DRUG FORMULATIONS AND CLINICAL METHODS

New Nonextractive and Highly Sensitive High-Performance Liquid Chromatographic Method for Determination of Paroxetine in Plasma After Offline Precolumn Derivatization with 7-Chloro-4-Nitrobenzo-2-Oxa-1,3-Diazole

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New nonextractive and simple offline precolumn derivatization procedures have been proposed, for the first time, for the trace determination of paroxetine (PXT) in human plasma by HPLC with fluorescence detection. Trimetazidine (TMZ) was used as an internal standard. Plasma samples were treated with acetonitrile for protein precipitation and then derivatized with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole in borate buffer of pH 8 at 70°C for 30 min. Separations of the derivatized PXT and TMZ were performed on a Nucleosil CN column using a mobile phase consisting of acetonitrile–10 mM sodium acetate buffer (pH 3.5)–methanol (47 + 47 + 6, v/v) at a flow rate of 1.0 mL/min. The derivatized samples were excited at 470 nm and monitored at an emission wavelength of 530 nm. Under the optimum chromatographic conditions, a linear relationship with good correlation coefficient ($r = 0.9998$, $n = 7$) was found between the peak area ratio and PXT concentrations in the range of 5–600 ng/mL. The LOD and LOQ were 1.37 and 4.14 ng/mL, respectively. The intraday and interassay precisions were satisfactory; the RSD did not exceed 4.2%. The accuracy of the method was proved by recovery of PXT from spiked human plasma at levels of 97.28–104.38 ± 0.41–3.62%. The proposed method had high throughput, as the analysis involved a simple sample pretreatment procedure and short run time (<10 min). The results demonstrated that the method would have a great value when it is applied in the therapeutic monitoring of PXT.

Paroxetine (PXT), 3S, 4R-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl) piperidine, is a potent selective seroto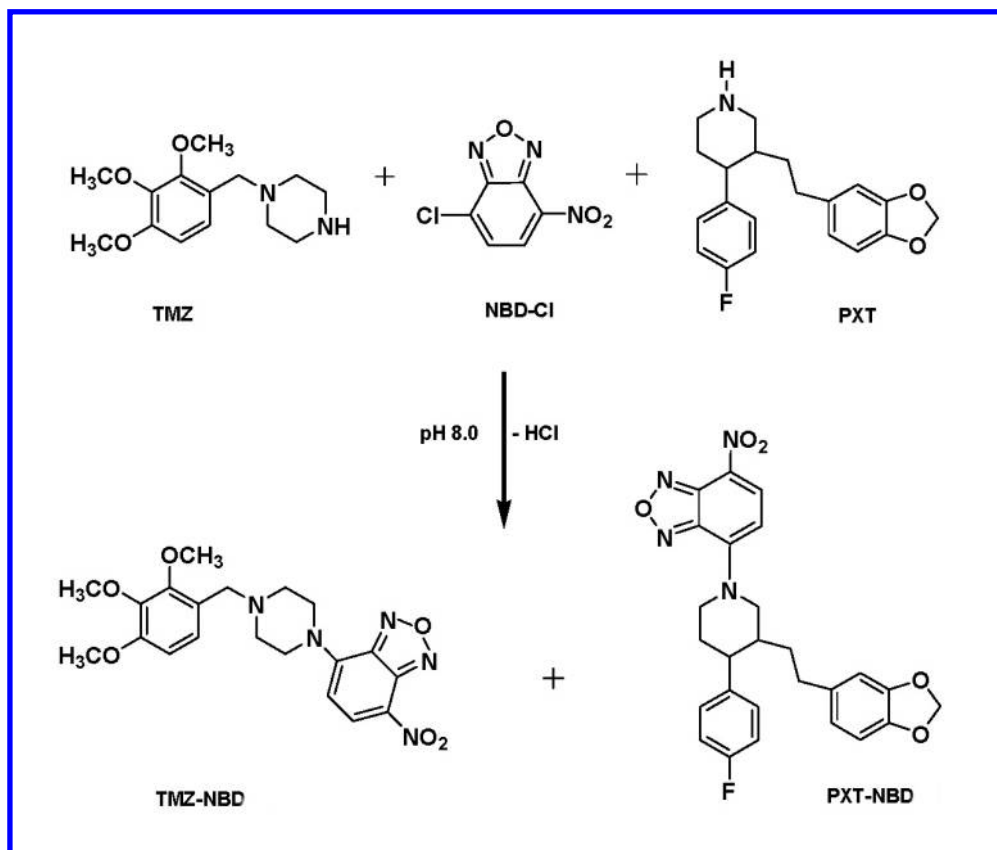nin reuptake inhibitor that is prescribed in the treatment of depression (1). It is also effective in the management of obsessive-compulsive disorder (1), panic attacks (2), social phobia, and post-traumatic stress (3), and in the prevention of depressive relapse during long-term treatment (4). Clinical studies indicated that PXT is comparable to tricyclic antidepressants (TCAs) in its clinical efficacy, but it is devoid of anticholinergic and antiaderenergic side effects and is remarkably safer than TCAs in overdose. The mean half-life of PXT is 24 h, which is consistent with a once-a-day dosing, and it has great acceptance by patients. For these reasons, PXT has become the most widely prescribed antidepressant (5). Most psychiatric patients are subjected to polypharmacotherapy to achieve successful control of the symptoms; therefore, therapeutic monitoring for PXT levels in the plasma of patients is advised. The maximum plasma level of PXT after normal daily doses was found to be in the range of 10–30 ng/mL (5). For reliable therapeutic PXT monitoring, suitable and sensitive analytical methods are required.

Numerous analytical methods have been developed for the quantitative determination of PXT in its dosage forms (6–10); however, these methods did not offer the adequate sensitivity for measuring PXT in plasma. Although some authors (8) claimed the spectrofluorometric determination of PXT in spiked human plasma, the spiked PXT concentration was higher than that which is normally found in the clinical samples, and the average recovery was less than 78%. The methods that have been devoted to the determination of PXT levels in plasma are GC (11–13) and, mostly, HPLC (14–23). These methods offered adequate sensitivity; however, the procedures involved liquid–liquid and/or solid-phase extraction of PXT from the plasma samples prior to their analysis. The multiple extraction procedures increased the overall analysis time (i.e., the methods are time-consuming) and negatively affected the accuracy of the method. LC/MS/MS (14, 15) offered adequate accuracy and sensitivity, but the cost and complexity of the instrumentation limits its applications in clinical laboratories. The present work describes the development of a new nonextractive and highly sensitive and accurate HPLC method with fluorescence detection for the determination of PXT in

Figure 1.   Scheme for the reaction of PXT and TMZ with NBD.

plasma. The method involved a very simple nonextractive isolation of PXT from plasma samples using one-step protein precipitation with acetonitrile, followed by precolumn derivatization with 7-chloro-4-nitrobenzo-2-oxa-1,3-dizole (NBD-Cl). The method was successfully applied to the determination of PXT in spiked human plasma samples.

Experimental

Chromatographic System

The HPLC apparatus consisted of a Shimadzu Corp. (Kyoto, Japan) system equipped with an LC-10AD VP pump with FCV-10AL VP low-pressure flow control valve, SCL-10A VP system controller, Rheodyne-7725 injection valve with 20 µL loop, SPD-10 Avp, UV-Vis detector, and RF-10A XL fluorescence detector. The chromatographic separations were performed on a Nucleosil CN analytical column (250 mm length × 3.9 mm id, 5 µm particle diameter) manufactured by Machery Nagel GmbH (Dueren, Germany). The column temperature was kept constant at 25 ± 2°C and separations were performed in the isocratic mode. The mobile phase consisted of acetonitrile–10 mM sodium acetate buffer (adjusted to pH 3.5 with 100% acetic acid)–methanol (47 + 47 + 6, v/v). The mobile phase was filtered by a Millipore Corp. (Billerica, MA) vacuum filter system equipped with a 0.45 µm filter, degassed in an ultrasonic bath, and by bubbling helium gas. The flow rate was 1.0 mL/min, and the sample injection volume was 20 µL. The fluorescence detector was set at

470 nm excitation wavelength and 530 nm emission wavelength. The system control and data acquisition were performed by Shimadzu CLASS-VP software, Version 5.032. The ratio of peak area of PXT to that of the internal standard (IS; trimetazidine, TMZ) was used for the quantitation.

Chemicals and Materials

PXT was obtained from SmithKline Beecham Pharmaceuticals (Brentford, UK). TMZ and NBD-Cl were purchased from Sigma Chemical Co. (St. Louis, MO). Human
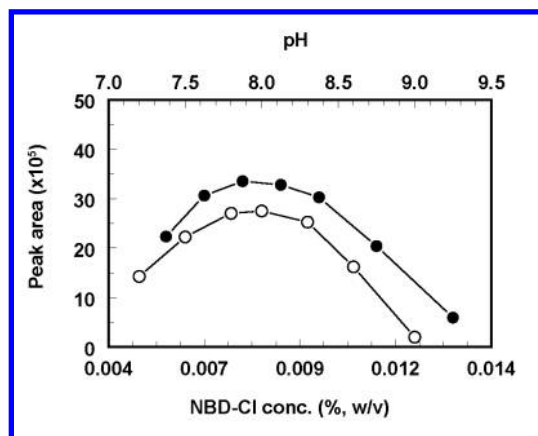

Figure 2.   Effect of NBD-Cl concentration (●) and pH (○) on the derivatization of PXT with NBD-Cl.
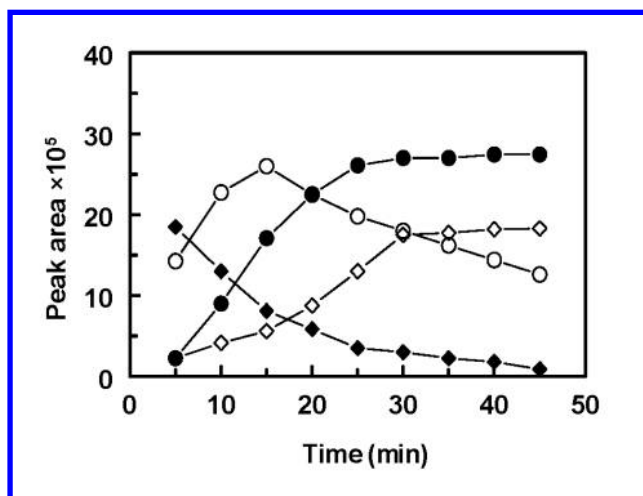
Figure 3. Effect of temperature and heating time on the derivatization of PXT with NBD-Cl. The temperatures investigated were 60 (◇), 70 (●), 80 (○), and 90°C (◆).

plasma samples were collected from a normal, healthy volunteer at King Khaled University Hospital (Riyadh, Saudi Arabia), and they were stored in a deep freezer at –20°C until analysis. All solvents were of HPLC grade (Merck, Darmstadt, Germany). All other materials were of analytical grade.

Preparation of Solutions

(**a**) *PXT standard solution.*—An accurately weighed amount (25 mg) of PXT was quantitatively transferred into a 50 mL volumetric flask, dissolved in 30 mL distilled water, and completed to volume with water to produce a stock solution of 0.5 mg/mL. This stock solution was further diluted with water to obtain a working standard solution of 600 ng/mL.

(**b**) *TMZ IS solution.*—An accurately weighed amount of TMZ (25 mg) was quantitatively transferred into a 50 mL volumetric flask, dissolved in 30 mL distilled water, and completed to volume with water to produce a stock solution of 0.5 mg/mL. This stock solution was further diluted with water to obtain a working standard solution of 40 ng/mL.

(**c**) *NBD-Cl derivatizing reagent.*—An accurately weighed amount of NBD-Cl (25 mg) was quantitatively transferred into a 50 mL volumetric flask, dissolved in 5 mL methanol, and completed to volume with methanol to produce a stock solution of 0.05% (w/v). The solution was freshly prepared daily and protected from light during use.

(**d**) *Borate buffer solution.*—Weighed amounts of 1.238 g boric acid and 1.490 g potassium chloride were dissolved in 100 mL distilled water. A volume of 8.0 mL of 0.2 M NaOH was added, and the mixture was diluted to 400 mL with distilled water. The pH of the solution was adjusted to 8 ± 0.1 by use of a calibrated pH meter (Microprocessor pH meter BT-500; Boeco, Hamburg, Germany).

General Derivatization Procedure

PXT standard solution (400 µL) and TMZ solution (200 µL) were transferred to a screw-capped reaction tube using micropipet. A volume of 1 mL borate buffer pH 8.0 and 300 µL NBD-Cl solutions was added. The tube was capped, swirled, and left to stand in a thermostatically controlled water bath (MLW type, Memmert GmbH, Co., Schwabach, Germany) at 70°C for 30 min. Then the tube was cooled rapidly, and a volume of 100 µL HCl was added. A volume of 20 µL of the resulting solution was injected into the HPLC system.

Sample Preparation and Construction of the Calibration Curve

The calibration standard samples were prepared by spiking blank human plasma with PXT to yield final concentrations of 10, 20, 40, 80, 150, 300, and 600 ng/mL. One milliliter of each sample was mixed with an equal volume of acetonitrile, mixed on a vortex mixer for 30 s, and centrifuged for 20 min at 13 000 rpm by a Heraeus Pico centrifuge (Thermo Fisher Scientific, Waltham, MA). The supernatants were isolated and manipulated as described under the *General Derivatization Procedure* section, and 20 µL was injected into the HPLC system. An eight-point calibration curve was constructed by plotting the peak area ratio of PXT to TMZ versus PXT concentration (x). Analysis of calibration samples at each concentration was performed in triplicate. Slope, intercept, and r values were calculated as regression parameters by linear regression. The linear regression equation was used to calculate the concentrations of PXT in spiked plasma based on their peak area ratios.

QC Samples

The QC plasma samples for determination of the accuracy and precision of the method were independently prepared at low (5 ng/mL), medium (80 ng/mL), and high (500 ng/mL) concentrations in the same manner as the calibration standards and stored at –20°C until use. A sample volume of 1 mL was thawed and analyzed at time intervals (0, 7, and 21 days) using TMZ as the IS. The system suitability parameters were evaluated each working day.

Results and Discussion

Design and Strategy for Assay Development

PXT contains a weakly absorbing chromophore in its molecule, thus its direct UV-based determination in plasma was not possible without preconcentration of the samples. Fluorescence-based HPLC has been used as a sensitive alternative approach. Therefore, the present work was directed to the development of an HPLC method with fluorescence detection. PXT has native fluorescence that was used in the development of HPLC methods with fluorescence detection for its determination in plasma (19–22). These methods offered adequate sensitivity; however, liquid–liquid extraction procedures were involved in preparation of the samples for avoiding the interferences coming from the
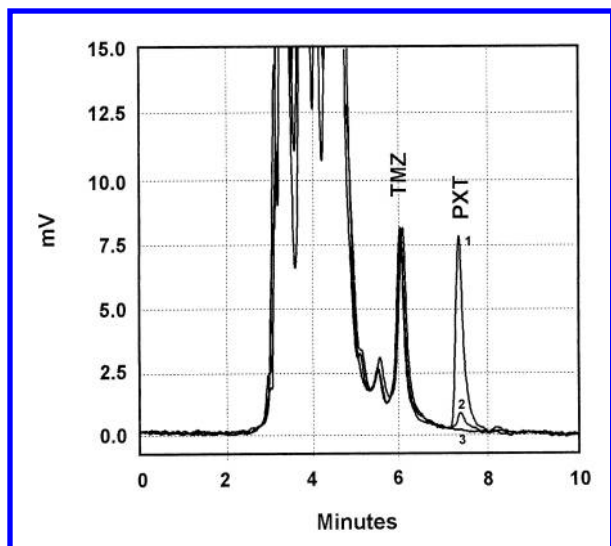
Figure 4. Representative chromatograms: (1) plasma spiked with PXT (40 ng/mL) and TMZ (5 ng/mL); (2) plasma sample spiked with PXT (5 ng/mL) and TMZ (5 ng/mL); and (3) blank plasma spiked with TMZ (5 ng/mL). The mV on the *y*-axis is the detector response in millivolts.

natively fluorescent coeluted plasma components. Therefore, precolumn derivatization of PXT was necessary for enhancement of the selectivity.

Derivatization with dansyl chloride was previously described for fluorescence-based HPLC determination of PXT, but the procedure still used a liquid–liquid extraction (23). For these reasons, the present work was directed to the development of a new HPLC method employing a nonextractive sample pretreatment procedure and an alternative derivatization reaction.

NBD-Cl is an activated halide derivative that has been used as a fluorogenic reagent for the determination of amino group-containing compounds (24–26). Darwish et al. (27) demonstrated the reactivity of PXT, via its secondary amino group, with NBD-Cl as a reagent. The PXT–NBD-Cl derivatives exhibited the highest fluorescence intensity at 530 nm after excitation at 470 nm. Therefore, the present study was devoted to adopting this reaction, for the first time, as a precolumn derivatization in the development of the method described herein. In our previous study (28), we demonstrated the high reactivity of NBD-Cl with TMZ via its piperazinyl N-H group, and the derivatized TMZ has the same excitation and emission fluorescence maxima as PXT. For these reasons, TMZ was adopted as an IS in the present study. Figure 1 shows the scheme of the reaction between PXT and TMZ with NBD-Cl reagent. The following sections describe the optimization of the experimental assay variables and validation of the assay performance.

Method Development

Optimization of the derivatization procedure.—Our experiments for the optimization of the derivatization procedure were initiated using a mobile phase consisting of acetonitrile–10 mM sodium acetate buffer (pH 3.5; 50 + 50, v/v). Optimum conditions for the reaction with respect to the concentration of the NBD-Cl reagent, pH of the reaction medium, type of buffer used, temperature, heating time, and the diluting solvent were investigated. Owing to the presence of labile chloride in the chemical structure of NBD-Cl, a daily fresh solution was used. In order to select the optimum NBD-Cl concentration, the reaction was carried out using varying concentrations (0.01–2%, w/v) of NBD-Cl reagent. The maximum detector signal was obtained when the concentration of NBD-Cl reagent in the reaction solution was 0.0075% (w/v; 300 μL of 0.05% solution; Figure 2). Therefore, this concentration was considered as optimum and used in all subsequent experiments. In order to generate the nucleophile from PXT, the reaction should be carried out in an alkaline medium. The dependence of the reaction on the pH of the reaction medium was studied using buffer solutions in the range of 7.2–9. The results indicated that the maximum detector signal was obtained at pH 8 ± 0.1 (Figure 2). Different buffer systems (borate, phosphate, and carbonate) of pH 8 were tested. The highest signals were obtained when the reaction was carried out using borate buffer. With carbonate buffer, precipitation of white colloid occurred upon addition of NBD-Cl reagent solution. This was attributed to the weak solubility of inorganic buffer components in organic solvent. With phosphate buffer, nonreproducible results and/or weak sensitivities were observed. Studies for optimization of the ionic strength of borate buffer revealed that the optimum concentration was 0.2 M. To determine the temperature and time required for completion of the reaction, the derivatization reaction was carried out at varying temperatures, and the induced signals were monitored at different time intervals. The results indicated that the reaction was dependent on temperature, and complete reactions were achieved after 15 and 30 min at 80 and 70°C, respectively (Figure 3). The signals at 80°C were not reproducible; thus, further experiments were carried at 70°C. As seen from the results (Figure 3), the maximum fluorescence intensities of the final solutions at 90°C were lower than those obtained at lower temperatures, and a rapid progressive decrease in the readings was observed as the reaction time increased. This was attributed to the degradation of the reagent at high temperature. This observation was coincident with the results reported by Aktas et al. (29). Under these conditions, significantly high fluorescence background was also observed. This was attributed to the hydrolysis of NBD-Cl to the corresponding hydroxy derivative, 7-hydroxy-4-nitrobenzo-2-oxa-1,3-diazole (NBD-OH; 30). The fluorescence of NBD-OH was found to be quenched by decreasing the pH of the reaction medium to less than 1 (31). Therefore, acidification of the reaction mixture prior to its injection into the HPLC system was necessary to remarkably decrease the background signal. Meanwhile, the reaction product was not affected; thus, the sensitivity was increased. The amount of HCl required for acidification was found to be 0.1 mL/2 mL reaction volume.

Table 1. Chromatographic parameters for determination of PXT in spiked human plasma

Parameter	Value
Retention time of PXT, min	7.46 ± 0.12
Retention time of TMZ (IS), min	6.13 ± 0.09
Capacity factor of PXT (k′), min	1.487
Resolution of PXT and IS peaks	2.9263
Peak asymmetry at 10% peak height	1.42
Number of theoretical plates	4311
Height equivalent to a theoretical plate, μm	57.99

Optimization of the chromatographic conditions.—Separation was performed in the isocratic mode. The separation of PXT and TMZ (IS) could be adjusted by changing the composition of the mobile phase. Several experiments have shown that use of an appropriate ratio of sodium acetate buffer solution in the mobile phase may improve the chromatographic peaks (32). Reciprocal variations of sodium acetate and acetonitrile contents in the mobile phase from 40 + 60 to 70 + 30 (v/v) were employed. These ratios produced retention times of 6.0–11.5 and 5.2–7.0 min for PXT and TMZ, respectively. Both PXT and TMZ were retained longer on the column as the aqueous content of the mobile phase increased, however PXT was affected more than TMZ. The best resolution was achieved when the content of aqueous sodium acetate–acetonitrile was 47 + 53 (v/v). It was found that the use of 6% methanol in the mobile phase gave better resolution and symmetrical peaks. Different concentrations of sodium acetate buffer solution at levels of 10, 20, 30, and 40 mM were tested in the mobile phase. The results showed that the lowest concentration of 10 mM sodium acetate buffer was able to improve the symmetry of the chromatographic peaks of both PXT and TMZ. The acidic pH of the mobile phase was beneficial to reduce the significantly high fluorescence background as mentioned above. TMZ was found to be quantitatively derivatized by NBD-Cl under the same conditions required for PXT, and it showed good resolution and chromatographic profile with the applied chromatographic conditions. Another reversed-phase column was also tested, μ-Novapak C18, 150 mm length × 3.9 mm id, 5 μm particle diameter (Waters, Milford, MA). Upon using this column and the optimized mobile phase composed of acetonitrile–10 mM sodium acetate (pH 3.5)–methanol (47 + 47 + 6, v/v), PXT was not eluted within the run time (10 min), but TMZ was eluted at about 4.3–4.6 min. Therefore, the Nucleosil CN column was selected for all subsequent work. Under these chromatographic conditions, the run time of the sample was 10 min, and the retention times were 7.46 ± 0.12 and 6.13 ± 0.09 min (n = 5) for PXT and TMZ, respectively (Figure 4).

Method Validation

Selectivity, linearity, LOD, and LOQ.—The selectivity of the method was evaluated by carrying out blank experiments in the mobile phase and different batches of blank human plasma to identify the reagent peaks and the peaks due to the derivatized biogenic plasma components. Typical chromatograms obtained from blank plasma (containing 5 ng/mL TMZ and plasma spiked with the same concentration of TMZ and PXT (0, 5, and 40 ng/mL) are shown in Figure 4. The chromatograms showed complete separation of PXT and TMZ from the reagent and endogenous plasma constituents. The chromatographic performance parameters of the PXT and TMZ are presented in Table 1.

Under the above optimum conditions, linear relationship with good correlation coefficient (r = 0.9998, n = 7) was found between the peak area ratio of PXT to that of TMZ (Y) versus PXT concentration (X) in the range of 5–600 ng/mL. The mean regression equation (mean ± SD, n = 7) of the calibration curve obtained from seven points was:

$$Y = -0.0097 \, (\pm 0.0041) + 0.0099 \, (\pm 0.0002)X$$

The RSD for the slopes of the calibration curves was 1.69% (n = 5). The LOD and LOQ were calculated according to the International Conference on Harmonization (ICH) guidelines for validation of analytical procedure based on the SD of the response and the slope of the calibration curve (33) using the formula:

$$\text{LOD or LOQ} = \kappa \times SDa/b$$

where κ = 3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and SDb is the slope of the calibration curve. Calculations for five replicate experimental injections gave LOD and LOQ of 1.37 and 4.14 ng/mL, respectively. The high sensitivity of the proposed method and its wide dynamic range allowed the analysis of the clinical specimens without preconcentration or dilution; the clinical therapeutic range of PXT concentrations in plasma is 10–30 ng/mL (5).

Precision.—Intra-assay precision was studied at three concentration levels (5, 80, and 500 ng/mL) of PXT and 5 ng/mL of TMZ (IS). Six aliquots were prepared at each concentration level and injected into the HPLC system. The RSD calculated for the values of the peak areas corresponding to the IS during the study was 3.17% (n = 18; Table 2). The RSDs calculated for the values of the retention time corresponding to the chromatographic peaks were 1.61 and 1.47% for PXT and the IS, respectively. The RSD of the determinations were 3.62, 0.41, and 0.86% for 5, 80, and 500 ng/mL, respectively.

Interassay precision evaluation was carried out on five different days at the same concentration levels for spiked plasma samples freshly prepared daily. The RSDs calculated for the values of the retention time corresponding to the chromatographic peaks were 2.33 and 1.78% for PXT and IS, respectively. The RSD of the determinations were 4.20, 1.10, and 1.95% for 5, 80, and 500 ng/mL, respectively.

Accuracy.—The accuracy of the proposed method was assessed by recovery studies. Plasma samples spiked at different concentration levels (5, 10, 40, 80, 120, 240, and

Table 2. Intra-assay and interassay precision and accuracy for determination of PXT in spiked human plasma

Nominal concn, ng/mL	Intra-assay		Interassay	
	Measured concn, ng/mL	Recovery ± RSD, %	Measured concn, ng/mL	Recovery ± RSD, %
5	4.86	97.28 ± 3.62	4.80	96.93 ± 4.20
10	10.44	104.38 ± 2.93	10.20	102.04 ± 4.00
40	40.37	100.92 ± 1.13	39.96	99.89 ± 2.67
80	79.94	99.92 ± 0.41	80.83	101.03 ± 1.10
120	120.58	100.48 ± 0.88	121.26	101.05 ± 1.74
240	238.84	99.52 ± 0.97	241.16	100.48 ± 1.48
500	498.19	99.64 ± 0.86	492.16	98.43 ± 1.95

500 ng/mL) of PXT and 5 ng/mL for the IS were analyzed by the proposed method. The values of the ratios between PXT and the IS were then interpolated using the linear regression equation computed in the linearity study to calculate the experimental concentration values. The recovery values were presented as percentages, calculated by the formula:

(Calculated concentration/nominal concentration) × 100

The recovery values ranged from 97.28 to 104.38 (± 0.41–3.62%), indicating the good accuracy of the method.

Interference studies.—As the proposed method was developed for the therapeutic monitoring of PXT in plasma, the potential possible interferences from co-administered drugs were studied. According to the current American Psychiatric Association practice guidelines, selective serotonin reuptake inhibitors are considered as the first-line agents in the treatment of panic disorder, as they have the most favorable balance of efficacy and adverse effects (34). However, recent studies (35, 36) reported evidence for the more rapid and earlier antipanic efficacy of PXT/clonazepam co-administration in the acute phase of panic disorder therapy. Also, König et al. (37) and Yamauchi et al. (38) described olanzapine and risperidone (atypical antipsychotic agents) in a combination therapy with PXT to control anxiety symptoms. The interferences from these co-administered therapeutic agents were investigated, and the results revealed that none of these agents causes any interference with PXT determination. This was attributed to the following facts: (*1*) olanzapine and risperidone (tertiary amines) and clonazepam (amide) are not derivatized by NBD-Cl reagent into fluorescent derivatives; (*2*) olanzapine and risperidone do not exhibit native fluorescence in the acidic mobile system employed in PXT analysis; and (*3*) clonazepam is weakly fluorescent in neutral or acidic medium (39). But the excitation and emission wavelengths (425 and 368 nm, respectively) are far away from those employed in the proposed PXT method.

Robustness and ruggedness.—In order to measure the extent of the method robustness, the most critical parameters were changed while keeping the other parameters unchanged. The following chromatographic parameters were varied within the range of 1–10% of the optimum recommended conditions: the pH of the mobile phase, percentage of methanol, concentration and pH of the sodium acetate buffer, pH of the derivatization reaction mixture, and column temperature. The chromatographic profile, including capacity factor (k'), retention time (R_t), peak asymmetry, resolution, column efficiency, and peak width, were calculated and compared with the system suitability parameters (Table 1). The results revealed that the method was robust for these small changes in methanol content, concentration of sodium acetate in the mobile phase, and pH in the range of 3.2–3.7. With respect to the pH of the derivatization reaction, the results did not significantly change in the range of 7.8–8.2. However, increasing the pH value above 8.3 resulted in a dramatic decrease in the detector signal. The increase in the column temperature generally decreased the k' values, and the column temperature had to be maintained at 25 ± 5°C.

The ruggedness of the method was evaluated by application of the recommended analytical procedures on the same HPLC system by two of the authors of the study, independently on different days, for the analysis of the series of PXT samples. The RSD values of the k', R_t, and peak areas obtained by the two authors were not more than 2%.

Stability of PXT in plasma.—The stability of PXT in plasma was studied under a variety of storage and handling conditions at low, medium, and high concentration levels (5, 40, and 120 ng/mL, respectively). The short-term temperature stability was assessed by analyzing three aliquots of each concentration level that were thawed at room temperature (25 ± 5°C) and kept at this temperature for 6 h. Freeze-thaw stability (–20°C in plasma) was checked through three cycles. Three aliquots at each concentration level were stored at –20°C for 24 h and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 24 h under the same conditions. The freeze-thaw cycles were repeated three times, and then samples were analyzed on the third cycle. The long-term stability was determined by analyzing three aliquots of each concentration level stored at –20°C for 21 days. The results revealed the stability of PXT under the tested conditions, as the RSD of the recovered PXT was 0.57–3.82%.

Post-preparative stability of PXT was tested by processing stock samples obtained from plasma samples spiked with

80 ng/mL PXT and stored at room temperature on the bench top. The samples were assayed immediately and 1, 3, 6, 12, and 24 h after processing. RSDs calculated for the recovered PXT concentration were 0.34, 1.28, 0.84, 1.61, and 2.19%, respectively. These results indicated the stability of derivatized PXT and the suitability of the system for processing a large number of samples without a negative effect on the analytical results.

Conclusions

This paper describes for the first time the development of a very simple, nonextractive HPLC method with fluorescence detection for the determination of PXT after its precolumn derivatization with a NBD-Cl reagent. The proposed method yielded reliable analytical results for even low concentrations of PXT. The derivatized sample was directly injected into the HPLC system in isocratic elution mode, and the 10 min run time resulted in high throughput for the method. The analytical results demonstrated that the proposed method is suitable for the accurate quantitation of PXT in human plasma. The performance of the method makes it valuable for the therapeutic monitoring of PXT in human subjects after oral administration of therapeutic doses.

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