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Short Communication

Determination of sertraline and desmethylsertraline in human serum using copolymeric bonded-phase extraction, liquid chromatography and gas chromatography–mass spectrometry

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Abstract

The determination of the new antidepressant drug sertraline and its main metabolite, desmethylsertraline, in human serum is described. A new solid-phase extraction method employing the dual functionality Clean Screen® cartridge is presented followed by reversed-phase liquid chromatographic (LC) analysis. The sample preparation yielded extremely clean extracts and absolute recoveries in excess of 90% for both drugs from human serum. The response of the LC system was linear over the concentration range 0.01–2.5 mg/l for both sertraline and desmethylsertraline with a limit of detection of 0.01 mg/l. A gas chromatographic–mass spectrometric (GC–MS) system is also described should confirmation of the drugs be necessary.

1. Introduction

Sertraline (Zoloft) is a relatively new antidepressant and has been prescribed as an alternative to fluoxetine (Prozac) when undesirable side-effects are observed. The pharmacology, metabolism and disposition of sertraline in animal species have been reported [1,2]. Sertraline, a secondary amine, with the chemical name (1*S-cis*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphthalenamine hydrochloride, is strongly protein bound and demethylates in the body to desmethylsertraline which has 10% of the activity of sertraline (Fig. 1). Since sertraline is a new clinically approved drug, the parent compound and its metabolite, desmethyls-

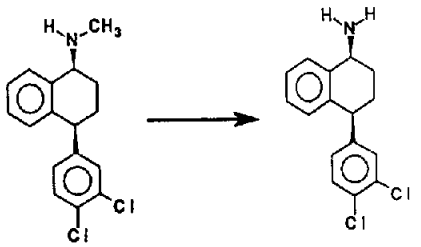

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Fig. 1. Structure of sertraline (I) and desmethylsertraline (II).

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ertraline, will require rapid and accurate determination in both clinical and post-mortem samples.

The determination of these drugs by reversed-phase LC [3], GC [4] and GC–MS [5] has been reported but none of these papers used solid-phase extraction in the sample preparation procedure. The advantages of bonded-phase extraction over conventional liquid–liquid methods have been well documented and include reduction of solvent waste and time of extraction as well as higher recoveries and ease of automation.

Recently reported drug levels observed in blood samples from post-mortem cases ranged from 0.44, to 0.84 mg/l for sertraline and 1.3–1.55 mg/l for desmethylsertraline [6]. Clinical trials have noted therapeutic sertraline levels of 0.03–0.19 mg/l.

In this rapid, simple method, the drugs are isolated from serum using the first reported solid-phase extraction method and analyzed by LC with UV detection. The detection limit of the method is 0.01 mg/l and the recoveries for both isolates is in excess of 90%. A GC–MS analysis procedure is also presented.

2. Experimental

2.1. Materials and methods

Sertraline (SER) and desmethylsertraline (DMS) were obtained from Pfizer (Groton, CT, USA). All solvents were LC grade; all reagents were analytical grade or better and were purchased from Thomas Scientific (Phillipsburg, NJ, USA) Copolymeric bonded-phase extraction columns (Clean Screen, Worldwide Monitoring) were from United Chemical Technologies (Bristol, PA, USA).

2.2. Extraction

Various concentrations of sertraline and desmethylsertraline (0.01, 0.05, 0.1, 0.25, 0.5, 1.0, and 2.5 mg/l) were spiked into drug free serum (1 ml) and 2 ml of 0.1 *M* phosphate buffer (KH_2PO_4, pH 6) was added. Protriptyline (2 mg/l; 250 μl) was added as the internal standard.

Clean Screen copolymeric solid-phase extraction columns (ZSDAU020; 200 mg sorbent/10 ml tube) equipped with Clean Thru tips were positioned into a twelve place vacuum manifold and conditioned with methanol (3 ml), flushed with deionized water (3 ml) and buffered with 0.1 *M* phosphate buffer (pH 6, 1 ml). The serum sample was loaded onto the column and drawn through slowly under vacuum. The columns were washed with deionized water (1 ml), 1.0 *M* acetic acid (1 ml) and methanol (3 ml). The column was allowed to dry under full vacuum for 5 min, then the retained drugs were eluted with methylene chloride–isopropanol–ammonium hydroxide (78:20:2, v/v; 3 ml). A drop of diethylamine was added and the eluent was evaporated to dryness of 40°C under a stream of nitrogen. The residue was reconstituted in mobile phase (100 μl) for analysis by LC, or ethyl acetate (100 μl) for analysis by GC–MS.

2.3. LC analysis

Reversed-phase liquid chromatography (LC) was performed on a Hewlett-Packard (Wilmington, DE, USA) HP1050 system incorporating a pump operating at a flow-rate of 1.5 ml/min, a twenty-place autosampler with an injection volume set to 50 μl, and a variable wavelength UV detector. The detection wavelength was set at 600 nm for 2 min then automatically altered to 214 nm for the remainder of the run. The column was 15 cm × 4.6 mm I.D. containing 5 μm LC-8 phase (Supelco, Bellefonte, PA, USA). The ion-pairing mobile phase consisted of acetonitrile–methanol–deionized water–diethylamine (1000:800:1200:2, v/v) and contained 1.5 g of pentane sulfonic acid (sodium salt). The final pH was adjusted to 5.5–6.0 with phosphoric acid.

2.4. GC–MS analysis

Gas chromatography–mass spectrometry (GC–MS) was performed on a Hewlett–Packard 5890-Series II GC interfaced with the 5971 MS. Data collection and integration was performed

by Target Software (UNIX) purchased from ThruPut Systems (Orlando, FL, USA). The GC analytical column was a RTx-5 15 m × 0.25 mm I.D. 0.25 μm film thickness (Restek, Bellefonte, PA, USA). The temperature parameters were programmed for an initial temperature of 100°C for 0.0 min then ramped at 50°C/min to a final temperature of 210°C. The injection temperature was 250°C and detector temperature was 300°C.

3. Results and discussions

The extraction procedure yielded recoveries of 91.2 ± 5.6% for SER and 90.7 ± 8.2% for DMS at concentrations of 0.1, 0.25, and 0.5 mg/l. The addition of diethylamine to the eluent stabilized the reproducibility of recovery by preventing the possible adsorption to glass. The use of the copolymeric bonded sorbent allowed for extremely clean extracts because it is possible to incorporate an organic wash step. This removes any non-ionized interferences which are soluble in methanol.

Using the LC system described, SER and DMS are well resolved from each other and from the internal standard, protriptyline (Fig. 2a). Fig. 2b shows a serum extract spiked with the same concentration. Standard curves for SER and DMS were linear over the concentration range 0.05–2.5 mg/l with regression coefficients of $r^2 = 0.9999$ (slope 0.36; y-intercept 0.0142) and $r^2 = 0.999$ (slope 0.696; y-intercept 0.0227) respectively.

Altering the detection wavelength from 600 nm to 214 nm after 2 min had the same affect as solvent delay in gas chromatography. The unretained compounds are effectively deleted from the chromatogram, even though they are actually still present, allowing low levels of drug to be detected.

A GC–MS analysis procedure is necessary for the determination of sertraline and its metabolite in forensic post-mortem cases [6] and so GC–MS analysis was included in this determination. The retention times for SER and DMS were 6.0 and 5.9 min respectively. In MS the selected ions for the underivatised drugs were 274, 159 and 246 for SER; 119, 246 and 274 for DMS.

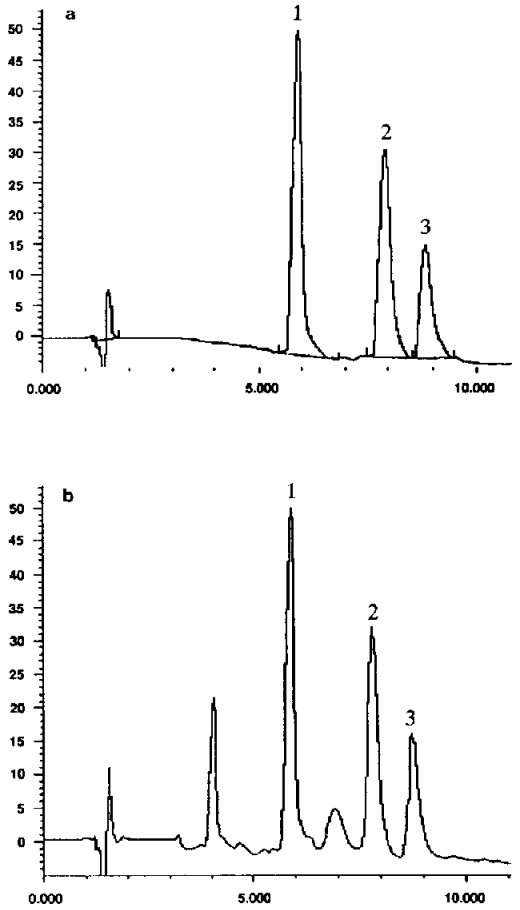

Fig. 2. (a) LC of (1) protriptyline (internal standard) (0.5 mg/l), (2) desmethylsertraline and (3) sertraline (0.5 mg/l). (b) LC of spiked serum extract of (1) protriptyline (internal standard) (0.5 mg/l), (2) desmethylsertraline and (3) sertraline (0.5 mg/l).

4. Conclusion

The assay described provided a rapid, simple and efficient extraction and analysis of sertraline and its major metabolite in human serum. No derivatization was necessary for either LC with UV detection or GC–MS identification. The addition of diethylamine to the final eluent aided the reproducibility of the extraction and incorpo-

ration of an automated wavelength change helped with the production of clean chromatograms, resulting in a detection limit of 0.01 mg/l.

5. References

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