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Note

Measurement of alphaprodine by selected-ion monitoring

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Alphaprodine (Nisentil) is a synthetic narcotic agent that is used during labor for management of pain. It is characterized by rapid onset and short duration of action. It has been the subject of several recent studies monitoring the effect of the drug on the fetus and mother [1–4]. However, none of these studies examined drug levels in conjuction with the observed effects. To do such a study, a sensitive analytical method was needed to quantitate levels of alphaprodine in plasma drawn at delivery and from the cord vein and cord artery.

All previously published methods of analysis by gas chromatography (GC) with flame ionization detection [5], microspectrophotometry [6], and thin-layer chromatography [7] were found to be unsuitable. The drawback to these methods was lack of sensitivity. The purpose of this paper is to report a sensitive analytical method for the analysis of alphaprodine by electron-impact mass spectrometry.

EXPERIMENTAL

Reference compounds

Reference crystals of alphaprodine, *cis*-1,3-dimethyl-4-phenyl-4-piperidinol propionate, were provided by Hoffmann-La Roche (Nutley, NJ, U.S.A.). The internal standard (I.S.), 2-(ethyl-*sec.*-butylamino)-N-(2,6-dimethylphenyl)acetamide, was obtained from Astra Pharmaceutical Products (Worcester, MA, U.S.A.). Stock solutions of these reference compounds were made up in 0.01 *M* hydrochloric acid. The stock solution of alphaprodine was made up to a concentration of 10 μg/ml and the internal standard was made up to a concentration of 1 μg/ml. The structures of these compounds are shown in Fig. 1.

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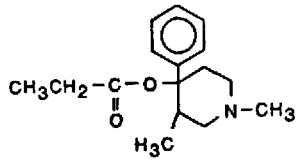
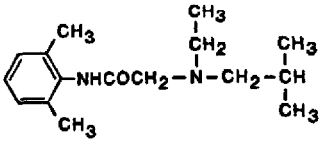


Alphaprodine



Internal Standard

Fig. 1. Structures of alphaprodine and the internal standard.

Chemicals

Methyl *tert.*-butyl ether was obtained from American Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). HPLC GC–MS grade methylene chloride and reagent grade sodium hydroxide were purchased from Fisher Scientific (Springfield, NJ, U.S.A.). Reagent grade hydrochloric acid was obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.).

Apparatus

A Hewlett-Packard 5995 A quadrupole table-top mass spectrometer equipped with a direct injection probe was used to obtain 70-eV electron-impact mass spectrum from crystals of the reference compounds. For selected-ion monitoring, the gas chromatograph was interfaced to the mass spectrometer with a jet separator. The chromatograph was fitted with 2 m×2 mm I.D. acid-washed dimethylchlorosilane-treated glass column packed with 3% SE 30-OV 17 (6:1) coated on 80–100 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). The instrument conditions for chromatography were: helium carrier gas flow-rate, 20 ml/min; injection port temperature, 250°C; oven temperature, programmed from 210°C (0 min) to 230°C at 16°C/min. The optics of the mass spectrometer were optimized by autotuning at *m/z* 169 of PFTBA (perfluorotri-*n*-butylamine). Using these conditions the entire run time for an analysis was 3.9 min. The ions monitored were the base peaks for each compound: *m/z* 172 for alphaprodine and *m/z* 114 for the I.S. The ion intensities at *m/z* 172 and 114 were monitored with dwell times of 200 and 100 ms, respectively, with a window width of 0.1 a.m.u.

Procedure

At the time of delivery, blood samples were collected from the maternal vein and from the umbilical artery and vein of a doubly clamped segment of the umbilical cord. The samples were collected in heparinized Vacutainers® (Becton-

Dickenson, Rutherford, NJ, U.S.A.). We have repeatedly found these tubes to be without interfering contamination since the company removed tris(2-butoxyethyl) phosphate from the stoppers [8]. The plasma was removed following centrifugation and frozen at −20°C until analysis. Blood bank plasma was used for standard curve preparation and repeatability tests. Batches were screened for potential interfering contaminants and verified to be clean before use.

Maternal and cord plasma samples and spiked plasma were extracted using a procedure similar to that described by Mather and Tucker [9]. A 40-ng amount of I.S. was added to 0.5 ml of plasma. The samples were made basic with 100 μl of 1 *M* sodium hydroxide and extracted with 3 ml of methyl *tert.*-butyl ether. This ether was substituted for the diethyl ether used by Mather and Tucker [9] because it is less likely to form hazardous peroxides. The samples were vortexed, centrifuged (5 min at 1000 *g*), flash frozen in a methanol dry ice bath, and the organic layer was transferred to a centrifuge tube containing 200 μl of 1 *M* hydrochloric acid. They were again vortexed and centrifuged as listed above. The organic layer was discarded following flash freezing. The aqueous layer was placed in a 60°C bath under nitrogen for a few minutes to remove any residual ether. The aqueous layer was then transferred to a 1-ml Reactivial (Pierce, Rockford, IL, U.S.A.). A 200-μl volume of 2 *M* sodium hydroxide was added. The samples were then extracted with 50 μl of methylene chloride, vortexed, and centrifuged. Of the methylene chloride layer, 2 μl were injected into the GC–MS system.

Meperidine and lidocaine, drugs commonly used for analgesia and anesthesia in obstetrics, and noralphaprodine, a metabolite of alphaprodine, were tested to ensure that they did not extract and interfere with alphaprodine or the I.S. Meperidine did produce an ion at *m/z* 172, however, its peak eluted at a shorter retention time than alphaprodine and was completely resolved at the baseline. Noralphaprodine also produced a relatively small peak on ion at *m/z* 172. Its retention time was longer than that of alphaprodine and it was easily resolved with the 2-m column. Lidocaine did not produce a peak at *m/z* 114 or 172.

RESULTS

The electron-impact mass spectrum of alphaprodine is shown in Fig. 2. The molecular ion is found at *m/z* 261. Loss of propionic acid from the molecular ion results in the fragment at *m/z* 187. Subsequent loss of a methyl radical produces the base peak ion, *m/z* 172. The spectrum of the I.S., 2-(ethyl-*sec.*-butylamino)-N-(2,6-dimethylphenyl)acetamide, was found to match the previously published spectrum [10].

Fig. 3 illustrates a selected-ion chromatogram from plasma extracts of a blank sample, a spiked standard containing 3 ng/ml alphaprodine, and a patient sample containing 21 ng/ml alphaprodine and 80 ng/ml I.S. At these drug levels, the ion chromatograms are virtually free from interfering contaminants. The retention time of alphaprodine was 1.8 min and that of the I.S. was 2.7 min. The full scale sensitivity shown in Fig. 3 is directly proportional to the abundance of ions detected by the mass spectrometer. This is inversely related to the amplification needed to plot the ion chromatogram.

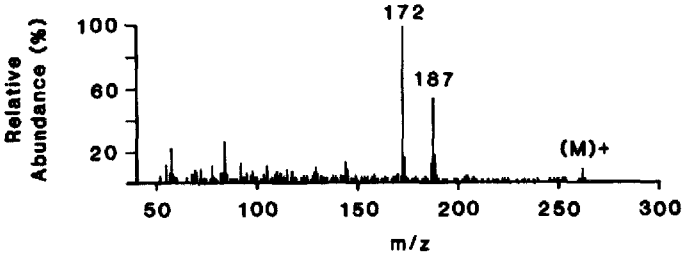


Fig. 2. Electron-impact (70 eV) mass spectrum of alphaprodine.

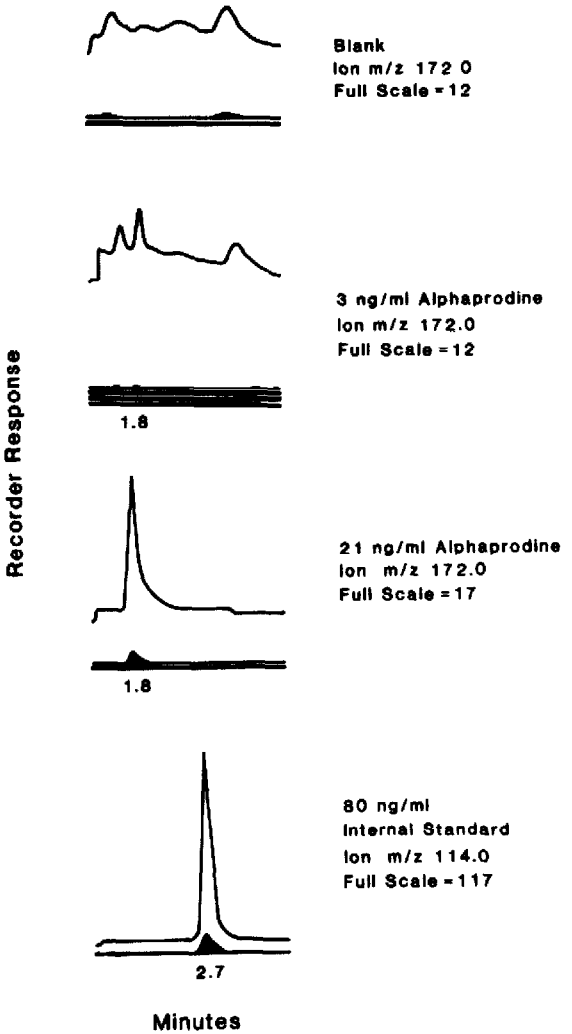


Fig. 3. Selected-ion chromatogram of plasma extracts of a blank sample, a spiked sample containing 3 ng/ml alphaprodine, and a patient sample containing 21 ng/ml alphaprodine and 80 ng/ml internal standard.

TABLE I

COEFFICIENTS OF VARIATION OBTAINED DURING PRECISION STUDIES

Procedure	Concentration (ng/ml)	n	Coefficient of variation (%)
Precision of instrument*	100	10	6
Precision of method**	100	10	10
	25	10	7
Day-to-day variability***	25	9	9
	50	9	8

*Multiple injections of the same sample into the GC–MS system.
**Extraction and analysis of multiple aliquots of the same sample on the same day.
***Extraction and analysis of multiple aliquots of the same sample over five days.

TABLE II

MATERNAL AND FETAL ALPHAPRODINE PLASMA CONCENTRATIONS AND PHARMACOKINETIC RATIOS FOUND AT DELIVERY

	Concentration (mean ± S.D.) (ng/ml)	n
Maternal vein	60.8 ± 19.5	9
Umbilical vein	31.0 ± 14.6	9
Umbilical artery	34.8 ± 11.9	6
Umbilical vein/maternal vein	0.52 ± 0.18	9
Umbilical artery/umbilical vein	1.06 ± 0.36	6

This method is sensitive to less than 2 ng/ml alphaprodine. Standard curves were linear from 2 to 200 ng/ml alphaprodine. Peak-area measurements were made using Hewlett-Packard's commercial software. Clinical samples were found to be within the range 12.5–200 ng/ml. A typical least-squares regression line for alphaprodine is $y = 0.558x + (-2.310)$ with a correlation coefficient (r) of 0.99844. In this equation, y is the dependent variable, peak area percentage (area alphaprodine divided by area of internal standard), and x is the concentration of alphaprodine.

The precision of the method was evaluated by repeat extraction and analysis of spiked plasma samples containing low (25 ng/ml) and high (100 ng/ml) concentrations of alphaprodine. Instrumental precision was determined by repeat injections of the same 100 ng/ml sample. Day-to-day variation was tested by running 25 and 50 ng/ml spiked plasma samples with each set of patient samples. The means for these samples were 24.6 ± 2.1 and 48.9 ± 4.0 ng/ml, respectively. The resulting coefficients of variation for these procedures are listed in Table I. Recovery was determined by comparing unextracted spiked standards in methanol with extracted spiked plasma samples. The mean recovery of alphaprodine (100 ng/ml) for ten spiked samples was 84%.

This method has been successfully used to quantitate samples from parturients receiving alphaprodine during labor. Umbilical venous samples ranged from 12 to 62 ng/ml, umbilical arterial samples ranged from 21 to 49 ng/ml, and maternal venous samples obtained at delivery ranged from 29 to 97 ng/ml. These data are presented in Table II. The clinical characteristics of these patients have been reported previously [11].

DISCUSSION

Previously published methods for the analysis of alphaprodine had problems with sensitivity. Limits of 250 ng/ml were reported by Burns [6] and 430 ng/ml by Gorodetzky [7]. The method of Fung et al. [5] was the previous method with the best sensitivity (30 ng). It was used to measure alphaprodine levels following intravenous administration of 35 mg/kg body weight to normal healthy male and female volunteers. In that study, mean subject plasma concentrations at 5 h post-injection were 70 ng/ml. In the study of pregnant women in labor, less drug was administered in some cases and often less that 1 ml of plasma was available for analysis [11]. Therefore, greater sensitivity was required. Results indicate that the sensitivity of our method (2 ng/ml) is sufficient for the measurement of levels of alphaprodine following doses commonly given to women in labor. The lowest patient value found was 12 ng/ml.

No interfering contaminants from clinically used drugs were noted. Noralphaprodine, the demethylated metabolite of alphaprodine described by Abdel-Monem et al. [12], was not seen in the clinical samples tested. Other principal metabolites of alphaprodine are conjugated compounds that would not co-extract with alphaprodine.

Fung et al. [5] used phencyclidine or meperidine as the I.S. for their samples. Since meperidine is routinely given to women in labor and there is a chance that phencyclidine is abused, those drugs could not be used as I.S. We have successfully used 2-(ethyl-*sec.*-butylamino)-N-(2,6-dimethylphenyl)acetamide as an I.S. for the analysis of meperidine [13]. Since alphaprodine is similar in structure to meperidine, this compound was a convenient choice as an I.S. This was particularly true since no interfering peaks were seen, results were reproducible, the compound is not used clinically, and no stable isotope analogue was needed to be synthesized. However, if available, a compound with a structure closer to that of alphaprodine that is not used clinically might be a more logical choice (i.e. deuterated meperidine).

This method provides the first practical means of alphaprodine analysis in small-volume, low-concentration samples from pregnant patients and/or neonates. It is sensitive, specific, and quantitative. It also has the advantage of using electron-impact MS. Due to their relative inexpensiveness, small mass spectrometers with electron-impact capabilities are increasingly common in laboratories not affiliated with large MS centers. In addition, the automatic quantitation feature of the Hewlett-Packard 5995A system greatly decreases analysis time. One technician can comfortably extract, analyze and quantitate eighteen samples and six standards in a day.

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