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Rapid high-performance liquid chromatographic measurement of venlafaxine and *O*-desmethylvenlafaxine in human plasma Application to management of acute intoxications

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Abstract

Venlafaxine, a second-generation antidepressant, acts by inhibition of the reuptake of presynaptic noradrenaline and serotonin. The main metabolite, *O*-desmethylvenlafaxine was found biologically active. For toxicological purpose, a rapid specific and accurate RP-HPLC assay was developed for the simultaneous determination of venlafaxine and *O*-desmethylvenlafaxine in human plasma. A linear response was observed over the concentration range 0.2–4 μg/ml. A good accuracy (<8%) was achieved for all quality controls, with intra-day and inter-day variation coefficient less than 10%. Finally, no interference was observed with other psychotic drugs encountered in acute poisoning. This rapid method (run time <10 min) was used to manage four voluntary intoxications involving venlafaxine.

Keywords: Venlafaxine; *O*-desmethylvenlafaxine

1. Introduction

Venlafaxine (VX) is a new phenethylamine bicyclic antidepressant which inhibits the reuptake of both serotonin and noradrenaline [1,2]. In human, VX is well absorbed and undergoes metabolism in the liver. Its major metabolite, *O*-desmethylvenlafaxine (ODV) (Fig. 1), has an antidepressant activity profile similar to that of the parent drug [3].

Many intoxications involving venlafaxine have been described [4–8]. Consequently, it is essential to use a specific and rapid method for the determination of VX and ODV in case of poisoning.

The published methods for both VX and ODV analyses in biological fluids included GC [6–8] and HPLC [9–12]. Among the different HPLC techniques, one requires a solid-phase extraction before the coulometric detection of drugs, which is not easy to run for routine applications. On an other hand, the use of another psychotic drug as internal standard described in some HPLC techniques [11,12] could be detrimental for the management of multiple drug intoxications. Indeed the use of an appropriate internal standard (IS) is essential to improve precision and accuracy of the HPLC method, but this I.S. should not interfere with other drugs involved in the poisoning cases. We selected opipramol as I.S. It is a

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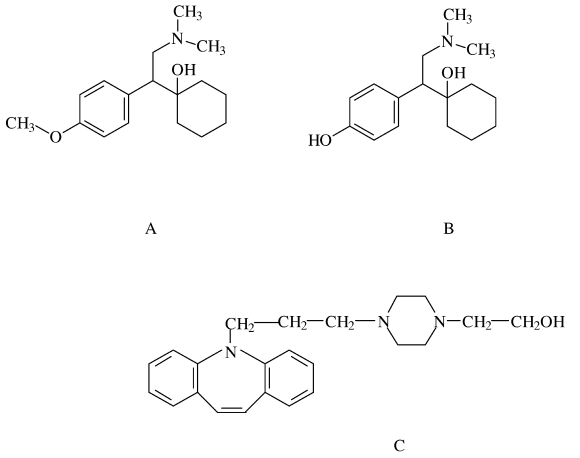

Fig. 1. Chemical structure of venlafaxine (A), ODV (B) and opipramol (C).

basic drug extracted in about the same conditions than those used for venlafaxine and ODV.

The aim of this study was to establish a simple and accurate RP-HPLC method for the measurement of VX and ODV in plasma involving an internal standard, opipramol. It presents several advantages, such as rapidity and selectivity and it is especially adapted for the management of VX poisoning cases leading to concentrations generally above the therapeutic range (0.2–0.5 μg/ml) [6]. Under the chromatographic conditions, VX, ODV and internal standard (IS) were well separated and sufficiently resolved from endogenous plasma compounds. Finally, the stability of these tested compounds was studied, particularly during the sample storage.

2. Experimental conditions

2.1. Chemicals

VX, ODV and opipramol were obtained from Lederle Laboratories (New York, USA). All reagents used for the assay were of HPLC or analytical grade. The reagent containing sulfonic pentane acid (Pic B5® Low UV) was a premixed product of Waters (Milford, MA, USA). The phosphate buffer was prepared by dissolving 9.08 g of KH_2PO_4 and 11.60 g of K_2HPO_4 in 1000 ml of water. Water was deionized and glass-distilled prior to use and human heparinized plasma of healthy volunteers was purchased from Aquitaine Establishment of Blood Transfusion (E.T.S.A, Bordeaux, France).

2.2. Equipment

The chromatographic apparatus (ThermoQuest™, San Jose, CA, USA) was equipped with a constant flow pump M 100, a Model 150 ultraviolet detector and a Datajet® integrator.

The chromatographic separation was performed at room temperature on a Spherisorb® S5 C8 analytical column (Waters) (4.6×150 mm; 5-μm particle size).

The mobile phase consisted of acetonitrile–phosphate buffer (6.24×10^{-2} *M*) (30:70, v/v). To this mixture, 500 μl of diethylamine and a vial of Pic B5® was added for 1 l. Finally, the pH of this eluent was adjusted to 5.5 with orthophosphoric acid. The mobile phase was filtered through a 0.5-μm filter and degassed prior to use. The flow-rate was maintained at 1.4 ml/min.

The compounds were chromatographed at 229 nm within 12 min. All data were processed by Datajet® integrator. The unknown concentrations of VX and ODV were quantified using linear regression of response (drug/IS peak height ratio) versus VX or ODV concentrations.

2.3. Standard solutions

Stock standard solutions of VX, ODV and I.S. were prepared at concentrations of 1 mg/ml in methanol and stored at −20°C. They were stable for at least 3 months. The internal standard (IS) stock solution was diluted daily in bidistilled water to yield a 0.1 mg/ml working solution.

From two stock solutions of ODV and VX, a working solution (80 μg/ml) containing ODV and the parent drug was made-up daily and the calibration standards were prepared freshly for each assay from this working solution. Calibration standards were added into drug-free human plasma to yield concentrations of 0.2, 0.4, 1, 2 and 4 μg/ml of both VX and ODV. In the same manner, plasma quality controls (QC) spiked with 0.3, 0.6, 1.2, 1.8 and 3.6 μg/ml were prepared to measure the accuracy and the precision of the method.

2.4. Sample preparation

To 1 ml of calibration or patient plasma was added 50 μl of I.S. (0.1 μg/ml) and 200 μl of 0.1 *M* NaOH. The mixture was extracted in 7 ml of hexane–isoamylic alcohol (99:1; v/v) by rotative shaking during 10 min. After centrifugation, the organic phase was added in 200 μl of 0.05 *M* HCl. The mixture was shaken during 10 min and centrifuged. The upper organic phase was discarded and 25 μl of aqueous phase was injected into the chromatograph.

2.5. Recovery, precision and accuracy

2.5.1. Recovery

Extraction recoveries from human plasma were determined by comparison of HPLC responses from extracted samples, containing known amounts (0.2, 1, 4 μg/ml) of VX and ODV, to those from unextracted and directly injected standards, spiked with the same amounts.

2.5.2. Precision

Plasma standard samples were prepared and analysed to obtain the standard curve. The same procedure was repeated on three consecutive days. The intra-day precision of the method was assessed by calculating coefficient of variation (C.V.) for replicates ($n=6$) of QC samples prepared as described above. The inter-day precision ($n=18$) was determined from QC samples obtained on three different days.

2.5.3. Accuracy

Accuracy, expressed as % bias, was calculated as the percentage difference between the amount of VX and ODV added and found.

3. Results and discussion

Among the identified metabolites of VX, ODV, which is the major one, presents an antidepressant activity [1]. Indeed, it was shown that 87% of absorbed venlafaxine is metabolised and excreted within 48 h in urine [7]. Consequently, analyses of both VX and ODV are necessary for pharmacokinetic studies.

The described RP-HPLC procedure is specific and easy to perform, leading to the simultaneous determination of VX and ODV plasmatic concentrations. It is especially adapted for the management of venlafaxine poisoning yielding concentrations generally above the therapeutic range (0.2–0.5 μg/ml) [6]. Under the chromatographic conditions, VX, ODV and opipramol (IS) were sufficiently resolved from endogenous plasma compounds (Fig. 2). Moreover, the analysis is rapid with the three compounds chromatographed within 10 min.

3.1. Precision, accuracy and linearity

The results obtained for precision and accuracy are listed in Table 1 and expressed as C.V. (%) and % bias, respectively. The method showed an intra-day precision with a C.V. ranging from 1.87 to 4.12 and from 3.45 to 6.50 for VX and ODV, respectively. The accuracy was less than 8% for venlafaxine and its metabolite. From inter-day study, precision and accuracy of the method showed values generally more elevated than those of intra-day study. But the mean concentrations of both compounds presented a C.V. and a bias less than 10%.

From seven calibration curves, constructed with five unique calibration points ranging from 0.2 to 4 μg/ml, a high correlation coefficient was found for ODV and VX (Table 2). The limit of quantification (LOQ) was 100 and 50 ng/ml for ODV and VX, respectively.

3.2. Extraction efficiency

Chloroform, ethyl acetate, diethyl ether and hexane were tested for their ability to extract VX and ODV and to decrease the extraction of endogenous plasma compounds. Among them, only hexane satisfied these two experimental conditions. Indeed, chloroform was found the most effective in terms of extraction efficiency, but the obtained chromatographs presented too many parasite peaks. For hexane by using chosen extraction conditions, the mean recoveries were 85 and 32% for VX and ODV, respectively. These extraction results prompted us to calculate the octanol–water partition coefficient ($C \log P$) for ODV and VX by a fragmental method available in Mac log P [13]. From the calculated

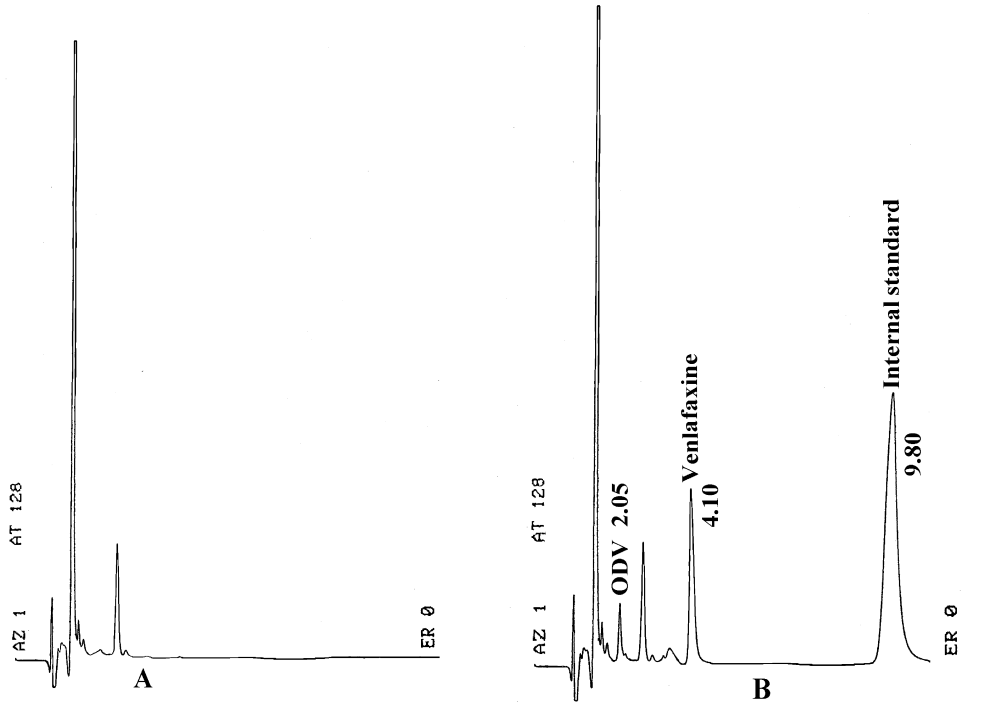

Fig. 2. Chromatogram of 0.70 μg/ml blood extract of venlafaxine and ODV standard with the internal standard.

Table 1
Precision and accuracy of the HPLC method for the analysis of venlafaxine and ODV in human plasma

Amount added (μg/ml)	Intra-day studies ($n=6$)						Inter-day studies ($n=18$)					
	Venlafaxine			ODV			Venlafaxine			ODV		
	Concentrations found±SD (μg/ml)	% C.V.	% Bias	Concentrations found±SD (μg/ml)	% C.V.	% Bias	Concentrations found±SD (μg/ml)	% C.V.	% Bias	Concentrations found±SD (μg/ml)	% C.V.	% Bias
0.3	0.280±0.011	4.12	6.67	0.278±0.010	3.45	7.33	0.279±0.016	5.82	7.00	0.283±0.028	9.76	5.67
0.6	0.608±0.020	3.35	−1.39	0.587±0.027	4.40	2.22	0.600±0.021	3.52	−0.07	0.610±0.032	5.17	1.58
1.2	1.150±0.025	2.20	4.17	1.198±0.059	4.92	0.16	1.153±0.038	3.31	4.04	1.181±0.051	4.36	1.63
1.8	1.725±0.048	2.78	4.17	1.865±0.089	4.78	−3.61	1.703±0.034	2.02	5.72	1.797±0.093	5.18	0.17
3.6	3.388±0.063	1.87	5.88	3.647±0.237	6.50	−1.29	3.359±0.074	2.19	7.15	3.459±0.259	7.48	3.82

Table 2
Statistical data ($n=18$) for linearity including standard deviation (SD)

	r^2	Slope (±SD)	Intercept (±SD)
Venlafaxine	0.9741±0.0501	1.064±0.004	0.0040±0.0069
ODV	0.8740±0.0224	0.204±0.017	−0.0167±0.0346

values (2.68 and 3.27 for ODV and VX, respectively), there appears to be a better solubility of venlafaxine in non-polar solvents. On the other hand, the ODV chemical structure (Fig. 1) provides a complementary explanation to the limited extraction coefficient. It contains an acidic phenolic group and a tertiary amine function, which could lead to an amphoteric behaviour. Under similar extraction conditions achieved in hexane, Levine et al. [6] had already reported differences in VX and ODV extraction efficiency.

3.3. Stability

To determine the influence of temperature on the stability of compounds, QC (quality control) samples spiked with ODV and VX were stored under different conditions: at −20°C during 15 days; at +4°C during 48 h; at +20°C during 24 h. No decomposition of ODV and VX was noted in the quick-frozen samples during 2 weeks. Indeed, at −20°C, the percent variation coefficient (C.V.) for both compounds was less than 5% and the accuracy showed a bias less than 7%.

The storage during 48 h at +4°C produced no significant decrease of ODV and VX concentrations (C.V. and % bias values less than 5).

Finally, the storage at room temperature during 24 h showed a good stability of both compounds, with C.V. and % bias values less than 8.

3.4. Clinical cases

In the case of voluntary drug intoxications, the ingested dose and the beginning of intoxication were often unknown. Nevertheless, the expected concentrations are generally above the therapeutic concentrations. The therapeutic concentrations of VX and ODV are in the range of 0.20–0.50 μg/ml [8].

By using the described method we were able to manage four cases of venlafaxine poisoning (Table 3). The obtained chromatogram for patient 2 is shown in Fig. 3.

In order to verify the specificity of detection, an absorption spectrum was carried out on the chromatographic peaks of VX and ODV obtained from drug-abuse patients. No interference was notified.

For the three first patients, the VX concentration was markedly above the therapeutic concentrations (4.53 μg/ml for case 3). Patients 1 and 2 were also characterized by elevated concentrations of ODV. For patients 2 and 4, the plasma ODV concentrations exceed those of VX. According the pharmacokinetic

Table 3
Concentrations of drugs involved in four cases of venlafaxine poisonings

Patients	Concentrations (μg/ml)			
	VX	ODV	Zolpidem	Diazepam
1	3.15	2.76	–	–
2	1.74	1.96	–	–
3	4.58	0.31	0.4^{TH}	–
4	0.08	0.73	–	3.3

TH = therapeutic concentrations.

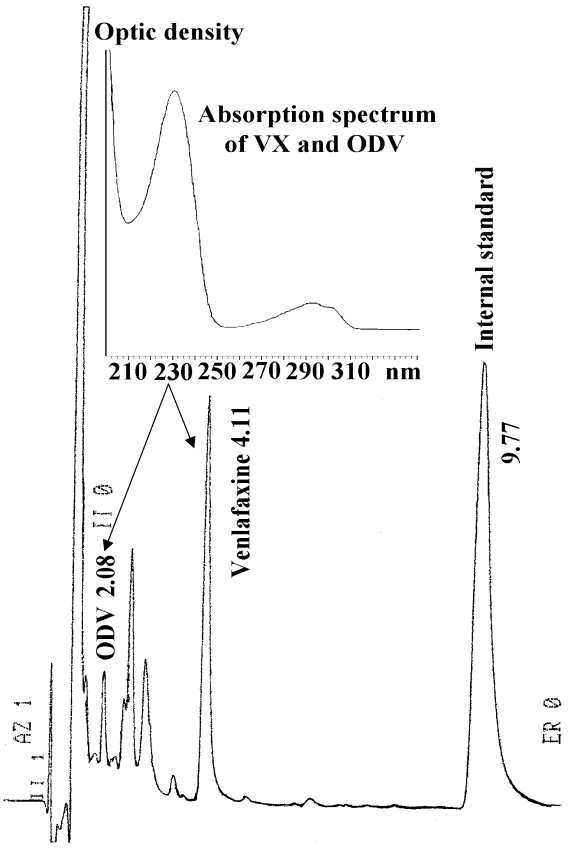

Fig. 3. Chromatograms of blank plasma (A) and patient 2 sample (diluted 1/2) (B). Specific absorption spectrum of VX and ODV performed on PDA detector (Model 996, Waters, Milford, MA, USA).

data, ODV, which is the the major metabolite, presents a 10-h terminal half-life versus 4 h for VX. Consequently, the apparent clearance of ODV is lower than that of the parent drug [14], which could explain these concentration values.

On an other hand, a therapeutic concentration of zolpidem was detected for patient 3, whereas diazepam at abuse concentration was measured for patient 4.

Finally, the HPLC method described for simultaneous determination of VX and ODV in human plasma proved to be simple and rapid for routine application. The LOQ and the total length of this assay are particularly adapted to the management of acute VX intoxication.

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