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Determination of *l*-α-acetylmethadol, *l*-α-noracetylmethadol and *l*-α-dinoracetylmethadol in plasma by gas chromatography–mass spectrometry

Brian F. Thomas*, A. Robert Jeffcoat, Mary W. Myers, James M. Mathews, C. Edgar Cook

Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27707, USA

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Abstract

A method is described for the simultaneous determination of *l*-α-acetylmethadol (LAAM) and its N-demethylated metabolites, *l*-α-noracetylmethadol (norLAAM) and *l*-α-dinoracetylmethadol (dinorLAAM), in plasma by gas chromatography–chemical ionization mass spectrometry. Deuterated internal standards for each analyte serve as carriers and control for recovery during sample purification on a solid-phase extraction column (C_{18}), and subsequent separation and analysis on a DB-17 capillary column. With this method, we have determined levels of LAAM, norLAAM, and dinorLAAM in small volumes of plasma (100 μl). The limit of quantitation for all analytes was approximately 1.0 ng/g plasma and the limit of detection was approximately 0.5 ng/g plasma. An experimental application is also described where these analytes are quantitated in plasma obtained from rats before, during, and after chronic administration of LAAM-HCl. Since this technique affords a selective and sensitive means of detection of LAAM and its active, N-demethylated metabolites in small samples of blood, it may enable patient compliance to be more easily assessed by allowing samples to be collected by a simple finger-prick technique.

1. Introduction

The abuse of heroin remains widespread throughout the United States. A 1988 Household Survey indicated that 1.9 million Americans had used heroin, and modeling methods have estimated that there were approximately 500 000 addicts in 1982 [1]. By 1990, the number of estimated heroin addicts had increased to *ca.* 750 000 [2]. Since acquired immune-deficiency syndrome (AIDS) among intravenous opiate abusers is contributing to the AIDS epidemic, treatment to reduce the abuse of heroin has taken on increased importance. Therapeutic modalities currently under investigation include maintenance agents other than methadone, long-term opiate blockade with opiate antagonists such as naltrexone, and medications aimed at detoxification and ameliorating withdrawal, such as clonidine.

With regard to replacement therapies, the long-acting synthetic narcotic *l*-alpha-acetylmethadol (LAAM, Fig. 1) is believed to possess several advantages over methadone

* Corresponding author.

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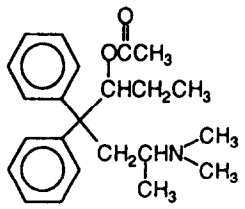


Fig. 1. Structure of *l*-α-acetylmethadol.

maintenance. Preliminary studies indicate that treatment with LAAM three times a week may be as effective as daily doses of methadone in maintenance treatment of heroin addicts. LAAM taken orally 3 times weekly establishes a more stable plasma concentration than methadone, a therapeutic steady-state which is both recognized and appreciated by patients [3–5]. Since LAAM is longer acting than methadone; it could decrease clinic visitations, increase treatment capacity, and facilitate breaking the drug-taking cycle. LAAM is currently in Phase III clinical trials and has been submitted to the FDA as a treatment Investigational New Drug (IND), which would allow patients to be treated with LAAM while it is still awaiting final FDA approval.

The detection and quantification of not only LAAM, but also its major metabolites norLAAM and dinorLAAM, are of particular interest because these two metabolites are formed rapidly from LAAM, and appear to persist for a longer duration of time than does the parent compound [6,7]. Furthermore, these metabolites are more potent than LAAM in substituting for codeine in rhesus monkeys, and norLAAM is more effective than dinorLAAM, LAAM, and even morphine in displacing ^{3}H-Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (DAMGO) binding in monkey brain membranes [8]. Thus, the longer duration of action of LAAM compared to methadone is due, in part, to the production of these active metabolites [6,7,9].

Previously reported analytical procedures for measuring LAAM and its metabolites in biological matrices have primarily involved multi-step liquid–liquid extraction, with either thin-layer chromatography [see ref. 10 for method], high-performance liquid chromatography [11], or gas chromatography [7,9,12,13] as the means of final separation. For maximum sensitivity, radiolabeled LAAM is used with thin-layer or high-performance liquid chromatographic methods. Since it is important to evaluate levels of LAAM and its metabolites in humans (*e.g.* to evaluate patient compliance), it would be preferable to have a sensitive, selective, and non-radiolabeled assay available. Although GC methods have been described for the determination of non-radiolabeled LAAM and its metabolites, each method has suffered from significant limitations. Specifically, the analytical method described by Billings *et al.* [9] utilizes electron capture to detect the trifluoroacetylated derivatives of LAAM metabolites, yet the technique is not applicable to the analysis of LAAM itself. The GC methodology of Kaiko *et al.* [12,13] allows the simultaneous determination of LAAM, norLAAM and dinorLAAM in plasma. However, it involves a multi-step solvent extraction to purify and concentrate the compounds, and utilizes a single internal standard (β-diethylaminoethyl diphenylpropyl acetate hydrochloride, SKF 525-A [12], or 2-allyl-5-ethyl-2′-hydroxy-9-methyl-6,7-benzomorphan [13]) to control for the recovery of LAAM and its metabolites. In addition, large volumes of plasma (4 ml) are required for this analysis.

GC–MS analytical methodologies offer several advantages over alternative means of quantitative analysis. Mass spectrometric assays have been shown to provide increased sensitivity and selectivity over a wide range of chemical classes, and have been successfully applied to the analysis of several opiates (for review, see ref. 14). GC–MS enables the use of deuterated internal standards which more closely control for sample loss. While the GC–MS method of Jennison *et al.* [15] has been successfully applied to a pharmacokinetic study of LAAM [16], this method again suffers from the utilization of a multi-step solvent extraction scheme, and also requires an intramolecular conversion of norLAAM and dinorLAAM under basic conditions (pH 13) to their amide derivatives. The recovery of LAAM, norLAAM and dinorLAAM by this procedure is not clearly specified, and it is known that this

derivatization procedure can result in alkaline hydrolysis of LAAM to methadol if not done extremely carefully. Furthermore, large volumes of plasma (2 ml) are required for this procedure.

As a result of the large volumes of plasma required by all of these procedures, and their extensive sample extraction schemes, there remained a need for an improved method allowing quantitation of LAAM, norLAAM and dinorLAAM with high sensitivity and selectivity. This report describes the validation and application of a novel solid-phase extraction method, coupled with an ammonia/methane GC–chemical ionization MS assay for the purification, detection and quantification of LAAM, norLAAM and dinorLAAM in small volumes (0.1 ml) of plasma.

2. Experimental

2.1. Chemicals and reagents

Deuterated ($^2H_3$) and non-deuterated LAAM, deuterated ($^2H_6$) and non-deuterated norLAAM, and deuterated ($^2H_6$) and nondeuterated dinorLAAM were provided by the National Institute on Drug Abuse (Bethesda, MD, USA) as the hydrochloride salts. All analytes were the *l*-α isomers. Trifluoroacetic anhydride (TFAA) was purchased from Pierce (Rockford, IL, USA). All solvents were Burdick and Jackson HPLC grade. Solid-phase extraction columns were Varian C_{18} Bond-Elut containing 200 mg of sorbent in a 3-ml liquid reservoir (Chromtech, Apple Valley, MN, USA). A 12-sample manifold (Supelco, Bellefonte, PA, USA) was used for processing the C_{18} columns under negative pressure (aspiration).

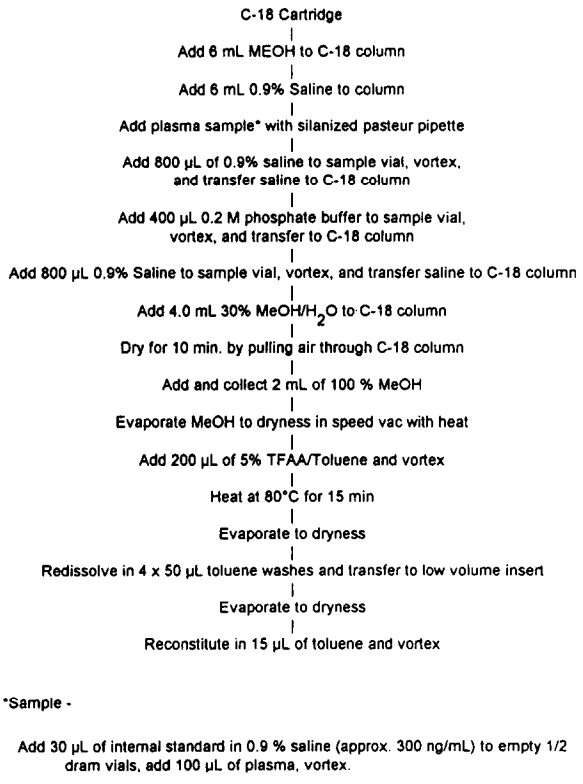
2.2. Analytical instrumentation

The Hewlett-Packard (Kennett Square, PA, USA) GC–MS system consisted of a 5890 gas chromatograph, a 7673 autosampler, a 5989A MS engine, and a Chemstation (HP-UX) for system control. The chromatographic system for this analysis was a J and W Scientific (Folsom, CA, USA) DB-1701 capillary column (30 m × 0.25 mm I.D., film thickness 0.25 μm) with helium at a flow-rate of approximately 1.5 ml/min serving as the carrier gas. The injection port temperature was held at 260°C. The initial oven temperature, 90°C (1.5 min), was increased at a ramp rate of 50°C/min to 260°C (2 min), and then at 25°C/min to 300°C. The temperature of the transfer line to the mass spectrometer was 270°C, and the mass spectrometer source and analyzer temperatures were maintained at 160°C and 100°C, respectively. A splitless injection of 2 μl was made with the purge valve turning on at 1.0 min. With this methodology, the order of elution of the analytes is LAAM (retention time approximately 6.0 min), dinorLAAM (retention time approximately 7 min), and norLAAM (retention time approximately 7.3 min).

The selected-ion monitoring (SIM) methodology is essentially that previously developed by Roger L. Foltz [17]. Chemical ionization (5% ammonia in methane) was used to generate intense M + 1 (protonated) and M + 18 (ammonia adduct) ions of LAAM and its N-demethylated metabolites, respectively. Single-ion monitoring occurred initially for LAAM, and after elution, changed to monitoring for dinorLAAM and norLAAM simultaneously. Deuterated LAAM, norLAAM, and dinorLAAM were monitored concomitantly with their respective analytes. Thus, the mass spectrometer was time-programmed to monitor *m/z* 354 (LAAM), 357 ($^2H_3$-LAAM), and then monitored 439 (dinorLAAM), 445 ($^2H_6$-dinorLAAM), 453 (norLAAM) and 459 ($^2H_6$-norLAAM). The dwell time for *m/z* 354 and 357 was 150 ms, whereas for the remaining ions it was set to 75 ms.

2.3. Analytical methods

The procedure used for the extraction of LAAM, norLAAM and dinorLAAM is shown in Scheme 1. The plasma samples were transferred to a "pre-wet" C_{18} solid-phase extraction column. The columns were then flushed with 30% MeOH in water and eluted with 2 ml of MeOH. The methanol was evaporated and the samples derivatized in 200 μl of 5% (v/v) TFAA in



Scheme 1. Flow chart depicting protocol developed for C_{18} solid-phase extraction of LAAM, norLAAM, and dinorLAAM from 100 μl sample of rat plasma.

toluene. After derivatization, the samples were concentrated to dryness, transferred to a low volume sample vial inserts in a series of 4×50 μl washes, and again concentrated to dryness. The residues were then redissolved in 15 μl of toluene for analysis by GC–MS. Since rearrangement of norLAAM to N-acetylnormethadol can occur, especially at high pH, it is necessary to keep the samples at −20.0°C before and after extraction in order to minimize this spontaneous intramolecular conversion.

2.4. GC–MS calibration

All standards used for generating calibration curves or quality control samples were formulated in plasma prepared fresh from blood obtained from CD rats (Charles River, Raleigh, NC, USA) by cardiac puncture. Fourteen plasma samples were generated containing mixtures of LAAM, norLAAM and dinorLAAM at various concentrations. These calibration standards were generated from two independently weighed samples, and covered a range extending from 0.1 to approximately 2000.0 ng/g plasma. A 100-μl aliquot of each plasma standard (in duplicate) was added to a half-dram vial containing 30 μl of an internal standard solution (300 ng/ml of deuterated LAAM, norLAAM and dinorLAAM in 0.9% saline) prior to extraction and analysis. After extraction and derivatization, the standards were kept at −20.0°C until analysis by GC–MS

GC–MS analysis of a complete set of calibration standards was performed prior to the analysis of experimental samples. Blank plasma samples were run to account for any interferences. Area ratios for each standard were plotted against the actual concentration and then fit by linear regression analysis using a weighting factor of 1 over the analyte concentration ($[\text{analyte}]^{-1}$) to control for the large dynamic range (high concentrations having an exaggerated effect on the fit). The calibration curve standards were also extracted and analyzed at the end of the study to determine whether the linearity of the mass spectrometer had remained constant.

2.5. Inter- and intra-assay variability

Inter-assay variability was determined with quality control standards (containing approximately 1.0, 10.0 and 200.0 ng of each analyte/g plasma) that were generated from a third, independently-weighed sample. Fifteen quality control samples were analyzed at the beginning of the analysis (5 standards at 1.0 ng/g, 5 standards at 10 ng/g, and 5 standards at 200 ng/g). In order to assess the variability of the assay over time the 15 quality control samples were extracted and analyzed again after approximately 3 weeks, and again after one month. Intra-assay variability was then calculated from these three determinations. These control samples were extracted and analyzed in the same

manner as all other samples. During the analysis of a set of experimental samples, control samples at each concentration (including a plasma blank) were extracted and analyzed along with the unknowns.

2.6. Experimental application

Blood samples were collected from the tail vein of 10 male and 10 female CD rats (weighing approx. 250 g) prior to dosing with LAAM for 30 days (day 0). The rats received food and water *ad libitum*, and were maintained on a 12 h light/dark cycle. Female rats were dosed with 5.6 mg/kg LAAM-HCl daily for 30 days, while male rats were dosed with 3.0 mg/kg LAAM-HCl for 30 days. Plasma samples were taken from the tail vein of each rat on days 3, 7, 14, 21, 25, and 28 (prior to those day's dosing). Plasma was prepared immediately after blood was taken and a 100-μl aliquot added to a half-dram vial containing 30 μl of an internal standard solution (300 ng/ml of deuterated LAAM, norLAAM and dinorLAAM in 0.9% saline). Each sample was vortex-mixed and stored at −20°C prior to extraction and GC–MS analysis. An entire day's plasma samples were analyzed as a batch. Thus each day's plasma samples (consisting of 20 rat plasma samples) were treated as a group in that they were extracted and analyzed in one batch, along with a plasma blank, containing internal standard but no analytes, and a set of three quality control samples (1 at each concentration).

3. Results and discussion

3.1. Chemical ionization mass spectrometry

The mass spectra of the deuterated internal standards are shown in Fig. 2. The ammonia/methane ionization of [$^2H_3$]LAAM resulted in a prominent M + 1 ion at 357. These conditions also produced abundant M + 18 ions with [$^2H_6$]norLAAM and [$^2H_6$]dinorLAAM. However, much more fragmentation occurred with these two compounds than with [$^2H_3$]LAAM. The high degree of isotopic purity for all of the deuterated internal standards is discernible in these spectra.

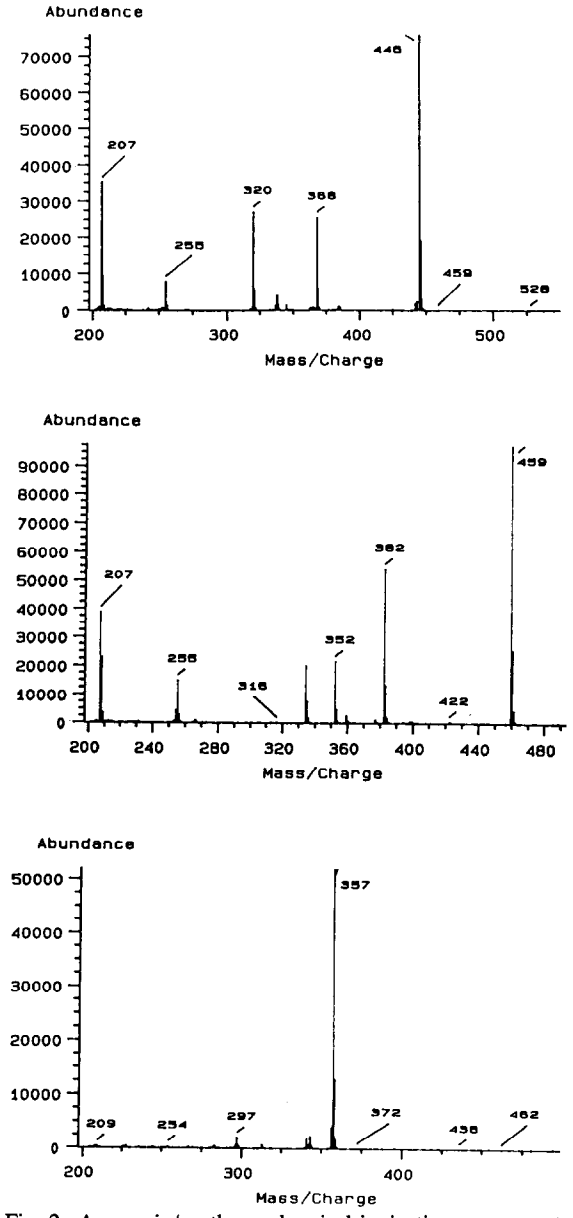


Fig. 2. Ammonia/methane chemical ionization mass spectra of [$^2H_3$]LAAM (bottom), [$^2H_6$]norLAAM (middle) and [$^2H_6$]dinorLAAM (top).

3.2. Extraction recovery

The extraction efficiency of the solid-phase system was optimized using [^{3}H]LAAM, and was determined to result in approximately 77% recovery of LAAM (data not shown). The system is relatively simple, and eliminates multiple liquid–liquid solvent extraction or the need for back extraction. Furthermore, the extraction efficiency of LAAM using this technique is greater than that achieved with a single extraction with *n*-butyl chloride, a common extraction solvent for LAAM and its metabolites. The extraction and derivatization procedure are enhanced by the addition of deuterated "carriers", which also serve to control for the total recovery of all analytes. It is important to note that the derivatization procedure requires a sample residue that is completely devoid of any water. Even a small amount of water was observed to hinder the derivatization of norLAAM and dinorLAAM.

3.3. GC–MS calibration

During sample extraction and analysis 2 calibration samples were lost, so the calibration curves were generated from a total of 26 calibration standards. As stated previously, these 26 standards covered 14 different concentrations of mixtures of LAAM, norLAAM and dinorLAAM, alternating between 2 independent stock solutions. The curves covered a wide dynamic range from approximately 0.1 to 2000.0 ng/g plasma with good linearity. The correlation coefficients obtained for each analyte were all greater than 0.95, indicating a good proportionality existed between the response (measured as integrated area) and concentration of analyte.

3.4. Inter- and intra-assay variability

The results of the initial quality control analysis are shown in Table 1 (standard set No. 1). In the blank sample, areas were recorded for LAAM and norLAAM, indicating that there is usually some interference when trying to quantitate at extremely low levels. At the lowest level, 1 ng/g plasma, the variation was greatest for dinorLAAM, and the level of dinorLAAM was underestimated by approximately 25%. At the higher levels of control (10 and 200 ng/g) the coefficient of variation was below 5% for all analytes. Approximately 3 weeks later a second set of quality control samples was run. The data obtained during this analysis (Table 1, standard set No. 2) showed greater error and variance in the estimates obtained for the 1 ng/g (low pool) than had been obtained previously. The mid- and high-pool estimates again showed much lower variation and error than was measured in the low pool. A final set of quality control samples (Table 1, standard set No. 3) was run after the analysis of experimental animal plasma samples had been completed. These samples indicated that the assay was still variable at the low end (1.0 ng/g plasma). The assay failed to detect dinorLAAM at this level, and at the mid-pool level (10.0 ng dinorLAAM/g plasma) overestimated the concentration by approximately 63%. The other analytes, at all levels, and dinorLAAM at 200 ng/g plasma, showed variability and error levels similar to that obtained in the previous quality control runs.

These three determinations of intra-assay variation were then used to calculate inter-assay variability (Table 2). The 1.0 ng/g plasma control had coefficients of variation of approximately 29, 24, and 100% for LAAM, norLAAM, and dinorLAAM, respectively. Low coefficients of variation for LAAM and norLAAM at 10.0 and 200.0 ng/g plasma were obtained, while dinorLAAM variation was still considerable (32.5%) at 10.0 ng/g. Only at the 200 ng/g level did the variability of dinorLAAM quantitation decrease below 10%.

The variation in the assay during the analysis of experimental samples is shown in Fig. 3. These plots illustrate that although LAAM and norLAAM variation remained relatively stable throughout the assay, the variation in the low-pool and mid-pool values of dinorLAAM reached unacceptable levels repeatedly at the second half of the analysis. The reason for the increase in variability for dinorLAAM after the 1st half of the study was the appearance of an

Table 1
Intra-assay variability

Analyte	Added (ng/g)	Measured (ng/g)		C.V. (%)	Difference[a] (%)
		Mean	S.D.		
Set 1					
LAAM	0.0	0.2			
	1.0	1.3	0.1	4.6	29.0
	10.1	11.5	0.3	2.6	13.0
	196.6	218.5	2.4	1.1	11.0
nor-LAAM	0.0	0.1			
	1.1	1.2	0.1	7.1	11.0
	10.5	10.4	0.2	2.3	−1.0
	203.2	199.0	2.5	1.3	−2.0
dinor-LAAM	0.0	0.0			
	1.0	0.8	0.3	33.0	−25.0
	10.1	10.0	0.4	4.4	0.7
	196.4	201.7	9.6	4.8	3.0
Set 2					
LAAM	0.0	0.0			
	1.0	1.8	1.7	55.8	80.0
	10.1	10.9	0.5	4.7	8.0
	196.6	221.8	3.1	1.4	13.0
nor-LAAM	0.0	0.0			
	1.1	2.0	1.1	57.4	81.0
	10.5	10.5	0.9	8.3	0.4
	203.2	200.5	0.3	0.2	−1.3
dinor-LAAM	0.0	0.0			
	1.0	1.7	0.6	33.7	68.2
	10.1	9.4	0.9	9.5	−6.6
	196.4	183.7	6.9	3.7	−6.5
Set 3					
LAAM	0.0	0.0			
	1.0	1.1	0.3	30.3	11.1
	10.1	11.9	0.3	2.5	18.2
	196.6	223.1	4.4	2.0	13.5
nor-LAAM	0.0	0.0			
	1.1	1.8	0.2	13.6	59.2
	10.5	10.3	0.5	4.7	−2.2
	203.2	196.4	1.2	0.6	−3.3
dinor-LAAM	0.0	0.0			
	1.0	0.0	0.3	−3076.2	−101.4
	10.1	16.5	0.3	2.0	63.5
	196.4	210.8	4.4	2.1	7.3

[a]Difference defined as (measured mean − actual)/actual.

Table 2
Inter-assay variability

Analyte	Added (ng/g)	Measured (ng/g)		C.V. (%)	Difference[a] (%)
		Mean	S.D.		
LAAM	0.0	0.1	0.1		
	1.0	1.4	0.4	28.6	40.0
	10.1	11.4	0.5	4.4	12.9
	196.6	221.1	2.4	1.1	12.6
nor-LAAM	0.0	0.1	0.0		
	1.1	1.7	0.4	24.0	54.5
	10.5	10.4	0.1	1.0	−1.0
	203.2	198.6	2.1	1.1	−2.3
dinor-LAAM	0.0	0.0	0.0		
	1.0	0.8	0.9	112.5	−20.0
	10.1	12.0	3.9	32.5	18.8
	196.4	198.7	13.8	6.9	1.2

[a]Difference defined as (measured mean − actual)/actual.

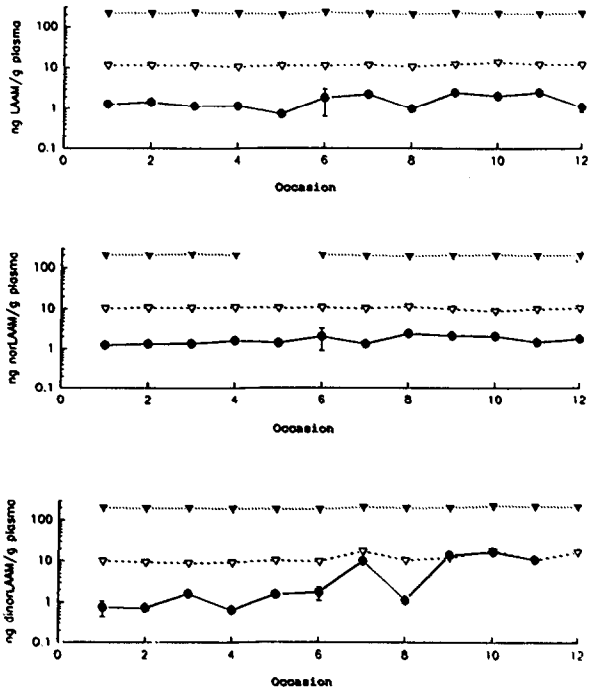


Fig. 3. Scatter graphs showing the variability over time for the analysis for LAAM (top), norLAAM (middle), and dinorLAAM (bottom). (●) Levels of each analyte determined in the low-concentration plasma controls. (△) Levels of each analyte determined in the mid-concentration plasma controls. (▲) Levels of each analyte determined in the high-concentration plasma controls. The error bars indicate the standard error of the mean ($n = 3$).

interfering peak that was not completely separated from the dinorLAAM signal. The source of this interference peak is unknown. The variability measured at the lowest level clearly indicates the importance of running control samples with experimentals. When a control sample is found to be estimated poorly by the method, the entire batch run can be reanalyzed after recalibration or elimination of the source of the error.

3.5. Experimental application

Fig. 4 shows the extracted ion-current profiles recorded for the analytes and their internal standards (I.S.) in a female rat (No. 3) measured on day 0 (prior to dosing), on day 3 (during dosing), and on day 32 (48 h after last dose). In the tracings recorded prior to dosing (day 0), the peaks for the internal standards are obvious, whereas no peaks are seen for the analytes. On day 3, the levels of LAAM, norLAAM and dinorLAAM had increased such that peaks for each analyte are apparent in this tracing. In particular, the levels of dinorLAAM had accumulated to such a degree that the peak height of the extracted ion-current profile for dinorLAAM approximated the peak height recorded for its internal standard. The tracings recorded 48 h after the last dose of LAAM indicated that only dinorLAAM persisted in the plasma.

The average data for both male and female rats are presented in Fig. 5 (error bars indicating the range of the standard error of the mean). These data show an increase in LAAM, norLAAM and dinorLAAM levels in plasma by day 3 due to the daily dosing of LAAM. However, by day 7, the levels of all three compounds had decreased from those recorded on day 3, suggesting mechanisms of tolerance had developed. On days 14–28, the trend toward plasma levels approaching lower, steady-state levels of all 3 analytes continued. The lower levels of LAAM, norLAAM and dinorLAAM in males compared

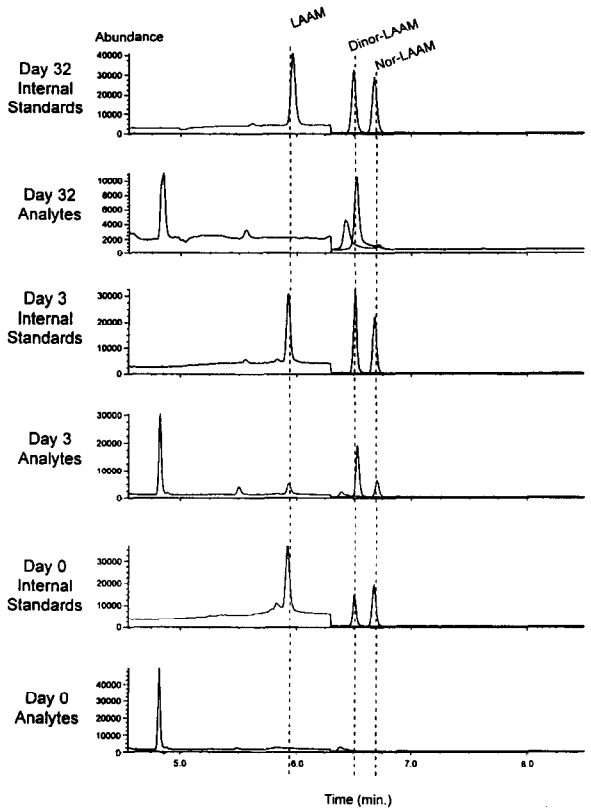


Fig. 4. Extracted ion-current profiles for LAAM, norLAAM and dinorLAAM and their internal standards measured in a female CD rat dosed with 5.6 mg/kg LAAM-HCl daily on day 0 (bottom two panels), day 3 (middle two panels), and day 32 (upper two panels).

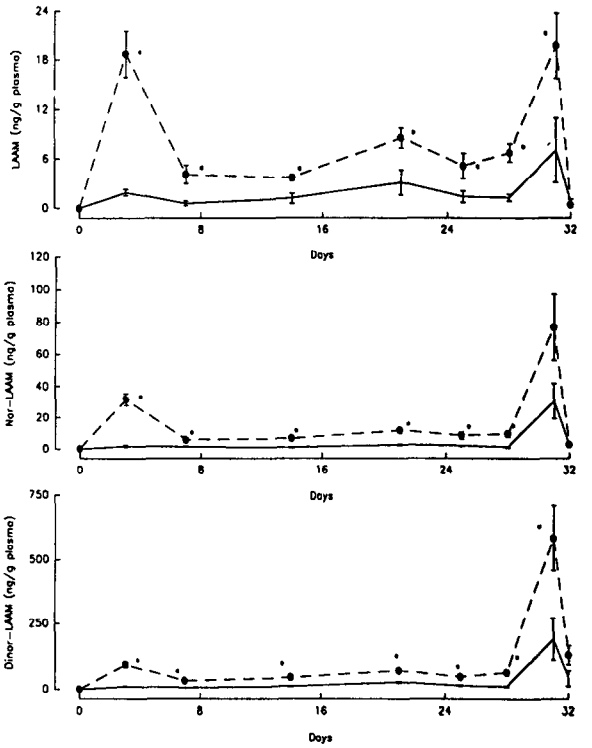


Fig. 5. Levels of LAAM (top), norLAAM (middle) and dinorLAAM (bottom) in males (○) and females (●) treated with LAAM-HCL for 30 days (mean value is plotted with standard error bars).

to females (Fig. 5) could be the result of the higher dose given to the females. However, the levels, when normalized for the different doses administered, still indicated that the females' plasma levels were higher than that of the males. On day 29 the rats were dosed, then cannulated, and on day 30 they received their last dose of LAAM. As can be seen in Fig. 5, the GC–MS analysis indicated that there was a remarkable increase in the trough levels of each analyte, especially the levels of dinorLAAM. Indeed, the levels of dinorLAAM in two female rats exceeded 1 μg/g plasma.

In general, higher levels of norLAAM and dinorLAAM compared with LAAM were detected at 24 h post administration (trough levels). This is consistent with a rapid N-demethylation of LAAM to the secondary amine and subsequent demethylation to the primary amine. This conclusion is supported by data which has been reported with other *in vivo* rat studies where the demethylation of LAAM to norLAAM was reported to occur rapidly, at a rate of *ca.* three times the rate of formation of dinorLAAM [6]. In both sexes of rats, an initial plasma level maximum was measured on day 3 for all analytes which decreased dramatically to a steady state at much lower levels. This observation suggests that a mechanism of tolerance had been elicited during the repetitive dosing regime. This observation is also supported by previous reports that chronic administration of LAAM [18] or norLAAM [19] induced their own metabolism and increased the elimination of their metabolites in rhesus monkeys. Additionally, sex-related differences in LAAM biodisposition

have previously been reported in the rat [20], which supports the data reported here (albeit when normalized for differing dose administration) that female rats, as compared with male rats, tended to have higher plasma levels of LAAM and its N-demethylated metabolites. Since the results obtained from the experimental study are consistent with previous findings, they serve to provide further support for the validity of the analytical methodology.

The plasma levels of all 3 analytes increased dramatically, especially the levels of dinorLAAM, after ketamine/xylazine anesthesia and jugular cannulation. This observation is disturbing in that ketamine/xylazine use is prevalent in laboratory procedures, and cannulated animals are frequently used for obtaining pharmacokinetic data. Even though the dosing was oral and not through the cannula, the extremely high plasma levels on day 31 may be an artifact caused by pulling the sample from the cannula rather than the tail-vein. However, other possibilities deserve further investigation, including: (1) that ketamine/xylazine treatment increases the absorption of LAAM from the gastrointestinal tract (reported to occur with oral administration of phenytoin and intraperitoneal ketamine [21]), (2) that microsomal enzymes are being inhibited by ketamine/xylazine [22], or (3) that cannulation produces physiological changes [23] (*e.g.* stress-related changes in hormones, *etc.*) affecting LAAM pharmacokinetics. Clearly, the effect of ketamine/xylazine treatment on the pharmacokinetics of LAAM, norLAAM and dinorLAAM requires further clarification.

4. Conclusion

The method described for the analysis of LAAM, norLAAM and dinorLAAM allows fully automated and rapid analysis of biological samples to be performed. With manual sample extraction, 40 samples can be processed and analyzed per day. Future research could be performed in order to determine if this method can be used for the analysis of other biological fluids for non-invasive monitoring. Additionally, refinement of this assay is necessary for extension of sensitivity to and below the picogram range, or decreasing the volume of plasma required for analysis. The later is particularly important because obtaining large quantities of blood from intravenous drug abusers often proves to be problematic.

5. Acknowledgements

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