The mean recovery of IS was determined as 81.38% for 200 ng/ml (Table 2).

Precision Intra- and inter-day precision and accuracy were evaluated by assaying plasma samples spiked with DE at three different concentrations (40, 80 and 160 ng/ml). Intra- and inter-day relative standard deviation values were found to be within 6.43–11.35% and 9.82–13.05%, respectively. The results are given in Table 3.

Applications of the methods

The developed method was applied to a pharmacokinetic study for DE as active metabolite of imipramine. Figure 3 shows the plasma concentration time profile after oral administration of imipramine medication (75 mg) to a 28-year-old healthy woman volunteer. The data obtained indicate that C_{max} 45.2 ng/ml was attained at 2 h (t_{max}) of dosing. Plasma concentrations were measurable up to 24 h. The preliminary data for the elimination half-life ($t_{½}$) was 18.2 h. $AUC_{0–24}$ (area under the curve) was 525 ng/h/ml (Table 4). The developed methods also have adequate sensitivity for the method to evaluate plasma concentration profile of DE in pharmacokinetic studies at the administered oral dose (Nelson et al. 1982, Kenney et al. 1989).

Table 1 Results of analytical parameters for proposed methods.

Parameter	
Linearity range[a] (ng/ml)	15–400
Regression equation[b]	
Slope±SD	0.0067±0.00015
Intercept±SD	0.08±0.0023
Correlation coefficient (r)	0.9988
LOD (ng/ml)	1.0
LOQ (ng/ml)	15

[a]Average of five determinations. [b]A=a+b C, where C is the concentration of the drug in ng/ml, A is the peak area ratios of DE versus IS.

Table 2 Absolute recovery of desipramine and IS from plasma.

Analyte	Concentration (ng/ml)		Recovery (%)	RSD[a] (%)
	Added	Found (mean±SD)		
Desipramine	35	31.14±3.13	88.98	10.06
	200	172.29±6.15	86.14	3.57
	400	347.61±10.08	86.90	2.90
Paroxetine	200	162.8±5.97	81.4	3.7

[a]RSD, relative standard deviation.

Table 3 Intra-day and inter-day precision and accuracy of desipramine in plasma.

Concentration (ng/ml)		RSD[a] (%)
Added	Found (mean±SD)	
Intra-day		
40.0	40.52±4.6	11.35
80.0	80.44±5.17	6.43
160.0	159.15±14.12	8.87
Inter-day		
40.0	40.13±5.24	13.05
80.0	80.61±8.17	10.13
160.0	157.58±15.47	9.82

[a]RSD, relative standard deviation.

Discussion

The reaction between DE and TCNQ is simple, 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 h. DE 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.

The proposed HPLC method has great advantage in terms of selectivity. Among numerous drugs tested, only six (nortriptyline, paroxetine, fluoxetine, maprotiline, sertraline and barbexaclone) yield purple derivative with TCNQ under the described experimental conditions. Among the above drugs, only nortriptyline and fluoxetine interfere with the analysis.

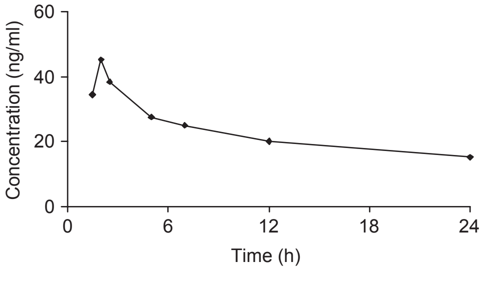

Figure 3 Plasma concentration time profile of desipramine as the active metabolite of imipramine in a healthy volunteer after a single oral administration of 75 mg of imipramine.

Table 4 Pharmacokinetic parameters of desipramine as active metabolite of imipramine in plasma.

Parameters	Results
C_{max} (ng/ml)	45.2
t_{max} (h)	2
$t_{1/2}$ (h)	18.2
AUC (ng/h/ml)	525
$AUC_{0-\infty}$	924

Conclusion

A fast, simple and sensitive method for the determination of DE as active metabolite of imipramine in human plasma was developed. The validated method allows determination of DE in the 15–400 ng/ml range. The assay is rapid. The analysis time is approximately 15 min. The precision and accuracy of the method are well within the limits required for bioanalytical assays. The limit of quantification permits the use of the method for pharmacokinetic studies.

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