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High-performance liquid chromatographic method to screen and quantitate seven selective serotonin reuptake inhibitors in human serum

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Abstract

A high-performance liquid chromatographic screening method (HPLC) is described for the determination of seven selective serotonin reuptake inhibitors (SSRIs) (fluvoxamine, milnacipran, paroxetine, sertraline, fluoxetine, citalopram, venlafaxine) and for three pharmacologically active *N*-demethylated metabolites (desmethylcitalopram, didesmethylcitalopram and norfluoxetine). A tricyclic antidepressant, clomipramine, was used as an internal standard. The method consists of liquid extraction of serum after alcalinisation at pH 9.50, followed by chromatography on a Beckman C_{18} reversed-phase column. Compounds were detected at 200.4 nm. The standard curves were linear over a working range of 50–1000 ng/ml for fluvoxamine, 15–1000 ng/ml for fluoxetine, 25–500 ng/ml for norfluoxetine, 50–500 ng/ml for sertraline, 20–500 ng/ml for paroxetine, 25–550 ng/ml for citalopram, 25–750 ng/ml for desmethylcitalopram, 25–800 ng/ml for didesmethylcitalopram, 25–650 ng/ml for milnacipran, and 25–500 ng/ml for venlafaxine. The quantitation limits of the method were 15 ng/ml for fluoxetine, 20 ng/ml for paroxetine, 25 ng/ml for venlafaxine, norfluoxetine and citalopram, and its metabolites, 40 ng/ml for sertraline and 50 ng/ml for fluvoxamine. No interferences were noted with this sensitive and specific method which can be used for therapeutic drug monitoring.

Keywords: Fluvoxamine; Milnacipran; Paroxetine; Sertraline; Fluoxetine; Citalopram; Venlafaxine

1. Introduction

Fluvoxamine, fluoxetine, paroxetine, citalopram, sertraline, milnacipran, venlafaxine and their pharmacologically active *N*-demethylated metabolites (desmethylcitalopram, didesmethylcitalopram and norfluoxetine) are selective serotonin reuptake inhibitors (SSRIs). They exhibit clinical efficacy comparable with classical tricyclic antidepressants, but are devoid of some of the adverse anticholinergic and cardiovascular effects commonly associated with these drugs [1–3]. Tricyclic antidepressants are not specific, they inhibit serotonin and noradrenalin reuptake but also block muscarinic and histaminic receptors [4]. However, adverse effects exist and acute intoxication can be observed [5–10].

Several methods have been described to measure SSRIs in serum or plasma, such as thin layer chromatography (TLC), with ultraviolet (UV) detection [9]; it is a rapid method but is not sensitive or specific. HPLC is also used to measure SSRIs using

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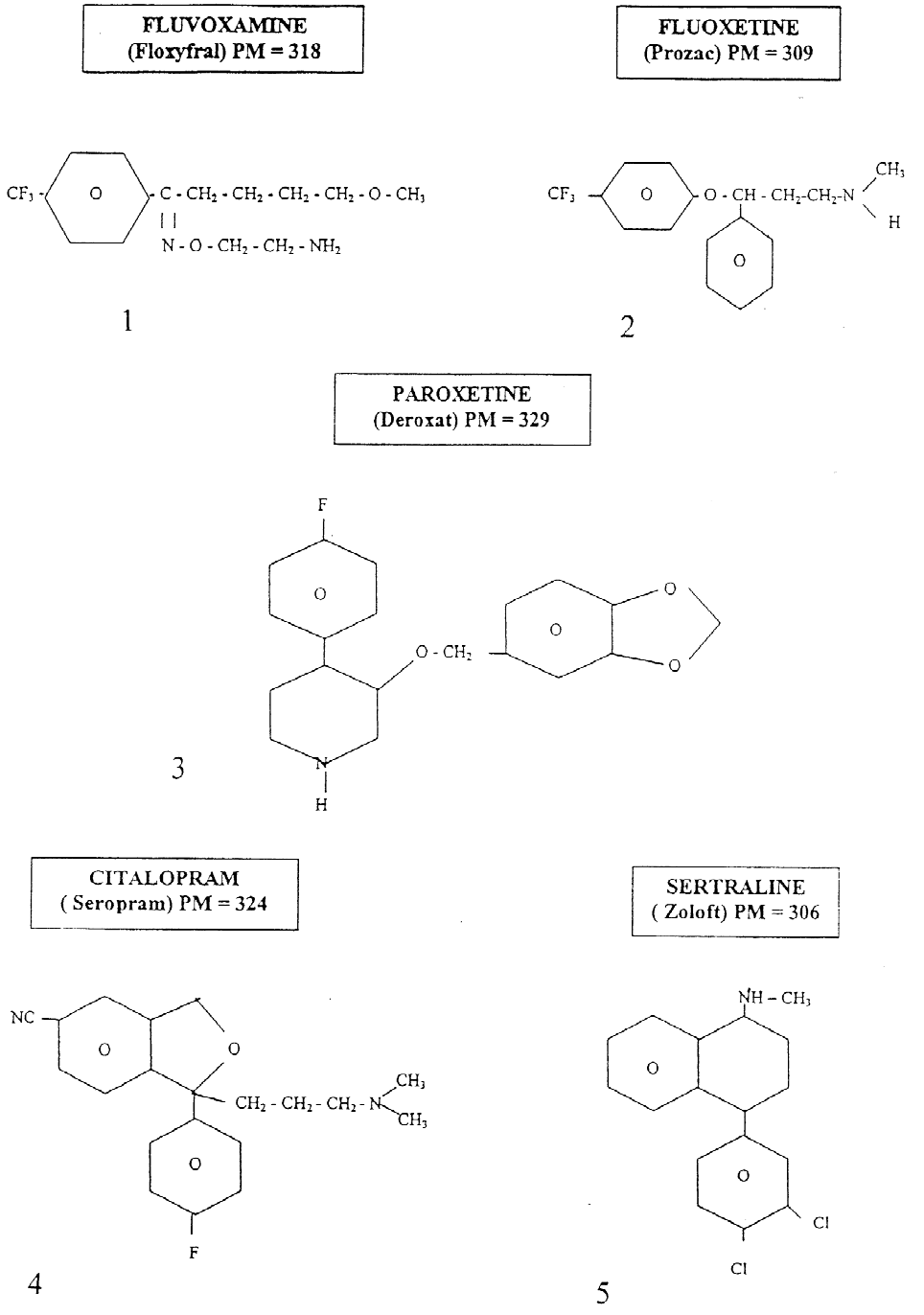

Fig. 1. Structure of selective serotonin reuptake inhibitors.

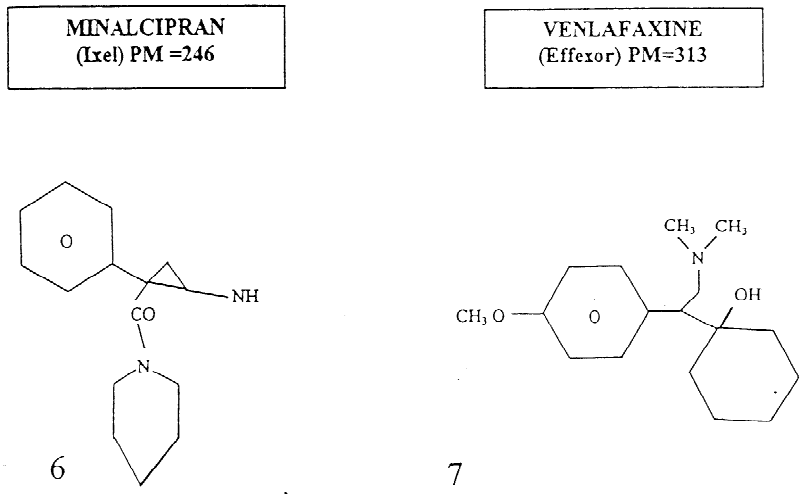

Fig. 1. (*continued*)

UV [11] or fluorometric detection [12], and gas chromatography–mass spectrometry is sometimes used to detect SSRIs [13–15]. The aim of this study is to propose a simple and rapid screening method to determine seven SSRIs and three metabolites by HPLC. The structures of these antidepressants are presented in Fig. 1.

2. Experimental

2.1. Chemicals and reagents

Clomipramine (tricyclic antidepressant, internal standard) was supplied by Ciba-Geigy (France); fluvoxamine Floxyfral® by Solva-Pharma (France); milnacipran Ixel® by Fabre (France); paroxetine Deroxat® by Beecham (France); sertraline Zoloft® by Pfizer (France); fluoxetine, norfluoxetine Prozac® by Eli Lilly (USA); citalopram, demethylcitalopram, didesmethylcitalopram Seropram® by H. Lundbeck (France); venlafaxine Effexor® by Wyeth Lederle (France). Acetonitrile, methanol, and chloroform were supplied by Carlo Erba (Italy); isopropanol and ammoniac by Merck (France); and *n*-heptane by Prolabo (France).

2.2. Standards

Concentrations of methanolic stock solutions of seven SSRIs and three metabolites were 100 mg/l. For analytical procedures, working solutions were prepared to concentrations of between 15 and 1000 ng/ml: 50–1000 ng/ml for fluvoxamine, 15–1000 ng/ml for fluoxetine, 25–500 ng/ml for norfluoxetine, 50–500 ng/ml for sertraline, 20–500 ng/ml for paroxetine, 25–550 ng/ml for citalopram, 25–750 ng/ml for desmethylcitalopram, 25–800 ng/ml didesmethylcitalopram, 25–650 ng/ml for milnacipran and 25–500 ng/ml for venlafaxine by dilution in methanol. These solutions were stable at −20°C for at least 3 months.

2.3. Apparatus

The extracts were analysed using a Hewlett-Packard 1090 chromatograph (HP, Les Ulis, France) equipped with an automatic sample injector and a diode array. Data analyses were performed using operating software and a Hewlett-Packard Vectrax M2 4/100 computer. The column was a Beckman (Gagny, France) ODS C_{18}, 5 μm (25 cm×4.6 mm ID), protected by a guard column of ultrasphere ODS, 5 μm (4.5 cm×4.6 mm). The mobile phase

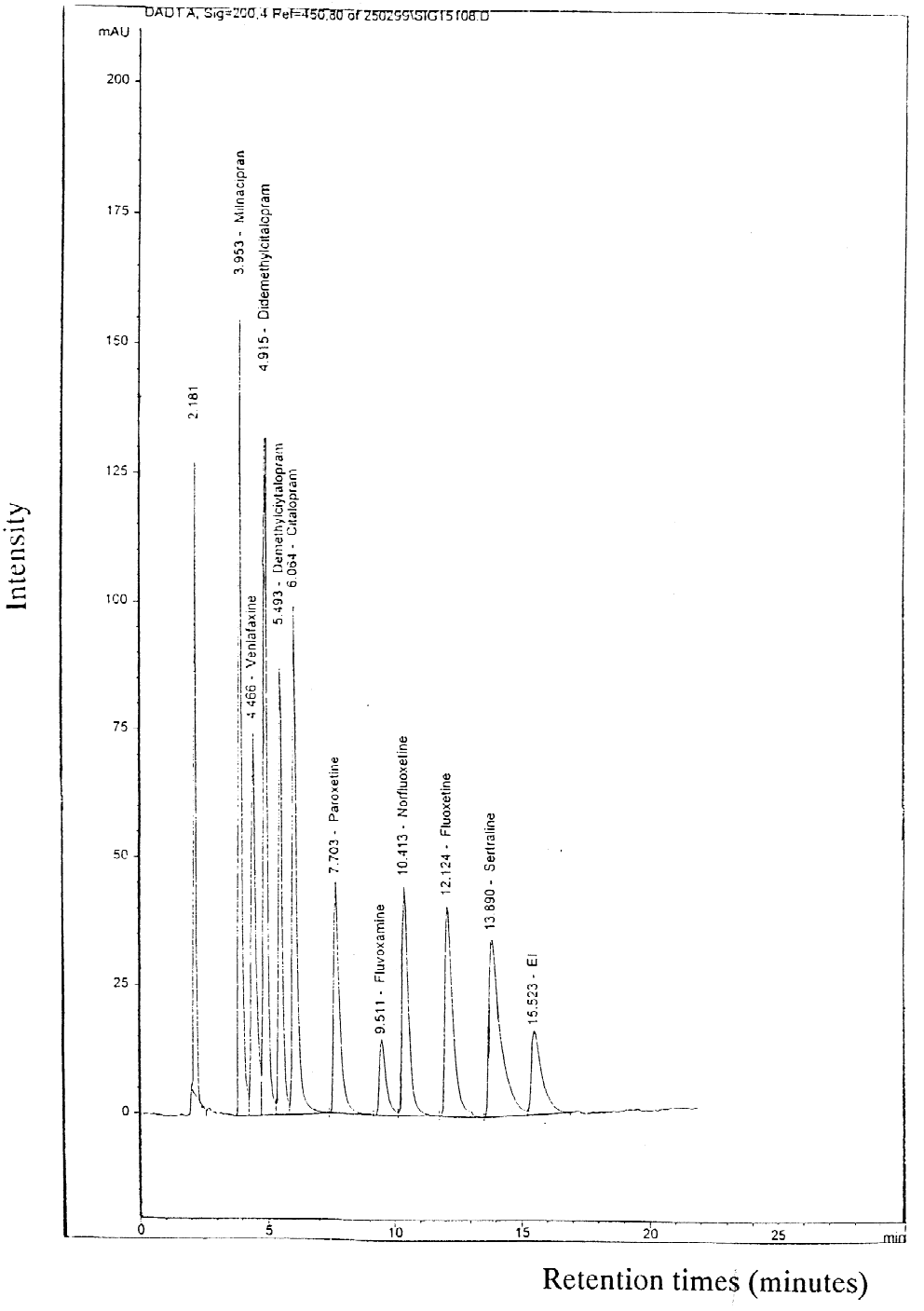

Fig. 2. A chromatogram of seven serotonin reuptake inhibitors and three metabolites.

contained (50%, v/v) acetonitrile in a sodium phosphate buffer (0.05 *M*, pH 3.80). The flow rate was set to 1 ml/min, with the oven maintained at 50°C.

2.4. Extraction procedure

SSRIs were extracted according to the reported method by Tracqui et al. [16] with minor modifications. Serum was obtained from healthy men, who were not receiving therapeutic SSRIs. A 200-μl amount of internal standard (clomipramine) was added to 1 ml of serum containing SSRIs. After shaking, 1.5 ml of saturated NH_4Cl, pH 9.50, was added. The mixture was shaken mechanically for 30 s, and extracted with a solution of chloroform/2-isopropanol/*n*-heptane (v/v/v: 60/14/26). After shaking for 2 min and centrifuging for 10 min at 2800 *g*, the organic layer was transferred to other tubes. The mixture was evaporated under a stream of nitrogen in a 40°C water bath. Dried extracts were dissolved in 100 μl acetonitrile (50:50 v/v), phosphate buffer (0.05 *M*, pH 3.80) and 50 μl was injected onto the column. Amounts of SSRIs were determined by peak–area ratios of compound to internal standard.

2.5. Validation procedure

Linearity was studied for each compound in the range 15–1000 ng/ml: 50–1000 ng/ml for fluvoxamine, 15–1000 ng/ml for fluoxetine, 25–500 ng/ml for norfluoxetine, 50–500 ng/ml for sertraline, 20–500 ng/ml for paroxetine, 25–550 ng/ml for citalopram, 25–750 ng/ml for desmethylcitalopram, 25–800 ng/ml for didesmethylcitalopram, 25–650 ng/ml for milnacipran and 25–500 ng/ml for venlafaxine. The lowest detection limit with extraction results was determined for each drug by extracting pure plasma samples of decreasing concentrations, until a response equivalent to three times the background was obtained. The day-to-day intra-laboratory variation in retention times (t_R) was investigated for the ten compounds by carrying out weekly analyses of 1 ml of blood containing 500 ng/ml of each SSRI, over a period of 5 months. The single day intra-laboratory retention times (t_R) were investigated for the ten compounds by carrying out analyses on 1 ml of blood containing 500 ng/ml 20 times over the period of 1 day. Each drug was monitored at 200.4 nm.

3. Results and discussion

Fig. 2 shows typical chromatographic separation obtained after the extraction of 2 ml serum containing 250 ng/ml of the antidepressants under study. The retention time is defined for each molecule: milnacipran (3.95 min), venlafaxine (4.46 min), didesmethylcitalopram (4.91 min), demethylcitalopram (5.49 min), citalopram (6.064 min), paroxetine (7.70 min), fluvoxamine (9.51 min), norfluoxetine (10.41 min), fluoxetine (12.12 min), sertraline (13.89 min) and internal standard (15.52 min).

Modifying a previously published assay [16], our goal was to develop a simple and fast procedure to determine several SSRIs at the same time, and to

Table 1
Extraction yields ($n=10$) of selective serotonin reuptake inhibitors

Commercial names	Denomination Commune Internationale (D.C.I.)	Metabolites	Extraction, %
Floxyfral	Fluvoxamine		92±4.5
Prozac	Fluoxetine		96.3±5.5
		Norfluoxetine	88.4±5.3
Zoloft	Sertraline		74.5±1
Deroxat	Paroxetine		83.7±3.9
Seropram	Citalopram		94.4±3.4
		Demethylcitalopram	87.8±0.2
		Didesmethylcitalopram	96.2±0.05
Ixel	Milnacipran		83±2
Effexor	Venlafaxine		86.4±1.7

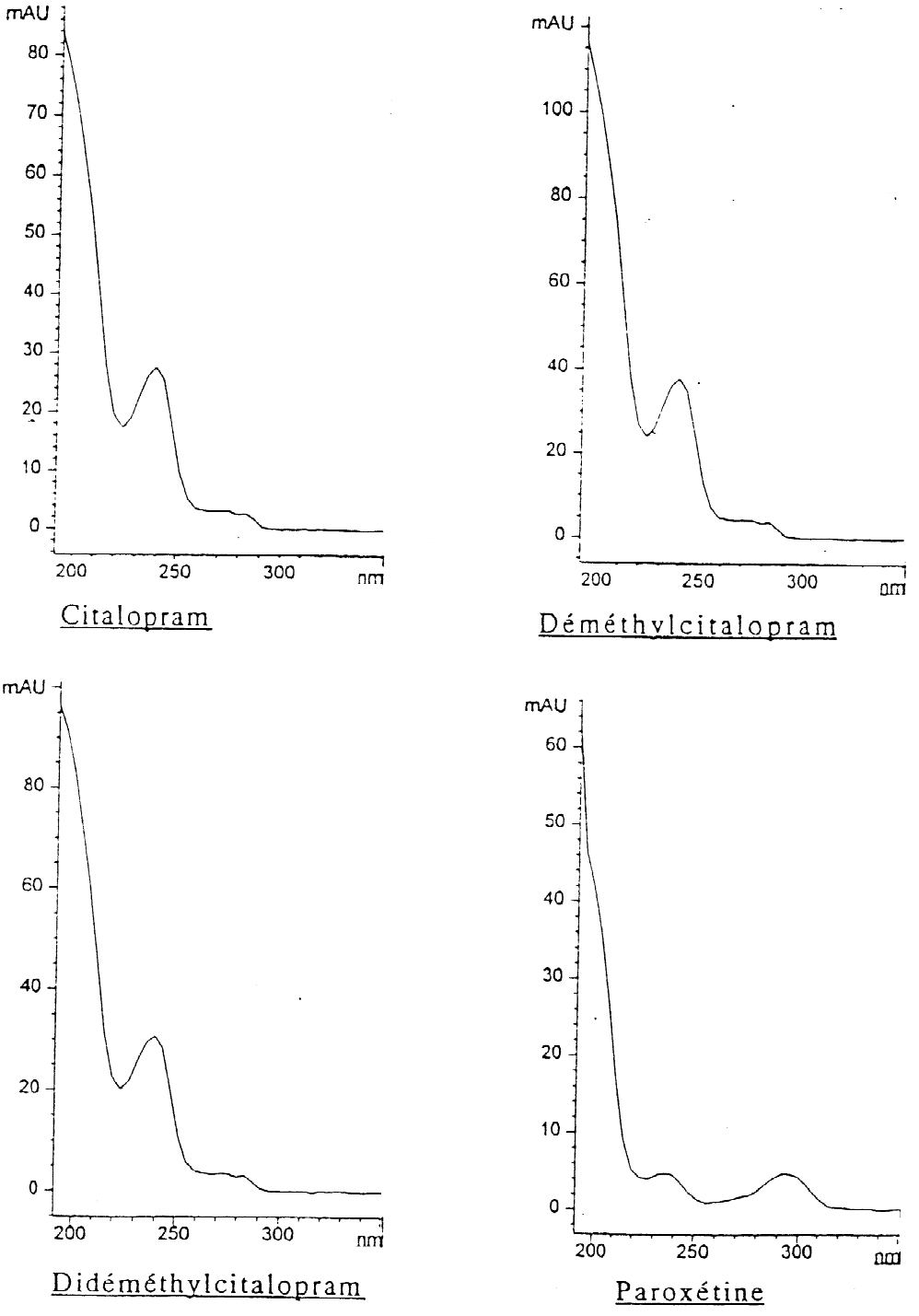

Fig. 3. Wavelength.

perform toxicological screening. This method and its simplicity make direct adaptation possible.

HPLC is performed in 20 min and the total screening procedure (including single step extraction, evaporation and chromatographic run) may be achieved in less than 90 min, making our method convenient not only for forensic determinations but also for poisoning cases and the determination of therapeutic levels. Extraction yields were in the range 83–96%. These results are presented in Table 1.

Several laboratories have used HPLC to detect one or two SSRIs: Oyehaug and Ostensen (citalopram) [17], Hicks et al. [18], Kosel et al. (venlafaxine) [19], Matsui et al. [20], Haupt [21], Carlson and Norlander [22], Akerman et al. (citalopram and metabolites) [23], Foglia et al. [24], Shin et al. (paroxetine) [25], Belmadini et al. (fluvoxamine) [26], Holladay et al. [27], Crifasi et al. (fluoxetine and norfluoxetine) [28], and Patel et al. [29]. Wavelength specifics were defined for each antidepressant studied [21,23, 26,27,29–32]. This method makes it possible to detect and analyse seven SSRI compounds and their metabolites. The simplicity of the procedure is evident in the use of a readily available C_{18} column and detection wavelength of 200.4 nm. At this wavelength, there is maximum absorbance, and

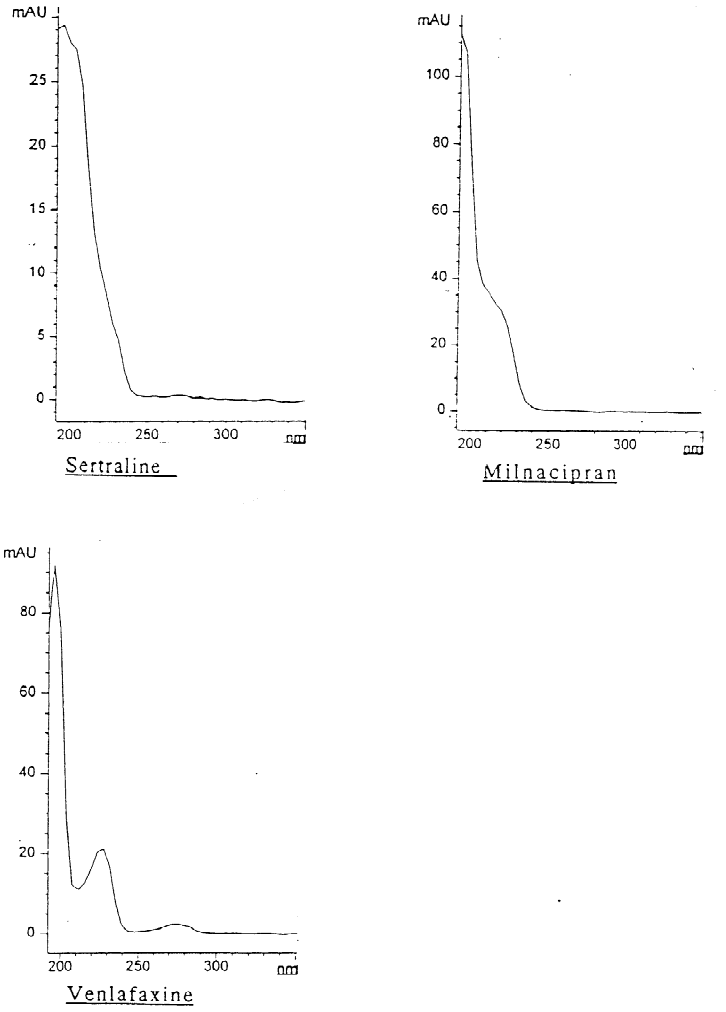

Fig. 3. (*continued*)

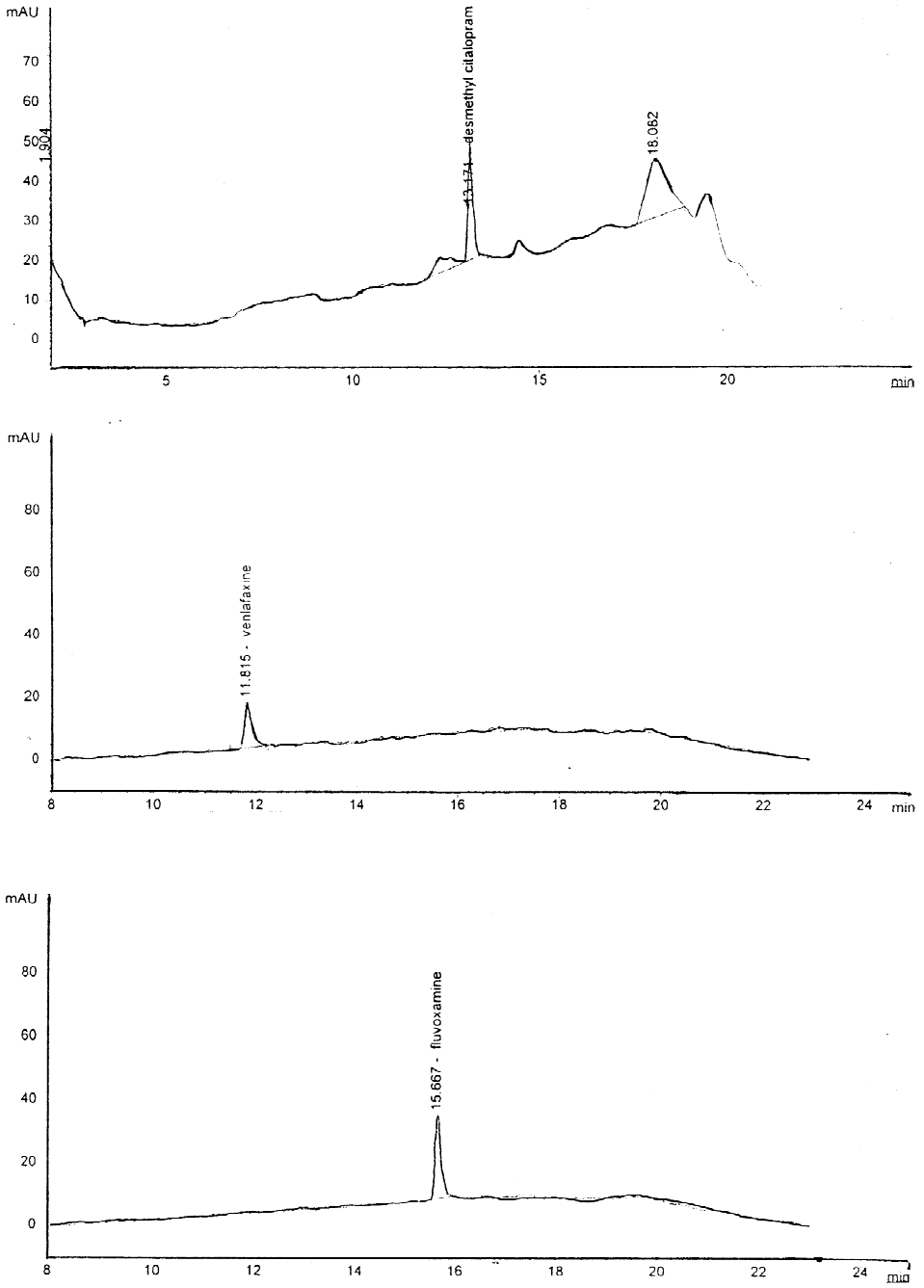

Fig. 4. Serum plus each sample alone.

interference from endogenous compounds is possible. But no endogenous interfering substances have occurred so far (Figs. 3 and 4).

In our procedure, clomipramine can be used as the internal standard, since this compound is never associated with SSRIs in therapeutics.

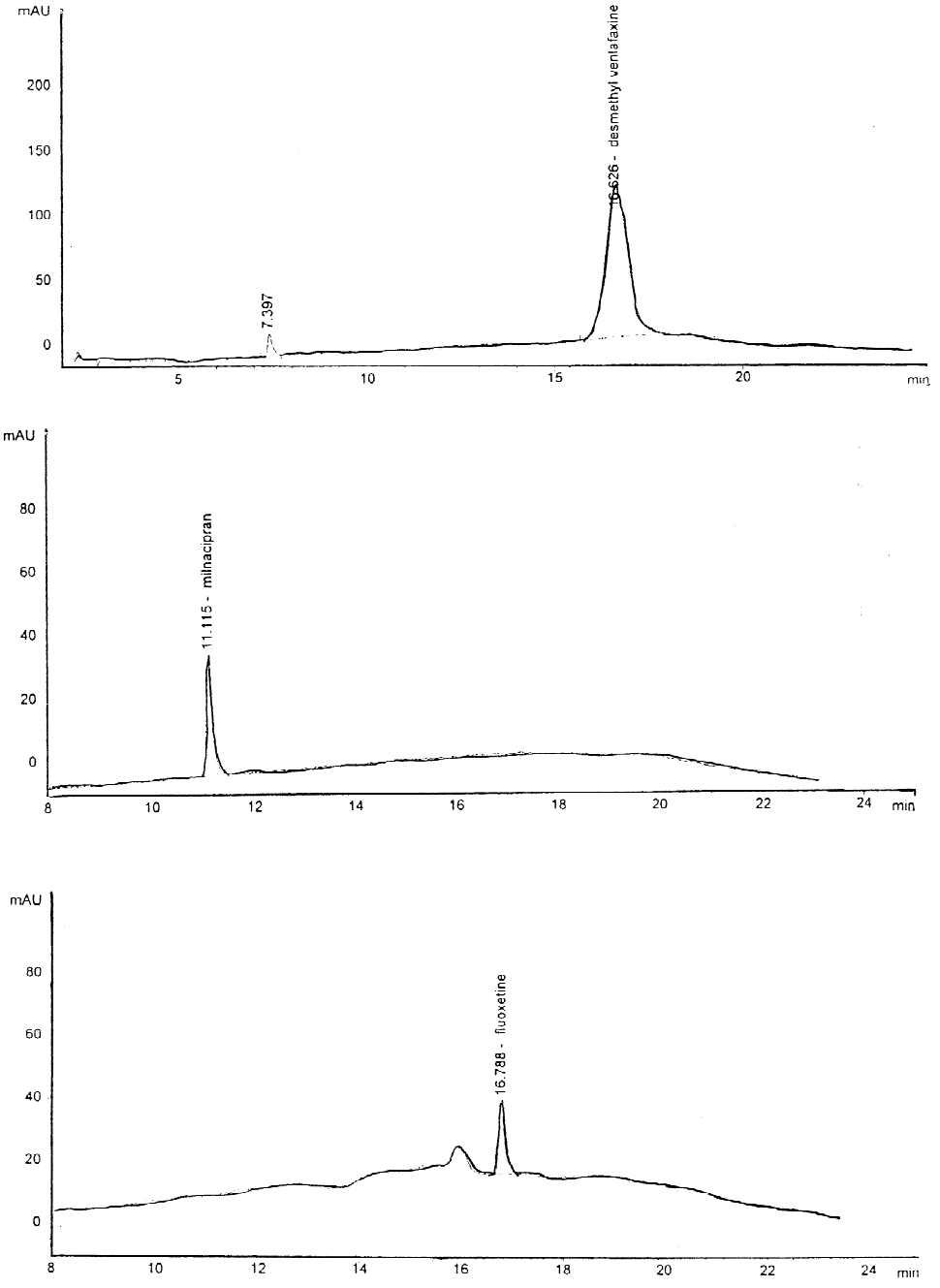

Fig. 4. (*continued*)

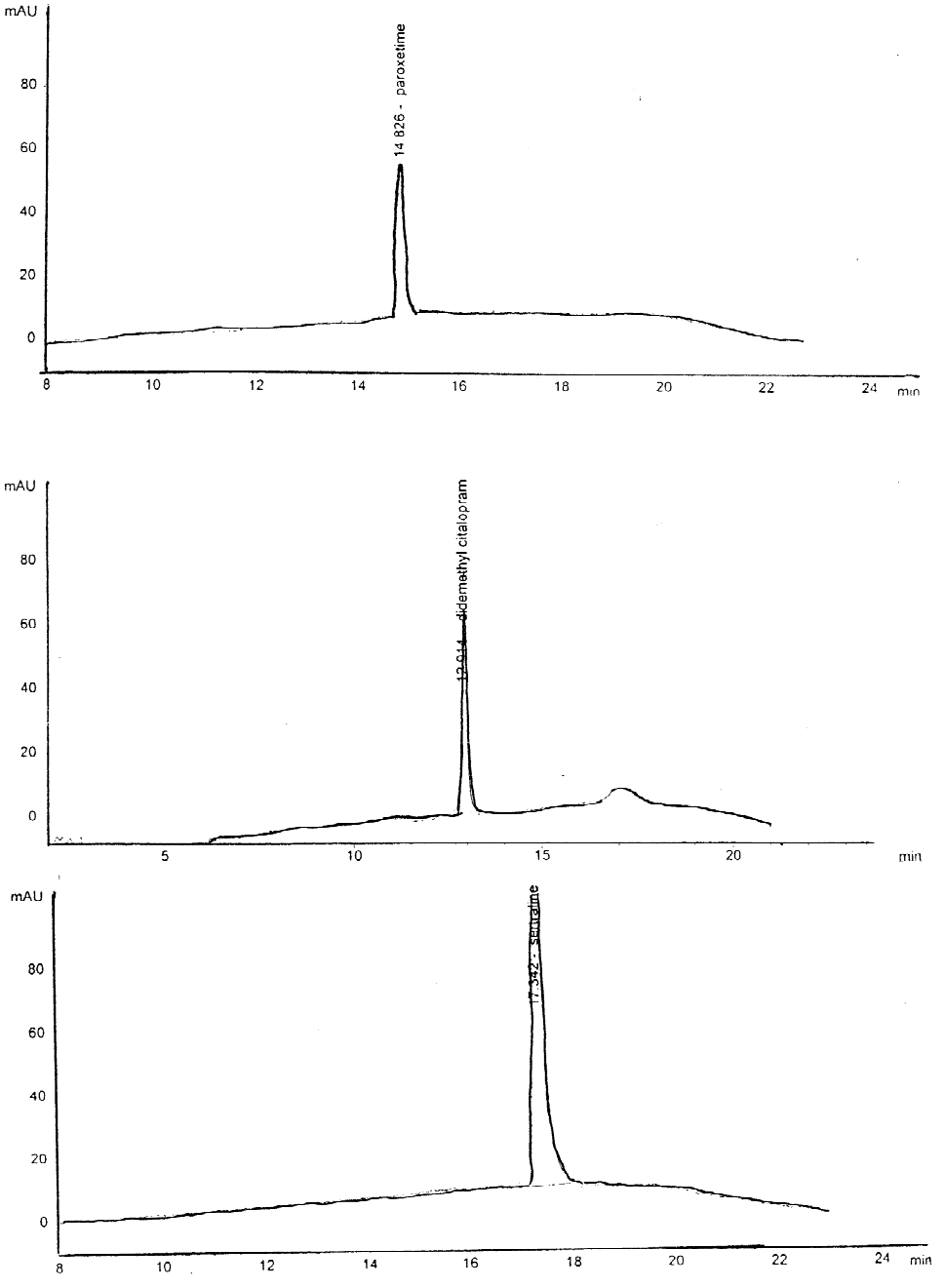

Fig. 4. (*continued*)

Table 2
Detection limits, therapeutic levels, and variations ($n=4$) of selective serotonin reuptake inhibitors

Commercial names	D.C.I.	Metabolites	Range (ng/ml)	Therapeutic levels (ng/ml)	Limits of detection, sensitivity (ng)
Floxyfral	Fluvoxamine		50–1000	50–300	50
Prozac	Fluoxetine		15–1000	30–500	15
		Norfluoxetine	25–500		25
Zoloft	Sertraline		50–500	50–300	40
Deroxat	Paroxetine		20–500	50–500	20
Seropram	Citalopram		25–550	30–300	25
		Demethylcitalopram	25–750		25
		Didesmethylcitalopram	25–800		25
Ixel	Milnacipran		25–650	100–300	25
Effexor	Venlafaxine		25–500	100–200	25

Chloroform–isopropanol–*n*-heptane (60:14:26, v/v/v) was chosen as the extraction solvent since it is not prone to emulsion formation and allows for good recoveries, not only for the drugs tested, but also for a great variety of pharmaceuticals and drugs of abuse [16].

The lowest detection limits are presented in Table 2. Several authors have established lower detection limits for one or two SSRIs [33–36], but ours can be used and are sufficient for therapeutic determination.

The coefficients of variation (C.V.%) of intra-laboratory day-to-day retention times ranged from 1.56% for demethylcitalopram to 9.91% for citalopram (Table 3).

The single day coefficients of variation (C.V.%) of retention times ranged from 0.79% for sertraline to 7.42% for desmethylcitalopram.

Extraction yields observed in our study were comparable to previously found results [33,36]. Detection limits and extraction yields make this method feasible for measuring therapeutic levels.

4. Conclusion

To conclude, the toxicological analysis is performed within 20 min. The method is simple, rapid and specific. It is the first assay to be described for the convenient screening of several SSRIs in one chromatographic separation. The method can be used for toxicological screening and therapeutic determination.

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Table 3
C.V.% of intra-laboratory results

Commercial names	D.C.I.	Metabolites	C.V.%, 1 day	C.V.%, day-to-day
Floxyfral	Fluvoxamine		5.41	7.2
Prozac	Fluoxetine		3.04	7.6
		Norfluoxetine	2.47	1.72
Zoloft	Sertraline		0.79	5.2
Deroxat	Paroxetine		2.29	9.73
Seropram	Citalopram		5.75	9.91
		Demethylcitalopram	7.42	1.56
		Didesmethylcitalopram	4.83	6.65
Ixel	Milnacipran		1.35	3.3
Effexor	Venlafaxine		3.61	8.1

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