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Short communication

Column liquid chromatographic determination of paroxetine in human serum using solid-phase extraction

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Abstract

A 0.5-ml aliquot of a serum sample, after the addition of a 100-μl aliquot of a 5 μg/ml solution of dibucaine as the internal standard, is vortex-mixed with 0.5 ml of acetonitrile and centrifuged. The supernatant is applied to a 1-ml BondElut C_{18} silica extraction column conditioned with subsequent washings with 1 *M* HCl, methanol and water. After passing the sample at a slow rate, the column is washed twice with water and once with acetonitrile. The desired compounds are then eluted with a 0.25-ml aliquot of 35% perchloric acid–methanol (1:40, v/v). A 7-μl aliquot of the eluate is injected onto a 150 × 4.6 mm I.D. column packed with 5-μm C_8 silica particles and eluted at ambient temperature with a mobile phase of 10 m*M* phosphate buffer–acetonitrile (2:1, v/v) (pH 3.2). The peaks are detected with a fluorescence detector (excitation at 295 nm, emission at 365 nm). The resulting chromatogram is clean with no extraneous peaks. Paroxetine and dibucaine give sharp peaks which are well separated from each other and from the solvent peaks. The extraction recovery of the drug and the internal standard is in the range of 90% which allows a highly sensitive determination of paroxetine.

1. Introduction

Paroxetine (Paxil), a selective serotonin-reuptake inhibitor, was recently approved in Canada for the treatment of depression. It has been claimed that paroxetine is as effective as tricyclic antidepressants, e.g. imipramine [1], but has much reduced side effects [2]. Paroxetine has no active metabolites [3]. No clear relationship, as yet, has been established between clinical or toxic response and serum drug concentration. However, paroxetine is a good candidate for therapeutic monitoring because of the lack of active metabolites and the wide range of serum drug concentrations observed in patients receiving the same dose of paroxetine [4]. Serum paroxetine concentration is potentially useful to identify patients who require higher than the standard 20-mg daily dose of the drug and to monitor compliance.

Brett et al. [5] have described a column liquid chromatographic (LC) procedure involving liquid–liquid extraction and pre-column preparation of a dansyl derivative for the sensitive fluorescence detection of paroxetine. The present study uses an alternative LC procedure which involves a rapid and convenient solid-phase extraction recently described for the determination of antidepressants [6], to assay paroxetine.

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2. Experimental

2.1. Reagents

All reagents were of analytical grade. Deionized water was distilled in an all-glass still.

Paroxetine hydrochloride hemihydrate (87.4% free base) was obtained as a gift from SmithKline Beecham Pharma (Oakville, Ont., Canada). Dibucaine hydrochloride was obtained from Sigma (St. Louis, MO, USA). Stock solutions of paroxetine and dibucaine were prepared in methanol at a concentration of 1 mg/ml. The solutions were stored at −10°C.

Serum standard of paroxetine (1 μg/ml) was prepared by mixing 50 μl of stock paroxetine solution in a 50-ml volumetric flask and making up to volume with drug free serum. This standard was serially diluted to prepare eight serum standards. The concentration of the most dilute standard was 7.8 ng/ml.

Working internal standard solution was prepared by mixing 50 μl of stock dibucaine solution with 10 ml of 1% potassium bicarbonate solution. The solution was stored at 4°C for one week and then discarded.

2.2. Extraction

A 0.5-ml aliquot of the sample was mixed with 100 μl of the working internal standard solution and 0.5 ml of acetonitrile in a 12 × 75 mm glass disposable tube. After centrifugation at 1500 *g* for 3 min, the supernatant was applied to a 1-ml BondElut C_{18} extraction column (Varian Associates, Harbor City, CA, USA) which had been previously activated by washing successively once with 1 *M* HCl, twice with methanol and once with water. The sample was passed slowly through the column by mild suction. The column was then washed successively twice with water and once with acetonitrile, making sure that each column was drained completely after every wash. The tips of the columns were wiped with tissue and placed on 16 × 100 mm glass tubes containing correspondingly labelled 1.5-ml plastic sample cups. An aliquot of 0.25 ml of methanol containing 2.5 ml/100 ml of 35% perchloric acid was applied to each column. The liquid was allowed to pass through the column bed by gravity and finally drained completely by centrifugation at 1000 *g* for 30 s. The cups were covered with aluminium foil and loaded in the autosampler. A 7-μl aliquot of the eluate was injected onto the chromatographic system.

2.3. Chromatography

A modular chromatographic system consisting of a Model LC-6A pump, a Model RF-535 fluorescence detector, a Model Sil-9A autosampler and a Model CR501 integrator plotter (all from Shimadzu Scientific Instrument Co., Columbia, MD, USA) was used. A 150 × 4.6 mm I.D. Ultrasphere Octyl reversed-phase silica column packed with 5-μm bonded silica particles (Beckman Instruments, San Ramon, CA, USA) protected by a 15 × 3.2 mm I.D. guard cartridge packed with 7-μm C_8-silica particles (Applied Biosystems, San Jose, CA, USA) was used as the analytical column. The mobile phase of 10 m*M* KH_2PO_4–acetonitrile (2:1, v/v), adjusted to pH 3.2 with 50% phosphoric acid was pumped at a flow-rate of 1.2 ml/min with an operating pressure of 8.2 MPa. Chromatography was performed at ambient temperature.

3. Results and discussion

3.1. Detection

The fluorescence detector Model Rf-535 used in this study is 5 to 6 times more sensitive than the detector Model RF-530 used by Brett et al. [5]. Paroxetine shows optimal fluorescence at an excitation wavelength of 295 nm and emission wavelength of 350 nm. On the other hand dibucaine shows optimal fluorescence at excitation and emission wavelengths of 330 and 410 nm, respectively. At the selected excitation and emission wavelengths of 295 and 365 nm, there is ca. 15% decrease in the fluorescence response of paroxetine and 90% decrease in that of dibucaine. Therefore, a relatively large amount of dibucaine (500 ng) is used as the internal stan-

dard for adequate sensitivity. Excess amount of dibucaine is helpful to minimize loss of the analytes due to adsorption. A number of commercially available fluorescent compounds were screened for use as the internal standard for the assay of paroxetine. Dibucaine appeared to be the most suitable. It seems that analogues of paroxetine have not been synthesized as Brett et al. used only maprotiline as the internal standard for the determination of paroxetine. Maprotiline does not have any native fluorescence under conditions for optimal detection of native fluorescence of paroxetine. Paroxetine can also be detected by measuring absorbance at 210 nm. For adequate sensitivity 25 μl of the eluate are injected. For UV detection, only 1 μg/ml of debucaine is used as the internal standard.

3.2. Method validation

The recovery of extraction was determined by comparing peak areas of serum extracts with those of unextracted standards prepared in the elution reagent. The recovery was ca. 90% (range = 88–94%). There is no change in the ratio of peak areas of drug/internal standard after extraction.

The standard curve is linear for the range tested, 7.8 ng/ml–1 μg/ml. The linear regression of peak-area ratios of drug/internal standard (y) vs. drug concentration (x) is excellent: $y = 0.003 + 0.651x$ ($r = 1.0$). Analysis of serum supplemented with paroxetine added at high concentration showed a within-batch C.V. of 2.9% (mean = 407 ng/ml) and a between-batch C.V. of 4% (mean = 400 ng/ml). Analysis of serum supplemented with low concentration of paroxetine showed a within-batch C.V. of 7% (mean = 22 ng/ml) and a between-batch C.V. of 9.1% (mean = 21 ng/ml). In all cases $n = 8$. Drug free serum spiked with a nominal concentration of 50 ng/ml of paroxetine, when analyzed in duplicate, gave an average value of 49 ng/ml. A negative bias of 2% between the spiked and the observed values indicate that the procedure is fairly accurate.

The chromatogram of an extract of drug free serum sample (Fig. 1A) shows a very stable base

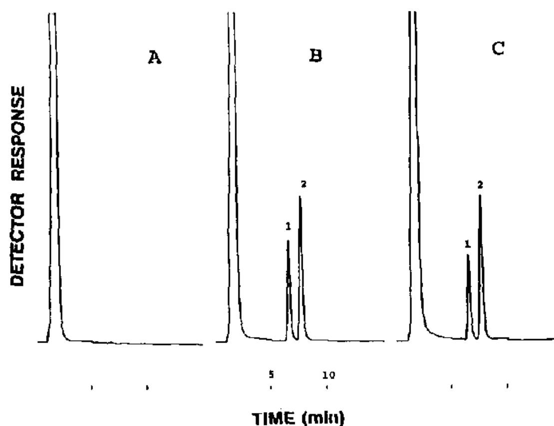

Fig. 1. Chromatograms of extracts obtained from (A) drug-free serum; (B) serum supplemented with 100 ng/ml of paroxetine; (C) patient serum receiving a daily dose of 30 mg of paroxetine. Peaks: 1 = paroxetine; 2 = dibucaine (internal standard). Detector: signal output = 1 mV; sensitivity = high; attenuation = 4; response time = slow. Integrator: attenuation = 2; chart speed = 4 mm/min.

line and virtually no extraneous peaks after the solvent peak. The chromatogram of an extract of a serum standard supplemented with 100 ng/ml of paroxetine (Fig. 1B) shows good separation of the drug and the internal standard from each other and from the solvent peak. A chromatogram of an extract of serum obtained from a patient receiving a daily dose of 30 mg of paroxetine for more than 3 weeks (Fig. 1C) does not show any additional peaks after the solvent peaks.

The limit of quantitation under the described conditions is 5 ng/ml which is adequate for therapeutic monitoring. However, the sensitivity of detection can be increased in a number of ways. The detector attenuation can be decreased without significantly affecting the baseline, a larger volume of eluate (upto 40 μl) can be injected without distorting the peaks, and a sample volume larger than 0.5 ml can be extracted without affecting recovery or producing extraneous peaks.

3.3. Selectivity of the assay

Acidic, neutral and weakly basic compounds are removed by the described extraction pro-

cedure. Thus, commonly prescribed drugs like acetamiophen, salicylate, benzodiazepines and antiepileptic drugs are completely eliminated. However, if present other strongly basic drugs, e.g. antidepressants, antihistamines, phenothiazines and antiarrhythmic drugs, are co-extracted with paroxetine. Measuring native fluorescence is much more specific than measuring absorbance at 210 nm or measuring fluorescence of dansyl derivatives. However, measuring native fluorescence is also not absolutely specific. Possible interference of a number of basic drugs, particularly other antidepressant drugs, was also investigated. Protriptyline, a rarely prescribed antidepressant interferes with the assay of paroxetine at any concentration by the described procedure. Up to 400 ng/ml the commonly prescribed drugs imipramine and desipramine do not interfere with the internal standard or with paroxetine. However, at concentrations higher than 400 ng/ml, desipramine interferes with paroxetine and imipramine with the internal standard. Trimipramine and its metabolite desmethyltrimipramine also show weak fluorescence response. However, they do not interfere with the paroxetine assay as they are well separated from the peaks of interest.

4. Conclusions

The described procedure presents a suitable and practical alternative LC procedure for the determination of paroxetine in serum. The availability of sensitive fluorescence detectors has obviated the need for the preparation of the dansyl derivative of paroxetine.

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