ELSEVIER

Journal of Chromatography B, 775 (2002) 163–168

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Determination of mirtazapine and its demethyl metabolite in plasma by high-performance liquid chromatography with ultraviolet detection
Application to management of acute intoxication

T. Romiguieres[a], F. Pehourcq[b,*], M. Matoga[a], B. Begaud[b], C. Jarry[a]

[a]*EA 2962, Pharmacochimie, Université Bordeaux 2 Victor Segalen, 33076 Bordeaux Cedex, France*
[b]*Laboratoire de Pharmacologie, Université Bordeaux 2 Victor Segalen, 146 rue Léo Saignat, 33076 Bordeaux Cedex, France*

Received 29 January 2002; received in revised form 29 April 2002; accepted 3 May 2002

Abstract

Mirtazapine is a new centrally acting noradrenergic and specific serotonin antidepressant, with an active demethyl metabolite. For toxicological purposes, a specific and accurate RP-HPLC assay was developed for the simultaneous plasma determination of these compounds. A linear response was observed over the concentration range 50–500 ng/ml. A good accuracy (bias <10%) was achieved for all quality controls, with intra-day and inter-day variation coefficients less than 8.3%. The lower limit of quantification was 20 ng/ml, without interferences with endogenous or exogenous components. This rapid method (run time <12 min) was used to manage three intoxications involving mirtazapine. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Mirtazapine; Demethylmirtazapine

1. Introduction

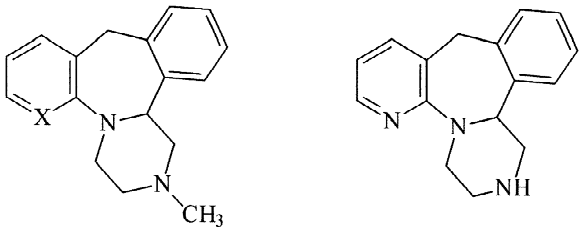
Mirtazapine (1,2,3,4,10,14b-hexahydro-2-methylpyrazino[2,1-a]pyrido[2,3-c]benzazepine) is a new and well tolerated antidepressant (Fig. 1). This drug, not related to any other class of psychotropic agents, has a unique pharmacological profile combining dual action on both the noradrenergic and serotonergic neurotransmitter systems. It blocks pre-synaptic α2-adrenergic receptors and postsynaptic serotonin type 2 and type 3 receptors [1]. After oral administration, mirtazapine is rapidly absorbed with a peak plasma concentration achieved within 2 h of dosing. Its major metabolite, demethylmirtazapine (Fig. 1) is formed in the liver and contributes 3–6% to the total pharmacodynamic profile of the parent drug [2,3].

Mirtazapine is efficacious in the short-term and continuation treatment of moderately and severely depressed hospitalized and out-patients. Many suicide attempts in patients on this antidepressant medication have been described [4–7]. Consequently, it appears essential to use a specific and rapid method for the determination of mirtazapine and its metabolite in case of poisoning.

The published methods for both mirtazapine and

*Corresponding author. Tel.: +33-5-5757-1560; fax: +33-5-5624-5889.
E-mail address: fabienne.pehourcq@pharmaco.u-bordeaux2.fr (F. Pehourcq).

1570-0232/02/$ – see front matter
PII: S1570-0232(02)00290-8



X = N, mirtazapine
X = CH, mianserin

demethylmirtazapine

Fig. 1. Chemical structures of mirtazapine, its demethyl metabolite and mianserine.

demethylmirtazapine analyses in biological fluids included GC [8] and HPLC [9,10]. Maris et al. [9] described an assay with fluorescence detection and consequently the limit of quantification was low (0.5 ng/ml). This method requiring a two-step extraction is not very suitable for a rapid poisoning management. A HPLC method, involving a solid-phase extraction procedure before the separation on a chiral column was recently published [10]. Such an enantioselective method is not generally used for routine applications.

The aim of this study was to establish a simple and accurate RP-HPLC method for the measurement of mirtazapine and demethylmirtazapine 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 poisoning cases leading to concentrations generally greater than the therapeutic range (20–75 ng ml^{-1}) [11]. Under the chromatographic conditions, mirtazapine, demethylmirtazapine and the internal standard were well separated and resolved from endogenous plasma compounds. Moreover, no interference was noticed with other studied psychotropic drugs. Finally, the stability of these tested compounds was studied, particularly during the sample storage at different temperatures.

2. Experimental conditions

2.1. Chemicals

Mirtazapine and demethylmirtazapine were kindly furnished by Organon (Oss, The Netherlands). The internal standard, opipramol, was obtained from Geigy (Paris, France). 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). 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. HPLC conditions

The chromatographic apparatus (Thermo-Finnigan™, San Jose, CA, USA) was equipped with a constant flow pump M100, a Model 150 ultraviolet detector and a Datajet® integrator.

Separation of compounds was carried out on an XTerra™ MS C_{18} analytical column (Waters) (3.9× 150 mm; 5 μm particle size). The mobile phase consisted of acetonitrile–phosphate buffer (6.24×10^{-2} *M*) (30:70, v/v). 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. 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 6.4 with orthophosphoric acid. The flow-rate was maintained at 0.8 ml/min.

The compounds were chromatographed at 294 nm within 12 min. All data were processed by Datajet®. The unknown concentrations of mirtazapine and its metabolite were quantified using linear regression of response (drug/I.S. peak height ratio) versus mirtazapine or metabolite concentrations.

2.3. Standard solutions

Stock solutions of mirtazapine, demethylmirtazapine and opipramol (1 mg/ml) were prepared in methanol and stored at −20 °C. They were stable for at least 4 months.

Dilutions of mirtazapine and demethylmirtazapine were freshly prepared into drug-free human plasma to provide concentrations of 50, 100, 250 and 500 ng/ml of both compounds.

The internal standard (I.S.) stock solution was diluted daily in bidistilled water to yield a 10 μg/ml

working solution. In the same manner, plasma quality controls (QC) spiked with 75, 150 and 300 ng/ml of both mirtazapine and demethylmirtazapine were prepared to measure the accuracy and the precision of the method.

2.4. Sample preparation

To 1 ml of calibration or patient plasma were added 100 μl of I.S. (10 μg/ml) and 200 μl of 0.1 N 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 N HCl. The mixture was shaken during 10 min and centrifuged. The upper organic phase was discarded and 40 μl of aqueous phase were injected into the chromatograph.

2.5. Accuracy, precision and recovery

The accuracy and intra-day and inter-day precision of the method were estimated by assaying six replicates of QC samples prepared as described above, in three analytical runs. The intra-day precision was defined by calculating coefficient of variation (C.V.) for replicates ($n=6$) of QC samples. The inter-day precision ($n=18$) was determined from QC samples obtained on 3 different days. Accuracy, expressed as % bias, was calculated as the percent difference between the amount of mirtazapine and demethylmirtazapine added and found.

Extraction recoveries from human plasma were determined by comparison of HPLC responses from extracted samples, containing low (75 ng/ml) and high (300 ng/ml) concentrations of mirtazapine and its metabolite, to those from unextracted and directly injected standards, spiked with the same amounts.

3. Results and discussion

3.1. Retention times, selectivity and linearity

Under the described RP-HPLC procedure, demethylmirtazapine eluted at ~2.6 min, mirtazapine and I.S. at 4.2 min and 10.3 min, respectively. Representative chromatograms of blank and spiked plasma samples are illustrated in Fig. 2. Blank plasma showed no interfering endogenous compounds with mirtazapine, its metabolite and the I.S, respectively. Potentially coadministered drugs had retention times that were different from mirtazapine, demethylmirtazapine and opipramol or were not detected or extracted with the described bioanalytical method. The relative retention times for these psychotic drugs tested for interference are shown in Table 1.

From 10 calibration curves, constructed with calibration points ranging from 50 to 500 ng/ml, a high correlation coefficient (r) was found for mirtazapine and demethylmirtazapine (Table 2).

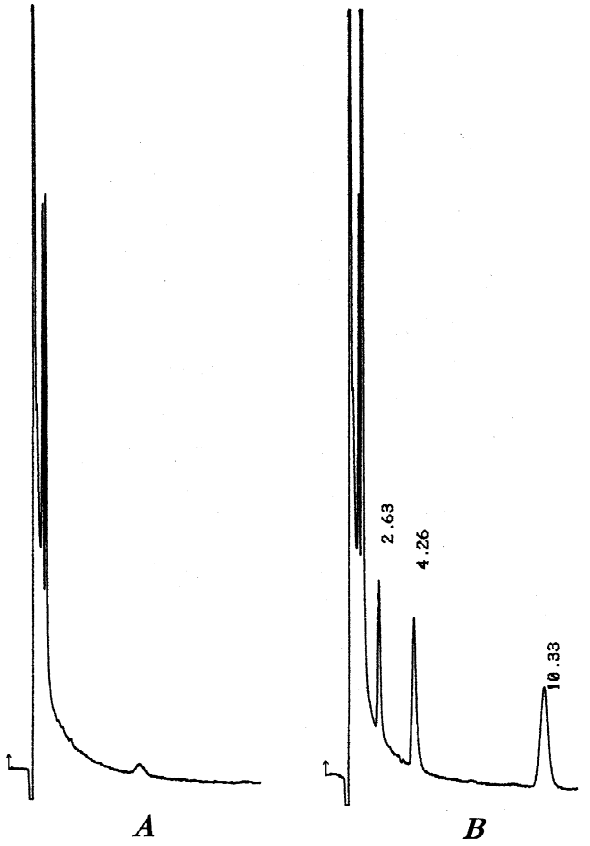


Fig. 2. Chromatograms of blank plasma (A) and 400 ng/ml plasma extract of mirtazapine ($t_r=4.26$ min) and demethylmirtazapine ($t_r=2.63$ min) with the internal standard ($t_r=10.33$ min) (B).

Table 1
Compounds studied for potential interferences (injected amount: 0.6 μg)

Compound	t_{R1}	t_{R2}	t_{R3}	Compound	t_{R1}	t_{R2}	t_{R3}
Zopiclone	1.3	0.8	0.3	Fluvoxamine	5.5	3.4	1.4
Bromazepam	2.0	1.3	0.5	Imipramine	6.4	3.5	1.5
Lorazepam	2.4	1.5	1.0[a]	Nordiazepam	7.6	4.7	1.9
Citalopram	3.0	1.9	0.8	Trimipramine	8.1	5.0	2.0
Amoxapine	3.1	1.9	0.8	Flunitrazepam	10.7	6.5	1.8
Zolpidem	3.5	2.1	0.9	Clomipramine	12.2	7.5	3.1
Venlafaxine	3.6	2.2	0.9	Diazepam	13.4	8.2	3.4
Desipramine	5.0	3.0	1.3	Nortriptyline	NR	NR	NR
Dosulepine	5.0	3.0	1.3	Amitriptyline	NR	NR	NR
Alprazolam	5.1	3.1	1.3	Minalcipran	NR	NR	NR
Paroxetine	5.1	3.1	1.3	Sertraline	NR	NR	NR
Mianserine	5.3	3.3	1.4				

t_{R1}, relative retention time to demethylmirtazapine (2.63 min); t_{R2}, relative retention time to mirtazapine (4.26 min); t_{R3}, relative retention time to opipramol (10.33 min). NR, no response.
[a] Compound not extracted in our chromatographic conditions.

Table 2
Statistical data (n=40) for linearity including standard deviation (SD)

	r	Slope (±SD)	Intercept (±SD)
Mirtazapine	0.962	3.904±0.183	−0.039±0.053
Demethylmirtazapine	0.961	3.777±0.178	−0.012±0.051

3.2. Precision and accuracy

The results obtained for precision and accuracy are listed in Table 3 and expressed as C.V. (%) and % bias, respectively. These results indicate that the method is precise: intra-day precision less than 8.3% and 6.1% for mirtazapine and demethylmirtazapine, and inter-day precision less than 5.7 and 6.0% for the parent compound and its metabolite, respectively. This method is accurate (bias ranged from 0.5 to 4.2% for mirtazapine and from 1.1 to 9.9% for demethylmirtazapine).

3.3. Limit of quantification (LOQ)

The LOQ was defined as the lowest mirtazapine and demethylmirtazapine concentration that could be determined with a precision less than 20 (% C.V.) and with an accuracy between 80 and 120%, as de-

Table 3
Precision and accuracy of results for plasma spiked with mirtazapine and demethylmirtazapine

		Theory (ng/ml)	n	Found±SD (ng/ml)	C.V. (%)	Bias (%)
Intra-day	Mirtazapine	75	6	74.6±2.9	3.9	0.5
		150	6	156.3±4.6	2.9	−4.2
		300	6	297.1±24.8	8.3	0.9
	Demethylmirtazapine	75	6	80.6±3.5	4.3	−7.5
		150	6	163.4±1.1	0.7	−8.9
		300	6	296.6±18.1	6.1	1.1
Inter-day	Mirtazapine	75	18	73.5±3.7	5.0	2.0
		150	18	152.7±7.8	5.1	−1.8
		300	18	297.2±17.0	5.7	0.9
	Demethylmirtazapine	75	18	79.7±3.0	3.8	−6.2
		150	18	164.9±3.3	2.0	−9.9
		300	18	310.0±18.6	6.0	−3.3

termined in the inter-day analytical runs. It was found to be 20 ng/ml for both mirtazapine and demethylmirtazapine, with a precision of 10.8% and 6.0%, respectively, and a bias of −18.5% and −14.4%, respectively.

3.4. Extraction efficiency

For mirtazapine as well as demethylmirtazapine, the recovery was determined at two different concentrations (75 ng/ml and 300 ng/ml). Whatever the concentration level, the recovery was good. The average values were 96.9±3.6% for mirtazapine and 102.9±1.9% for the metabolite.

3.5. Stability

To determine the influence of temperature on the stability of compounds, two quality control samples spiked with mirtazapine and demethylmirtazapine (75 ng/ml and 150 ng/ml, respectively) were stored in different conditions: at −20 °C during 21 days; at +4 °C during 48 h; at +20 °C during 24 h.

No tendency for decomposition was noticed in the quick frozen samples during 3 weeks. Indeed, at −20 °C, for both compounds the percent variation coefficient (C.V.) was less than 5.1% and the accuracy presented a bias percent less than 10. The storage during 48 h at +4 °C produced no significant decrease of mirtazapine and demethylmirtazapine concentrations (C.V. and % bias values less than 10). Finally, a good stability of both compounds was found after a storage at room temperature during 24 h, with C.V. and % bias values less than 7.5.

3.6. Clinical cases

In case of drug voluntary intoxication, the ingested dose and the beginning of intoxication were often unknown. Nevertheless, the expected concentrations are generally higher than the therapeutic range. By using the described method, we were able to manage three cases of voluntary mirtazapine poisoning. The measured concentrations of both mirtazapine and demethylmirtazapine were greater than the therapeutic ones (Table 4).

Table 4
Concentrations of mirtazapine and its demethyl metabolite in three voluntary intoxications

Patient	Mirtazapine (ng/ml)	Demethylmirtazapine (ng/ml)
1	325	160
2	171	100
3	140	60

According to the literature, no serious adverse effect of overdose with mirtazapine was reported. The clinical signs generally encountered are amnesia, somnolence and minor tachycardia [5]. In our study, patient 1 presented concentrations fivefold greater than the therapeutic one after ingestion of 150 mg of mirtazapine. He showed somnolence and an impaired memory of recent events, but no cardiovascular effect was recorded. Patient 2, a young depressive woman, had ingested mirtazapine in combination with another structurally related tetracyclic antidepressant, mianserine. As depicted in Table 1 and despite the chemical similarity (Fig. 1), no interference was observed on the plasma extract chromatogram for patient 2. The mianserine concentration was measured by HPLC at 408 ng/ml. This value is sixfold greater than the therapeutic range [12]. Nevertheless, no hypertension or cardiac toxicity was noticed by the physician.

Finally, the HPLC method described for simultaneous determination of mirtazapine and demethylmirtazapine in human plasma was simple and rapid enough for a routine application. The LOQ and the total length of this assay are particularly adapted to management of acute intoxications.

References

[1] T. de Boer, F. Nefkens, A. van Helvoirt, Eur. J. Pharmacol. 253 (1994) R5.
[2] C.J. Timmer, J.E. Paanakker, M. Vrijmoed-de Vries, Pharm. Res. 14 (1997) 98.
[3] C.J. Timmer, J.M. Ad Sitsen, L.P. Delbressine, Clin. Pharmacokinet. 38 (2000) 461.
[4] A.W. Gerritsen, J. Clin. Psychiatry 58 (1997) 271.
[5] J.D. Bremner, P. Wingard, T.A. Walshe, J. Clin. Psychiatry 59 (1998) 233.
[6] R. Holzbach, H. Jahn, F.-G. Pajonk, C. Mähne, Biol. Psychiatry 44 (1998) 925.

[7] D.T. Anderson, K.L. Fritz, J.J. Muto, J. Anal. Toxicol. 23 (1999) 544.
[8] J.E. Paanakker, H.J.M. Van Hal, J. Chromatogr. 417 (1987) 203.
[9] F.A. Maris, E. Dingler, S. Niehues, J. Chromatogr. B 721 (1999) 309.
[10] S. Dodd, G.D. Burrows, T.R. Norman, J. Chromatogr. B 748 (2000) 439.
[11] S.H. Preskorn, J. Clin. Psychiatry 58 (Suppl. 6) (1997) 3.
[12] S. Mongomery, R. McAuley, D.B. Mongomery, Br. J. Clin. Pharmacol. 5 (1978) 71S.