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Simultaneous stereoselective analysis of venlafaxine and *O*-desmethylvenlafaxine enantiomers in human plasma by HPLC-ESI/MS using a vancomycin chiral column

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Abstract

A high-performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESI/MS) method for simultaneous stereoselective analysis of venlafaxine (VEN) and its major metabolite *O*-desmethylvenlafaxine (ODV) enantiomers in human plasma has been developed and validated. Chiral chromatography is performed on the CHRIOBIOTIC V ™ (5 μm, 250 mm × 4.6 mm) column with mobile phase constituted of 30 mmol/l ammonium acetate–methanol (15:85, pH 6.0) at a flow rate of 1.0 ml/min and a postcolumn splitting ratio of 3:1. The compounds were ionized in the electrospray ionization (ESI) ion source of the mass spectrometer and detected using the selected ion recording (SIR) mode. Calibration curves obtained from spiked plasma were linear in the range of 5.0–400 ng/ml for *S*-(+)-VEN and *R*-(−)-VEN, 4.0–280 ng/ml for *S*-(+)-ODV and *R*-(−)-ODV, respectively, with linear correlation coefficient all above 0.999. The average extraction recoveries for all the four analytes were above 76%. The methodology recoveries were higher than 92%. The limit of detection were 1.0 ng/ml for *S*-(+)-VEN and *R*-(−)-VEN, 1.5 ng/ml for *S*-(+)-ODV and *R*-(−)-ODV, respectively. The intra- and inter-day variation coefficients were less than 9%.
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Keywords: Venlafaxine; Enantioseparation; Vancomycin chiral column; HPLC-MS/ESI

1. Introduction

Chiral discrimination is frequently encountered in biological systems. Chirality is also an important issue in the pharmaceutical industry due to the potential of different activities and toxicities of drug enantiomers [1]. The pharmacological, pharmacodynamic, and toxicological behavior of the enantiomers of chiral drugs can differ widely. It is therefore of importance to develop enantioselective separation methods for studies on stereoselective pharmacokinetics and metabolism [2]. Chiral high-performance liquid chromatography is a fast, selective and effective technique, successfully employed for determination of enantiomers of drugs.

Vancomycin, a macrocyclic antibiotic, which is an amphoteric glycopeptide produced by *streptomyces orinetalis*, has been introduced by Armstrong et al. as a powerful chiral selector in liquid chromatography [3], thin-layer chromatography [4], and capillary electrophoresis [5]. A variety of racemic compounds have been resolved on it, such as promethazine, mirtazapine, benidipine, vesamicol, flurbiprofen, ketoprofen, citalopram and so on [6–12]. High-performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESI/MS) is a powerful tool for quantitative analysis when evaluated on the basis of speed, specificity, reliability and sensitivity. The present paper explored the feasibility of HPLC-ESI/MS coupled with a vancomycin chiral phase for the quantitative analysis of the enantiomers of venlafaxine (VEN) and its major metabolite, *O*-desmethylvenlafaxine (ODV) in human plasma.

Venlafaxine (1-[2-(dimethylamino)-1-(4-methoxy-phenyl) ethyl]cyclohexanol hydrochloride) (Fig. 1A) is a particularly effective second generation antidepressant chiral drug, administered as a racemic mixture, exerting a dual mechanism of action on the monoaminergic system [13,14]. The two enantiomers exhibit different activity. The *R*-(−) enantiomer inhibits both the noradrenalin and serotonine synaptic re-uptake whereas the *S*-(+) enantiomer inhibits only the serotonine one [15]. *O*-Desmethylvenlafaxine (Fig. 1B) is the main metabolite

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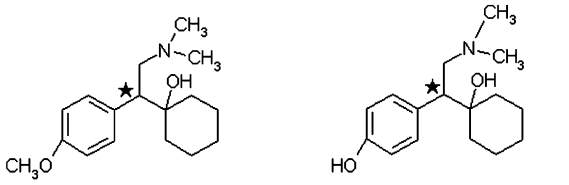

A. venlafaxine(VEN) B. o-desmethylafaxine(ODV)

Fig. 1. The structure of venlafaxine (VEN) and *O*-desmethylvenlafaxine (ODV)–the star represent the chiral centre.

produced by biotransformation in humans presenting a pharmaceutic activity similar to that of venlafaxine, which also has a chiral centre and now the racemic ODV is being developed to a new antidepressant utilized in the depression management [16]. So, the determination of the enantiomeric concentration of venlafaxine and *O*-desmethylvenlafaxine in human's plasma is important to understand the mechanism of action of each enantiomer and their pharmacokinetic and pharmacodynamic relation.

Analytical methods for determination of the enantiomers of VEN and/or ODV include high performance liquid chromatography (HPLC) [17], which only determined the venlafaxine enantiomers by utilizing a chiral reagent, and capillary electrophoresis (CE), which have a relative low sensitivity (LOD > 20 ng/ml) [18,19].

The aim of the present paper is to establish a specific, reliable and sensitive LC–ESI/MS method coupled with vancomycin chiral column for simultaneous determination of two enantiomers of VEN and ODV in human plasma.

2. Experimental

2.1. Equipments and reagents

A system of HPLC (Waters2690, USA)-MS with a Micromass ZQ mass spectrometer (Wythenshawe, Manchester, UK) with mass-selective detector equipped with an electrospary ionisation (ESI) ion source was used. COMPAQ Deskpro Workstation and MassLynxTM3.5 software were utilized.

S-(+)-VEN (>99.8%), *R*-(−)-VEV (>99.8%), *S*-(+)-ODV (>99.8%), *R*-(−)-ODV (>99.8%) were purchased from TRC (Toronto Research Chemicals Inc., Canada), Sildenafil (I.S) (99.8%) was obtained from Sigma (Steinheim, Germany).

HPLC grade reagents (methanol, acetontritile, 2-propanol, *tert*-butyl methyl ether) were purchased from Caledon Laboratory LTD (Georgetown Ont., Canada). Other AR grade reagents (aqueous ammonia, 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 *R*-(−)-VEN (80 μg/ml) *S*-(+)-VEN (120 μg/ml), *R*-(−)-ODV (68.4 μg/ml), *S*-(+)-ODV (630 μg/ml) were prepared by dissolving appropriate amount 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.

Routine daily calibration curves were prepared in drug-free serum. Appropriate volume of working solutions and drug-free human plasma were added to each test tube. Final concentration were 5.0, 10.0, 25, 50, 100, 200 and 400 ng/ml for *S*-(+)-VEN; 5.2, 10.3, 25.8, 51.5, 103, 206 and 412 ng/ml for *R*-(−)-VEN; 3.5, 7.0, 17.9, 35.8, 71.5, 171 and 342 ng/ml for *S*-(+)-ODV; 4.3, 8.5, 21.4, 42.7, 85.4, 143 and 286 ng/ml for *R*-(−)-ODV. Quality control samples that were run in each assay, were prepared in the same way.

2.3. Chromatographic conditions

The analytic column was a CHIROBIC V ™ (5 μm, 250 mm × 4.6 mm) (Astec, USA, Cat. #11024, Ser. #20444) with column temperature 25 °C. The mobile phase for chiral HPLC analysis consisted of 30 mmol/l ammonium acetate–methanol (15:85, pH 6.0) at a flow rate of 1.0 ml/min and a postcolumn splitting ratio of 3:1.

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, 3.0 kV; cone voltage, 26 V for VEN, 21 V for ODV, 42 V for Sildenafil (I.S); 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

One hundred microliters internal standard working solution (Sildenafil, 2.4 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 *tert*-butyl methyl ether was added to the sample. After 2 min vortex-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 *tert*-butyl methyl ether. The upper layer was put together with former, and the *tert*-butyl methyl ether was evaporated under a stream of nitrogen at 40 °C. The residue was reconstituted in 100 μl mobile phase. Twenty microliters solution was injected for analysis through the auto-injector. This procedure was applied to both the spiked plasma samples and clinical samples.

2.6. Validation of method

The extraction recoveries were determined at three concentration levels by comparing the analytes peak areas obtained from the quality control samples ($n = 5$) after extraction with

Table 1
Intra- and inter-day precision

Added drug	Concentration (ng/ml)	Inter-day precision (%, $n=5$)		Intra-day precision (%, $n=5$)	
		Found ± SD	RSD (%)	Found ± SD	RSD (%)
S-(+)-VEN	10.5	10.3 ± 0.6	6.2	9.8 ± 0.3	3.0
	35	33.3 ± 1.7	5.0	31.3 ± 1.4	4.4
	105	103.4 ± 1.6	1.5	103.4 ± 1.7	1.7
R-(−)-VEN	10.5	9.5 ± 0.7	7.0	8.9 ± 0.8	8.8
	35	37.5 ± 1.5	4.0	31.6 ± 0.7	2.2
	105	103.9 ± 1.7	1.6	102.2 ± 2.0	1.9
S-(+)-ODV	10.7	9.6 ± 0.4	4.2	9.5 ± 0.5	5.2
	35.7	30.9 ± 1.3	4.2	29.9 ± 0.6	1.9
	107	102.9 ± 5.7	5.5	104.4 ± 2.0	1.9
R-(−)-ODV	10.7	10.1 ± 0.2	2.0	9.4 ± 0.3	3.6
	35.7	31.7 ± 0.4	1.3	30.3 ± 1.3	4.2
	107	101.5 ± 2.3	2.2	102.3 ± 1.2	1.2

Table 2
Matrix effect of VEN and ODV enantiomers, and I.S. in water or control plasma ($n=5$)

Added drug	Concentration (ng/ml)	Water		Control plasma	
		AME[a] (%)	RSD (%)	AME[a] (%)	RSD (%)
S-(+)-VEN	10.5	97.1	3.2	94.3	4.6
	35	95.4	4.6	93.6	3.1
	105	95.3	5.8	96.8	1.9
R-(−)-VEN	10.5	97.0	8.2	97.8	3.4
	35	103.5	7.6	97.7	2.5
	105	98.8	2.1	100.5	1.4
S-(+)-ODV	10.7	95.2	4.4	89.5	4.3
	35.7	95.4	3.4	91.2	1.7
	107	96.0	4.1	95.6	2.2
R-(−)-ODV	10.7	94.2	5.6	88.1	6.1
	35.7	96.6	2.2	90.3	4.9
	107	104.4	5.5	94.3	3.2
I.S. (Sildenafil)	24	93.0	3.7	90.1	5.8
	240	94.5	5.1	95.5	4.5
	2400	93.3	4.9	99.2	2.2

[a] The average matrix effect.

those obtained from the corresponding unextracted reference standards prepared at the same concentrations. 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 1) on the same day and over five different days.

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

2.7. Evaluation of matrix effect

In correspondence to the strategy applied by Matuszewski et al. [20], matrix effects were evaluated by comparing the MS responses of known amounts of working standards (A) with those measured in a blank water or control plasma extract spiked with the same analyte amount after extraction (B). Differences observed in MS response could thus be attributed to the effect of sample matrix on the ionization efficiency only. The ratio (B/A × 100) is defined as absolute matrix effect (ME%). The absence of absolute matrix effect is indicated by a value of 100%, i.e. the response in the mobile phase and in the extract was the same. A value of >100% indicates an ionization enhancement and a value of <100% indicates an ionization suppression. If the ratio <85% or >115%, a matrix effect is implied. The results showed there was no matrix effect of the analytes observed in present study (Table 2).

3. Results

The HPLC-MS/ESI in the SIR mode provided a highly selective method for the determination of *S*-(+)-VEN, *R*-(−)-VEN, *S*-(+)-ODV, *R*-(−)-ODV and Sildenafil. The retention times of them were approximately 11.8, 12.8, 11.2, 11.9 and 4.6 min, respectively. The chromatograms of control human plasma, standards in control human plasma and patient samples were shown

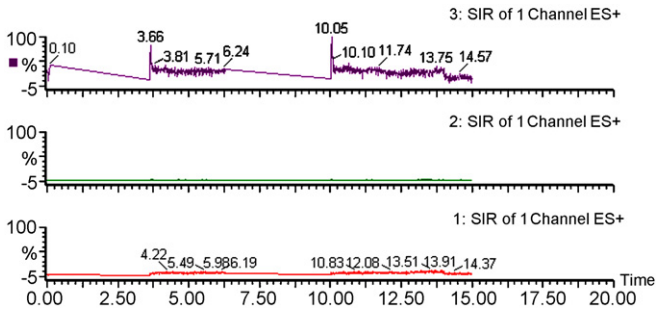

Fig. 2. Chromatograms of control human plasma. Channel 1: ODV, Channel 2: I.S., Channel 3: VEN. The mobile phase was methanol–ammonium acetate buffer (30 mM, pH 6.0) (85:15, v/v).

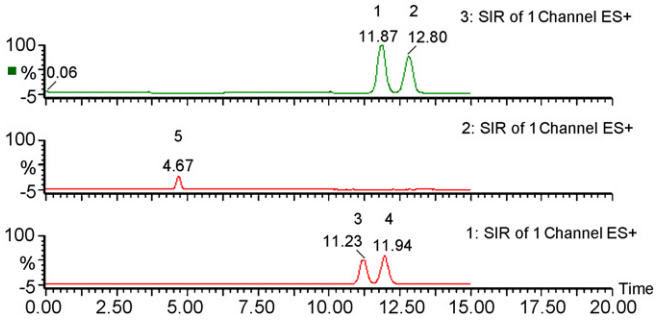

Fig. 3. Chromatograms of compounds and I.S. in the control human plasma Channel 1: ODV, Channel 2: I.S., Channel 3: VEN. Figs. 1–5 represent the *S*-(+)-VEN, *R*-(−)-VEN, *S*-(+)-ODV, *R*-(−)-ODV and Sildenafil, respectively The mobile phase was methanol–ammonium acetate buffer (30 mM, pH 6.0) (85:15, v/v).

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* 278.2 for VEN (channel 3), 475.4 for I.S. (channel 2), 264.1 for ODV (channel 1) (Fig. 5).

3.1. Selectivity

Selectivity was assessed by comparing the chromatograms of the blank plasma with the corresponding spiked plasma. Figs. 2–4 showed the typical chromatograms of blank plasma, spiked plasma sample with VEN, ODV and I.S., and plasma sample from the patient 4.0 h after an oral administration.

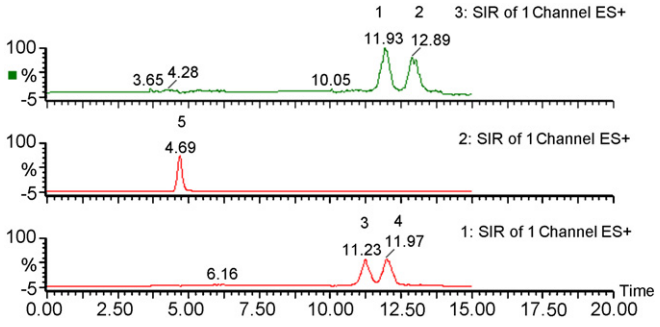

Fig. 4. Chromatograms of the patient sample. Channel 1: ODV, Channel 2: I.S., Channel 3: VEN. Figs. 1–5 represent the *S*-(+)-VEN, *R*-(−)-VEN *S*-(+)-ODV, *R*-(−)-ODV and I.S, respectively. The mobile phase was methanol–ammonium acetate buffer (30 mM, pH 6.0) (85:15, v/v).

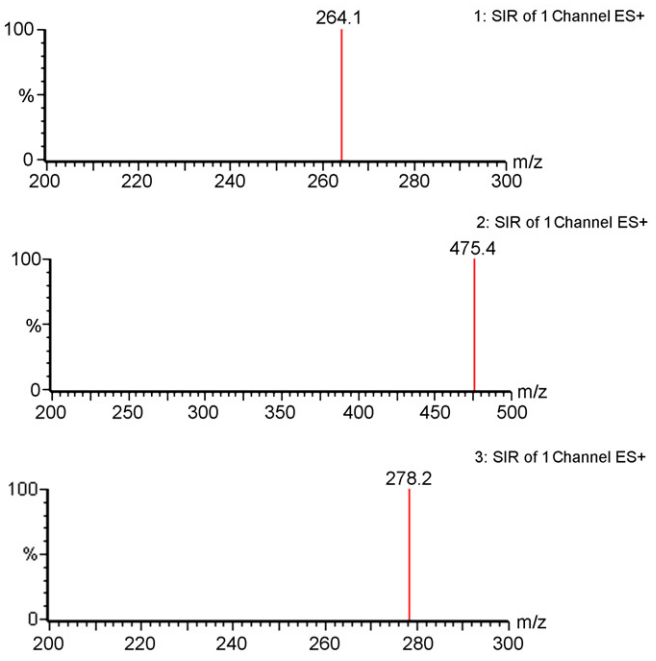

Fig. 5. The mass-spectrogram of compounds and I.S. in the control human plasma Channel 1: ODV, Channel 2: S I.S., Channel 3: VEN.

Interferences from the matrices at the expected retention times of the target ions were not observed.

3.2. Calibration curves

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. The concentration range were 5.0–400 ng/ml for *S*-(+)-VEN and *R*-(−)-VEN, 4.0–300 ng/ml for *S*-(+)-ODV and *R*-(−)-ODV. The area ratio of each analyte to I.S. was well related to the concentration. The data were based on five replicates of a seven-point calibration curve. The linear relationships were described by following equations:

$$\text{S-(+)-VEN}: \ Y = 0.0896 \times X - 0.461, \quad r = 0.9997$$

$$\text{R-(−)-VEN}: \ Y = 0.0691 \times X - 0.308, \quad r = 0.9996$$

$$\text{S-(+)-ODV}: \ Y = 0.0499 \times X - 0.144, \quad r = 0.9997$$

$$\text{R-(−)-ODV}: \ Y = 0.0614 \times X - 0.400, \quad r = 0.9991$$

The lower limit of quantification (LLOQ), defined as the minimum concentration at which the analyte could be quantified with acceptable accuracy and precision (RSD < 15%), was determined by the experimental analysis of different samples with known concentrations of the analyte. And the LLOQ of *S*-(+)-VEN, *R*-(−)-VEN, *S*-(+)-ODV and *R*-(−)-ODV in present method were 5.0, 5.2, 4.3 and 3.5 ng/ml, respectively, which were the lowest concentration of the calibration curves and the RSD all were below 9%. Similarly, the upper limit of quantification were 400, 412, 342, and 286 ng/ml for *S*-(+)-VEN, *R*-(−)-VEN, *S*-(+)-ODV and *R*-(−)-ODV, respectively.

Table 3
Mean extraction recoveries (±SD), methodology recoveries (±SD) and RSD

Added drug	Concentration (ng/ml)	Mean extraction recoveries (%, $n=5$)		Mean methodology recoveries (%, $n=5$)		
		Mean recoveries ± SD	RSD (%)	Found ± SD	Recoveries (%)	RSD (%)
S-(+)-VEN	10.5	85 ± 6.5	7.9	10.1 ± 0.2	96.2	2.0
	35	91 ± 4.0	4.3	33.5 ± 1.8	95.7	5.4
	105	92 ± 3.9	4.5	102 ± 2.9	97.1	2.8
R-(−)-VEN	10.5	83.5 ± 5.7	6.8	9.8 ± 0.6	93.3	6.1
	35	91.5 ± 3.8	4.2	36.9 ± 1.8	105.4	4.9
	105	93.7 ± 4.1	4.3	102.6 ± 1.7	97.7	1.7
S-(+)-ODV	10.7	76.5 ± 5.9	7.7	9.9 ± 0.6	92.5	6.0
	35.7	85 ± 4.3	5.0	33.4 ± 1.3	92.4	3.0
	107	87 ± 5.8	6.7	108.5 ± 6.2	101.4	5.8
R-(−)-ODV	10.7	79.0 ± 5.5	6.9	10.1 ± 0.3	93.4	2.9
	35.7	88.7 ± 6.5	7.3	36.8 ± 1.5	103.1	4.0
	107	94.2 ± 4.0	4.2	102.5 ± 3.6	95.8	3.5

3.3. Recovery and precision

The mean extraction recoveries (means ± SD), methodology recoveries (means ± SD), intra- and inter-day precision for the four analytes were shown in Tables 1 and 3. The average extraction recoveries for all the four analytes were at least above 76%. The average methodology recoveries were higher than 92% for the analytes. The intra- and inter-day RSD are less than 9%.

The precision assays were carried out on five continuous days before and after the analyzing of the samples. The samples were stored at −70 °C in ultra cold freezer until they were analyzed.

3.4. Sensitivity

Five quality control plasma samples were utilized to determine the sensitivity. The limit of detection (LOD) were 1.0 ng/ml for *S*-(+)-VEN and *R*-(−)-VEN, 1.5 ng/ml for *S*-(+)-ODV, and *R*-(−)- DDV, respectively (S/N = 3).

3.5. Analysis of patients plasma

Plasma samples were obtained from five depressed patients under depression therapy with venlafaxine. The specimen collection from human subjects was approved by the Ethical Committee of Xiang Ya Second Hospital of Central South University.

Table 4
The plasma concentrations of enantiomers of VEN and ODV in depressed patients

Patient no.	Gender	Drug administered	Drug dose (mg/day)	Analyte	Concentration (ng/ml)	
					C_{min} [a]	C_{max} [b]
1	Female	Venalfaxine	200	*S*-(+)-VEN	34.4	45.8
				R-(−)-VEN	31.5	38.7
				S-(+)-ODV	53.8	66.7
				R-(−)-ODV	47.1	59.0
2	Female	Venlafaxine	75	*S*-(+)-VEN	15.8	21.7
				R-(−)-VEN	13.2	17.9
				S-(+)-ODV	47.0	64.2
				R-(−)-ODV	43.7	64.7
3	Male	Venlafaxine	75	*S*-(+)-VEN	8.5	13.2
				R-(−)-VEN	5.1	9.2
				S-(+)-ODV	59.7	62.4
				R-(−)-ODV	58.2	67.7
4	Male	Venlafaxine	75	*S*-(+)-VEN	37.0	52.4
				R-(−)-VEN	33.5	46.8
				S-(+)-ODV	50.7	69.8
				R-(−)-ODV	44.8	60.7
5	Male	Venlafaxine	75	*S*-(+)-VEN	23.5	28.1
				R-(−)-VEN	18.9	25.3
				S-(+)-ODV	52.1	71.0
				R-(−)-ODV	48.6	65.4

[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.

The patient's plasma drug concentrations determined by the method are shown in Table 4. In accordance with previously published data [19], the VEN and ODV enantiomers can be significantly different from patient to patient leading to a difficult interpretation of VEN pharmacokinetic and pharmacodynamic processes.

4. Discussion

Enantioselective separation on vancomycin chiral stationary phase (CSP) are affected by the buffer concentration, the type and concentration of organic modifiers and the pH of the mobile phase. These parameters were systematically studied in the development of the enantioselective separation. Temperature also plays a role in separation on a CSP. However, in this study, the temperature was maintained at 25 °C and this parameter was not adjusted.

4.1. Selection of the buffer concentration

The buffer selected for this study was ammonium acetate because of its compatibility in LC–MS application. Buffer concentrations of 100, 50, 30 and 10 mM were investigated and there was no significant influence of buffer concentration on the enantioselective separation. However, a 30 mM concentration of ammonium acetate could acquire the highest signal response of MS. Therefore, the 30 mM concentration of ammonium acetate was chosen for the study.

4.2. Selection of the organic modifier

The mobile phase concentration of methanol was varied between 80 and 95%, the optimum enantioselective separation of the two compounds were achieved with methanol–buffer (85:15, v/v). Under these conditions, the observed selectivity factor (α) were 1.18, 1.16 and the resolution factor (Rs) were 2.7, 2.17 for the VEN and ODV, respectively. With increasing of methanol concentration, the Rs was improved, α unaffected, While in the lower concentration of methanol, the Rs deteriorate and the α has no significant change. It was strange that the retention time extended with the increase of methanol, which was conflicting in the RP-HPLC on a C18 column. The possible explanation was that the enantioseparation of VEN and ODV on the vancomycin chiral column was the combination of several mechanisms, and more researches should be done on it.

Other organic modifiers have also been studied in the present paper. Acetonitrile couldn't separate the enantiomers of VEN and ODV at any concentration. While 2-propanol has the similar property with methanol. The addition of 2-propanol to the mobile phase has been shown to acquire the similar enantioselectivity. In the mobile phase (methanol:2-propanol:buffer = 75:10:15, v/v) the α and Rs were 1.16 and 2.13, respectively. While the system pressure increased greatly. The reason was that the viscosity of 2-propanol was bigger than methanol. So, the mobile phase selected for the validation and clinical studies did not contain 2-propanol.

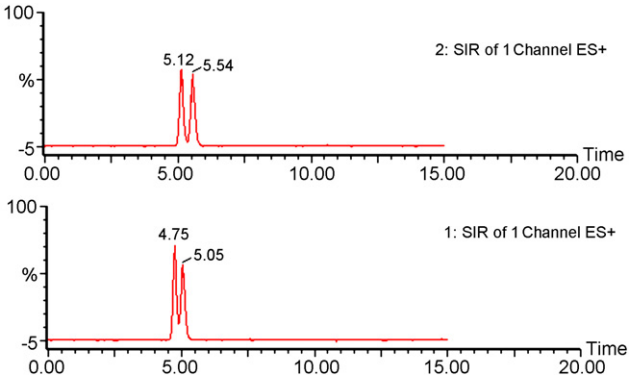

Fig. 6. Chmmatograms of compounds in the control human plasma. Channel l: ODV, Channel 2: VEN. The mobile phase was methanol–ammonium acetate buffer (30 mM, pH 5.5) (85:15, v/v).

4.3. Optimization of buffer pH

The effect of pH on the enantioselective separation of VEN and ODV was studied using the aforementioned mobile phase composition of methanol–ammonium acetate buffer (30 mM) (85:15, v/v). The safest and most stable pH range specified for the Chirobiotic V phase was 3.5–7.0 (ASTEC, 2004). In present study, all pH values recommended by the manufactor were tested in intervals of 0.5 unit. An adequate enantioseparation of ODV was hard to achieve when pH was below 5.5 (Fig. 6).

Although the best chromatographic separations were achieved at pH 7.0, the stability of the Chirobiotic V phase would reduce when the pH of the mobile phase was close to 7.0 according to the operating instructions of Chirobiotic V because the only critical operating parameter detrimental to the column was extreme of pH. Thus, the selected pH was a compromise between chromatographic separation and column life.

Based upon these results, the mobile phase composition for the validation and clinical study was set at methanol–ammonium acetate buffer (30 mM, pH 6.0) (85:15, v/v). Under these conditions, the analysis was completed in less than 13 min. The retention time of *S*-(+)-VEN and *R*-(−)-VEN were 11.8 and 12.8 min, and the observed α and Rs were 1.18 and 2.7, respectively. While the retention time of *S*-(+)-ODV and *R*-(−)-ODV were 11.2 and 11.9 min, with the α 1.16, Rs 2.17, respectively. This method was stable and reproducible, allowing us to analyze the patient samples on a single analytical Chirobiotic V column with a guard column.

5. Conclusions

The bioananlytical assay reported in this manuscript is a simple, sensitive and reproducible method for the enantioselective and simultaneous determination of VEN and its major metabolite, ODV in human plasma by LC–MS. The assay has greater sensitivity than previously reported methods. The method is accurate, sensitive and reliable and has been utilized in the analyses of plasma samples from a clinical study of racemic venlafaxine in antidepression management.

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