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Journal of Chromatography B, 701 (1997) 124–128

JOURNAL OF CHROMATOGRAPHY B

Short communication

Simple and sensitive method for determination of nicotine in plasma by gas chromatography

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Received 12 May 1997; accepted 26 June 1997

Abstract

Smoking and other forms of nicotine consumption are among the most important risk factors for cardiovascular disease and cancer. Many of the cessation therapies require administration of nicotine. Accordingly, precision analysis for nicotine in plasma has become increasingly important. Several of the recently published methods require elaborate sample handling and/or processing. We report a simple, rapid and reliable gas chromatography method with a high sensitivity for determination of unchanged nicotine in plasma, which can be used in the processing and quantification of large series of nicotine samples, e.g., in clinical trials of nicotine-based smoking or tobacco cessation drug delivery systems. © 1997 Elsevier Science B.V.

Keywords: Nicotine

1. Introduction

Smoking and other forms of nicotine consumption are among the most important risk factors for cardiovascular disease and cancer [1,2]. Several novel cessation therapies such as transdermal systems, chewing gum and spray require administration of sufficient doses of nicotine to counteract the craving for tobacco [3–5]. As a result, precision analysis for nicotine in plasma has become increasingly important in the design of effective nicotine drug delivery systems.

Current nicotine assays include gas chromatography (GC), high-performance liquid chromatography (HPLC) or gas chromatography–mass spectrometry (GC–MS) for determination of unchanged nicotine in biological samples, mainly plasma [6–11]. Several of the recently published methods require elaborate sample handling and/or processing, which may preclude their use in large scale applications.

The aim of the present work was to design a rapid GC method with a high sensitivity for determination of unchanged nicotine in plasma.

2. Experimental

2.1. Chemicals and reagents

Aqueous solutions were prepared of nicotine-base, $C_{10}H_{14}N_2$ (Mw 162.24) (Aldrich, Steinheim, Germany) and quinoline-base, C_9H_7N (Mw 129.15) (Aldrich). Sodium hydroxide solution was prepared

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0378-4347/97/$17.00
PII S0378-4347(97)00349-6

from pellets (EKA, Bohus, Sweden). Toluene, glass distilled, and *n*-hexane, HPLC-grade, were obtained from Rathburn (Walkerburn, UK).

Vials, inserts and caps used in the GC system were acquired from Brown chromatography supplies, Skandinaviska GeneTec (Kungsbacka, Sweden).

Gases used were synthetic air 5.0, helium plus 4.6 and hydrogen 5.7 (all from AGA Gas, Sundbyberg, Sweden).

2.2. Extraction procedure

A 1 ml volume of human plasma from a non-smoking volunteer and internal standard (125 ng quinoline in water) were pipetted into a 4 ml polypropylene tube fitted with a cap. The sample was made alkaline with 1 ml of 5 *M* sodium hydroxide. One-step single extraction with 0.2 ml toluene–*n*-hexane (1:1) was performed using a horizontal shaker at 200 rpm for 8 min. After centrifugation 5 min at 3600 rpm the tube was kept in a freezer (−70°C) for 30 min. The organic layer was then separated from the aqueous phase and transferred into a small conical vial. Toluene–*n*-hexane was evaporated, by keeping the vial in a heating block at 90°C, until less than 100 μl of the solution remained. A 1 μl aliquot of this toluene–*n*-hexane solution was injected onto the chromatographic column.

Chromatograms of extracts of blank plasma samples and of samples spiked with nicotine and quinoline are given in Figs. 1 and 2.

2.3. Instrumentation

The gas chromatographic analyses were performed on a Hewlett-Packard 5890 II instrument equipped with a nitrogen–phosphorus detection system and an automatic injector HP 7673 (Hewlett-Packard, Wilmington, DE, USA).

Data were stored and processed using a Hewlett-Packard Vectra PC 386/25N and Hewlett-Packard Chemstation 3365.

The column used was a widebore HP-1, methylsilicone, 10 m×0.53 mm I.D. with 2.65 μm film thickness.

The flow-rates were 40 ml/min for the carrier gas (helium), 4 ml/min for the hydrogen and 100 ml/min for the air.

The temperatures were 100°C for the column oven, 200°C for the injector and 275°C for the detector.

Using the chromatographic conditions described

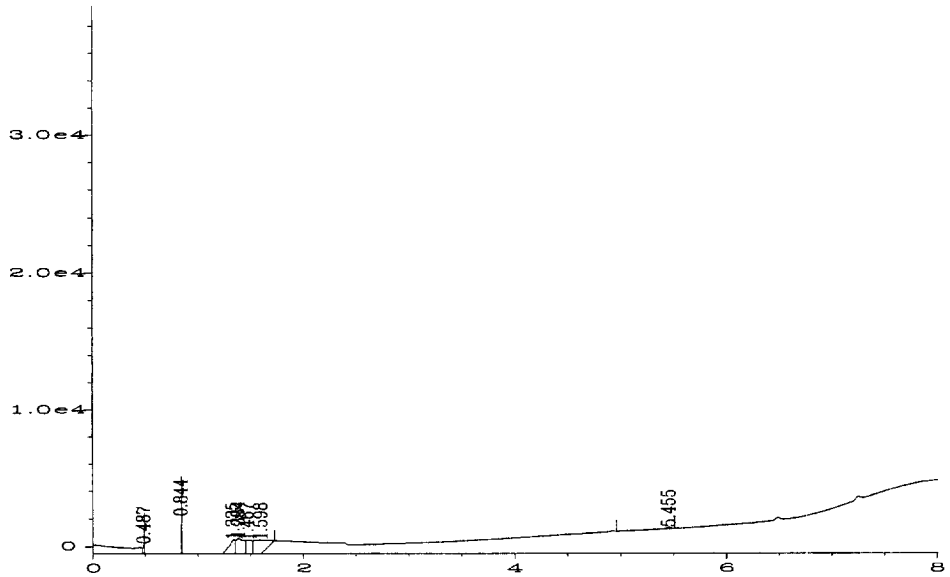

Fig. 1. Extract of 1 ml non-smoker human plasma blank.

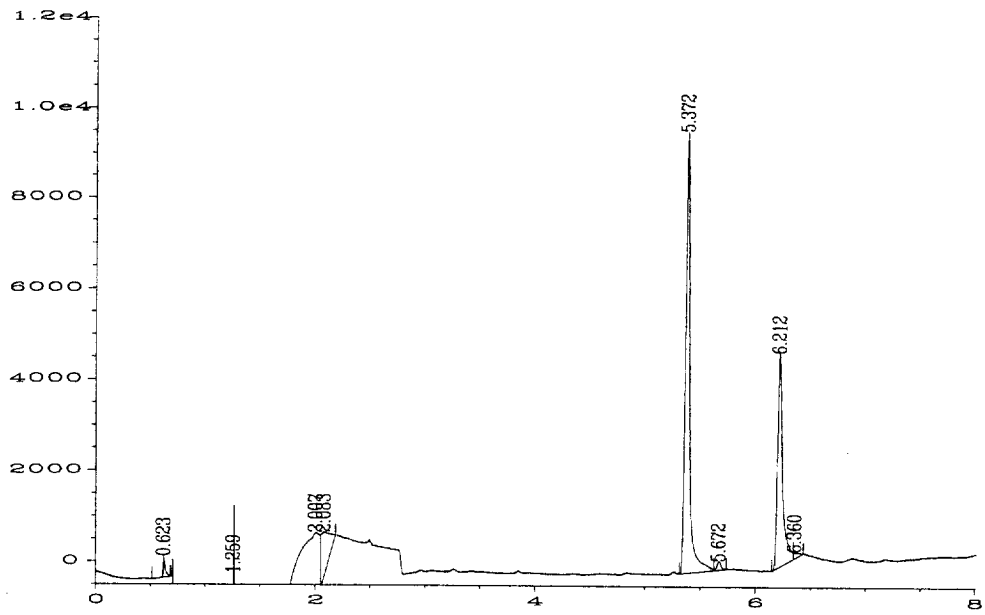

Fig. 2. Extract of 1 ml non-smoker human plasma blank spiked to produce 125 ng/ml of quinoline as internal standard, t_R 5.37 min and 62.5 ng/ml of nicotine, t_R 6.21 min.

above the retention times for nicotine and quinoline were 6.2 and 5.4 min, respectively, at a pressure of 10 kPa helium.

2.4. Stability

Stock solutions of nicotine and quinoline in water were prepared new every second day. Sodium hydroxide solution and toluene–*n*-hexane (1:1) solution were prepared freshly every week.

2.5. Contamination

Stock solutions of nicotine and quinoline should be strictly prepared and stored in a well-separated room to avoid contamination of the human plasma samples.

To avoid nicotine contamination during the analytical procedure all rooms used for blood collection and for analytical work must be kept smoke-free. In addition, all personnel handling the samples or the glassware must be non-smokers.

3. Results and discussion

3.1. Limits of quantification, detection and recoveries

The limit of quantification (LOQ) was estimated to be 2 ng per ml (12 pmol) (Fig. 3) and the limit of detection (LOD) to be approximately 0.5 ng/ml (3 pmol).

Aliquots of an aqueous solution of nicotine were added to blank plasma samples from non-smoking volunteers. The samples were analysed according to the described method. The recovery was 101% (±17%) in the range 2 ng/ml to 31.3 ng/ml and 100% (±6%) in the range 31.3 ng/ml to 125 ng/ml. The coefficient of variation (C.V.) was 16.8% ($n=11$) in the concentration range 2 ng/ml to 31.3 ng/ml

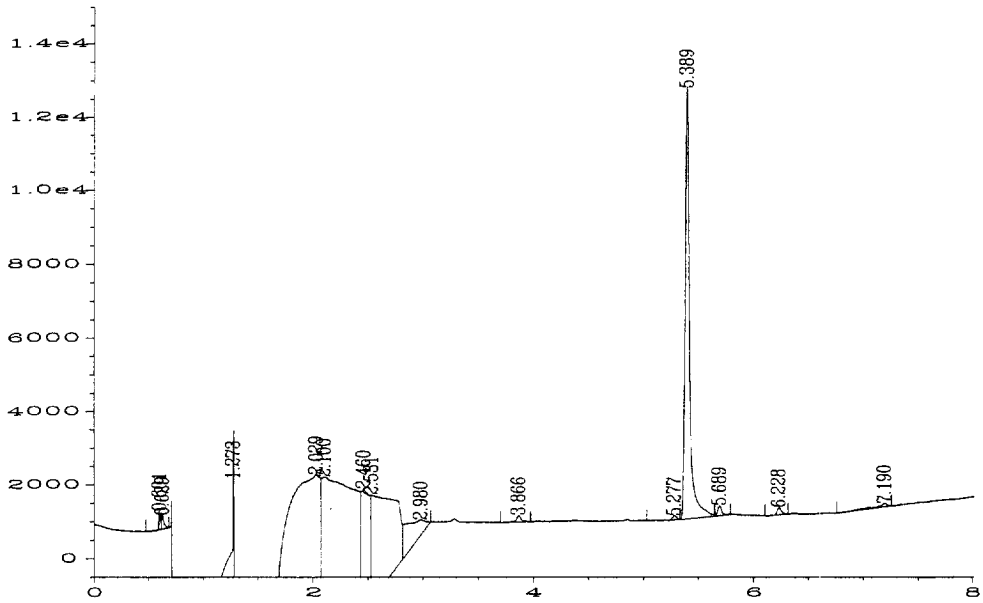

Fig. 3. Extract of 1 ml plasma containing 2 ng (12 pmol) nicotine.

and 6.3% (n=11) in the range 31.3 ng/ml to 125 ng/ml.

3.2. Calibration curves

A calibration curve was prepared by addition of 2–125 ng nicotine and 125 ng quinoline, as internal standard, to 1 ml blank plasma samples. The samples were extracted as described above and chromatographed. The area ratios (nicotine to quinoline) were plotted versus the original nicotine concentrations. The calibration graph was linear in the given concentration range (Fig. 4).

3.3. Application

The nicotine assay described was used to analyse achieved steady state plasma concentrations in a group of healthy male habitual oral snuff users. In this group (n=20) the mean plasma concentration of nicotine was 23.2±3.43 ng/ml (range 5.1–61.0 ng/ml). The mean oral snuff consumption in this group was 24.3±14.0 g during the day of experimentation.

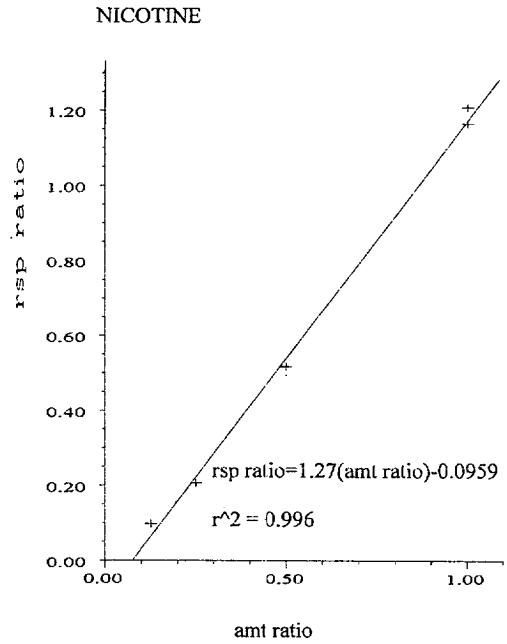

Fig. 4. Calibration graph: nicotine 2 ng/ml to 125 ng/ml vs. quinoline 125 ng/ml. y=response ratio, x=amount nicotine.

4. Conclusions

This method for determination of nicotine proved to be a simple and reliable assay. It can be used in the processing and quantification of large series of plasma nicotine samples, e.g., in clinical trials of nicotine-based smoking or tobacco cessation drug delivery systems.

Acknowledgements

This study was supported by an educational grant from CIBA (Västra Frölunda, Sweden) and by the Medical Faculty (LUA), Gothenburg University, Sweden.

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