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Simultaneous determination of mecamylamine, nicotine, and cotinine in plasma by gas chromatography-mass spectrometry

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Abstract

The nicotine receptor antagonist mecamylamine has been shown to increase the efficacy of transdermal nicotine as a pharmacotherapy for tobacco addiction. A product for simultaneous transdermal administration of nicotine and mecamylamine is undergoing clinical trials. In order to carry out pharmacokinetic studies, quantitation of low nanogram per milliliter levels of mecamylamine and nicotine was required. This paper describes a method for simultaneous determination of mecamylamine, nicotine, and the nicotine metabolite, cotinine, in human plasma using gas chromatography-mass spectrometry (GC-MS). Limits of quantitation for mecamylamine, nicotine and cotinine are 2, 1 and 2 ng/ml, respectively.

Keywords: Mecamylamine; Nicotine; Cotinine; Plasma; Gas chromatography; Mass spectrometry

1. Introduction

Tobacco-related diseases are the major preventable cause of death in most developed countries. Many strategies for the treatment of tobacco addiction have been advanced, but most people who attempt to quit using tobacco are unsuccessful. Nicotine replacement medications such as polacrilex gum, trandermal patches and, more recently, nasal sprays have proven to be efficacious in promoting smoking cessation; nevertheless, in most studies success rates are below 30% [1,2].

Recent studies have shown that the combined administration of the nicotine antagonist mecamylamine with nicotine replacement medications significantly reduces tobacco craving and increases success rates for smoking cessation [3]. A transdermal patch for simultaneous administration of nicotine and mecamylamine is currently undergoing clinical trials.

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In order to carry out studies of the pharmacokinetics and pharmacodynamics of combined nicotine and mecamylamine administration, a method for the quantitation of nicotine, mecamylamine, and the nicotine metabolite, cotinine, in plasma was needed. A number of methods suitable for the determination of nicotine and cotinine in plasma have been developed [4,5], but sensitive methods for quantitation of mecamylamine have not been reported. In this paper, we report a gas chromatographic-mass spectrometric (GC-MS) method for determination of low nanogram per milliliter concentrations of mecamylamine, nicotine, and cotinine in plasma.

2. Experimental

2.1. Standards and reagents

Mecamylamine hydrochloride, USP reference standard, was obtained from USPC, Inc. (Bethesda, MD). Nicotine bitartrate [6] and cotinine perchlorate [7,8] were synthesized as described previously. The internal standards, nicotine-d_4 and cotinine-d_9 were synthesized in our laboratory [9,10]. Normecamylamine (3-aminoisocamphane) was synthesized by the method of Kochelkov et al. [11]. The internal standard mecamylamine-d_3 was synthesized as described below. Toluene and 1-butanol used in the extractions were Optima and HPLC grade, respectively, from Fisher Scientific (Pittsburgh, PA). Other chemicals were analytical reagent grade.

2.1.1. Synthesis of mecamylamine-d_3

2.1.1.1. N-Carbobenzyloxy-3-aminoisocamphane. A solution of 3-aminoisocamphane (normecamylamine) hydrochloride (1 g, 5.3 mmol) in 10 ml of diisopropylethylamine was stirred while cooling in an ice bath, and 1.4 g (8.2 mmol) of benzyl chloroformate was added. The cooling bath was removed, and the solution was stirred overnight at room temperature. The reaction mixture was poured into 100 ml of 5% (v/v) aqueous sulfuric acid, and then extracted with 100 ml of methyl *tert*-butyl ether. The solvent was removed from the extract using a rotary evaporator, and the product was distilled bulb-to-bulb (Kugelrohr, oven temperature 150–160°C, 0.1 mmHg) to give 1.9 g of a viscous liquid.

2.1.1.2. Mecamylamine-d_3 (3-trideuteromethylaminoisocamphane) hydrochloride. N-Carbobenzyloxy-3-aminoisocamphane (1 g, 3.1 mmol) in 5 ml dry tetrahydrofuran was added to 0.5 g (12 mmol) of lithium aluminum deuteride (Aldrich, 98 at.% d) in 5 ml tetrahydrofuran under an argon atmosphere. The mixture was heated under reflux for 1 h. The mixture was cooled with an ice bath, and the excess lithium aluminum deuteride was decomposed by cautious addition of 5 ml isopropyl alcohol. Five millilters of 10% aqueous sodium hydroxide was added, and the precipitated salts were removed by filtration. The solvents were removed from the filtrate using a rotary evaporator, 50 ml of 5% sodium hydroxide was added to the residue, and the mixture was extracted with 50 ml of petroleum ether (30–60°). The petroleum ether solution was extracted with 50 ml of 5% sulfuric acid, the acid layer was separated, and then made basic with sodium hydroxide. This was extracted with 50 ml of petroleum ether, and the extract was washed with 50 ml water. The petroleum ether solution was extracted with 50 ml methanol containing 2 ml of conc HCl. The acidic methanol solution (lower layer) was separated, evaporated using a rotary evaporator, and then dried under a vaccum of 0.1 mmHg to give a white solid. This was stirred with 50 ml diethyl ether, filtered, washed with ether, and air dried to give 0.3 g of white crystalline solid, m.p. (dec) 244–245°C. Lit m.p. for mecamylamine-d_0 hydrochloride, 246–247°C [12]. The electron ionization mass spectra of mecamylamine-d_0 and mecamylamine-d_3 are presented in Fig. 2.

2.1.2. Instrumentation

Gas chromatographic analyses were carried out using a Hewlett–Packard (Palo Alto, CA)

model 5890A instrument with a capillary inlet system and a 7673A automatic sampler. Injections were made in the splitless mode onto a 25 m × 0.31 mm ID column with a 5% phenyl methyl silicone stationary phase (HP-5). GC-MS was carried out using a Hewlett–Packard 5890 GC with a 7673 automatic sampler interfaced to a Hewlett–Packard 5970B mass selective detector. Injections were made in the splitless mode onto a 12 m × 0.2 mm ID fused silica capillary column with a 5% phenyl methyl silicone stationary phase (HP-5), 0.33 μm film thickness. Instrument control and data acquisition were carried out with a Hewlett–Packard 59970 MS ChemStation. Data were processed using a Hewlett–Packard model 4930 ChemServer running Target3 data analysis software, obtained from Thru-put (Orlando, FL).

2.1.3. Extraction procedure

To 1 ml of plasma sample or standard contained in a 13 × 100 mm glass culture tube was added the internal standards, 50 ng of mecamylamine-d_3, 20 ng nicotine-d_4, and 200 ng cotinine-d_9 in 100 μl of 0.01 M HCl. The tubes were mixed for 5 min using a vortex mixer, and then 0.5 ml of 2 M sodium hydroxide in 0.2 M ammonia was added. Three milliliters of a 70:30 (v/v) mixture of toluene and 1-butanol was added, the tubes were capped, and then vortex-mixed for 5 min. After centrifugation to break emulsions, the tubes were placed in a dry ice-acetone bath to freeze the aqueous layers. The organic layers were poured into tubes containing 0.5 ml of 1 M sulfuric acid, which were then capped and vortex-mixed for 5 min. The tubes were centrifuged, then placed in a dry ice-acetone bath to freeze the aqueous layers. The organic layers were poured off, 0.5 ml of 50% (w/v) potassium carbonate in 0.2 M ammonia and 150 μl of a 90:10 (v/v) mixture of toluene and 1-butanol were added to each tube. The tubes were vortex-mixed for 5 min, centrifuged, and placed in a dry ice-acetone bath to freeze the aqueous layers. The organic layers were poured into 300 μl autosampler vial inserts, which were placed in a heating block at 85°C to evaporate the extracts to a volume of ~25 μl. The vials were capped and placed in an autosampler tray for GC-MS analysis.

2.1.4. GC-MS analysis

A 2 μl aliquot of the extract was injected in the splitless mode into a glass injection port liner containing a small plug of polyethylene glycol deactivated glass wool, prepared as described previously [8]. The injection port temperature was 250°C, the carrier gas (helium) flow rate was 1 ml/min, and septum purge on-time was 0.8 min. The initial column temperature was 70°C. After a 1 min hold, the temperature was programmed to 123°C at 60°C/min. The temperature was brought to 155°C at 5.8°C/min, and then raised to 200°C at 8°C/min. The column temperature was then raised to 275°C at 60°C/min. The temperature of the transfer line to the mass spectrometer was 280°C. The mass spectrometer was calibrated with perfluorotributylamine (PFTBA) using the system software 'Autotune' program. Following this, the abundance of the m/z 69 fragment of PFTBA was maximized by adjusting the focus ion setting. Ionization was in the electron impact (EI) mode at 70 eV. The electron multiplier was programmed to carry out analyses at 600 V above the Autotune values. The ions were monitored with a mass peak width of 0.9 amu and a dwell time of 50 ms, in three groups: m/z 98 and 101 (mecamylamine and mecamylamine-d_3) from 4.4 to 5.7 min, m/z 84 and 88 (nicotine and nicotine-d_4) from 6.2 to 7.1 min, and m/z 176 and 185 (cotinine and cotinine-d_9) from 12.1 to 13.0 min.

Quantitation was carried out by integration of the ion chromatograms and constructing eight-point standard curves of concentration versus peak area ratio of analyte/internal standard by linear regression. Standard curves were linear (Table 1) from 0 to 500 ng/ml for mecamylamine, from 0 to 50 ng/ml for nicotine, and from 0 to 500 ng/ml for cotinine, which spans the ranges of concentrations generally found in plasma. Separate low-level standard curves were used for concentrations below 20 ng/ml for mecamylamine, below 2 ng/ml for nicotine, and below 20 ng/ml for cotinine to circumvent the bias for higher values that results from nonweighted linear regression.

Due to the difficulty in obtaining plasma completely free of nicotine and cotinine [8], standards were made up in 0.01 M HCl. These were stored frozen at −20°C until used. The use of aqueous standards, rather than spiked plasma, was validated by the analysis of spiked plasma samples. The concentrations found were close to the expected values (Tables 2 and 3).

2.1.5. Precision and accuracy

Precision and accuracy were determined by analyzing plasma from nonsmokers spiked with mecamylamine, nicotine, and cotinine at concentrations spanning the expected range (Tables 2 and 3).

2.2. Clinical study

Representative plasma concentrations of mecamylamine, nicotine, and cotinine are presented for one subject who participated in a clinical study of the pharmacokinetics of mecamylamine and nicotine, conducted by Sano Pharmaceutical Corporation (Pembroke Pines,

Table 1
Equations for typical standard curves

Analyte	Concentration range (ng/ml)	Slope[a]	Intercept	Correlation coefficient (r^2)
Mecamylamine	Low	1.11	0.0295	0.99911
	High	1.12	0.0260	0.99965
Nicotine	Low	1.33	0.0398	0.99644
	High	1.30	0.0412	0.99963
Cotinine	Low	0.886	0.0051	0.99992
	High	0.991	−0.000214	0.99954

[a] Equations were determined by linear regression: response ratio = $a \times$ (amount ratio) + intercept.

Table 2
Intraday precision and accuracy for the determination of mecamylamine, nicotine, and cotinine in plasma

Analyte	Actual concentration (ng/ml)	Measured mean (ng/ml)	Accuracy (%)	Coefficient of variation (%)	Replicate analyses
Mecamylamine	2	1.94	97	9.9	6
	5	4.76	95	2.3	8
	10	9.97	98	1.6	7
	20	19.8	99	0.65	7
	50	50.1	100	1.3	7
	100	102	102	1.1	7
	500	503	101	0.9	7
Nicotine	1	0.95	95	7.4	7
	2	1.94	97	2.3	7
	5	5.73	115	5.2	7
	10	11.4	114	1.6	7
	50	55.8	112	7.2	7
Cotinine	2	2.32	116	6.1	6
	5	5.26	105	6.0	8
	10	9.49	95	2.8	7
	20	17.8	89	2.5	7
	50	48.9	98	1.8	7
	100	101	101	1.4	7
	500	532	106	3.7	7

Table 3
Interday precision and accuracy for the determination of mecamylamine, nicotine, and cotinine in plasma

Analyte	Actual concentration (ng/ml)	Measured mean (ng/ml)	Accuracy (%)	Coefficient of variation (%)	Days
Mecamylamine	1	1.11	111	12	7
	2	206	103	6.7	7
	5	5.02	100	7.6	7
	10	10.2	102	10	6
	20	20.1	101	4.0	7
	50	50.4	101	2.1	7
	100	101	101	2.7	7
	500	497	99	2.7	7
Nicotine	1	1.12	112	17	5
	2	2.05	103	11	6
	5	5.35	107	13	6
	10	10.7	107	8.3	6
	50	56.5	113	8.2	6
Cotinine	2	2.30	115	17	6
	5	5.37	107	14	6
	10	10.0	100	11	5
	20	20.3	101	7.3	6
	50	51.9	104	4.5	6
	100	104	104	4.1	6
	500	557	111	3.7	6

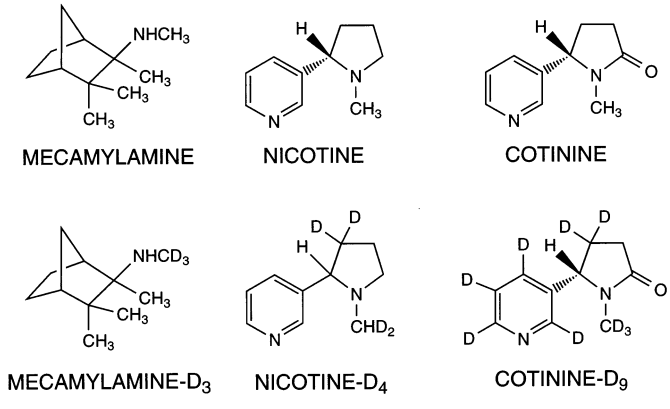

Fig. 1. Structures of mecamylamine, nicotine, cotinine, and deuterium-labeled analogs.

FL). The healthy volunteer subject was instructed to abstain from tobacco use for a minimum of 24 h. At 09:00 h, a Habitrol 21 mg transdermal nicotine system (Novartis, Summit, NJ) was applied, and a 5 mg mecamylamine tablet was administered at 09:00 and 21:00 h. The patch was removed after 24 h. Frequent plasma samples for analysis of mecamylamine, nicotine, and cotinine concentrations were obtained over 48 h.

3. Results and discussion

Concentrations of mecamylamine in human plasma following therapeutic doses have not been reported. Based on the doses employed in clinical trials, it was predicted that a method with a limit of quantitation of 1–2 ng/ml would be required for adequate pharmacokinetic characterization. Mecamylamine (Fig. 1) is a relatively low molecu-

lar weight, nonpolar compound with no significant chromophore, which suggested that GC methods would be most promising. Both GC with nitrogen-phosphorus detection and GC-MS with electron ionization were evaluated for sensitivity. Better sensitivity was obtained with GC-MS, so it was selected for assay development.

Previously, a method for simultaneous determination of nicotine and cotinine in human plasma was developed in our laboratory [8]. Since mecamylamine is a basic compound with physical properties similar to nicotine, simultaneous extraction and GC-MS determination of mecamylamine, nicotine, and cotinine using selected ion monitoring appeared feasible. A deuterium-labeled analog of mecamylamine, 3-trideuteromethylaminoisocamphane (Fig. 1) was synthesized for use as an internal standard.

The electron ionization mass spectrum of mecamylamine is shown in Fig. 2. The molecular

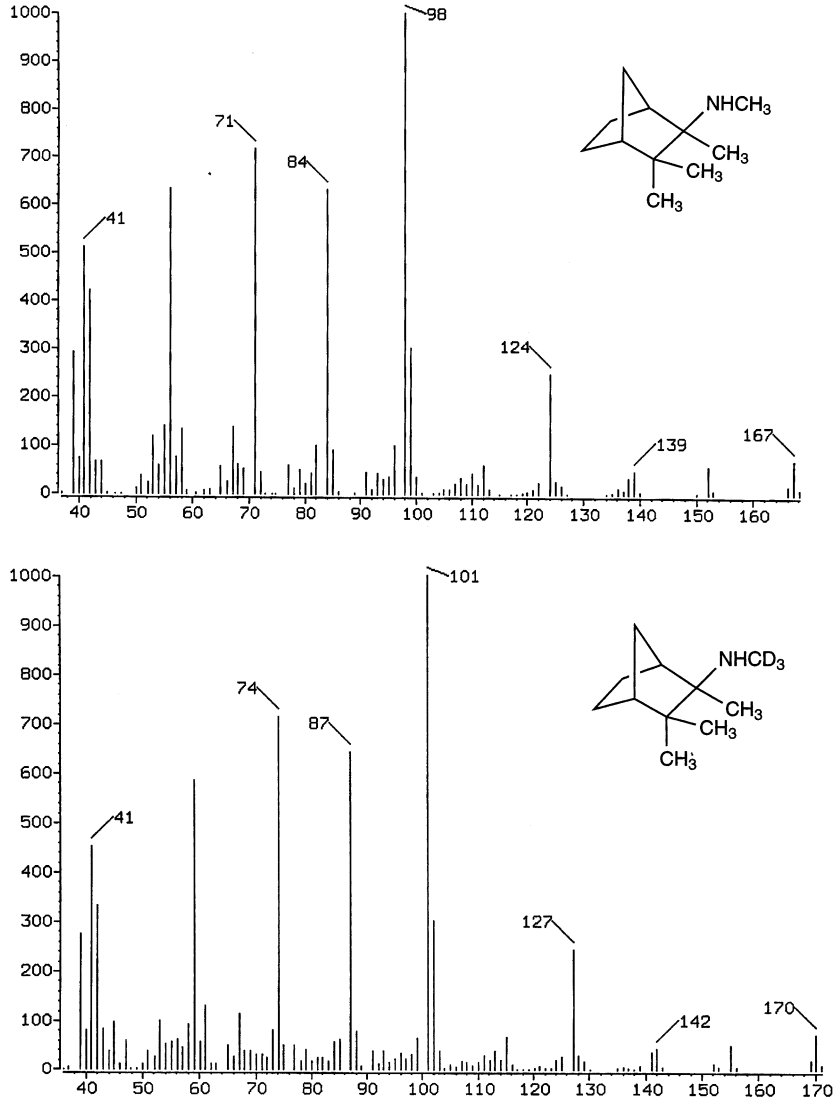

Fig. 2. Mass spectra of mecamylamine (upper panel) and mecamylamine-d_3 (lower panel).

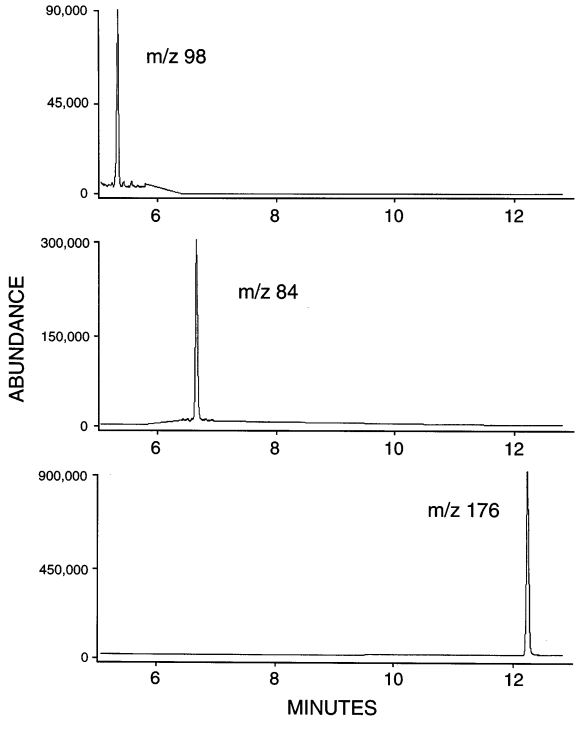

Fig. 3. Ion chromatograms for analytes in a plasma extract from a human subject following administration of mecamylamine and nicotine: m/z 98, mecamylamine (9.7 ng/ml); m/z 84, nicotine (12.1 ng/ml); m/z 176, cotinine (251 ng/ml).

ion m/z 167 has relatively low abundance (7.5%) and was not adequate for quantitation. The most abundant ion, m/z 98, presumably resulting from loss of cyclopenyl, allowed detection at 1 ng/ml in the selected ion monitoring mode. Because the m/z 98 ion maintains the *N*-methyl group, the corresponding ion m/z 101 from the internal standard, mecamylamine-d_3 (Fig. 2), is suitable for quantitation. Furthermore, there were no substances in extracts of blank plasma that produced significant interfering peaks at the retention time of mecamylamine in the m/z 98 and 101 ion chromatograms.

Extraction of the three analytes from plasma was carried out using mixtures of toluene and 1-butanol, as described previously for nicotine and cotinine [8]. These solvents remain liquid at −78°C, which allows separation of phases by freezing the aqueous layers in a dry-ice bath, greatly facilitating the extraction and analysis of large numbers of samples.

The three analytes are well-separated on a (5% phenyl)methylpolysiloxane (HP-5) capillary column. Deuterium-labeled analogs are used as internal standards for each analyte (Fig. 1). Mass spectral data are acquired in the selected ion monitoring mode in three time-programmed groups: group 1, m/z 98 and 101 for mecamylamine and mecamylamine-d_3; group 2, m/z 84 and 88 (loss of pyridyl) for nicotine and nicotine-d_4; and group 3, m/z 176 and 185 (molecular ions) for cotinine and cotinine-d_9 (Figs. 3–5).

Precision and accuracy were determined by replicate analysis of plasma spiked with mecamylamine, nicotine, and cotinine over the expected concentration ranges (Tables 2 and 3). Good precision and accuracy were obtained for mecamylamine over the range of 2–500 ng/ml, for nicotine over the range of 1–50 ng/ml, and for cotinine over the range of 2–500 ng/ml.

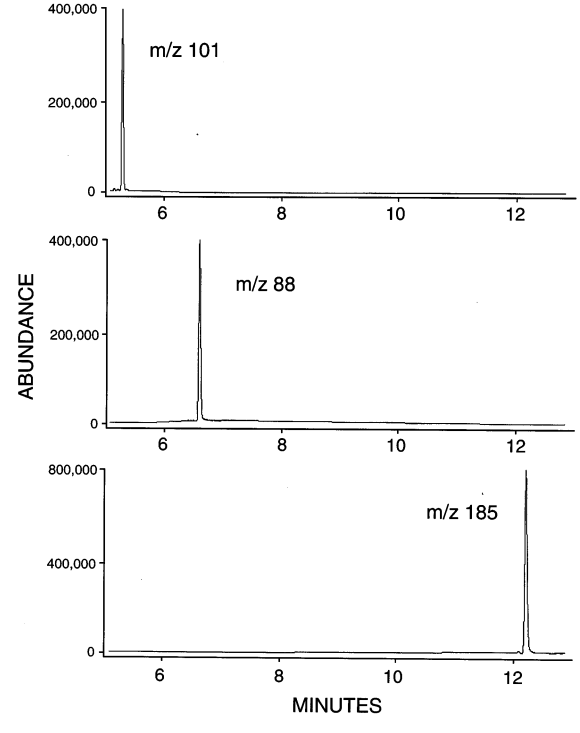

Fig. 4. Ion chromatograms for internal standards in a plasma extract from a human subject following administration of mecamylamine and nicotine: m/z 101, mecamylamine-d_3 (50 ng/ml); nicotine-d_4 (20 ng/ml); cotinine-d_9 (200 ng/ml).

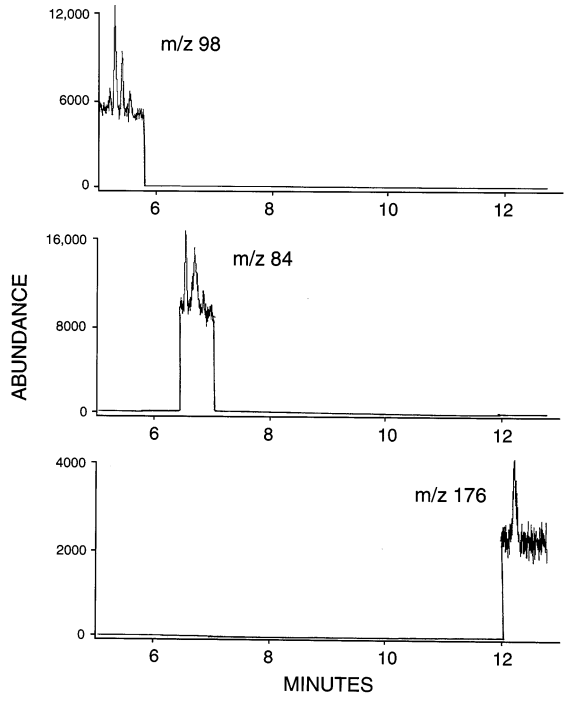

Fig. 5. Ion chromatograms at m/z values for for analytes in an extract of blank plasma.

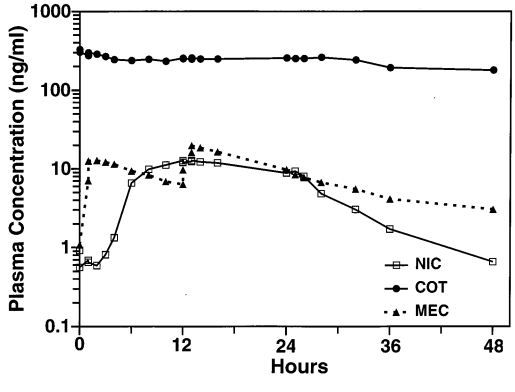

Fig. 6. Plasma concentration–time curves for mecamylamine (MEC), nicotine (NIC), and cotinine (COT) from a human subject following oral administration of mecamylamine and transdermal administration of nicotine.

Plasma concentrations of mecamylamine, nicotine, and cotinine from a subject in the clinical study are shown in Fig. 6. Plasma mecamylamine concentrations peaked at 12.8 ng/ml 2 h after the first oral dose and at 19.8 ng/ml 1.5 h after the second dose. The terminal half-life of mecamylamine in this individual was 16.5 h. Plasma nicotine concentrations were below 1 ng/ml prior to patch application, and peaked at 12.8 ng/ml 12 h after patch application. Plasma cotinine concentrations started at 328 ng/ml reflected the residual from previous smoking, and remained at about 250 ng/ml for most of the day as cotinine was being generated from nicotine.

4. Conclusion

In summary, a sensitive GC-MS method for simultaneous quantitation of mecamylmine, nicotine, and cotinine in plasma has been developed. To our knowledge, this is the first method reported that is suitable for quantitation of mecamylamine in human plasma following therapeutic doses. The method has been applied to clinical trials of combined mecamylamine and nicotine administration, a new drug product being developed for the treatment of tobacco addiction.

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