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Simultaneous measurement of venlafaxine and its major metabolite, oxydesmethylvenlafaxine, in human plasma by high-performance liquid chromatography with coulometric detection and utilisation of solid-phase extraction

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Abstract

Venlafaxine, oxydesmethylvenlafaxine and an internal standard (paroxetine) were extracted from plasma by a solid-phase extraction technique. Chromatography was performed using isocratic reversed-phase high-performance liquid chromatography (HPLC) with coulometric endpoint detection. The standard curves were linear over the range 0–200 ng/ml for both venlafaxine and oxydesmethylvenlafaxine in plasma. The mean inter- and intra-assay coefficients of variation over the range of the standard curves were less than 10%. The absolute recovery averaged 74% for venlafaxine and 67% for oxydesmethylvenlafaxine. The sensitivity was 0.5 ng for both the analytes. Plasma profiles of the analytes following oral administration of venlafaxine, are presented. © 1998 Elsevier Science B.V.

Keywords: Venlafaxine; Oxydesmethylvenlafaxine

1. Introduction

Venlafaxine (Wy-45,030), 1-[2-(dimethylamino)-1-(4-methoxy-phenyl)ethyl] cyclohexanol hydrochloride, is a novel antidepressant which has a neuropharmacologic profile distinct from that of existing antidepressants, including tricyclic compounds, selective serotonin reuptake inhibitors and monoamine oxidase inhibitors [1,2].

In humans, venlafaxine (V) is well absorbed and undergoes extensive metabolism in the liver [3]. Its major metabolite is oxydesmethylvenlafaxine (ODV) which has an antidepressant activity profile similar to that of venlafaxine [1]. A previous method for the measurement of both V and ODV in plasma has been described [4]. This method utilised high-performance liquid chromatography (HPLC) with ultra violet (UV) endpoint detection. The authors described this as a rapid, accurate and sensitive method for simultaneous determination of V and ODV. However, this technique utilised liquid–liquid extraction procedures which are both slow and generally yield lower working recoveries, this is not unusual since these type of extraction methods are known to give variable recoveries.

The aim was to establish an assay that was quick, accurate, sensitive, and at the same time easy to use – indeed a method that could be set up in any

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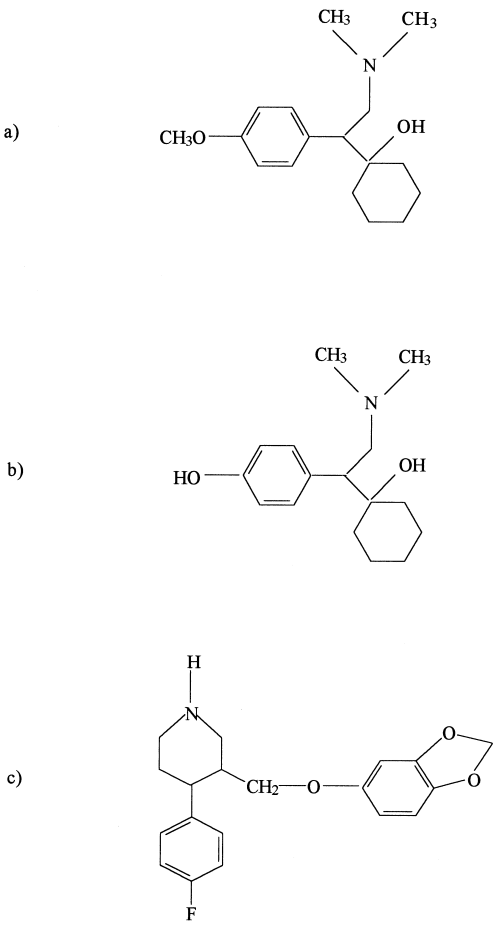

Fig. 1. Structures of (a) venlafaxine (b) oxydesmethylvenlafaxine and the internal standard, (c) paroxetine.

clinical laboratory. We describe here a method which we feel satisfy these criteria. The method uses HPLC with coulometric endpoint detection, solid-phase extraction (SPE) and an internal standard (I.S.), paroxetine. The structures for both the analytes and the I.S. are shown in Fig. 1.

2. Experimental

2.1. Materials

Venlafaxine (WY-45,030) and oxydesmethylvenlafaxine (WY-45,233) were kindly donated by Wyeth-Ayerst Research, Princeton, USA. Paroxetine was obtained from SmithKline Beecham, Harlow, UK. All reagents used for the assay were of the highest grade available. Water was deionised and glass distilled prior to use. Drug-free plasma for the preparation of calibration standards was obtained from normal healthy volunteers.

Stock standard solutions of venlafaxine, oxydesmethylvenlafaxine and paroxetine were prepared at concentrations of 100 μg/ml in methanol and stored at 4°C. These were stable for at least three months. The assay standards were prepared freshly for each assay from the stock solutions.

2.2. Chromatography

The HPLC system consisted of a Jasco PU-980 HPLC pump (Jasco Corporation, Tokyo, Japan), a GBC Model LC 1610 autosampler (GBC Scientific Equipment, Victoria, Australia), a 250×4.6 mm I.D. column (Phenomenex, Macclesfield, UK) packed with a 5 μm Spherisorb ODS/CN [a mixed mode combination of C_{18}(octadecyl) and cyanonitrile material] and a Model 5100A coulometric detector consisting of a Model 5010 analytical cell and a Model 5020 guard cell (ESA, Bedford, MA, USA). A Varian Model 4400 integrator (Varian Associates, Harbour City, CA, USA) was used for quantification purposes.

The selected operating potentials for the detectors 1, 2 and guard cell were 0.65 V, 0.95 V and 0.98 V, respectively as indicated by the voltammogram (see Fig. 2). The response time was 10 s. The mobile phase consisted of 0.05 *M* potassium phosphate buffer (pH 4.8)–methanol (30:70, v/v). The mobile phase was filtered through a 0.2-μm filter and degassed prior to use. The flow-rate was 1 ml/min with a back pressure of 160 kg/cm^2. The chromatography was carried out at room temperature. Peak heights rather than peak areas were measured by the integrator. Unknown plasma concentrations of V and ODV were quantified using linear regression of response (drug/internal standard peak height ratio) versus V or ODV concentrations [5].

2.3. Procedure

Blood samples were collected into tubes containing lithium heparin as anticoagulant and spun at

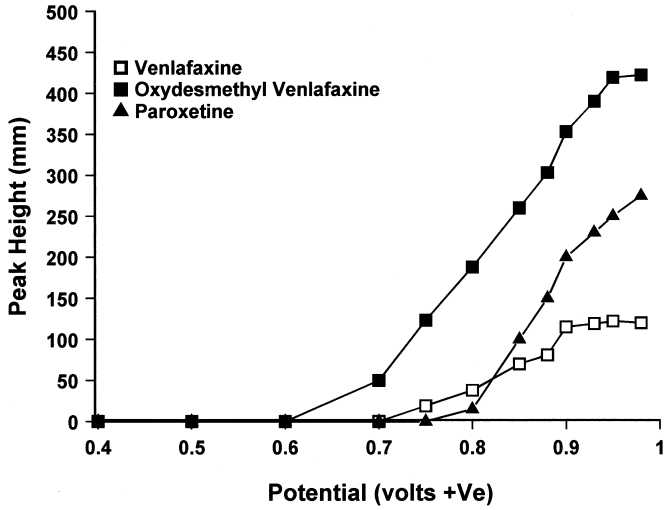

Fig. 2. Voltammogram of venlafaxine, oxydesmethylvenlafaxine and paroxetine for detector 2 (the analytical electrode) at different potentials. The voltammogram was determined when the guard cell and detector 1 were at zero potential.

1500 *g* in a refrigerated centrifuge at 4°C for 15 min. The plasma was separated and stored at −20°C until required for assay.

Standards for each assay were prepared freshly in drug-free plasma and consisted of five concentration points over the range of 10–200 ng/ml and blanks, in duplicate. To each 0.5 ml of standard/sample was added 20 ng of the paroxetine.

Carboxymethyl (CBA) sorbent columns containing silica carboxymethyl resin (50 mg Isolute, Jones Chromatography, Hengoed, UK) were conditioned with full column volumes (1 ml) of methanol followed by 0.025 *M* phosphate buffer (pH 6.8). The vacuum on the vacuum manifold system (VacMaster, IST, MID Glamorgan, UK) was diverted to prevent the columns from drying out and the standards and samples were loaded on to the columns. The vacuum was again applied to allow the complete passage of the materials through the column. Each column was washed with two column volumes of 0.025 *M* phosphate buffer (pH 6.8) and taken to dryness under vacuum. The vacuum was again diverted, the manifold needles were wiped dry and a collection tray containing 75×10 mm glass tubes was inserted into the vacuum manifold. Compounds were eluted with a single column volume of 1% ammonia in methanol. Eluates were evaporated to dryness under vacuum at 40°C. The residue was reconstituted in methanol (100 μl), vortex-mixed and made ready for injection onto the HPLC system. A 25-μl aliquot of the reconstituted extract was injected into the autosampler.

3. Results

The assay resolution and sensitivity was determined by injection of extracted drug-free plasma spiked with known amounts of V and ODV, respectively (see Fig. 3). The retention times of ODV, V and the I.S. were 5.4, 8.8 and 9.6 min, respectively. The linearity of extraction procedure and the detector response were verified over the standard range for both ODV and V (0–200 ng/ml). This was determined by measuring pooled drug-free plasma spiked with known amounts of ODV and V. Calibration graphs were calculated for both ODV and V and the peak-height ratio over the concentration range studied. The mean slope±standard error of the mean (S.E.M.) were 0.013±0.0006 for ODV (n=10) and 0.0039±0.0002 for V (n=10), respectively. The correlation coefficients for both compounds were 0.999. The absolute recoveries for V and ODV were 74% and 67%, respectively.

Samples extracts were stable for at least one week when stored at 4°C and out of light. A number of

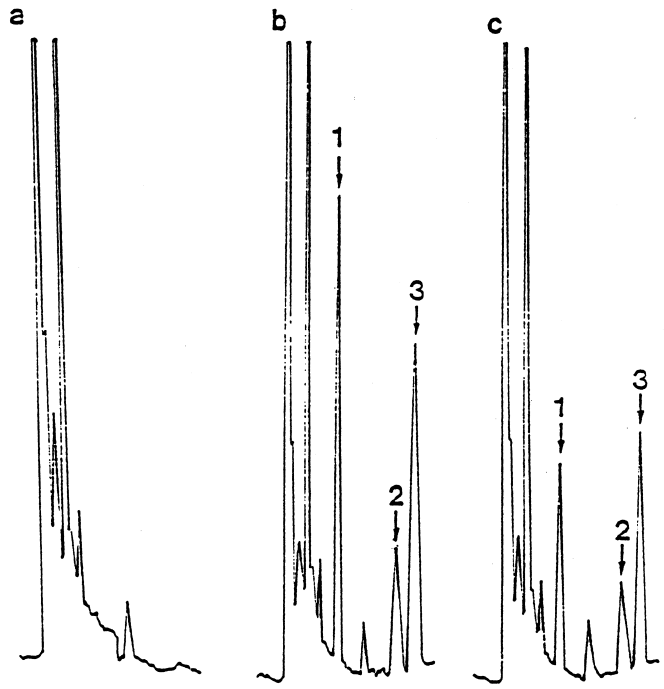

Fig. 3. Chromatograms of (a) blank drug-free plasma, (b) drug-free plasma spiked with 10 ng/ml of each, V and ODV. (c) Sample from a normal volunteer following oral administration of venlafaxine, both V and ODV estimated to be 5 ng/ml. Peaks 1, 2 and 3 represent ODV, V and paroxetine, respectively. The retention time for peaks 1, 2 and 3 were 5.4, 8.8 and 9.6 min, respectively. The amount of internal standard added to each sample extract was 20 ng.

Table 1
Inter- and intra-assay coefficients of variation (C.V.s) and precision data for the determination of venlafaxine and its major metabolite, oxydesmethylvenlafaxine in plasma ($n=5$)

Actual value (ng/ml)	Observed value (ng/ml)		C.V.[a] (%)	
	V	ODV	V	ODV
Inter-assay				
15	12.7±1.4	16.0±1.8	11.8	11.1
50	57.4±6.0	50.5±4.3	8.5	10.4
150	146±9.6	157±9.6	6.1	6.5
Intra-assay				
15	12.6±1.6	16.6±0.9	5.6	12.7
50	46.6±4.3	46.1±2.7	5.5	7.1
150	148.8±2.7	152±2.6	1.8	1.8

[a] The C.V.s were calculated from results obtained from drug-free plasma spiked with known amounts of V or ODV. For precision data the results were compared with externally prepared standards.

common psychotropic drugs were tested for assay interference, however, none of these were found to cause problems except *m*-chlorophenyl piperazine (mCPP, the metabolite of trazodone) which was found to interfere with resolution of V.

Precision was estimated from inter- and intra-assay coefficients of variation (C.V.s). The data for inter- and intra-assay variation are shown in Table 1. Mean plasma profiles of V and ODV following oral administration of venlafaxine in 10 normal healthy volunteers are shown in Fig. 4.

4. Discussion

We describe here a simple, sensitive and highly selective HPLC assay procedure for the measurement of V and ODV which utilises solid-phase extraction (SPE), an I.S., paroxetine and coulometric detection. The CBA material used in the SPE columns is a

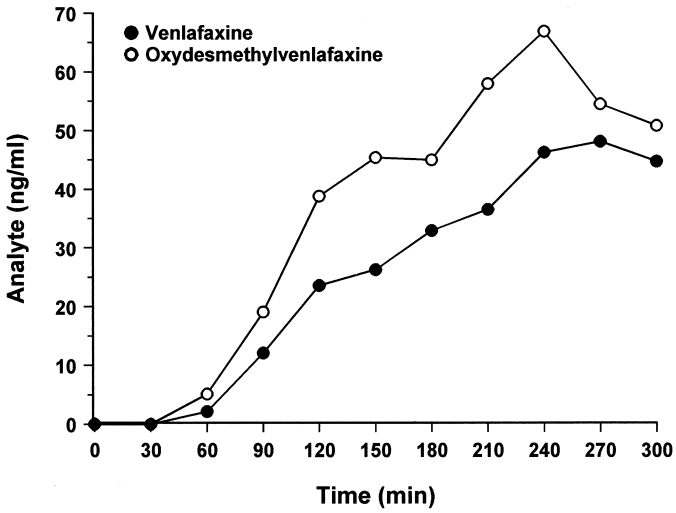

Fig. 4. Plasma concentrations of venlafaxine and oxydesmethylvenlafaxine in volunteers following oral administration of 37.5 mg venlafaxine. Each point is a mean of 10 subjects.

weak cation exchanger ($pK_a = 4.8$). Because of this characteristic, the columns are more selective in that only the more highly charged analytes may be bound. The use of 1% ammonia in methanol also increased the selectivity of the extraction, since our experience is that any enhancement in the concentration of ammonia in this reagent causes increased elution of unwanted compounds bound to the CBA sorbent. The detection limits for V and ODV were 0.5 ng on HPLC column (with a signal-to-noise ratio of 3:1), this represents 2 ng/ml in plasma, thus being approximately 2.5-fold more sensitive than the UV detection method quoted previously [4]. Moreover, we have found that coulometric detection gives more selective measurement of the analytes than UV detection method. The precision studies in human plasma have shown that mean C.V.s for both V and ODV were less than 10% (see Table 1) indicating that the measurements of V and ODV were both accurate and reliable using this method. Additionally the SPE technique used for extraction of the analytes in the procedure was shown to be quicker than that of the solvent extraction procedure used in a previously described method [4] and in our hands SPE gave consistently higher extraction recoveries of both V and ODV than did the solvent extraction procedure.

It has been established that the ratio between the analytical recovery of the analytes and that of the I.S. submitted to the same operations was constant over a wide range of concentrations. Furthermore, the detector response was linear for both analytes and the I.S.. The requirement for an I.S. assay procedure was therefore satisfied. Although we found the analytical recovery of the analytes to be generally consistent, we did occasionally get low recoveries from some plasma samples. We therefore concluded that the use of an I.S. to monitor the recovery was an essential requirement.

The voltammogram shown in Fig. 2 indicates that V has a much lower response to electrochemical oxidation than that of ODV and paroxetine, consequently a higher voltage setting was required to attain the necessary sensitivity for V to be measured simultaneously. The use of a high voltage (with a back pressure of 160 kg/cm^2) did not pose any problems for the determination of the V, ODV and paroxetine although operation at this high potential reduces the working life of the analytical cell.

Paroxetine was selected as an I.S. for two reasons. Firstly, V and paroxetine are both 5-HT reuptake inhibitors and it is unlikely that these would be administered together to subjects and secondly, paroxetine is well separated from V and ODV (see Fig. 3). We do sometimes use the tricyclic antidepressant, desipramine (DMI) as an I.S. which has a

retention time of 11.5 min as compared to V, 8.8 min, this also has similar characteristic to V.

This method has demonstrated substantial improvement in comparison with other assay systems. High recoveries for both analytes, lower sample volume and reduced sample preparation time have all been attained by utilisation of SPE technology. It has also facilitated better resolution of analytes from other psychotropic drugs that might possibly interfere chromatographically, together with greater detector response, thus allowing for lower concentrations of V and ODV to be resolved.

Finally, the usefulness and validity of this method is demonstrated by the fact that we have successfully determined V and ODV plasma profile in normal volunteers following oral administration of venlafaxine (see Fig. 4).

5. Conclusion

A simple, robust, highly selective and reproducible method for the measurement of venlafaxine and oxydesmethylvenlafaxine has been described. This is cheap to run and may easily be set up in a clinical laboratory. The method has clear advantages over previously described method.

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