Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 805 (2004) 141–146
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

# Determination of human plasma levels of levo-α-acetylmethadol and its metabolites by gas chromatography–mass spectrometry

C.B. Eap[a,*], G. Bouchoux[b], N. Scherbaum[c], M. Gastpar[c], K. Powell Golay[a], P. Baumann[a]

[a] *Unité de Biochimie et Psychopharmacologie Clinique, Département Universitaire de Psychiatrie Adulte, Hôpital de Cery, CH-1008 Prilly-Lausanne, Switzerland*
[b] *Département de Chimie, Laboratoire des mécanismes réactionnels, Ecole Polytechnique, F-91128 Palaiseau Cedex, France*
[c] *Klinik fuer Psychiatrie und Psychotherapie, Universitaetsklinikum/Rheinische Kliniken Essen, Virchowstrasse 174, D-45147 Essen, Germany*

Received 23 May 2003; received in revised form 24 February 2004; accepted 24 February 2004

**Abstract**

A gas chromatography–mass spectrometry (GC–MS) method is presented which allows the simultaneous determination of the plasma concentrations of the levo-α-acetylmethadol (LAAM) and of its active metabolites (NorLAAM and DiNorLAAM), after derivatization with the reagent trifluoroacetic anhydride (TFAA). No interferences from endogenous compounds were observed following the extraction of plasma samples from 11 different human subjects. The standard curves were linear over a working range of 5–200 ng/ml for the three compounds. Recoveries measured at three concentrations ranged from 47 to 67% for LAAM, from 50 to 69% for NorLAAM and from 28 to 50% for DiNorLAAM. Intra- and interday coefficients of variation determined at three concentrations ranged from 5 to 13% for LAAM, from 3 to 9% for NorLAAM and from 5 to 13% for DiNorLAAM. The limits of quantitation of the method were found to be 4 ng/ml for the three compounds. No interference was noted from methadone. This sensitive and specific analytical method could be useful for assessing the in vivo relationship between LAAM's blood levels, clinical efficacy and/or cardiotoxicity


*Keyword:* Levo-α-acetylmethadol

## 1. Introduction

Levo-α-acetylmethadol (LAAM) is an opioid agonist which can prevent opioid withdrawal symptoms for a longer period of time than methadone due to the long half-lives of its demethylated and didemethylated metabolites, Levo-α-acetylnormethadol hydrochloride (NorLAAM) and levo-α-acetyldinormethadol hydrochloride (DiNorLAAM), respectively. Three doses a week of LAAM are thus sufficient to stabilise opioid addicted patients and several studies have demonstrated that LAAM could be as effective as methadone in maintaining abstinence in patients with opioid addiction [1]. However, the recent report that the administration LAAM may result in rare case (less than 1%) of life-threatening cardiac rhythms disorders [2] resulted in the withdrawal of this drug in the countries of the European Union where it was previously marketed. On the other hand, LAAM can presently still be prescribed in the United States if some necessary steps (i.e. electrocardiograms) are taken in order to ensure its safety. However, in late August 2003, the US manufacturer of LAAM announced plans to discontinue the product.

Cardiotoxicity of LAAM is primarily due to its interference with the rapid component of the delayed rectifier potassium current, an effect which is concentration dependant [3]. Analytical methods allowing to measure blood concentrations of LAAM and its metabolites could therefore be useful for assessing the in vivo relationship between LAAM and metabolite's blood levels and cardiotoxicity and/or clinical efficacy. Several methods have been published for the determination of LAAM in biological fluids, including radioimmunoassay [4], HPLC with UV detection [5], gas chromatography (GC) with flame ionisation detection [6–8], an electron capture detection [7], nitrogen detection [9], or chemical ionisation mass spectrometry (MS) [10,11], generally after a derivatization step. A gas chromatograph–electron impact mass spectrometry

* Corresponding author. Tel.: +41-21-643-64-38; fax: +41-21-643-64-44.
*E-mail address:* c-b.eap@inst.hospvd.ch (C.B. Eap).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.02.034

(GC–MS) for the determination of LAAM, NorLAAM and DiNorLAAM is described.

## 2. Experimental

### 2.1. Reagents

Levo-α-acetylmethadol hydrochloride was kindly provided by Siegfried (Zofingen, Switzerland). Levo-α-acetylnormethadol hydrochloride and levo-α-acetyldinormethadol hydrochloride were kindly provided by the Research Triangle Institute (North Carolina, USA). Methylmaprotiline hydrochloride, maprotiline hydrochloride and desmethylmaprotiline methansulphonate (Internal standards MMP, MP and DMP, respectively) were provided by Ciba-Geigy (Basel, Switzerland). Trifluoroacetic anhydride (TFAA) was from Supelco (Buchs, Switzerland)

Stock solutions of LAAM, NorLAAM and DiNorLAAM were made at 1 mg/ml in methanol. Stock solutions of MMP, MP and DMP were made at 1 mg/ml in 0.01 M HCl and a working solution was made at 2 ng/μl in 0.01 M HCl. All solutions were stored at −20 °C until used. Fresh working solutions of LAAM, NorLAAM and DiNorLAAM were made at 10 and 1 ng/μl in 0.01 M HCl. Water was obtained from a Milli Q-RG apparatus (Millipore, Le Mont-sur-Lausanne, Switzerland), and all other reagents were of analytical or HPLC grade.

### 2.2. Instrumentation and chromatographic conditions

A Hewlett-Packard HP 5890 Series II Gas chromatographic system coupled to an HP 5972 Mass Spectrometer (Agilent, Geneva, Switzerland) was used with a 15 m Supelco SPB-5 (5% diphenyl/95% dimethylsiloxane) "Fused silica" column (0.25 mm i.d., 0.25 μm film thickness, no. 2-4032). Standard operating conditions were as follows; the initial oven temperature was 160 °C with 0 min initial time; the oven ramp was 30 °C with a final temperature of 260 °C and final time 3.2 min; the injector temperature was 250 °C and detector temperature, 280 °C. The gas vector was helium purity 50 (Carbagas, Switzerland) and the flow 50 ml/min. The septum purge was set at 3 ml/min. The inlet was at constant flow of pressure 2 p.s.i. and at 160 °C. A splitless injection was made during 60 s. The detection was made in selected ion monitoring mode for the ions of *m*/*z* 72 (LAAM), 207 (NorLAAM and DiNorLAAM), 345 (MP), 291 (MMP), 331 (DMP). Confirmation ions of LAAM, NorLAAM and DiNorlaam (207, 154 and 345, respectively) were also quantified. The dwell time for all ions was 70 ms.

### 2.3. Extraction and derivatization conditions

For the determination of LAAM, NorLAAM and DiNorLAAM, 200 ng of the internal standards MMP, MP and DMP, 0.5 ml 1 M carbonate buffer (pH 9.4) and 6 ml *n*-heptane–ethyl acetate (80:20, v/v) were added to a 1 ml aliquot of heparinized plasma. The extraction was then performed on a shaker for 20 min. After centrifugation (8 min, 3300 × *g*, 8 °C), the organic layer was transferred to another tube containing 1.2 ml 0.1 N HCl. After 20 min of shaking and 8 min of centrifugation, the organic phase was discarded, and the aqueous phase was transferred to another tube containing 150 μl toluene–isoamyl alcohol (85:15, v/v) and 1 ml carbonate buffer (pH 9.4). After 15 min of shaking and 3 min of centrifugation (2250 × *g*), the organic phase was transferred to a home-made micro tube, which allowed a good visualisation of the two phases. The organic phase was then pipetted with care to avoid contamination by the aqueous phase and gently transferred to an injection vial. The organic phase was then dried under a stream of nitrogen at 40 °C. To the residue was added 50 μl of pure toluene and 50 μl of trifluoroacetic anhydride, the vial capped and vortexed for 15 s. The resulting mixture was derived for 60 min at 64 °C in a heating block. The derived mixture was then again thoroughly dried under a stream of nitrogen. 20 μl of toluene–isoamyl alcohol (85:15, v/v) was added and the vial capped and vortexed for 15 s. A 3 μl portion was automatically injected into the GC–MS system.

## 3. Results and discussion

Fig. 1 shows the chemical structures of LAAM, NorLAAM, DiNorLAAM, MMP, MP and DMP and the probable fragmentation pathways of the molecular cations (the mass spectrum of MMP having been described elsewhere [12], only the dominant fragmentation leading to *m*/*z* 58 is indicated). Table 1 shows a summary of the statistical data on the analysis. In summary, the mean coefficients of correlation of the calibration curves obtained from four separate experiments were 0.998, 0.998, 0.992 for LAAM, NorLAAM and DiNorLAAM, respectively. It should be mentioned that no values are given for the intercepts, as the option "force through the origin" was chosen for the calibration curves with which better results are obtained for control plasma samples of low concentration (data not shown). As pure standards of the derivatized compounds are not available, recovery was calculated by dividing the mean areas ($n = 10$) obtained after the complete extraction and derivatization procedure of plasmas containing low, medium and high concentrations of the drugs by the mean areas obtained after direct derivatization of the same quantities of the pure standards. Recoveries ranged from 47 to 67% for LAAM, from 50 to 69% for NorLAAM, from 28 to 50% for DiNorLAAM, from 56 to 65% for MMP (internal standard of LAAM), from 49 to 61% for MP (internal standard of MP) and from 32 to 47% for DMP (internal standard of DiNorLAAM). Although coefficients of variations up to 30% were calculated for the recoveries, a good concordance of the recoveries between LAAM, NorLAAM and DiNorLAAM and their respective internal standards allows that

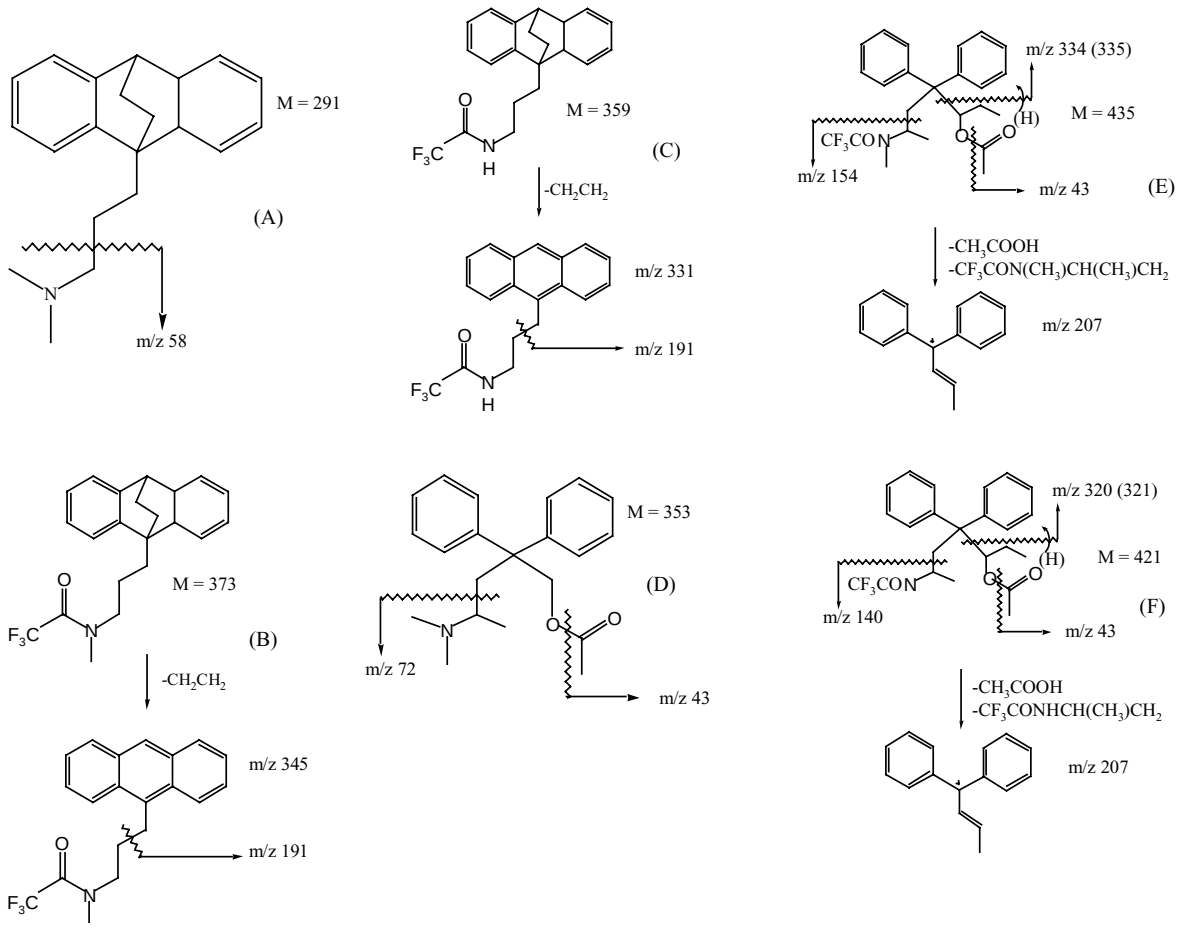


Fig. 1. Chemical structures and most probable fragmentation pathways of the molecular cations of (A) methyl maprotiline, (D) levo-α-acetylmethadol or LAAM and of the (B) trifluoroacetyl derivatives of maprotiline, (C) desmethyl maprotiline, (E) desmethyl levo-α-acetylmethadol or NorLAAM and (F) didesmethyl levo-α-acetylmethadol or DiNorLAAM.

this does not affect the precision of the method. Thus, the variability of the assays for the intra- ($n = 8$) and the inter-day experiments ($n = 8$), as assessed by the coefficients of variation (C.V.), measured at three concentrations for each substance, ranged from 5 to 13% for LAAM, from 3 to 9% for NorLAAM and from 5 to 13% for DiNorLAAM. The percent theoretical concentrations, which represent the accuracy of the method, were all within ±5% for LAAM, within ±7% for NorLAAM and within ±11% for DiNorLAAM. The limits of quantitation, defined as the concentrations for which the mean value of replicate determinations ($n = 9$) is within 15% of the actual value, the coefficient of variation less than 15%, and which gives a signal-to-noise ratio of at least 4, were found to be 4 ng/ml for the three substances.

It has also been controlled that methadone, an opioid agonist with a chemical structure close to LAAM, and which is the most frequently prescribed opioid agonist for maintenance therapy of opioid addicts, does not interfere with the determination of LAAM and of its metabolites (see Fig. 3). No interferences were observed from endogenous compounds following the extraction of plasma samples from 11 different human controls who were not receiving any medication. It should also be mentioned that MMP, which is used as the internal standard of LAAM, is not a metabolite of maprotiline and is not detected in patients receiving this drug [13]. The stability of LAAM and its metabolites was evaluated by analysing spiked plasma samples stored at −20 °C for different periods of time. No loss was noted after storage of up to 1 month. Finally, the stability of the derivatized forms of these three substances was evaluated: no change was noted after storage of one day at room temperature (data not shown).

Fig. 2 shows the SIM tracing of a blank plasma. Fig. 3 shows a chromatogram obtained from the analysis of an opioid addicted patient in methadone maintenance treatment not receiving LAAM. Fig. 4 shows an example of chromatogram obtained from the analysis of a 500 μl plasma sample drawn from an opioid addicted patient during LAAM maintenance therapy. The measured concentrations of LAAM, NorLAAM and DiNorLAAM were 65, 168 and 144 ng/ml, respectively. Plasma concentrations of LAAM and of its metabolites were measured in a group of 10 opioid addicted patients after 4 weeks of treatment with an unchanged dose of LAAM (mean ± S.D. dose on monday, wednesday, friday and of

Table 1
Statistical data concerning the analysis of LAAM, NorLAAM and DiNorLAAM

| Parameter | LAAM | NorLAAM | DiNorLAAM |
|---|---|---|---|
| Calibration ($n = 8$) | | | |
| Range (ng/ml) | 5–200 | 5–200 | 5–200 |
| Slope: mean ± S.D. (95% CI) | 26 ± 7.8 (19–32) | 1.9 ± 0.68 (1.4–2.5) | 1.0 ± 0.11 (0.91–1.09) |
| Coefficient of correlation: mean ± S.D. (range) | 0.998 ± 0.002 (0.995–1.000) | 0.997 ± 0.002 (0.992–1.000) | 0.992 ± 0.008 (0.979–0.999) |
| Recovery ($n = 10$) | | | |
| Concentration used (ng/ml) | 10 | 10 | 10 |
| Recovery (in %): mean ± S.D. (C.V.) | 47 ± 10 (20) | 50 ± 8 (15) | 28 ± 8 (30) |
| Recovery (in %) of the internal standard: mean ± S.D. (C.V.) | MMP: 56 ± 11 (20) | MP: 49 ± 10 (19) | DMP: 32 ± 8 (25) |
| Concentration used (ng/ml) | 30 | 30 | 30 |
| Recovery (in %): mean ± S.D. (C.V.) | 67 ± 13 (19) | 68 ± 7 (10) | 50 ± 10 (20) |
| Recovery (in %) of the internal standard: mean ± S.D. (C.V.) | MMP: 62 ± 5 (8) | MP: 57 ± 4 (8) | DMP: 43 ± 6 (15) |
| Concentration used (ng/ml) | 100 | 100 | 100 |
| Recovery (in %): mean ± S.D. (C.V.) | 65 ± 14 (22) | 69 ± 15 (22) | 48 ± 14 (29) |
| Recovery (in %) of the internal standard: mean ± S.D. (C.V.) | MMP: 65 ± 16 (25) | MP: 61 ± 13 (21) | DMP: 47 ± 10 (22) |
| Within-day variation ($n = 8$) | | | |
| Theoretical values (ng/ml) | 10 | 10 | 10 |
| Measured values (ng/ml): mean ± S.D. (C.V.) | 10.0 ± 0.6 (6) | 9.9 ± 0.4 (5) | 10.4 ± 1.3 (13) |
| Percentage of theory | 100 | 99 | 104 |
| Theoretical values (ng/ml) | 30 | 30 | 30 |
| Measured values (ng/ml): mean ± S.D. (C.V.) | 28.4 ± 1.8 (6) | 28.2 ± 1.0 (4) | 30.3 ± 3.4 (11) |
| Percentage of theory | 95 | 94 | 101 |
| Theoretical values (ng/ml) | 100 | 100 | 100 |
| Measured values (ng/ml): mean ± S.D. (C.V.) | 94.9 ± 6.7 (7) | 93.4 ± 3.3 (4) | 107.7 ± 5.5 (5) |
| Percentage of theory | 95 | 93 | 108 |
| Day-to-day variation ($n = 8$) | | | |
| Theoretical values (ng/ml) | 10 | 10 | 10 |
| Measured values (ng/ml): mean ± S.D. (C.V.) | 10.4 ± 1.3 (13) | 10.2 ± 0.5 (5) | 10.8 ± 1.0 (9) |
| Percentage of theory | 104 | 102 | 108 |
| Theoretical values (ng/ml) | 30 | 30 | 30 |
| Measured values (ng/ml): mean ± S.D. (C.V.) | 30.2 ± 1.6 (5) | 29.1 ± 0.9 (3) | 33.2 ± 2.8 (8) |
| Percentage of theory | 101 | 97 | 111 |
| Theoretical values (ng/ml) | 100 | 100 | 100 |
| Measured values (ng/ml): mean ± S.D. (C.V.) | 98.7 ± 9.8 (10) | 94.4 ± 8.6 (9) | 105.7 ± 7.6 (7) |
| Percentage of theory | 99 | 94 | 106 |
| Limit of quantitation ($n = 9$) | | | |
| Theoretical values (ng/ml) | 4 | 4 | 4 |
| Measured values (ng/ml): mean ± S.D. (C.V.) | 4.0 ± 0.5 (12) | 3.9 ± 0.2 (5) | 4.2 ± 0.4 (10) |

Standard deviation (S.D.), coefficient of variation (C.V., %). See text for other abbreviations.

the week: 98 ± 33 mg/day (40–130 mg/day); 98 ± 33 mg/day (40–130 mg/day); 121 ± 41 mg/day (50–160 mg/day); 316 ± 106 mg/week (130–420 mg/week), respectively). The mean (±S.D., range, in pg × week/ml × mg) trough concentrations corrected for the dose of LAAM of the week were for LAAM, NorLAAM and DiNorLAAM, of 181 ± 248 (45–876); 401 ± 186 (128–656); 390 ± 110 (261–583), respectively.

In summary, this method which is both sensitive and selective, allows the simultaneous quantification of LAAM, NorLAAM and DiNorLAAM in plasma samples. The use of a mass spectrometry detection method is an advantage in opioid addicted patients in maintenance treatment because of the frequent polymedications received by these patients as well as the possible use of illicit drugs. This method could be useful for assessing the in vivo relationship between LAAM

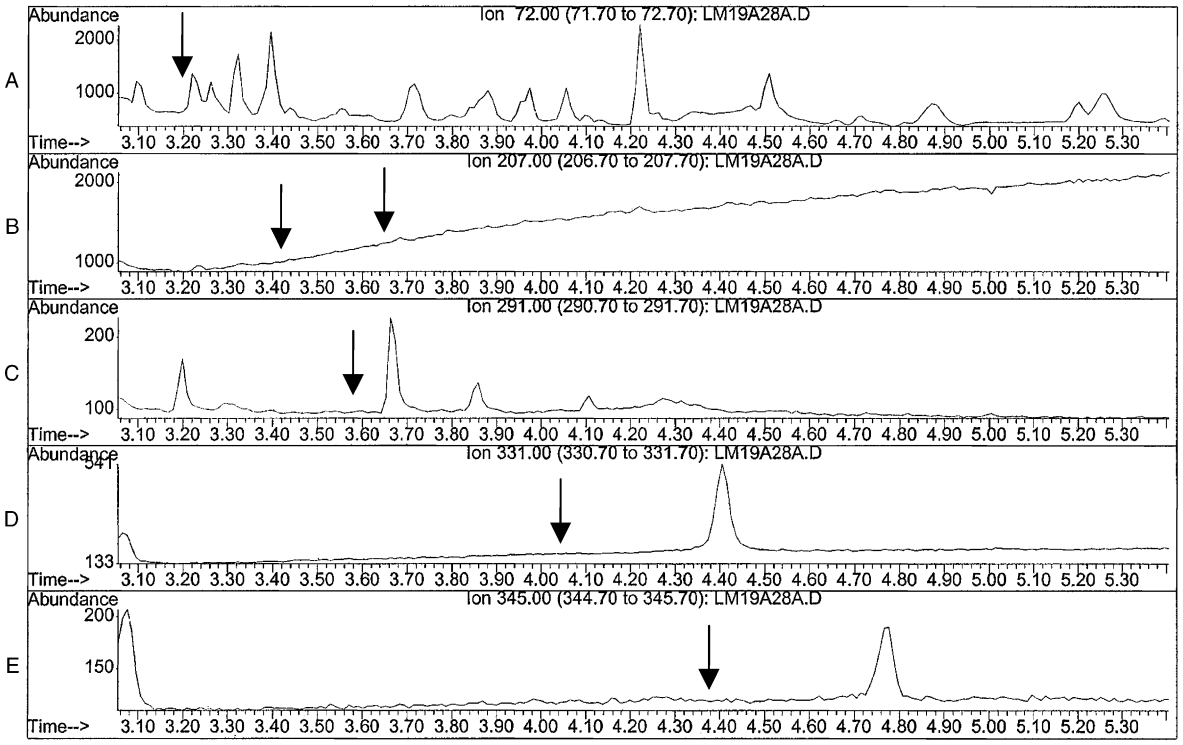


Fig. 2. SIM tracing of a 1 ml blank plasma. The arrows indicate the position of LAAM (trace A, ion 72), NorLAAM (trace B, ion 207), DiNorLAAM (trace B, ion 207), methylmaprotiline (MMP, internal standard for LAAM, trace C, ion 291), maprotiline (MP, internal standard for NorLAAM, trace E, ion 345) and desmethyl maprotiline (DMP, internal standard for DiNorLAAM, trace D, ion 331).

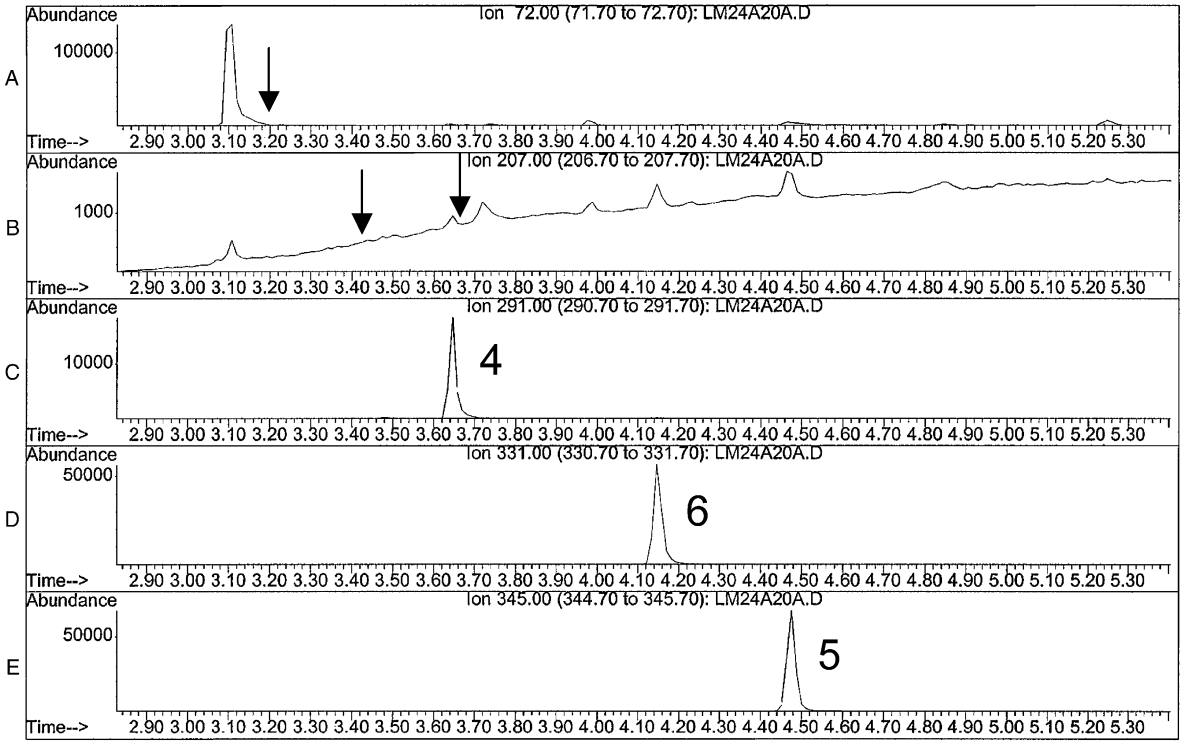


Fig. 3. SIM tracing of a 1 ml plasma from a patient in methadone maintenance therapy without LAAM. Methylmaprotiline (MMP, internal standard for LAAM, trace C, peak 4, ion 291, 3.65 min), maprotiline (MP, internal standard for NorLAAM, trace E, peak 5, ion 345, 4.48 min), desmethyl maprotiline (DMP, internal standard for DiNorLAAM, trace D, peak 6, ion 331, 4.15 min). The arrows indicate the position of LAAM (trace A, ion 72), NorLAAM (trace B, ion 207) and DiNorLAAM (trace B, ion 207).

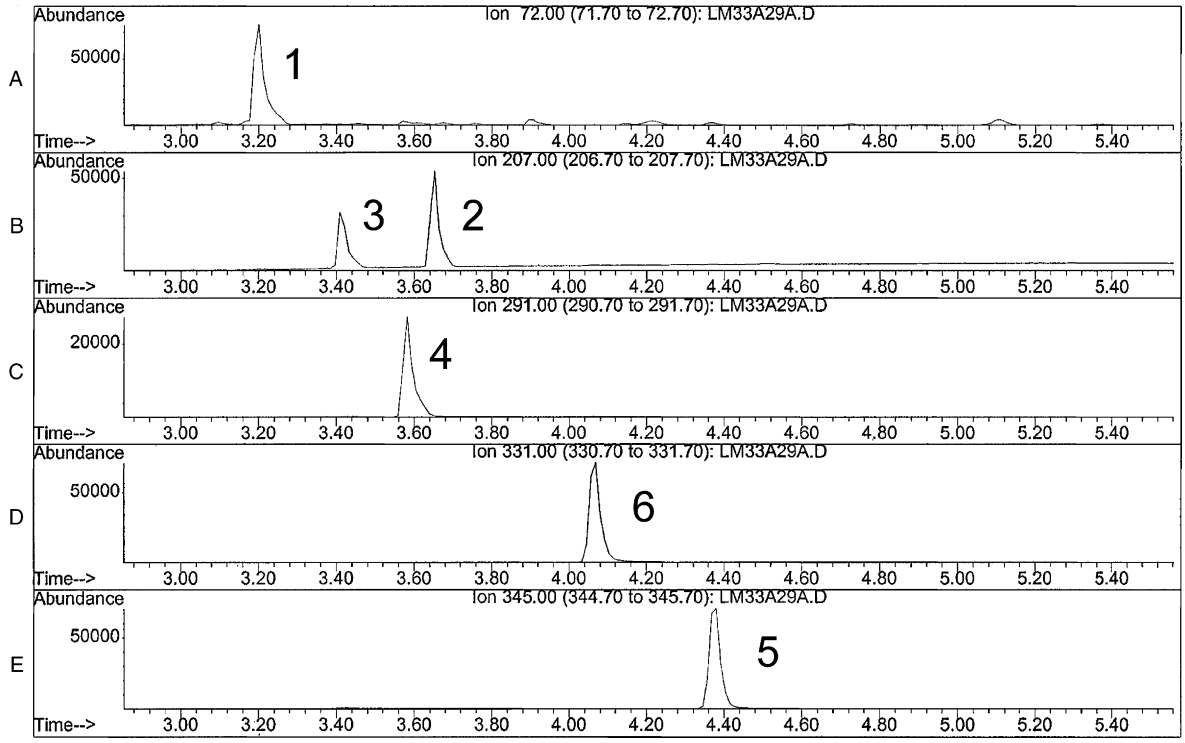


Fig. 4. SIM tracing of a 1 ml plasma from a patient in levo-α-acetylmethadol (LAAM) maintenance therapy. LAAM (trace A, peak 1, ion 72, 3.20 min), NorLAAM (trace B, peak 2, ion 207, 3.66 min), DiNorLAAM (trace B, peak 3, ion 207, 3.42 min), methylmaprotiline (MMP, internal standard for LAAM, trace C, peak 4, ion 291, 3.59 min), maprotiline (MP, internal standard for NorLAAM, trace E, peak 5, ion 345, 4.38 min), desmethyl maprotiline (DMP, internal standard for DiNorLAAM, trace D, peak 6, ion 331, 4.07 min).

and metabolite's blood levels and cardiotoxicity and/or clinical efficacy.

## Acknowledgements

We gratefully acknowledge the editorial assistance of Mrs V. Sari, and the bibliographic help of Mrs J. Rosselet, Mrs M. Gobin and Mrs E. Ponce. We thank the firm Ciba-Geigy for providing us with methylmaprotiline, maprotiline and desmethylmaprotiline, the firm Ziegfried for levo-α-acetylmethadol and the Research Triangle Institute for desmethyl levo-α-acetylmethadol and didesmethyl levo-α-acetylmethadol.

## References

[1] R.E. Johnson, M.A. Chutuape, E.C. Strain, S.L. Walsh, M.L. Stitzer, G.E. Bigelow, N. Engl. J. Med. 343 (2000) 1290.
[2] R.L. Deamer, D.R. Wilson, D.S. Clark, J.G. Prichard, J. Addict. Dis. 20 (2001) 7.
[3] A.N. Katchman, K.A. McGroary, M.J. Kilborn, C.A. Kornick, P.L. Manfredi, R.L. Woosley, S.N. Ebert, J. Pharmacol. Exp. Ther. 303 (2002) 688.
[4] K.L. McGilliard, G.D. Olsen, J. Pharmacol. Exp. Ther. 215 (1980) 205.
[5] C.-H. Kiang, S. Campos-Flor, C.E. Inturrisi, J. Chromatogr. Biomed. Appl. 222 (1981) 81.
[6] F.L.S. Tse, P.G. Welling, Biopharm. Drug Dispos. 1 (1980) 203.
[7] D.H.M. Lau, G.L. Henderson, J. Chromatogr. 129 (1976) 329.
[8] F.L.S. Tse, P.G. Welling, J. Chromatogr. 135 (1977) 205.
[9] K. Verebey, A. DePace, S.J. Mulé, J. Chromatogr. Biomed. Appl. 343 (1985) 339.
[10] T.A. Jennison, B.S. Finkle, D.M. Chinn, D.J. Crouch, J. Chromatogr. Sci. 17 (1979) 64.
[11] R.F. Kaiki, N. Chatterjie, C.E. Inturrisi, J. Chromatogr. 109 (1975) 247.
[12] C.B. Eap, G. Bouchoux, M. Amey, N. Cochard, L. Savary, P. Baumann, J. Chromatogr. Sci. 36 (1998) 365.
[13] G. Gabris, P. Baumann, M. Jonzier-Perey, P. Bosshart, B. Woggon, A. Küpfer, Biochem. Pharmacol. 34 (1997) 409.