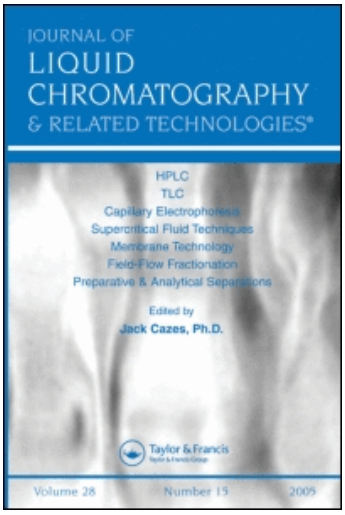

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Development and Validation of an HPLC Method, with Fluorescence Detection, for Simultaneous Determination of Paroxetine and its Metabolites in Plasma

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Development and Validation of an HPLC Method, with Fluorescence Detection, for Simultaneous Determination of Paroxetine and its Metabolites in Plasma

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Abstract: An isocratic reversed-phase high performance liquid chromatography (HPLC) method for simultaneous determination of paroxetine, a potent selective serotonin reuptake inhibitor and its metabolites {(-)-trans-4-(4-fluorophenyl)-3-(4-hydroxy-3-methoxyphenoxymethyl) piperidine, BRL 36610 A} (metabolite A) and {(-)-trans 4-(4-fluorophenyl)-3-(3-hydroxy-4-methoxyphenoxymethyl) piperidine, BRL 36583 A} (metabolite B) in plasma has been developed. The method utilizes a Zorbax Eclipse XDB-C18 5-μm column, a mobile phase composed of acetonitrile-phosphate buffer (KH_2PO_4 0.04 M; pH = 3.5) (30:70, v/v) at a flow rate of 1.0 mL/min and protriptyline as internal standard. The total analysis time was 12 min and paroxetine eluted at 10 min. A fluorescence detector was used with excitation and emission wavelength adjusted at 295 and 350 nm, respectively. All the experiments were performed at room temperature.

The isolation of paroxetine and its metabolites from plasma samples has been achieved from pH = 12 with liquid-liquid extraction using ether. The organic layer was removed, evaporated at 40°C under a nitrogen steam, and the residue was dissolved in 200 μL mobile phase and injected into the HPLC system.

The method was linear over the concentration range of 7–200 ng/mL for paroxetine, 12–200 ng/mL for metabolite A, and 27–200 ng/mL for metabolite B. The

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quantification limits were 7, 12, and 27 ng/mL, respectively, proving the suitability of the method for paroxetine drug level monitoring in the therapeutic range. The extraction recovery was about 84% for paroxetine. Within day precision of the method (RSD%) for paroxetine, metabolite A, and metabolite B were 0.26–7.5, 3.4–13, and 5.7–15%, respectively, while between day precision of the method was 4.9, 5.5, and 6.0%, correspondingly. No endogenous compounds were found to interfere with the analysis. Twenty six substances were studied for interference and none of them interfered in the determination.

The method was applied successfully in the quantitative determination of paroxetine in plasma samples of patients receiving 20–40 mg of Seroxat^{R} (paroxetine) and the concentrations were in the therapeutic range. Metabolites A and B were not detected in the plasma samples of the patients.

Keywords: Paroxetine, Paroxetine metabolites, Plasma, HPLC

INTRODUCTION

Paroxetine (Par) (Seroxat^{R}-SmithKlineBeecham), (-)-trans-4-(4-fluorophenyl)-3-(3,4-methylenedioxy-phenoxymethyl) piperidine, is a potent selective serotonin reuptake inhibitor, used widely as an antidepressant drug alone, or in combination, with other drugs.[1,2] It is administered as tablets in daily doses of 20–60mg. Paroxetine (Par) is mainly metabolized in the liver by cytochrome P450 2D6 isoform (CYP2D6) in two active metabolites {(-)-trans-4-(4-fluorophenyl)-3-(4-hydroxy -3- methoxyphenoxymethyl) piperidine, BRL 36610 A} (metabolite A) and {(-)-trans-4-(4-fluorophenyl)-3-(3-hydroxy-4- methoxyphenoxymethyl) piperidine, BRL 36583 A} (metabolite B).[1–3]

Several methods[4–17] have been published for the determination of Par in plasma, including reversed phase high performance liquid chromatography (HPLC) with UV,[4,5] fluorescence[6–9] (without[6,7] or with derivatization using dansylchloride,[8,9]) MS,[10–12] diode array[13,14] detection, or gas chromatography with MS detection,[15–17] and applied therapeutic drug level monitoring[4,5,7–11,13–17] or bioequivalence studies.[6,12] Only a few methods have been published for the simultaneous determination of Par and its metabolites in plasma, including HPLC with UV,[18,19] fluorescence[18] or MS [20] detection and applied to pharmacokinetic,[20] therapeutic drug level monitoring[18,19] and toxicological screening or forensic cases.[18,19] Liquid–liquid[4,7,9,12,20] or solid phase[18,19] extraction techniques have been used for the isolation of Par and/or its metabolites from plasma samples before chromatographic determinations.

Therapeutic concentrations of Par in plasma are ranging between 20–190ng/mL[18] or 10–75 ng/mL[21] and little information exists[18] whether Par metabolites are detected after administration of therapeutic doses. The purpose of this study was to develop an HPLC method with fluorescence detection for the simultaneous determination of Par and its metabolites in plasma, further improving the extraction recovery and the

sensitivity of previously described methods by optimization of the experimental conditions, and to apply the methodology for analyzing plasma samples of patients receiving the drug in order to study if paroxetine plasma levels remain in the therapeutic range when other drugs are coadministered to the depressive patients.

EXPERIMENTAL

Apparatus

Chromatographic analysis was performed with an Agilent 1100 HPLC modular system, consisting of a control unit (Controller), a pump (Agilent 1100 G1311A Quant Pump) fitted with a Ternary Gradient Unit, which was used under isocratic conditions, a Rheodyne PN 7725i manual injector, and a 20 μL sample loop. An Agilent variable wavelength UV/Vis detector (VWD Agilent 1100 G1314A) and an Agilent 1100 fluorescence detector (FLD Agilent 1100 G1321A) were fitted to the HPLC system. Samples were chromatographed on a Zorbax Eclipse XDB-C_{18} (150 × 4.6 mm)–5 μm particle size chromatography column. The column was stored in methanol when not in use, at room temperature. The manipulation of chromatograms, as well as the control of the chromatography system, was achieved using the Agilent Chemstation software.

A Millipore filtration system (Millipore, Bedford, M.A. USA) with type HV Millipore filters (diameter 47 mm, pore size 0.45 μm) was used, for degassing mobile phase under vacuum before analysis, while on line degassing of the mobile phase was performed with the Agilent 1100 G1379A system.

The organic phases (ether) from the extraction were evaporated on a Bioblock Scientific isotherm dry bath under nitrogen at 40°C.

A vortex (Falc Model Mix 20) set at speed 5 was used for the mixing of plasma samples and standards. The analog pH-meter used was a METROHM product with a glass combination electrode.

Reagents and Standards

Paroxetine hydrochloride hemidrate of 87.1% purity as free base, (-)-trans-4-(4-fluorophenyl)-3-(4-hydroxy, 3-methoxy-phenoxymethyl) piperidine hydrochloride, BRL 36610A (metabolite A) of 84.6% purity as free base, and (-)-trans-4-(4-fluorophenyl)-3-(3-hydroxy, 4-methoxy-phenoxymethyl) piperidine hydrochloride, BRL 36583A} (metabolite B) of 82.1% purity as free base, were kindly provided by SmithKlineBeecham. Protriptyline hydrochloride (Pro) was provided from Sigma and was used as internal standard.

Potassium dihydrogenphosphate, dipotassium hydrogenphosphate and orthophosphoric acid were obtained from Merck. All organic solvents

(methanol, acetonitrile) were HPLC grade, (LABSCAN®) and water prepared by Milli-Q Plus® (Millipore Watford, Herts., UK) was used throughout the study.

Phosphate buffer (pH 3.5; 0.04 M) for mobile phase was prepared by dissolving the appropriate quantity of KH_2PO_4 in water and adjusting the pH with the addition of orthophosphoric acid using the pH meter. Phosphate buffer (pH 12; 0.12 M), for the extraction of plasma samples, was prepared by dissolving the appropriate quantity of Na_2HPO_4 in water and adjusting the pH with the addition of NaOH 4 M using the pH meter.

Pooled drug free plasma (blanc plasma) was obtained from healthy volunteers, who had not received any medication prior to their blood donation, and was used for the preparation of spiked plasma standards.

Stock standard solutions of Par (1.0 mg/mL as free base), metabolite A (0.40 mg/mL as free base), metabolite B (0.40 mg/mL as free base), and Pro (1.0 mg/mL as free base) were prepared in methanol and when stored at -20°C were stable for 12 months. A more diluted methanolic stock standard solution of Pro (100 μg/mL) was prepared by diluting the stock standard solution 10-fold with methanol, and when stored at -20°C was stable for 12 months. Two aqueous Pro solutions of 3.0 and 1.5 μg/mL were prepared from the methanolic 100 μg/mL Pro solution, and when stored at 4°C were stable for 4 months. An aqueous Pro solution of 0.030 μg/mL was prepared with 100-fold dilution of the 3.0 μg/mL aqueous Pro solution, and when stored at 4°C were stable for 4 months. Two combined methanolic reference solutions containing Par, A, and B at final concentrations of 100 and 50 μg/mL, were prepared from the stock methanolic solutions of each compound; they were stable for 12 months when stored at -20°C.

Eight working solutions in mobile phase containing 0.050, 0.075, 0.100, 0.150, 0.250, 0.500, 0.750, and 1.00 μg/mL of Par, A, and B and 0.015 μg/mL of Pro were prepared by mixing the appropriate volumes (10–200 μL) of the combined methanolic reference solution containing Par, A, and B to 50 μg/mL with 100 μL of the Pro aqueous solution 1.5 μg/mL, and diluting up to 10 mL with mobile phase. All working solutions in mobile phase when stored at 4°C were stable for at least 4 months.

Eight working aqueous solutions, for spiking of plasma samples, containing 0.10, 0.15, 0.20, 0.30, 0.50, 1.00, 1.50, and 2.00 μg/mL of Par, A, and B were prepared by diluting the appropriate volumes (10–200 μL) of the combined methanolic reference solution containing Par, A, and B to 100 μg/mL, up to 10 mL with water. All the above mentioned working aqueous solutions when stored at 4°C were stable for at least 4 months.

Plasma standards for calibration curves were prepared by spiking 1.0 mL aliquots of pooled drug free plasma with 100 μL of the above mentioned aqueous working solutions, to make plasma standards corresponding to Par, A, and B in the concentration range of 10 to 200 ng/mL.

Calibration graphs, based on the peak area ratio (P.A.R) or the peak height ratio (P.H.R) of analyte/internal standard against analyte concentration, were prepared for each day of analysis to check linearity and to be used for the calculation of the analyte concentration in the patient samples.

Procedures

Chromatographic Conditions

The optimized mobile phase consisted of acetonitrile-phosphate buffer (pH 3.5; 0.04 M) at a ratio of 30:70, v/v. The mixture, prior to use, was filtered and degassed under vacuum. A flow rate of 1.0 mL/min under isocratic mode was used at ambient temperature, resulting in a pressure of about 160 kg/cm^2. The fluorescence detector was set at $\lambda_{ex} = 295$ nm and $\lambda_{em} = 350$ nm.

Extraction Procedure

In 10-mL glass conical tubes with glass stopper, 1.0 mL of plasma samples or the prepared spiked plasma standards and 100 μL of Pro 0.030 μg/mL (3 ng) internal standard aqueous solution were added and vortex mixed for 15 s. Consequently, 0.5 mL of phosphate buffer (pH 12; 0.12 M) was added to the tube and the contents were vortex mixed for another 30 s. Each sample was extracted with 5.0 mL of ether with vortexing at speed 5 for 3 min (2500 rpm). The sample tube was centrifuged for 5 min at 7000 rpm. After careful removal and transfer of the upper organic layer into a 10-mL conical glass tube, the organic layer was evaporated to dryness at 40°C in a dry bath, under a gentle stream of nitrogen. The residue was reconstituted in 200 μL of mobile phase (preconcentration × 5) and an aliquot of about 70 μL was injected into the HPLC system.

In Vivo Study

The method was applied in 13 female and male subjects aged 38–62 years old who were receiving Seroxat^R (paroxetine) at daily doses of 20–40 mg, administered to them early in the morning. All patients were under treatment with Seroxat^R for more than 6 months and, therefore, plasma concentrations of paroxetine were considered as steady state concentrations. Blood samples were collected 6 hours after dosing in unstoppered tubes containing sodium heparin as anticoagulant from all the subjects. Samples were centrifuged immediately after collection and plasma stored at −20°C until analysis.

RESULTS AND DISCUSSION

Methodology

The HPLC method, based on the modification of previously described methods, was optimized for the experimental conditions: composition and flow rate of mobile phase, internal standard and its concentration, and extraction of plasma samples. The chromatographic response factor (CRF)[22] was used for the optimization of mobile phase composition. Retention times of A, B, Par, and Pro were 3.433, 4.167, 10.004, and 10.849 min, respectively, and remained constant from day to day. Figure 1 shows a typical chromatogram obtained from the injection of a standard solution containing Par, A, B, and Pro at 1/1/1/0.015 μg/mL, respectively, (loop of 20 μL, referring to 20 ng of Par, A, B, and 0.3 ng of Pro). Within day precision (repeatability, RSD%) of the chromatographic system for Par, A, and B was calculated after repeated injections of the standard solution (n = 5) and was equal to 0.14, 0.13, and 0.12% for retention time, respectively, 7.1, 3.1, and 7.0% for area, correspondingly, 6.8, 3.7, and 6.2% for height, 3.1, 1.2, and 4.2% for P.A.R and 4.9, 4.9, and 3.4% for P.H.R, respectively. Similar RSD% values were received for Par, A, and B, concerning between day precision of the chromatographic system. Figure 2 shows a typical chromatogram obtained from an extracted drug free plasma. As can be seen from Figure 2, endogenous plasma compounds were eluted before 3 min. In order to investigate, in this study, if analytes need some time to equilibrate with plasma after spiking, we analyzed freshly-spiked plasma samples and plasma samples allowed to equilibrate with analytes after spiking for 24 hours. In both cases, we received the same results. Figure 3 shows a chromatogram of an extracted spiked plasma sample containing Par/A/B/Pro at 100/100/100/3 ng/mL, respectively. Figure 4 shows a chromatogram of an

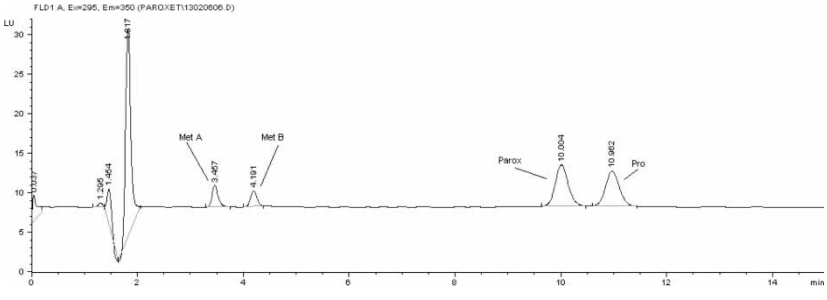

Figure 1. Typical chromatogram of a standard solution containing Paroxetine (Parox), metabolite A (Met A), metabolite B (Met B), and Protriptyline (Pro) (internal standard) in mobile phase at 1.0, 1.0, 1.0, and 0.015 μg/mL, respectively, (λ_{ex} = 295 nm, λ_{em} = 350 nm).

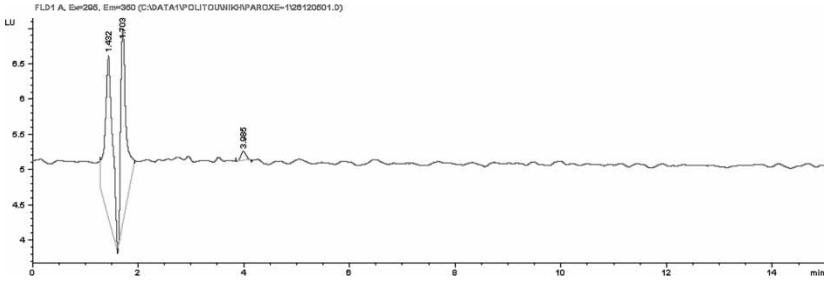

Figure 2. Typical chromatogram from an extracted drug free plasma sample ($\lambda_{ex} = 295$ nm, $\lambda_{em} = 350$ nm).

extracted plasma sample of a patient under treatment with Seroxat^{R}. Resolution of peaks in all cases was excellent {$Rs_{(A,\ B)} = 3.33$, $Rs_{(Par,\ B)} = 17.88$, $Rs_{(Pro,\ Par)} = 2.09$} and there were no interfering peaks of sample matrix observed by analyzing the patients plasma samples.

Extraction Procedure

Several solvent systems were tested for the extraction of Par, A, and B from spiked plasma samples (ethylacetate, ethylacetate/hexane 20:80 and 50:50, dichlormethane/pentane 40:60, dichlormethane/isopropylalcohol 90:10, pentane/amylalcohol 20:1, pentane/isopropylalcohol 95:5 v/v and ether). Phosphate buffer solution (pH 12; 0.12 M) was used for the pH adjustment of plasma during the extraction (pKa value for paroxetine is 9.9[21]). Among the extraction systems examined, ethlylacetate–hexane 20:80 and 50:50 v/v, as well as ether, gave the highest extraction recoveries. Therefore, three point calibration curves for paroxetine in plasma, in the concentration range of 50–200 ng/mL, were constructed using these three

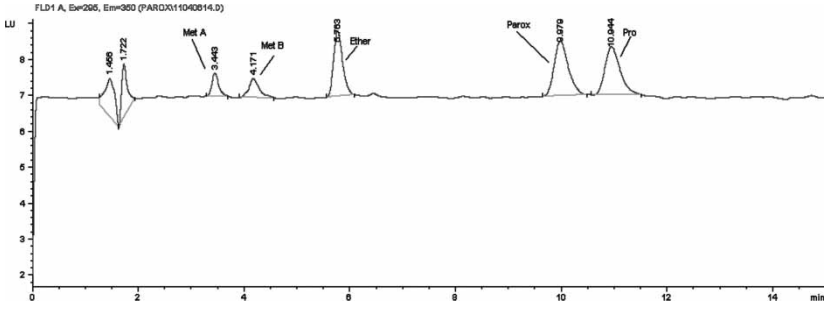

Figure 3. Typical chromatogram from an extracted spiked plasma sample containing Paroxetine, metabolite A, metabolite B, and Protriptyline in concentrations 100, 100, 100, and 3 ng/mL, respectively, ($\lambda_{ex} = 295$ nm, $\lambda_{em} = 350$ nm).

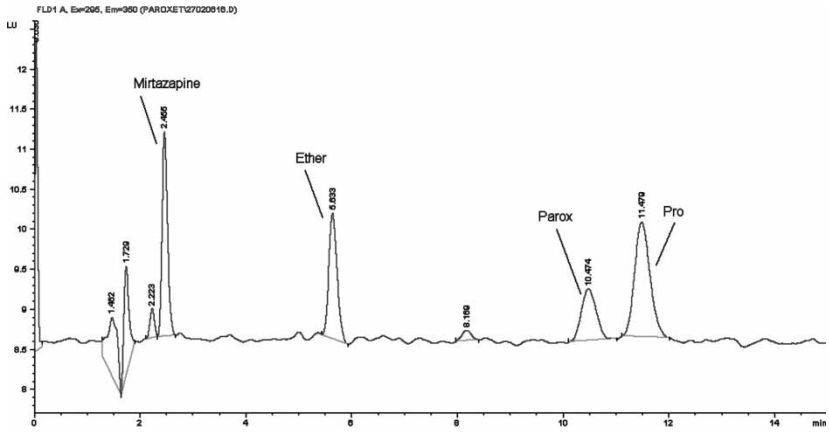

Figure 4. Typical chromatogram from an extracted plasma sample of a patient receiving 30 mg Seroxat daily (Saroten *και* Remeron were coadministerd). Plasma paroxetine concentration 43 ng/mL ($\lambda_{ex} = 295$ nm, $\lambda_{em} = 350$ nm).

solvent systems for extracting spiked plasma samples and the slopes of the calibration curves received, based on P.A.R or P.H.R, were quite similar. Therefore, ether has been chosen for the extraction of the analytes from plasma, due to its high volatility (easy evaporation). A back extraction (reextraction of the organic solvent with an acidic aqueous phase) was also tried without any improvement of the extraction recovery. Suitable chromatograms were received from spiked plasma samples with this procedure (absence of peak tailing, satisfactory resolution, and absence of baseline noise).

Recovery

Absolute and relative recoveries were calculated from the ratios of slopes of the corresponding calibration curves of standard solutions prepared in mobile phase (concentration expressed in μg/mL) and in plasma (concentration expressed in ng/mL) in the absence or in the presence of internal standard, respectively, taking into account the 5 × preconcentration (Table 1).

Linearity

Calibration curves of standard solutions and of spiked plasma standards based on area, height, P.A.R, or P.H.R for Par, A, and B, showed excellent linearity. The plasma calibration curves of Par, A, and B, were linear in the range of 7–200, 12–200, and 27–200 ng/mL, respectively. The correlation coefficient for each individual standard curve of Par, A, and B in plasma based on P.A.R was greater than 0.995, 0.995, and 0.9996, respectively, while based on P.H.R was greater than 0.994, 0.998, and 0.9991, correspondingly. Intercept values of

Table 1. Mean absolute and relative % recovery of paroxetine, metabolite A and metabolite B calculated from the slopes of calibration curves in plasma and in standard solutions prepared in mobile phase

	% Absolute recovery (no use of internal standard)		% Relative recovery (use of internal standard)	
	Area	Height	Peak area ratio	Peak height ratio
Paroxetine	78.4	70.9	82.6	84.0
Metabolite A	88.1	72.0	88.9	102
Metabolite B	102	87.3	108	103

each individual curve were not statistically different from zero (t-test) for Par, A, and B and, therefore, lines pass through the origin. Mean regression equations of 3 calibration curves of Par, A, and B in plasma over a period of 4 months are presented in Table 2, and showed a RSD% of slopes for Par equal to 2.6 % and 4.9% when using P.A.R or P.H.R, correspondingly.

Sensitivity

The detection (D.L) and the quantification limits (Q.L) for the determination of Par, A, and B in plasma were validated by independent analysis of 5 spiked plasma samples per analyte, prepared at 10, 20, and 30 ng/mL for Par, A, and B, respectively. The D.L and the Q.L (based on the 3.3 and 10 times the SD, respectively) were estimated from the slopes of the regression equations of the

Table 2. Mean equations of 3 calibration curves of paroxetine, metabolite A, metabolite B in plasma over a period of 4 months calculated from peak area ratio (P.A.R) or peak height ratio (P.H.R)

y = P.A.R	y = P.H.R
Paroxetine	
$y = (0.01164 \pm 0.00031)\ C - (0.020 \pm 0.0060)$	$y = (0.01224 \pm 0.00060)\ C - (0.016 \pm 0.009)$
RSD of slopes = 2.6%	RSD of slopes = 4.9%
Metabolite A	
$y = (0.00227 \pm 0.00011)\ C - (0.030 \pm 0.013)$	$y = (0.00572 \pm 0.00015)\ C + (0.027 \pm 0.017)$
RSD of slopes = 1.2%	RSD of slopes = 5.5%
Metabolite B	
$y = (0.003394 \pm 0.000071)\ C - (0.0518 \pm 0.0097)$	$y = (0.00606 \pm 0.00019)\ C - (0.124 \pm 0.025)$
RSD of slopes = 3.4%	RSD of slopes = 6.0%

corresponding calibration curves and the standard deviations of the P.H.R or P.A.R of the above mentioned low concentration spiked plasma samples. The D.L and the Q.L for the determination of Par in plasma were 2 and 7 ng/mL, respectively, based on P.A.R or P.H.R measurment (mass detection and quantification limits are equal to 40 and 140 pg, correspondingly). The D.L and the Q.L for the determination of metabolite A in plasma were lower (4 and 12 ng/mL, respectively) when using P.H.R than when using P.A.R (13 and 39 ng/mL, correspondingly). The D.L and the Q.L for the determination of metabolite B in plasma based on P.A.R were 9 and 29 ng/mL, respectively, while based on P.H.R were 9 and 27 ng/mL, correspondingly. For therapeutic drug level monitoring, both ways (P.H.R or P.A.R) are of equal value and they give the same D.L and Q.L for Par. When quantification of metabolites is

Table 3. Within run precision and accuracy data of paroxetine, metabolite A and metabolite B from spiked plasma samples using peak area ratio (P.A.R) or peak height ratio (P.H.R) (protriptyline – internal standard at 3.0 ng/mL)

	Concentration added (ng/mL)	Concentration found Mean ± SD (ng/mL)	RSD (%)	Er (%)
Using P.A.R				
Paroxetine	10.0 (n = 5)	8.46 ± 1.0	12	−15.4
	30.0 (n = 5)	34.3 ± 0.96	2.8	+14.3
	100.0 (n = 8)	90.0 ± 6.8	7.6	−10
	200.0 (n = 5)	211.4 ± 8.3	3.9	+5.7
Metabolite A	20.0 (n = 5)	16.8 ± 1.8	11	−16.0
	100.0 (n = 8)	95 ± 10	11	−5.0
	200.0 (n = 5)	205.2 ± 4.7	2.3	+2.6
Metabolite B	50.0 (n = 5)	59.2 ± 6.8	11	+18.4
	100.0 (n = 8)	111 ± 15	14	+11
	200.0 (n = 5)	175 ± 29	17	−12.5
Using P.H.R				
Paroxetine	10.0 (n = 5)	8.13 ± 0.61	7.5	−18.7
	30.0 (n = 5)	33.4 ± 0.087	0.26	+11.3
	100.0 (n = 8)	88.0 ± 4.2	4.8	−12.0
	200.0 (n = 5)	208.6 ± 5.3	2.5	+4.3
Metabolite A	20.0 (n = 5)	16.50 ± 2.1	13	−17.5
	100.0 (n = 8)	94 ± 12	13	−6.0
	200.0 (n = 5)	203.9 ± 6.9	3.4	+2.0
Metabolite B	50.0 (n = 5)	59.2 ± 3.4	5.7	+18.4
	100.0 (n = 8)	102 ± 15	15	+2.0
	200.0 (n = 5)	170 ± 14	8.2	−15.0

required, e.g. in overdose cases, P.H.R is preferable because it provides a lower Q.L for metabolite A.

Precision and Accuracy

Precision and accuracy were determined by analyzing plasma samples spiked with Par, at 10, 30, 100, and 200 ng/mL, with A at 20, 100, and 200 ng/mL, and with B at 50, 100, and 200 ng/mL, and the results are presented in Table 3. Within-run RSD% for Par was ranging from 2.8 to 11.8% (P.A.R) and 0.26–7.5% (P.H.R) all over the calibration curve, while for metabolites A and B from 3.4 to 13% and from 5.7 to 15 %, respectively (P.H.R).

Table 4. Results of interference study of co-administerd drugs

Compound	Trade name	Category	Retention time (min)
Alprazolam	Xanax	Anxiolytic	N.D[a]
Amitriptyline	Saroten	Antideppressant	N.D[a]
Atenolol	Tenormin	Antihypertensive	N.D[a]
Bromazepam	Lexotanil	Tranquilliser	N.D[a]
Camazepam		Tranquilliser	N.D[a]
Clonazepam	Rivotril	Antiepileptic	N.D[a]
Clorazepate	Tranxene	Tranquilliser Hypnotic	N.D[a]
Diazepam	Stedon	Tranquilliser Hypnotic, Muscle relaxant	N.D[a]
Hydrochlorothiazine	Ividol	Antihypertensive	2.352
Flunitrazepam	Hipnosedon	Hypnotic	N.D[a]
Fluoxetine	Ladose	Antideppressant	N.D[a]
Lorazepam	Tavor	Tranquilliser Hypnotic	N.D[a]
Medazepam			N.D[a]
Midazolam	Dormicum	Hypnotic	N.D[a]
Mirtazapine	Remeron	Antideppressant	2.475
Nirtrazepam		Antiepileptic	N.D[a]
Nortryptiline	Nortrilen	Antideppressant	N.D[a]
Oxazepam			N.D[a]
Oxcarbazepine	Trileptal	Antiepileptic	N.D[a]
Penfluridol	Flupidol	Antipsychotic	N.D[a]
Pimozide	Pirium	Antipsychotic	2.059
Prazepame	Centrac	Tranquilliser	N.D[a]
Risperidone	Risperdal	Antipsychotic	2.063
Temazepam	Normison	Hypnotic	N.D[a]
Triazolam	Halcion	Hypnotic	N.D[a]
Venlafaxine	Efexor	Antideppressant	3.179

[a]Not detected.

Note: Retention times of metabolite A, metabolite B, Par and Pro were 3.433, 4.167, 10.004, and 10.849 min, respectively.

Table 5. Plasma paroxetine concentrations of 13 subjects treated with Seroxat^{R}

α/α	Sex (F: female) (M: male)	Age (years)	Daily dose (mg)	Treatment time (months)	Coadministered drugs and daily doses	Plasma paroxetine (ng/mL)
1	F	48	40	> 12	Efexor XR 75 mg (2 × 1) Lexotanil 3 mg (3 × 1) Ladose 20 mg (1 × 1)	142
2	F	62	30	6	Remeron 30 mg (1 × 1) Saroten 10 mg (1 × 1)	43
3	F	58	30	24	Thirormon 0.1 mg 1 × 1) Lexotanil 3 mg (1 × 1) Tenormin 25 mg (1 × 1)	34
4	M	52	20	> 12	Vitamins	27
5	F	60	20	> 12	—	130
6	M	50	20	> 12	Tavor 2.5 mg (1 × 1) Pirium 4 mg (1 × 1) Flubidol 20 mg (1/7)	26
7	F	38	20	> 12	Lexotanil 1.5 mg (3 × 1)	31
8	F	55	20	> 12	Lexotanil 1.5 mg (3 × 1)	13
9	F	59	20	> 12	Risperdal 1 mg (6 × 1) Xanax 1 mg (3 × 1) Trileptal 300 mg (3 × 1)	43
10	M	63	20	> 12	—	100
11	F	60	20	> 12	—	30
12	F	48	30	> 12	—	56
13	M	54	20	> 12	Lexotanil 3 mg (1 × 1)	10

Study for Interferences

Twenty six compounds (drugs) were studied for possible interference, including several of those that might be administered to anxious or depressed patients (Table 4). Most of the compounds tested were not

detected with the fluorescence detector used in the present methodology. Hydrochlorthiazide, mirtazapine, pimozide, risperidone and venlafaxine were detected, but eluted in retention times not interfering with the retention times of the analytes.

In Vivo Study

A plasma paroxetine concentration as well as coadministerd drug to the 13 patients included in the study is presented in Table 5. Par plasma concentration ranged from 10–142 ng/mL in all subjects and, therefore, was within the therapeutic range of the drug (20–200 ng/mL), except patient 13. Linear correlation between plasma concentration and dose was not observed, and despite the fact that the coadministerd drugs might interfere with the rate of Par biotransformation, almost all Par plasma levels measured were within the therapeutic range. Metabolites A and B were not detected in any of the patients. This conclusion is in accordance with a previous publication[18] where it is stated that, after administration of therapeutic doses of Par, Par plasma levels ranged between 0.048–0.25 μmol/L (16–82 ng/mL), while both metabolites of the drug were not detected.

CONCLUSIONS

The analytical method developed in this report is simple, rapid, accurate, and sensitive. In comparison with previously reported methods[4,6,7,13,14] concerning the determination of Par in plasma, it is at least of equal value. In comparison with previously reported methods[18,19] concerning the simultaneous determination of Par and its metabolites in plasma, the proposed methodology is more sensitive. It can be used for monitoring of plasma Par levels in patients receiving therapeutic doses of the drug or in bioavailability studies. The method developed can also be used to monitor Par and its metabolites in overdose or in forensic cases, and can be further modified to correspond to the desirable concentration range.

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