Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 850 (2007) 405–411
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

# High performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-MS/ESI) method for simultaneous determination of venlafaxine and its three metabolites in human plasma

Wen Liu, Hua-lin Cai, Huan-de Li *

*Clinical Pharmaceutical Research Laboratory, Second XiangYa Hospital, Central South University, ChangSha, Hunan 410011, PR China*

Received 10 August 2006; accepted 12 December 2006
Available online 21 December 2006

**Abstract**

A high-performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-MS/ESI) method for simultaneous determination of venlafaxine (VEN) and its three metabolites *O*-desmethylvenlafaxine (ODV), *N*-desmethylvenlafaxine (NDV) and *N*,*O*-didesmethylvenlafaxine (DDV) in human plasma has been developed and validated. Estazolam was used as the internal standard. The compounds and internal standard were extracted from plasma by a liquid–liquid extraction. The HPLC separation of the analytes was performed on a Thermo BDS HYPERSIL $C_{18}$ (250 mm × 4.6 mm, 5 μm, USA) column, using a gradient elution program with solvents constituted of water (ammonium acetate: 30 mmol/l, formic acid 2.6 mmol/l and trifluoroacetic acid 0.13 mmol/l) and acetonitrile (60:40, V/V) at a flow-rate of 1.0 ml/min. All of the analytes were eluted within 6 min. The compounds were ionized in the electrospray ionization (ESI) ion source of the mass spectrometer and were detected in the selected ion recording (SIR) mode. Calibration curves in spiked whole blood were linear from 4.0–700 ng/ml, 2.0–900 ng/ml, 3.0–800 ng/ml and 2.0–700 ng/ml for VEN, ODV, NDV and DDV, respectively, all of them with coefficients of determination above 0.9991. The average extraction recoveries for all the four analytes were above 77%. The methodology recoveries were higher than 91%. The limits of detection were 0.4, 0.2, 0.3, and 0.2 ng/ml for VEN, ODV, NDV and DDV, respectively. The intra- and inter-day variation coefficients were less than 11%. The method is accurate, sensitive and reliable for the pharmacokinetic study of venlafaxine as well as therapeutic drug monitoring (TDM).


*Keywords:* Venlafaxine; Metabolite; HPLC-MS/ESI

## 1. Introduction

Venlafaxine (VEN)-chemical structure 1-[2-(dimethylamino)-1-(4-methoxy-phenyl)ethyl]cyclohexanol hydrochloride is a new antidepressant, which selectively inhibits re-uptake of norepinephrine and serotonin, and sightly inhibits re-uptake of dopamine, without significant affinity for muscarinic, histaminergic or $\alpha_1$-adrenergic receptors [1]. In humans VEN is metabolised into two minor metabolites, *N*-desmethylvenlafaxine (NDV) and *N*,*O*-didesmethylvenlafaxine (DDV), and the major active metabolite, *O*-desmethylvenlafaxine (ODV) which presents an activity profile similar to that of VEN [2]. Desvenlafaxine succinate (DVS), the succinate salt of the isolated major active metabolite of venlafaxine, is currently under clinical development and may be a new serotonin and norepinephrine inhibitor in recent future [3]. Different studies have provided evidence that increasing dose is associated with increased therapeutic response [4,5], and increased occurrence of adverse reactions such as nausea, anticholinergic effects, headache, somnolence, asthenia and dry mouth. The biotransformation of VEN into ODV is controlled by CYP2D6, CYP2C19 and CYP2C9, with estimated contributions of 89%, 10% and 1%, respectively. Formation of *N*-desmethylvenlafaxine is attributed to CYP3A4, CYP2C19 and CYP2C9 [6]. Though there is no clinical importance ascribed to the minor metabolites NDV and DDV, measurement of the two analytes in human plasma can help us to understand the metabolism of venlafaxine.

The published methods for both VEN and ODV assay in biological fluids include CE, GC and HPLC [7–9]. Among the

* Corresponding author. Tel.: +86 731 5292121; fax: +86 731 4436720.
*E-mail addresses:* liuwen0917@163.com (W. Liu), li53hd@medmail.com.cn (H.-d. Li).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.12.019

different HPLC methods, the HPLC couple with mass spectrometry or coulometric detection has high sensitivity but uses the solid-phase exaction (SPE) [9,10], which is laborious and unsuitable for routine application, while others using fluorescence or UV detection have low sensitivity or long analysis time (>10 min) [11–13]. By now there is only one paper reported for simultaneous determination of VEN, ODV, NDV and DDV [14]. The aim of the present study is to establish a simple, rapid and accurate HPLC-MS/ESI method for the measurement of the four analytes in the pharmacokinetic study of venlafaxine as well as therapeutic drug monitoring (TDM).

## 2. Experiment

### 2.1. Equipments and reagents

A system of HPLC (Waters 2690, USA) with a Micro mass ZQ mass spectrometer (Wythenshawe, Manchester, UK) with mass-selective detector equipped with an electrospary ionization (ESI) ion source was used. COMPAQ Deskpro Workstation and MassLynx$^{TM}$3.5 software were utilized.

Venlafaxine (>99.8%), *O*-desmethylvenlafaxine (>99.8%), *N*-desmethylvenlafaxine (>99.8%), *N*,*O*-didesmethylvenlafaxine (>99.8%) were purchased from TRC (Toronto Research Chemicals Inc., Canada), Estazolam (99.8%) from Sigma (Steinheim, Germany). The structures of these compounds are presented in Fig. 1.

HPLC grade reagents (methanol, acetontritile) were purchased from Caledon Laboratory LTD. (Georgetown, Ont., Canada). Other AR grade reagents (ether, aqueous ammonia, formic acid, trifluoroacetic acid and ammonium acetate) were purchased from Chemical Reagent Factory of Hunan (Changsha, Hunan, China). Distilled water was prepared by a laboratory purification system. Drug free human plasma was obtained from the Blood Center of Shanghai (Shanghai, China).

### 2.2. Standard solutions

The primary stock solutions of VEN (14.1 μg/ml), ODV (8.9 μg/ml), NDV (10.8 μg/ml), DDV (7.4 μg/ml) and Estazolam (13.6 μg/ml) were prepared by dissolving appropriate amounts of pure substance in methanol. Working solutions were obtained by diluting the stock solutions with methanol. All the standard solutions were stored at 0 °C.

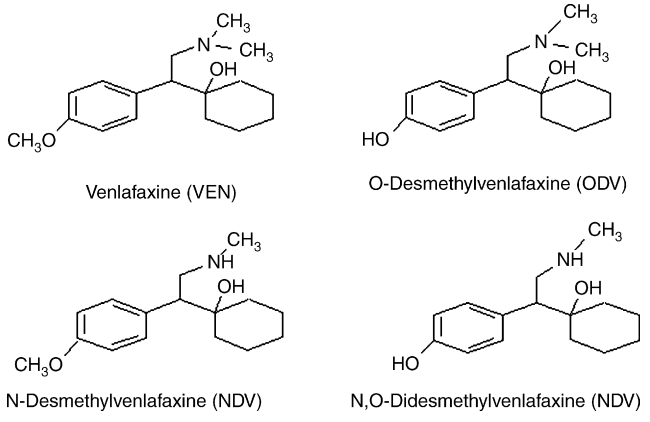


Fig. 1. Chemical structure of each compound.

Table 1
The gradient elution program

| Time (min) | Solvent A (%) | Solvent B (%) | Flow (ml/min) |
|---|---|---|---|
| 0.00 | 60.0 | 40.0 | 1.0 |
| 7.00 | 46.0 | 54.0 | 1.0 |
| 7.10 | 60.0 | 40.0 | 1.5 |
| 8.50 | 40.0 | 60.0 | 1.5 |

Solvent A: ammonium acetate 30 mmol/l, formic acid 2.6 mmol/l and trifluoroacetic acid 0.13 mmol/l; Solvent B: acetonitrile.

Routine daily calibration curves were prepared in drug-free serum. Appropriate volumes of working solution and drug-free human plasma were added to each test tube. Final concentrations were 3.5, 17.6, 35.3, 70.6, 176.5, 353 and 706 ng/ml for VEN; 2.2, 22.2, 44.4, 111, 222, 444 and 888 ng/ml for ODV; 2.7, 27, 54, 135, 270, 540 and 810 ng/ml for NDV; 1.9, 18.6, 37.2, 93, 186.0, 372 and 744 ng/ml for DDV. Quality control samples, which were run in each assay, were prepared in the same way.

### 2.3. Chromatographic conditions

Chromatographic separation of the analytes was performed on a BDS HYPERSIL $C_{18}$ (5 μm, 250 mm × 4.6 mm, USA) column with column temperature 50 °C. The mobile phase consisted of water (ammonium acetate: 30 mmol/l, formic acid 2.6 mmol/l, and trifluoroacetic acid 0.13 mmol/l) and acetonitrile (60:40, V/V) at a flow-rate of 1.0 ml/min and a postcolumn splitting ratio of 3:1, and was filtered using 0.45 μm filters in a Millipore solvent filtration apparatus. The gradient elution program conditions were given in Table 1.

### 2.4. MS/ESI detection conditions

The compounds were ionized in the positive electrospray ionization ion source ($ESI^+$) of the mass-spectrometer. Selected ion recording (SIR) mode was used for quantitation by the protonated molecular ions of each analyte.

The final optimized detection conditions were as follows: capillary voltage, 3.50 kV; cone voltage, 26 V for VEN, 21 V for ODV and NDV, 20 V for DDV, and 37 V for estazolam (IS); extractor voltage, 2 V; source temperature, 105 °C and desolvation temperature, 290 °C. Cone gas flow, 110 l/h; desolvation gas flow, 300 l/h.

### 2.5. Sample preparation

Thirty microliters internal standard working solution (Estazolam, 1.36 μg/ml) was added to the sample. The sample (0.5 ml) was alkalinized by adding 30 μl aqueous ammonia then shaken for 1 min. Two milliliters of ether was added to the sample. After 2 min vortical mix, the mixture was centrifuged at 3000 × *g* for 5 min at room temperature (20 °C), the upper layer was carefully aspirated and the remainder was extracted once again with 2 ml

Table 2
Accuracy, intra- and inter-day precision

| Added drug | Concentration (ng/ml) | Inter-day precision (%, $n=5$) | | Accuracy[a] | Intra-day precision (%, $n=5$) | |
|---|---|---|---|---|---|---|
| | | Found ± S.D. | R.S.D. (%) | Bias % | Found ± S.D. | R.S.D. (%) |
| VEN | 3.5 | 3.6 ± 0.1 | 2.8 | 2.8 | 3.3 ± 0.1 | 3.0 |
| | 70.6 | 68.1 ± 4.6 | 6.7 | −3.5 | 69.5 ± 5.9 | 8.5 |
| | 706 | 694.1 ± 18.0 | 2.6 | −1.6 | 704 ± 29.5 | 4.2 |
| ODV | 2.2 | 2.1 ± 0.1 | 4.8 | −4.5 | 1.9 ± 0.1 | 5.3 |
| | 44.4 | 45.8 ± 2.5 | 5.5 | 3.1 | 40.2 ± 3.2 | 8.0 |
| | 444 | 443.0 ± 15.2 | 3.4 | −0.2 | 439.1 ± 18.5 | 4.2 |
| NDV | 2.7 | 2.5 ± 0.2 | 8.0 | −7.4 | 2.3 ± 0.15 | 6.5 |
| | 54 | 53.4 ± 3.8 | 6.7 | −1.1 | 54.3 ± 5.5 | 10.1 |
| | 540 | 541.1 ± 14.3 | 2.5 | 0.2 | 532.0 ± 22.3 | 4.2 |
| DDV | 1.9 | 1.7 ± 0.1 | 5.9 | −9.4 | 1.6 ± 0.1 | 6.3 |
| | 37.2 | 36.6 ± 2.5 | 6.8 | −1.6 | 37.6 ± 2.3 | 6.2 |
| | 372 | 371.9 ± 7.7 | 2.1 | −0.1 | 366.2 ± 11.8 | 3.2 |

[a] (Found − nominal)/nominal × 100.

ether. The upper layer was put together with the former layer, and the ether was evaporated under a stream of nitrogen at 50 °C. The residue was reconstituted in 100 μl mobile phase. Thirty microliters of the solution were injected for analysis through the auto-injector.

### 2.6. Validation of method

The extraction recoveries were determined at three concentration levels by comparing peak-area ratios of the extracts of spiked plasma (three different concentration, $n=5$) with those obtained by direct injection of an aqueous solution of the compounds. The methodology recoveries were measured as the percentage difference from theoretical according to the equation:

$$\text{Methodology recovery}\,(\%) = \left(\frac{\text{concentration}_{\text{measured}}}{\text{concentration}_{\text{theoretical}}}\right) \times 100$$

Precision assays were carried out five times using three different concentrations (Table 2) on the same day and over five different days.

Calibration was performed by a least-squares linear regression of the peak-area ratios of the drugs to the I.S. versus the respective standard concentration.

### 2.7. Selectivity

Various antidepressants (fluoxetine, citalopram, paroxetine, duloxetine, milnacipram, and reboxetine) used in management of depression were evaluated for interference with the assay for VEN and its three metabolites.

## 3. Results

### 3.1. HPLC-MS/ESI

The column temperature was 50 °C in order to reduce pressure of column and improve resolution. Thirty microliters reconstituted solution was injected for analysis in order to improve the sensitivity.

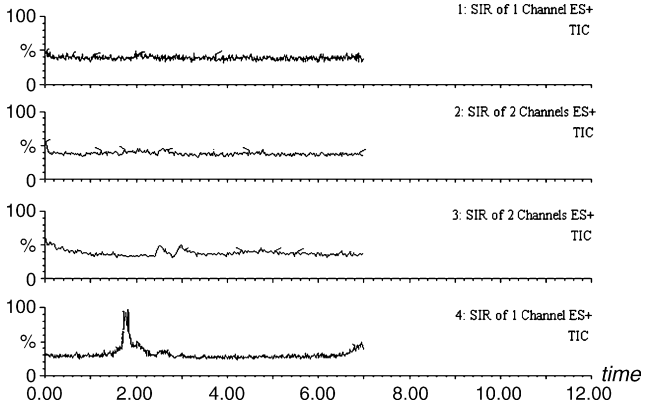


Fig. 2. Chromatograms of control human plasma.

The HPLC-MS/ESI in the SIR mode provided a highly selective method for the determination of VEN, ODV, NDV and DDV. The gradient elution program shortened the run time of all analytes. The retention time of VEN, ODV, NDV and DDV were approximately 4.43, 3.01, 3.95, 2.88 and 5.50 min, respectively. Compared with the published methods (the chromatographic

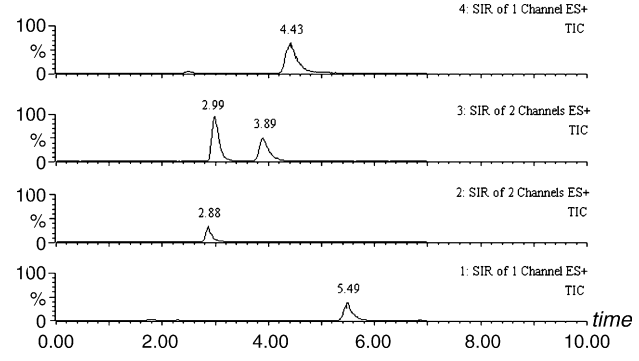


Fig. 3. Chromatograms of standards and I.S. in control human plasma. Channel 1: Estazolam, Channel 2: DDV, Channel 3: ODV and NDV, Channel 4: VEN.

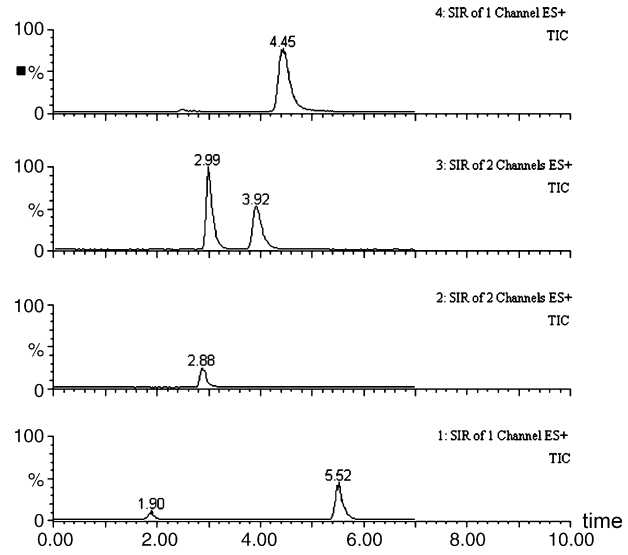


Fig. 4. Chromatgrams of patient samples. The represented patient is NO. 7.

run was as long as 10–20 min [7,8,10–13]), the run time of this method was shorter, i.e. the complete elution was obtained in less than 6.0 min. The chromatograms of control human plasma, standards in control human plasma and patient samples were shown in Figs. 2–4, respectively. The protonated molecules of the standards of $ESI^+$ mass spectrum (SIR) in control human plasma were identified at *m/z* 295.0 for I.S. (channel 1), 250.4 for $[DDV + H]^+$, 232.3 for $[DDV - OH]^+$(channel 2), 246.4 for $[ODV/NDV - OH]^+$, 264.4 for $[ODV/NDV + H]^+$(channel 3), 260.4 for $[VEN - OH]^+$ (channel 4). An $ESI^+$ mass spectrum (SIR) of standards in controlled plasma was illustrated in Fig. 5.

### 3.2. Calibration curves

The concentration range were 4.0–700 ng/ml for VEN, 2.0–900 ng/ml for ODV, 3.0–800 ng/ml for NDV and 2.0–700 ng/ml for DDV. The area ratio of each analytes to I.S. was well related to the concentration. The data were based on five replicates of a seven-point calibration curve and the linear

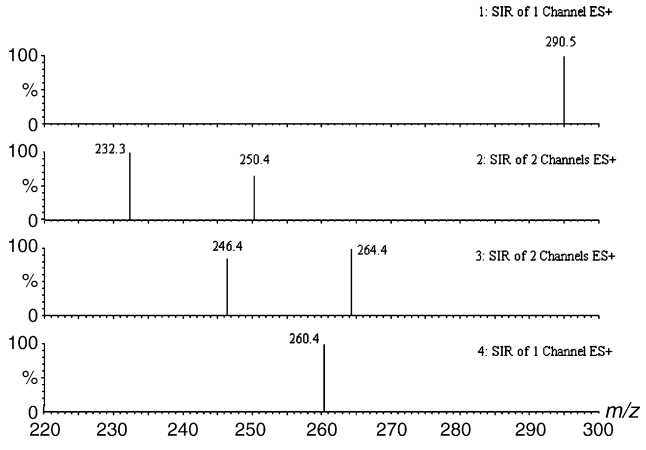


Fig. 5. $ESI^+$ mass spectra of standard in controlled plasma. Channel 1: Estazolam, Channel 2: DDV, Channel 3: ODV and NDV, Channel 4: VEN in this Figure (same in Figs. 3–5).

relationships were described by following equations:

$$\text{VEN}: \quad Y = 0.035X + 0.078 \quad r = 0.9997$$

$$\text{ODV}: \quad Y = 0.042X + 0.037 \quad r = 0.9991$$

$$\text{NDV}: \quad Y = 0.026X + 0.048 \quad r = 0.9992$$

$$\text{DDV}: \quad Y = 0.010X + 0.098 \quad r = 0.9993$$

### 3.3. Validation: accuracy, precision, recovery, selectivity and LOD/LLOQ

The accuracy, mean extraction recoveries (mean ± S.D.), methodology recoveries (mean ± S.D.), intra- and inter-day precision for the four analytes were shown in Tables 2 and 3. The average extraction recoveries for all the four analytes were at least above 77%. The average methodology recoveries were higher than 91% for the analytes. The intra- and inter-day R.S.D. were less than 11%.

The precision assays were carried out on five continuous days before and after analyzing of the samples. The samples were stored at −70 °C in ultra cold freezer during the time that they were not being analyzed.

Five quality control plasma samples were utilized to determine the sensitivity. The limit of detection (LOD) was 0.4 ng/ml for VEN, 0.2 ng/ml for ODV, 0.3 ng/ml for NDV, and 0.2 ng/ml for DDV, respectively (S/N = 3).

The standard solutions of various antidepressants like fluoxetine, citalopram, paroxetine, duloxetine, milnacipram, and reboxetine were determined by using the present method. It was approved that there weren't any interferences with VEN and its three metabolites.

The lower limit of quantification (LLOQ), defined as the minimum concentration at which the analyte could be quantified with acceptable accuracy and precision (R.S.D. < 15%), was determined by the experimental analysis of different samples with known concentrations of the analyte. And the LLOQs of VEN, ODV, NDV and DDV in the present method were 3.5, 2.2, 2.7 and 1.9 ng/ml, respectively, which was the lowest concentration of the calibration curves and the R.S.D. all were below 11% ($n = 5$). Similarly, the upper limit of quantification were 706, 888, 810, and 744 ng/ml for VEN, ODV, NDV, and DDV (R.S.D. < 12%, $n = 5$).

### 3.4. Stability

Standard solutions of VEN (14.1 μg/ml), ODV (8.9 μg/ml), NDV (10.8 μg/ml) and DDV (7.4 μg/ml) in methanol were stored at 0 °C for 3 months. All analytes appeared to be stable as the publications described [14].

Three batches of quality control samples spiked with the four analytes (VEN: 3.5, 70.6 and 706 ng/ml;ODV: 2.2, 44.4 and 444 ng/ml; NDV: 2.7, 54 and 540 ng/ml; DDV: 1.9, 37.2 and 372 ng/ml) were stored at −20 °C for 30 days, at 20 °C for 24 h or experienced three cycles of freeze–thaw, respectively. Then, the quality control samples were determined using the present

Table 3
Mean extraction recoveries (±S.D.), methodology recoveries (±S.D.) and R.S.D.

| Added drug | Concentration (ng/ml) | Mean extraction recoveries (%, $n=5$) | | Mean methodology recoveries (%, $n=5$) | | |
|---|---|---|---|---|---|---|
| | | Mean recoveries ± S.D. | R.S.D. (%) | Found ± S.D. | Recoveries (%) | R.S.D. (%) |
| VEN | 3.5 | 85 ± 7.2 | 8.4 | 3.6 ± 0.1 | 100.5 | 2.8 |
| | 70.6 | 88.4 ± 9.0 | 10.1 | 70.8 ± 5.7 | 100.3 | 8 |
| | 706 | 91.7 ± 3.4 | 3.7 | 692.7 ± 20.2 | 98.1 | 2.9 |
| ODV | 2.2 | 77.5 ± 8.4 | 10.9 | 2.1 ± 0.1 | 99.1 | 4.7 |
| | 44.4 | 91.7 ± 6.7 | 7.3 | 45.9 ± 3.0 | 103.4 | 6.6 |
| | 444 | 82.1 ± 4.8 | 5.8 | 440.3 ± 19.4 | 99.2 | 3.9 |
| NDV | 2.7 | 88.6 ± 3.8 | 4.2 | 2.5 ± 0.2 | 93.3 | 8.0 |
| | 54 | 85.0 ± 6.0 | 7.0 | 56.4 ± 5.6 | 98.9 | 9.9 |
| | 540 | 89.5 ± 3.4 | 3.8 | 541.1 ± 14.2 | 100.2 | 2.6 |
| DDV | 1.9 | 91.9 ± 5.7 | 6.2 | 1.7 ± 0.1 | 91.4 | 3.7 |
| | 37.2 | 82.1 ± 8.3 | 10.1 | 36.6 ± 2.5 | 98.4 | 6.9 |
| | 372 | 78.6 ± 5.4 | 6.9 | 371.9 ± 7.7 | 99.9 | 2.1 |

Table 4
Stability results for VEN, ODV, NDV and DDV ($n=5$)

| Conditions | VEN[a] | | ODV[a] | | NDV[a] | | DDV[a] | |
|---|---|---|---|---|---|---|---|---|
| | $Con._T$ | $Con._M$ | $Con._T$ | $Con._M$ | $Con._T$ | $Con._M$ | $Con._T$ | $Con._M$ |
| −20 °C for 30 days | 3.5 | 3.3 ± 0.1 | 2.2 | 2.1 ± 0.1 | 2.7 | 2.6 ± 0.1 | 1.9 | 1.8 ± 0.1 |
| | 70.6 | 67.5 ± 5.1 | 44.4 | 43.5 ± 2.5 | 54 | 52.3 ± 4.3 | 37.2 | 35.1 ± 2.6 |
| | 706 | 681 ± 29.7 | 444 | 435.2 ± 21.1 | 540 | 530.6 ± 19.1 | 372 | 368.1 ± 15.0 |
| 20 °C for 24 h | 3.5 | 3.2 ± 0.1 | 2.2 | 2.0 ± 0.1 | 2.7 | 2.5 ± 0.1 | 1.9 | 1.7 ± 0.1 |
| | 70.6 | 65.2 ± 4.0 | 44.4 | 41.2 ± 2.7 | 54 | 50.1 ± 4.5 | 37.2 | 34.3 ± 2.8 |
| | 706 | 678.5 ± 21.1 | 444 | 430.3 ± 26.6 | 540 | 528.9 ± 22.6 | 372 | 359.0 ± 20.1 |
| 3 Cycles of freeze–thaw | 3.5 | 3.4 ± 0.1 | 2.2 | 2.1 ± 0.1 | 2.7 | 2.6 ± 0.1 | 1.9 | 1.8 ± 0.1 |
| | 70.6 | 69.2 ± 4.1 | 44.4 | 43.0 ± 3.3 | 54 | 52.1 ± 4.3 | 37.2 | 34.0 ± 2.8 |
| | 706 | 691.5 ± 21.1 | 444 | 438.2 ± 24.0 | 540 | 527.8 ± 21.0 | 372 | 361.5 ± 17.1 |
| 0 °C for 3 months | 3.5 | 3.2 ± 0.1 | 2.2 | 2.0 ± 0.1 | 2.7 | 2.5 ± 0.2 | 1.9 | 1.7 ± 0.2 |
| | 70.6 | 66.7 ± 5.6 | 44.4 | 42.7 ± 3.1 | 54 | 49.1 ± 4.8 | 37.2 | 33.5 ± 2.5 |
| | 706 | 675.5 ± 30.1 | 444 | 427.9 ± 28.8 | 540 | 480.5 ± 35.1 | 372 | 350.0 ± 25.6 |

$Con._T$: theoretical concentration; $Con._M$: measured concentration.
[a] Mean (±S.D.); Concentration (ng/ml).

method. All analytes showed good stability with R.S.D. less than 12% (see Table 4).

### 3.5. Analysis of patient plasma

Plasma samples were obtained from seven depressed patients who were administered multi-dose of venlafaxine (the dosage is listed in Table 4) for one week and didn't receive other antidepressants comedication. At day 8, the patients' plasma samples were collected before and after administration of venlafaxine, respectively. The specimen collection from human subjects was approved by the Ethical Committee of Xiang Ya Second Hospital of Central South University.

The patients' plasma drug concentrations determined by the method are shown in Table 5. For all patients, the concentration of ODV was higher than VEN whenever in $Css_{min}$ or $Css_{max}$ which agreed with the previous report [12]. The major reason was that the half-life of ODV (11 h) was larger than VEN (5 h) at steady state [15]. While the concentration of NDV in all patients is lowest except for the patient No. 7. Thus, further work should be done to investigate the metabolism of venlafaxine in Chinese suffering from depression. And it is necessary to monitor the concentration of both VEN and ODV simultaneously during the therapeutic drug monitoring.

## 4. Discussions

At present, high performance liquid chromatography-electrospray mass spectrometry (HPLC-MS/ESI) has emerged as a powerful analytical technique for the determination of drugs and metabolites in biological fluids. It can detect the concentration by using one *m/z* or several *m/z* of one compound. There is an individual detecting channel for each compound in the selected ion recording mode (SIR), so the compounds with different *m/z*, which had not been separated under HPLC condition, can be separated and not interfere with each other through different detecting channels. In the present study, we discovered that the molecular ion of VEN was unstable and the intensity of peak of

Table 5
Plasma concentrations in depressed patients

| Patient No. | Gender | Drug administered | Drug dose (mg/day) | Analyte | Concentration (ng/ml) | |
|---|---|---|---|---|---|---|
| | | | | | $Css_{min}$ [a] | $Css_{max}$ [b] |
| 1 | Female | Venalfaxine | 200 | VEN | 52.3 | 144.4 |
| | | | | ODV | 173.4 | 234.6 |
| | | | | NDV | 19.3 | 29.4 |
| | | | | DDV | 45.8 | 74.5 |
| 2 | Female | Venlafaxine | 75 | VEN | 12.2 | 55.9 |
| | | | | ODV | 27.7 | 59.4 |
| | | | | NDV | 5.1 | 11.0 |
| | | | | DDV | 17.3 | 32.8 |
| 3 | Male | Venlafaxine | 75 | VEN | 13.7 | 22.2 |
| | | | | ODV | 46.2 | 59.3 |
| | | | | NDV | 4.0 | 8.5 |
| | | | | DDV | 11.6 | 27.1 |
| 4 | Male | Venlafaxine | 75 | VEN | 3.4 | 42.0 |
| | | | | ODV | 47.2 | 80.2 |
| | | | | NDV | 3.3 | 4.5 |
| | | | | DDV | 6.7 | 17.1 |
| 5 | Male | Venlafaxine | 75 | VEN | 26.0 | 34.4 |
| | | | | ODV | 39.0 | 51.4 |
| | | | | NDV | 3.6 | 5.2 |
| | | | | DDV | 6.8 | 22.9 |
| 6 | Female | Venlafaxine | 200 | VEN | 74.0 | 150.7 |
| | | | | ODV | 150.5 | 276.6 |
| | | | | NDV | 50.7 | 92.4 |
| | | | | DDV | 101.0 | 180.1 |
| 7 | Female | Venlafaxine | 75 | VEN | 12.3 | 14.0 |
| | | | | ODV | 216.0 | 317.2 |
| | | | | NDV | 302.3 | 431.7 |
| | | | | DDV | 14.0 | 37.5 |

[a] The values were determined before their administration of the drug when they had got their steady plasma-drug concentrations.
[b] The values were determined after the administration of the drug according to the $T_{max}$ of the drug when they got their steady plasma-drug concentrations.

*m/z* 278.2 was relatively low, so we selected the ion *m/z* 260.4 for its quantification (channel 4). The responses of ODV, NDV and DDV were all relatively weak, so we selected two strong and stable ions for the detection in both channel 2 and 3.

Compared with other HPLC methods, HPLC-MS/ESI improved the specificity, shortened the analysis time, and simplified the preparation of the sample. However compounds with the same *m/z*, like ODV and NDV, must be separated completely under HPLC condition to avoid interferences with each other, thus there was a choice of the mobile phase during the experiment.

## 5. Conclusions

Compared with others methods, HPLC-MS/ESI improved the specificity and sensitivity, the gradient elution program shortened the analytical time of sample. The main aim of the study was to establish a HPLC-MS/ESI method that was suitable for simultaneous determination of venlafaxine and its three metabolites in plasma of patients undergoing antidepressant therapy with venlafaxine. The method described has been found to be specific and accurate in application. To the best of our knowledge, this method meets the request of the present pharmacokinetic study of the drug. As for the TDM, the greatest advantage of the present method is the shortening of the analysis time of the compounds. Thus, the method suits for pharmacokinetic study of venlafaxine, and for the analysis of samples when undertaking TDM.

## Acknowledgement

The authors would like to thank Deng Mengxian, the chief nurse of Second XiangYa Hospital for her aid in the collection of plasma of the depressed patient.

## References

[1] J.M. Andrews, P.T. Ninan, C.B. Nemeroff, Depression 4 (1996) 48.
[2] D.R. Hicks, D. Wolaniuk, A. Russell, N. Cavanaugh, M. Kraml, Ther. Drug Monit. 16 (1994) 100.
[3] D.C. Deecher, C.E. Beyer, G. Johnston, et al., J. Pharmacol. Exp. Ther. 318 (2006) 657.
[4] J.E. Kelsey, J. Clin. Psychopharmacol. 16 (1996) 21s.
[5] D. Hackett, E. Salinas, Eur. Psychiatry 12 (Suppl. 4) (1997) 301s.
[6] S.M. Fogelman, J. Schmider, K. Venkatakrishnan, L.L. Von Moltke, J.S. Harmatz, R.I. Shader, D.J. Greenblatt, Neuropsychopharmacology 20 (1999) 480.

[7] C. Long, J. Crifasi, D. Maginn, M. Graham, S. Teas, J. Anal. Toxicol. 21 (1997) 166.
[8] S. Paterson, R. Cordero, S. Burlinson, J. Chromatogr. B 813 (2004) 323.
[9] J. Bhatt, A. Jangid, G. Venkatesh, G. Subbaiah, S. Singh, J. Chromatogr. B Analyt. Technal. Biomed. Life Sci. 829 (2005) 75.
[10] E.M. Clement, J. Odontiadis, M. Franklin, J. Chromatogr. B Biomed. Sci. Appl. 705 (1998) 303.
[11] R. Waschqler, W. Moll, P. Koniq, A. Conca, Int. J. Clin. Pharmacol. Ther. 42 (2004) 724.
[12] M. Matoga, F. Pehourcq, K. Titier, F. Dumora, C. Jarry, J. Chromatogr. B 760 (2001) 213.
[13] C. Duverneuil, G.L. de la Grandmaison, P. de Mazan Court, J.C. Alvarez, Ther. Drug Monit. 5 (2003) 565.
[14] M. Reis, J. Lundmark, H. Bjork, F. Bengtsson, Ther. Drug Monit. 24 (2002) 545.
[15] H. Veefkind Adrian, Haffmans P.M. Judith, Hoencamp Erick, Ther. Drug Monit. 22 (2000) 202.