Case Report

Comparison of Analytical Methods in the Determination of Two Venlafaxine Fatalities

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Abstract

Two venlafaxine (Effexor)-related deaths are reported with comparison of results from the analysis of specimens using capillary gas chromatography with a nitrogen-phosphorous detector (GC–NPD) and high-performance liquid chromatography (HPLC) using a UV-vis detector. Blood concentrations in Case 1 were 7.27 µg/mL venlafaxine and 5.03 µg/mL *O*-desmethylvenlafaxine, and Case 2 had 89.67 µg/mL venlafaxine with 3.44 µg/mL of the desmethyl metabolite. A comparison of analytical methods and specimen concentration is presented.

Introduction

Venlafaxine hydrochloride (Effexor, Wyeth-Ayerst, Philadelphia, PA) is a relatively new antidepressant derived from phenethylamine that has a chemical structure of (*R/S*)-1-(2-dimethylamino)-1-(4-methoxyphenyl)ethyl cyclohexanol.

Venlafaxine acts by inhibiting the reuptake of norepinephrine, serotonin, and, to a lessor extent, dopamine. This drug lacks monoamine oxidase inhibitor action, anticholinergic action, and the sedative effects associated with other antidepressants (1,2). Clinical manifestations of toxicity and untoward side effects include anxiety, nervousness, and insomnia, which are similar to those of fluoxetine (3). In an overdose, death can result from adrenergic stimulation inducing seizures, cardiac dysrhythmias, hypertension, or hypotension (4).

The elimination half-life is approximately 5 h. Steady state is achieved within 3 days. Approximately 87% of absorbed venlafaxine is eliminated within 48 h in the urine. Approximately 29–48% of the drug is eliminated as the *O*-desmethylvenlafaxine (ODV) metabolite, which appears to have significant antidepressant activity and a half-life of approximately 12 h (5,6).

Reports in the scientific literature detailing toxicologic analyses of fluid and tissues from lethal ingestions of venlafaxine are few (7,8). We report two fatal cases of venlafaxine ingestion.

Table I. Venlafaxine and ODV Concentrations in Fatal Case 1

	Venlafaxine µg/mL		ODV µg/mL	
	HPLC	GC	HPLC	GC
Blood	6.52	7.27	6.90	6.03
Vitreous	6.73	QNS*	1.23	QNS
Urine	–†	35.67	–	16.12

* Quantity not sufficient for analysis.
† Quantitation was unreliable because of multiple drug interference or poor chromatography.

Case History

Case 1

A 41-year-old female was found at 2:17 A.M. in a van parked behind a school. The van and the deceased were reported missing by her husband the afternoon before she was found. The patient had a prescription for 270 25-mg venlafaxine

Table II. Body Distribution of Venlafaxine and ODV in Fatal Case 2

	Venlafaxine µg/mL		ODV µg/mL	
	HPLC	GC	HPLC	GC
Blood	89.67	84.30	3.09	3.44
Vitreous	42.48	43.82	N.D.*	2.10
Urine	143.31	124.55	7.85	9.75
Brain	–†	542.70	–	14.82
Liver	–	405.10	–	18.80
Kidney	–	420.30	–	20.40
Bile	229.29	200.20	6.55	7.70
Gastric	10.4 mg	10.6 mg	N.D.	N.D.

* N.D. = Not detected.
† Quantitation was unreliable because of multiple drug interference or poor chromatography.

tablets, which was written by her husband, a psychiatrist, that was filled 16 days earlier. The prescription bottle was found at the scene and contained 12 tablets. A cup of wine and corresponding wine bottle were found on the floor of the van.

Postmortem findings

The body demonstrated well-developed rigor and livor mortis. The deceased weighed 187 lbs. (84 kg), and no injuries were apparent. An autopsy was not performed. Blood and urine specimens were collected for toxicological analysis.

Case 2

A 58-year-old male was found unresponsive at home by his wife; he was last seen 12 h previously. He had been treated for depression during the previous year and had seen his psychiatrist the week before. At this visit his death, he requested a month's supply of medication because he was leaving town on vacation. The evening before his death, his wife counted 170 tablets of 75-mg venlafaxine in the prescription bottle.

Postmortem findings

The autopsy was unremarkable except for obesity, cardiomegaly, marked pulmonary edema, and congestion. The deceased weighed 285 pounds (130 kg) and was 71-in. tall.

Toxicological analysis

The urine was screened by EMIT (Behring Diagnostics, Cupertino, CA) and thin-layer chromatography (ToxiLab, Ansys, Irvine, CA), and the blood was tested with GC–FID for alcohol and with dual column gas chromatograph with nitrogen-phosphorous detectors (GC–NPD) for basic and neutral drugs (9). The ToxiLab chromatogram was treated following the manufacturer's procedure, and venlafaxine was demonstrated an R_f of 0.55, which is consistent with the standard (10).

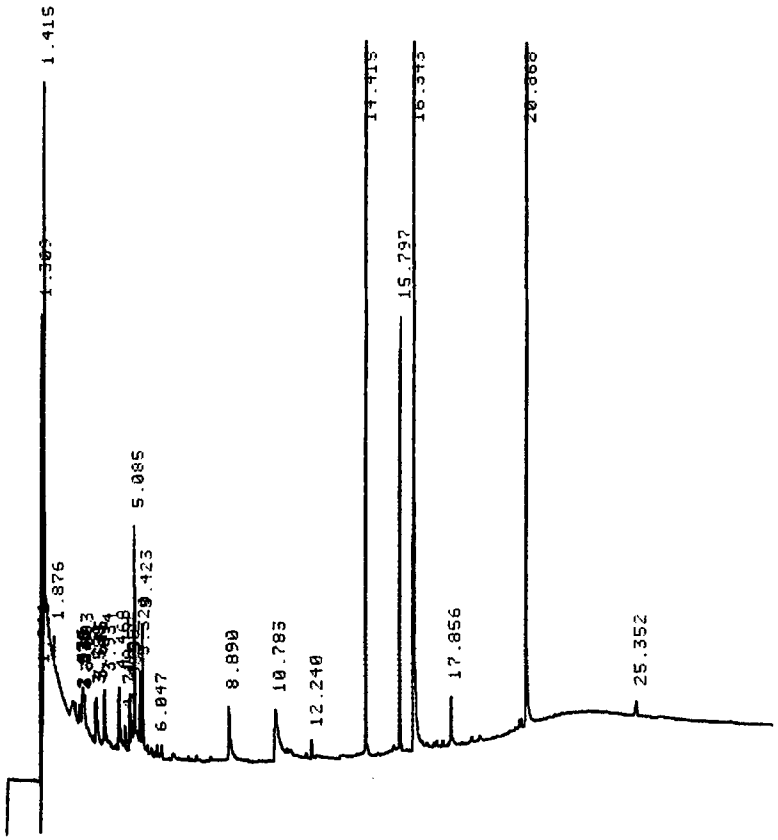
Gas chromatographic quantitation was performed with 2 mL of sample and 50 µL of SKF-525A (100 µg/mL) as the internal standard, then buffered with 100 µL of concentrated ammonium hydroxide. Six milliliters of *n*-butyl chloride (NBC) was added to all tubes, placed on a rotator for 10 min, centrifuged, and the NBC removed by glass pipette. The NBC was placed into a clean test tube and evaporated to dryness; then 100 µL of pyridine and 100 µL of acetic anhydride were added for derivatization. One microliter was injected into the GC.

Calibration used negative blood spiked with 0, 100, 200, 500, 1000, and 2000 ng/mL of both the venlafaxine and norvenlafaxine. The coefficients of correlation were 0.9995 and 0.9987 for parent and metabolite, respectively. Controls were also prepared by spiking negative blood.

Both screening and quantitation were performed using a Hewlett Packard (Avondale, PA) 5890 GC equipped with nitrogen-phosphorous detectors, an HP 7673 autoinjector, and dual HP 3396A integrators. The injector temperature was set at 250°C. The detectors were set at 285°C, and the column was set at 120°C for 1 min and increased at 8°C/min to 280°C, where it was then held isothermally for 10 min.

Venlafaxine was detected on a GC–NPD screen using a DB-17 column (30 m, 0.32-mm i.d., 0.25-µm film thickness, J&W Scientific, Folsom, CA) and an HP-5 column (5% Ph Me Silicone, 30 m, 0.32-mm i.d., 0.25-µm film thickness, Hewlett Packard) with retention indices of 2821 (14.5 min) and 2824 (16 min), respectively (9).

The tissues were homogenized on a Ultra-Turrax T-25 (VWR Scientific, St. Louis, MO)



Sample name: 938 BLX4 Sample #15
Method name: M:Venlafax.met.
Identifier: DB-17
Venlafax
ISTD-area

RT	Type	Area	Width	Cal #	ng/mL	Name
14.415	PB	21490	.034	1	1404.280	Venlafaxine
15.797	BB	9640	.035	2	1215.491	Norvenlafaxine
20.868	PB	40604	.035	36		Maprotaline

Total area = 171610
Mul factor = 1.0000E + 00
ISTD amount = 1.0000E + 00

Figure 1. Maprotiline internal standard.

with 10 g tissue and 30 mL distilled water for a 1:4 dilution. The same homogenates were used for both the GC–NPD and HPLC quantitations. Further dilutions were required on Case 2 and were either 1:10 or 1:50 and prepared in a similar fashion. Negative tissue samples (brain and liver) were also run as controls. Maprotiline was substituted for SKF-525A in GC–NPD reanalysis quantitations to eliminate possible nonspecific binding during tissue extractions.

Liquid chromatography quantitation used 2 mL of biological fluid or diluted tissue homogenates with 200 µL of nitrazepam (20 µg/mL) as the internal standard. Extracts were buffered with 2 mL pH 11 carbonate buffer extracted with 6 mL of NBC. The NBC was evaporated as in the gas chromatography method but without the derivatization. Extracts were reconstituted with 100 µL of mobile phase, and 25 µL was injected into the Perkin Elmer (PE, Norwalk, CT) HPLC.

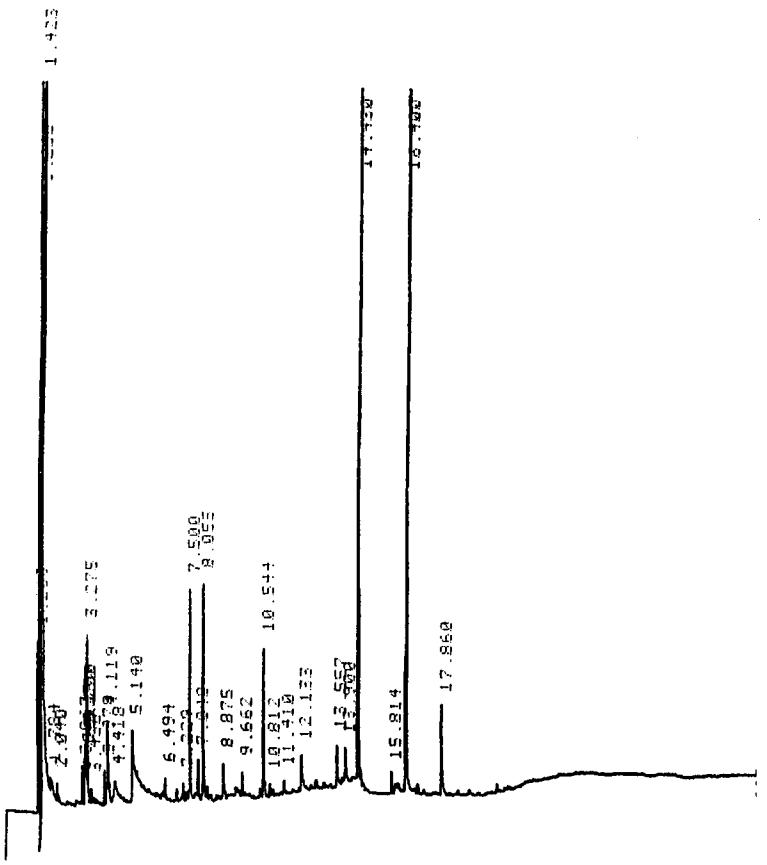
The HPLC consisted of a PE 250 binary pump equipped with a PE ISS 100 autosampler and a PE 235 diode array detector set to monitor at 220 nanometers. The mobile phase was 71% phosphate buffer (pH 4.0) with 29% methanol at 1 mL/min flow on a ODS 5 µm 4.6 × 150-mm reversed-phase column (Beckman Ultrasphere, PJ Cobert Associates, St. Louis, MO) (10).

In Case 1 (Table I), the blood also contained carbamazepine and the dihydroxy metabolite of carbamazepine at 12.96 and 1.66 µg/mL, respectively, as quantitated by HPLC (11). These were considered to be present in therapeutic concentrations. Ethanol was not detected at the 0.01 g% concentration. The specimens were subsequently analyzed by HPLC for venlafaxine and ODV; these results were found to agree with the original testing. The cause of death was certified as venlafaxine intoxication.

In Case 2 (Table II), the comparison of the HPLC and the GC–NPD method, which used an acetylated derivative, agreed within 10% with the exception of the tissues. Suspecting nonspecific binding and loss of the internal standard (12), SKF-525A, an alternate internal standard, maprotiline, was evaluated for the GC–NPD method. Maprotiline, which also forms an acetylated derivative, was preferred to SKF-252A because it would monitor the recovery through the extraction and the derivatization process. Brain, liver and kidney concentrations were comparable with maprotiline and SKF-525A internal standard.

Maprotiline had a retention time (RT) of 20.8 min (Figure 1), whereas SKF-525A had an RT of 16.4 min on the HP-5 column (Figure 2). The metabolite had an RT of 15.8 min in this system. Even though the actual quantitations were within 10%, maprotiline was chosen as the better internal standard because it requires acetylation along with norvenlafaxine for adequate chromatography. GC–NPD, using maprotiline as the internal standard with derivatization, was determined as our method of choice.

The two analytical methods (GC–NPD and HPLC) agreed within 10–15% of each for all tissues, except the brain, liver, and kidney. HPLC was tested in the hopes of avoiding derivatization because gas chromatographic column efficiency can be decreased with changes of derivatizing agents. The HPLC method yielded results significantly less than the GC–NPD testing. This discrepancy was attributed to poor HPLC of the venlafaxine. The chromatography was identified as the problem because the extractions for both procedures were analogous and the same tissue homogenates were aliquoted for analysis. This was further supported by manual calculations using area instead of peak height. The HPLC method did not provide an adequate separation of the norvenlafaxine

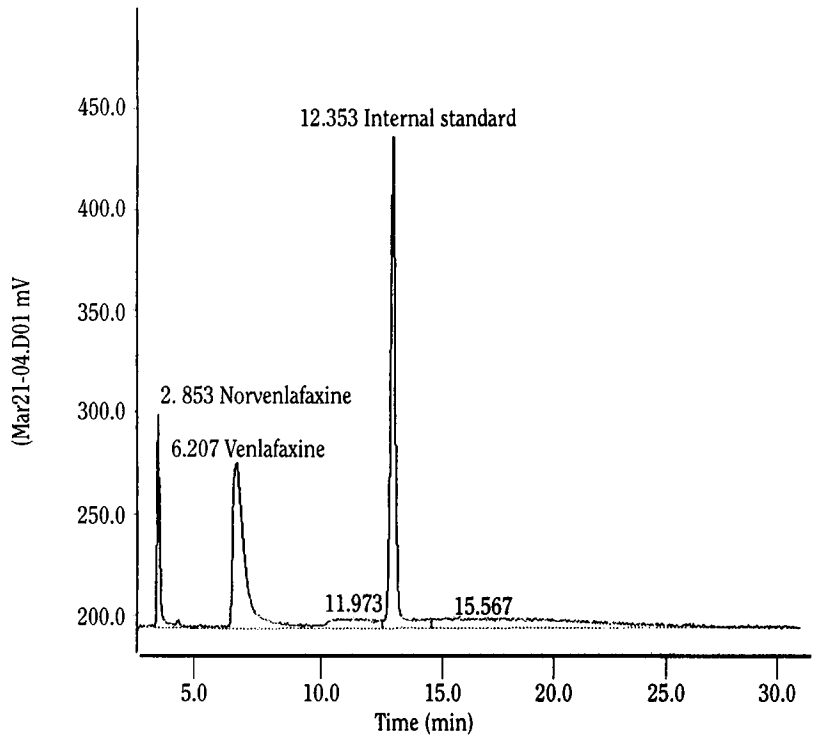


Sample name: 1760BLX40 Sample #10
Method name: M:Venlafax.met.
Identifier: DB-17
Venlafax
ISTD-area

RT	Type	Area	Width	Cal #	ng/mL	Name
14.430	VB	47592	.033	1	2074.696	Venlafaxine
15.814	PP	519	.035	2	78.932	Norvenlafaxine
16.400	PB	60394	.034	36		SKF

Total area = 210122
Mul factor = 1.0000E + 00
ISTD amount = 1.0000E + 00

Figure 2. SKF-525A internal standard.



File: Mar21-04.D01 1000 standard dm/jac
Run: 01 Queue: Mar21- Set number: 1 Type: CalibBrkC
Path: C:/Chrom
Collection: 16:42:41 Mar 21 1996 Method: VNLFXN (15:42:11 Mar 21 1996)
Integration: 16:42:41 Mar 21 1996 Method: VNLFXN (15:42:11 Mar 21 1996)
Report: 11:39:41 Mar 25 1996 Method: VNLFXN (08:58:14 Mar 25 1996)
Sample amount: 1.00000e+0 Standard amount: 1.00000e+0 Dilution: 1.00000e+0

Internal standard (height)

ExpRT	Height	Area	ng/mL	Name
3.039	104.667	955056	1136.5636	Norvenlafaxine
6.232	80.968	2766780	992.6979	Venlafaxine
12.853	243.010	3528944	1.0000	IR Internal standard

Figure 3. HPLC analysis.

from the "dead volume" of the instrument, and the chromatography was generally marginal for analysis in this solvent system (Figure 3).

Ingestions ranging from 125 to 6750 mg have been reported (13) to produce toxic manifestations. When presented at the hospital, symptoms ranged from lethargy to convulsions; however, no blood concentrations were reported. All these patients recovered fully.

Fantaskey and Burkhart (14) found 6.10 and 1.80 µg/mL for venlafaxine and metabolite, respectively, in an overdose. This patient also made a complete recovery.

At the 1995 Society of Forensic Toxicology conference (7), two cases were presented with fatal concentrations of venlafaxine and ODV. The respective results for venlafaxine and ODV were 6.6 and 31 µg/mL in Case 1, whereas Case 2 had 84 and 15 µg/mL, respectively.

The mechanism of action of venlafaxine and the active ODV could produce hypertension or hypotension. Consequently, the physiology of the patient may result in lower concentrations of venlafaxine being fatal.

Two cases of confirmed venlafaxine overdose with no significant other drugs or pathology are presented with alternate analytical techniques for analysis in human biological materials.

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