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# Quantitative determination of paroxetine in plasma by high-performance liquid chromatography and ultraviolet detection

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**Abstract**

An accurate, reliable procedure was developed for kinetic and therapeutic monitoring of paroxetine in human plasma. Steady-state plasma levels of paroxetine were measured for 18 geriatric patients (mean age 75) in a double-blinded study. Paroxetine doses ranged from 10 to 40 mg/day. The assay was suitable for patients on concurrent medications, and a small sample volume (1 ml) of patient plasma was used with sufficient sensitivity and specificity. After extraction and separation on a Beckman, Ultrasphere 5-μm $C_{18}$ column (150×2 mm I.D.), the recovery (mean±S.D.) for paroxetine was determined to be 86.5±5.2%. The limit of quantitation for paroxetine in this assay was 5 ng/ml. Inter-assay reproducibility (C.V.) for the patient samples and quality controls ranged from 3.7 to 7.6%.

*Keywords:* Paroxetine

## 1. Introduction

Therapeutic drug monitoring (TDM) of tricyclic antidepressants (TCAs) is quite common, because it has been shown to enhance therapeutic efficacy and prevent serious adverse effects [1,2]. The benefit of TDM with selective serotonin reuptake inhibitors (SSRIs) is still a matter of debate. Paroxetine, a new SSRI antidepressant, which has the highest activity in the inhibition of the reuptake of serotonin is now used for a variety of psychiatric conditions [3]. Paroxetine's chemical structure is unrelated to the TCAs or other SSRIs and as a result can have the potential for different drug interactions [3]. Unlike other SSRIs, paroxetine has a comparatively high affinity for muscarinic receptors in vitro, clinically, anticholinergic side effects are dose-related [4]. The metabolism of paroxetine is to a unstable catechol with further methylation, while the major metabolite in humans is rapidly conjugated to glucuronide and sulfate esters [5,6]. This metabolite is found only in small amounts in the body and has insignificant serotonin reuptake inhibition. Sometimes the metabolite can accumulate in the body indicating abnormal phase II metabolism which could be caused by liver disease [7] or drug interactions [8]. Previous methods have been reported for analysis of paroxetine and its metabolites including gas chromatography (GC) [9] and high-performance liquid chromatography (HPLC) [10,11]. These methods are very laborious, time consuming and require complex equipment to achieve results. Previous procedures have also required derivatization and extensive sample pretreatment before any analysis could be done [9,10].

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We have designed a simple extraction and sensitive HPLC method to analyze paroxetine with adequate sensitivity for TDM or kinetic analysis. The method could be modified to separate other SSRIs or any potential interfering TCAs in plasma.

## 2. Experimental

### 2.1. Materials

Ethyl acetate, *n*-heptane, acetonitrile and methanol were obtained from Burdick and Jackson (Obetz, OH, USA). Potassium phosphate and 85% phosphoric acid, HPLC grade, were from Fisher Scientific (Pittsburgh, PA, USA) and *n*-octylamine was purchased from Fluka (Ronkonkoma, NY, USA). The remaining chemicals used were analytical grade. Paroxetine HCl was purchased from SmithKline Beecham (Worthing, UK) and fluoxetine HCl was purchased from Alltech-Applied Science (State College, PA, USA).

### 2.2. Apparatus

The analysis was performed on a Applied Biosystems Model 759A absorbance detector with a Perkin-Elmer series 200 HPLC pump. The wavelength is set at 205 nm, with a range of 0.01 and a rise time of 1.0. A straight, stainless steel column 150×2 mm I.D., packed with 5 μm Ultrasphere $C_{18}$, Beckman column (Rainin Instrument, Woburn, MA, USA) was used at room temperature. The autosampler was a Perkin Elmer ISS-200 (Perkin-Elmer, Pittsburgh, PA, USA) which was fitted with a 7126 Rheodyne injector with peak tubing and a 50-μl loop (Rainin Instruments). A Spectra-Physics 4400 integrator was used for data gathering and reduction.

### 2.3. Human plasma samples

Human plasma samples were obtained from elderly patients with depression who were treated with paroxetine. Patients were initially placed on 10 mg of paroxetine for 3 days and were titrated to an appropriate dose according to adverse side effects or Hamilton ratings. Plasma was separated by centrifugation in an EDTA tube for 10 min at 3000 *g* and stored at −20°C.

### 2.4. Extraction procedure for plasma samples

The analytical method for HPLC determination of paroxetine is as follows: 1 ml of plasma is added to a 15-ml screw-capped polypropylene tube (Fred Morrow Scientific, New Milford, NJ, USA) containing 10 μl of a 1 mg/ml stock of fluoxetine as the internal standard. The stock standards were stored in methanol at −20°C, then diluted in 0.025 *M* $KH_2PO_4$ at a pH of 2.4 for the addition to the standard curve. To each sample, 0.5 ml of carbonate buffer pH 10.5 was added and 5 ml ethylacetate–*n*-heptane (2:8). This mixture was shaken for 2.5 min and centrifuged at 3000 *g* for 10 min. The organic layer was transferred to another 15-ml conical polypropylene Falcon tube containing 125 μl of 0.025 *M* $KH_2PO_4$ at a pH of 2.4. This mixture was again shaken for 2.5 min and centrifuged for 10 min at 3000 *g*. The top organic layer was discarded by aspiration and the remaining aqueous phosphate layer was placed in a savant evaporator for 90 min or until dryness. The paroxetine is reconstituted in 125 μl of 0.025 *M* $KH_2PO_4$ and transferred into autosampler vials for 50-μl injections into the autosampler.

### 2.5. Chromatography conditions

The mobile phase for paroxetine analysis was 0.02 *M* potassium dihydrogen phosphate buffer, filtered, pH 2.5 with 85% phosphoric acid (HPLC grade), acetonitrile and 125 μl/l octylamine (62:38). The flow rate was maintained at 0.35 ml/min. The column was maintained at room temperature. The retention times of paroxetine and fluoxetine HCL were 4.22 and 6.44 min, respectively.

### 2.6. Recovery and linearity

The linearity of the assay was determined between 5 and 200 ng/ml for paroxetine. The recovery of paroxetine was performed at three different concentrations in blank plasma. The recovery from spiked plasma would be calculated by standards which were made in 125 μl of 0.025 *M* $KH_2PO_4$.

Table 1
Assay recovery for paroxetine from spiked plasma at different concentrations ($n=5$)

| Concentration (ng/ml) | Recovery (%) | C.V. (%) |
|---|---|---|
| 7 | 85.20 | 11.00 |
| 40 | 92.20 | 8.24 |
| 100 | 82.00 | 9.00 |
| Fluoxetine (100) | 85.17 | 4.85 |

## 3. Results

Ultraviolet detection of paroxetine by this method is sensitive enough for therapeutic monitoring of patients dosed as low as 10 mg per day. Optimization of the HPLC conditions was done by using different detector wavelengths and various aqueous to organic mobile phase ratios to achieve maximum peak height and resolution of paroxetine and the internal standard. The use of a mini-bore column was to enhance sensitivity, reduce the separation time, and conserve the total mobile phase volume. The addition of octylamine to the mobile phase was to increase resolution between the peak of interest and internal standard. The limit of quantitation of paroxetine was 5 ng/ml with a signal-to-noise ratio of 6:1. The recoveries (Table 1) at three different concentrations had an average of 86.5%±5.2. The linearity of the ultraviolet assay over a range of 5 to 200 ng/ml for paroxetine was shown with a correlation coefficient of 0.9994 ($y=0.0156x-0.0102$). The inter-assay reproducibility of spiked plasma from the standard curve (Table 2) C.V. range for paroxetine was 5.4 to 3.4%. The reproducibility of controls is shown in Table 3, where the C.V. range for paroxetine was 6.1 to 7.6%. The inter-assay reproducibility of patient plasma levels at steady-state on 10 mg for three days then on a 20-mg dose is described in Table 4, where the C.V. range for paroxetine was 3.7 to 5.0%. Since our total patient plasma volume was 3 ml, the same patient sample was run on various days to determine the inter-assay reproducibility. These elderly patients were part of a double-blinded study in which subjects were randomized to either nortriptyline or paroxetine in their treatment of depression. All patient plasma samples analyzed were from patients receiving 0, 10, 20, 30 or 40 mg of paroxetine daily. Potential drug interferences were investigated and Table 5 lists those drugs that had retention times and peak heights that could distort the chromatography for this assay. Alteration of the mobile phase to avoid these interfering drugs would distort the chromatographic separation, resolution and sensitivity of paroxetine and the internal standard, fluoxetine. Drugs that were tested under the same conditions that did not produce any detectable peak heights to interfere with paroxetine are as follows: sertraline, rantidine, theophylline, propranolol, chlorpheniramine, diltizam, clomipramine, doxepin, mesoridazine, metoprolol and perphenazine.

Table 2
Inter-assay reproducibility of paroxetine from spiked plasma ($n=5$)

| Concentration (ng/ml) | Peak-height ratio (mean) | C.V. (%) |
|---|---|---|
| 5 | 0.0773 | 5.40 |
| 10 | 0.1877 | 3.59 |
| 20 | 0.3132 | 4.11 |
| 50 | 0.7415 | 4.98 |
| 100 | 1.6424 | 4.00 |
| 200 | 3.1078 | 3.38 |

Table 3
Interassay reproducibility of quality control spiked plasma at 3 different concentrations ($n=7$)

| Actual | Mean (ng/ml) | S.D. | C.V. (%) |
|---|---|---|---|
| 15 | 14.71 | 1.11 | 7.55 |
| 75 | 73.00 | 5.54 | 7.59 |
| 150 | 161.00 | 9.80 | 6.09 |

Table 4
Interassay reproducibility of paroxetine from patients on 20 mg daily

| Patient code | Assay 1 | Assay 2 | Assay 3 | C.V. (%) |
|---|---|---|---|---|
| 1 | 83 | 85 | 79 | 3.71 |
| 4 | 50 | 53 | 48 | 5.00 |

Table 5
Possible paroxetine drug interferences

| Drug | Time (min) |
|---|---|
| Verapamil | 4.26 |
| Haloperidol | 3.31 |
| Triazolam | 4.30 |
| Alprazolam | 3.96 |
| Amitriptyline | 4.60 |
| Desipramine | 3.48 |
| Lorazepam | 3.90 |

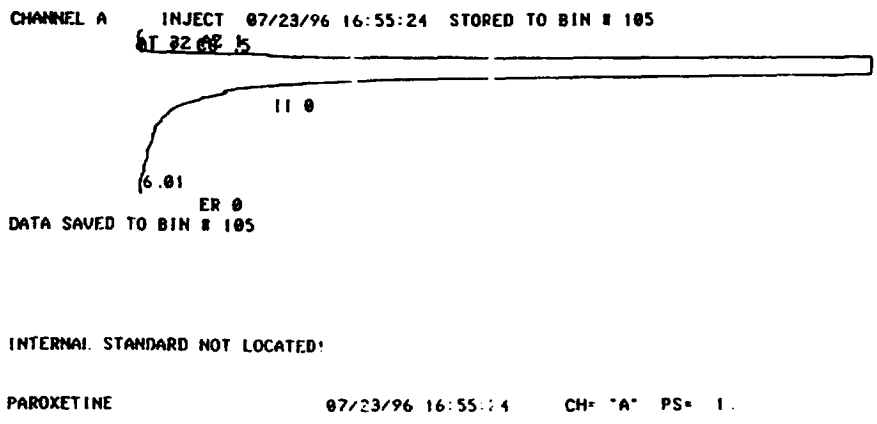


Fig. 1. Chromatogram of a blank plasma extract.

Figs. 1–3 show chromatograms of an extracted blank, low control (15 ng/ml) and a patient receiving 20 mg/day of paroxetine to demonstrate the resolution and detection of our drug of interest.

## 4. Discussion

Paroxetine is a potent new SSRI antidepressant and the monitoring of the plasma levels of this drug in depressed geriatrics is potentially important for the interpretation of issues concerning therapeutic efficacy, adverse effects and compliance. The assay is both reproducible and accurate for clinical and kinetic samples. The paroxetine assay had an average recovery of 86.5% at three different concentrations. The limits of quantitation were sufficiently low enough for the doses (10 to 40 mg/day) of paroxetine administered, which is the usual dosages for most clinical situations. The inter-assay reproducibility was consistent for all dosages, however, the plasma levels were not correlated to clinical response in this small sample. The patients on paroxetine at 20 mg/day had a wide range (20–110 ng/ml) of plasma levels which could be the result of differential metabolism by the CYP2D6 isozyme. The plasma sample volumes of the assay were low (1 ml) for steady-state, which is advantageous for studies involving the geriatric population. The assay has high specificity, even when patients were found to be on multiple medications. The monitoring of paroxetine plasma levels is important to prevent side effects since the drug is both a substrate/inhibitor of CYP2D6 with the potential for saturation kinetics

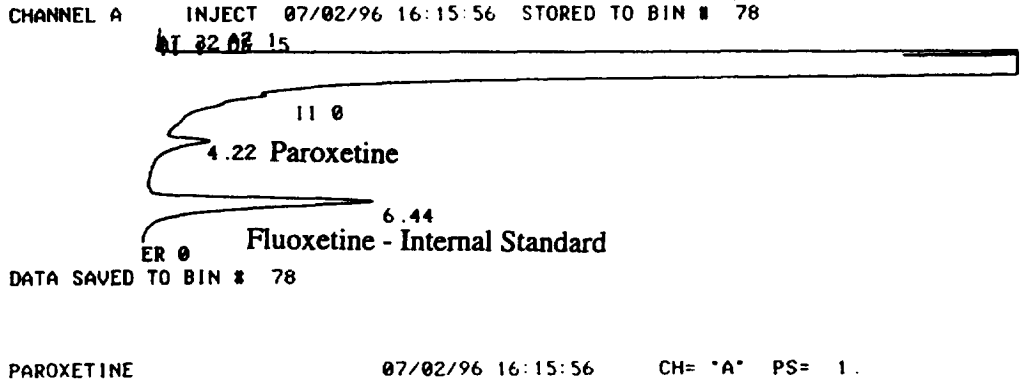


Fig. 2. Chromatogram of a plasma extract of a paroxetine low control shown at the concentration of 15 ng/ml.

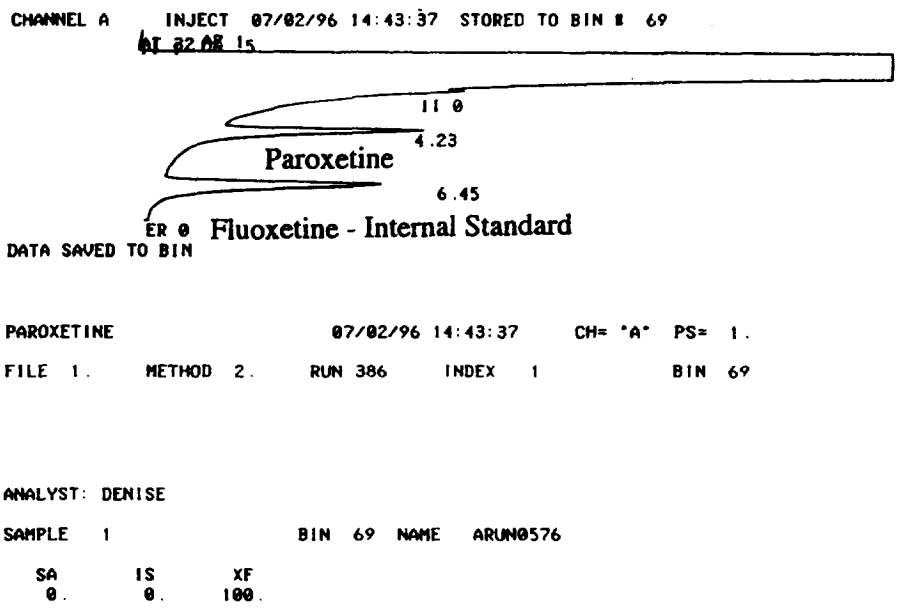


Fig. 3. Chromatogram of a plasma extract of a patient given 20 mg/day of paroxetine. A paroxetine plasma level of 61 ng/ml was detected.

and increased liability to anticholinergic side effects in the geriatric population.

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