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Short communication

Rapid solid-phase extraction method for automated gas chromatographic–mass spectrometric determination of nicotine in plasma

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Abstract

The aim of this study was to develop a simple, rapid and sensitive assay of nicotine in plasma for automated gas chromatographic–mass spectrometric analysis. Biological samples were extracted using pre-packed Extrelut-1 columns with 5 ml of ethyl acetate. Quantitative analysis was done using deuterium-labelled nicotine as internal standard. The limit of quantitation was 0.5 ng in 1-ml plasma samples. Precision was ranging from 13.3% to 1.64% (R.S.D.) depending on the concentration, while the deviation was 4.16%. This method has been used for determination of nicotine bioavailability from new, low-dosage, nicotine chewing gum strips. © 1998 Elsevier Science B.V.

Keywords: Nicotine

1. Introduction

Research and analytical laboratories are increasingly using mass spectrometry as a detection system for quantitative gas chromatography. This analytical choice reflects the fact that it is possible to use the isotopic labelled internal standard technique, which assures a high level of confidence in analytical data [1]. Commercial gas chromatographs–mass spectrometers (GC–MS) are now relatively inexpensive and are reliable for routine analysis, so automatic injectors are often used when large numbers of samples are to be analysed or for overnight instrument time. The aim of this work was to develop a method, for routine nicotine determination, specifically designed for automatic GC–MS instrumentation.

At first we tried to extract nicotine from plasma using a liquid–liquid dichloromethane extraction procedure, as a general approach described in most of the published papers dealing with nicotine analysis [2–4]. Though this technique is known to give good results, some practical difficulties affect the final extract quality as regards automatic injector requirements. During the dichloromethane extraction of the plasma samples, proteins denature and may form an amorphous gel between the two phases [4,5]. This needs to be broken, for example by freezing and thawing the sample a couple of times using a dry ice/acetone bath. The final result is time loss and a not perfectly clear organic extract, not optimum for automatic injection.

Solid-phase extraction of nicotine, based on

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diatomaceous earth adsorption, has been described for GC–FID [6] and recently for HPLC analysis [7]. We describe here the development, results and application of a new isotopic dilution, solid-phase extraction procedure with Extrelut-1 pre-packed glass columns and deuterated internal standard, using the requirements of an automatic GC–MS instrument.

2. Experimental conditions

2.1. Chemicals

Nicotine was purchased from Aldrich (Milan, Italy) and was 99.5% purity. Deuterated nicotine (*N*-methyl d_3) was 98% purity and was from Sigma. Helium was from Air Liquid (Liscate, Milan, Italy). Chewing gum strips containing 0.4, 1 and 4.0 mg per 3 g were prepared by Perfetti (Lainate, Italy). All reagents and solvents were analytical grade. All standard solutions were prepared fresh the same day of the analysis.

2.2. Glassware

Glassware was silanized, using *N,O-bis*-(trimethylsilyl)-trifluoracetamide (BSTFA) in toluene, except Extrelut 1 glass columns, that were used as received from Merck (Bracco, Milan, Italy). Silanization was done by dissolving 1 ml of BSTFA in 50 ml of toluene, washing all glassware with this solution and then rinsing with methanol.

2.3. Instrumental

Samples were injected into a Hewlett Packard 5890 gas chromatograph equipped with an HP 5890 MSD (Hewlett Packard, Avondale, PA, USA), and with a Hewlett Packard 7673 automatic liquid sampler. The column was an Easy Wax (bonded polyethylene glycol liquid phase), 25 m, 0.25 mm ID, film thickness 0.25 μm (Analytical Technology, Milan, Italy). A 2 μl sample aliquot was injected splitless. GC temperature conditions were isothermal at 100°C for 1 min followed by 20°C min^{-1} increases up to 220°C. The final temperature was maintained for 2 min. Injector temperature was 240°C. The carrier gas was helium and the pressure on the top of the column was 20 kPa. Septum purge was 1 ml min^{-1}. Samples were all injected in triplicate.

Mass spectrometry conditions were selective ion monitoring (SIM) of the ions m/z 162 and 84 for nicotine, m/z 165 and 87 for deuterated nicotine. These are the molecular ions (m/z 162 and 165) and the base peak (m/z 84 and 87) corresponding to the loss of the pyridil moiety [8]. Since deuterium labelling was on the *N*-methyl group, the base peak retains the 3 a.m.u. difference in molecular mass compared to the protonated molecule, and is used as a qualitative confirmation of the analyte.

2.4. Sample preparation

To a sample volume of 1 ml, we added 5 μl of a 1 ng μl^{-1} solution of deuterium labelled nicotine in ethyl acetate and 500 μl of a solution of 1 *M* NaOH in distilled water to ensure that the pH was basic enough to allow adsorption of nicotine to the solid-

Table 1
Precision and accuracy of the method described

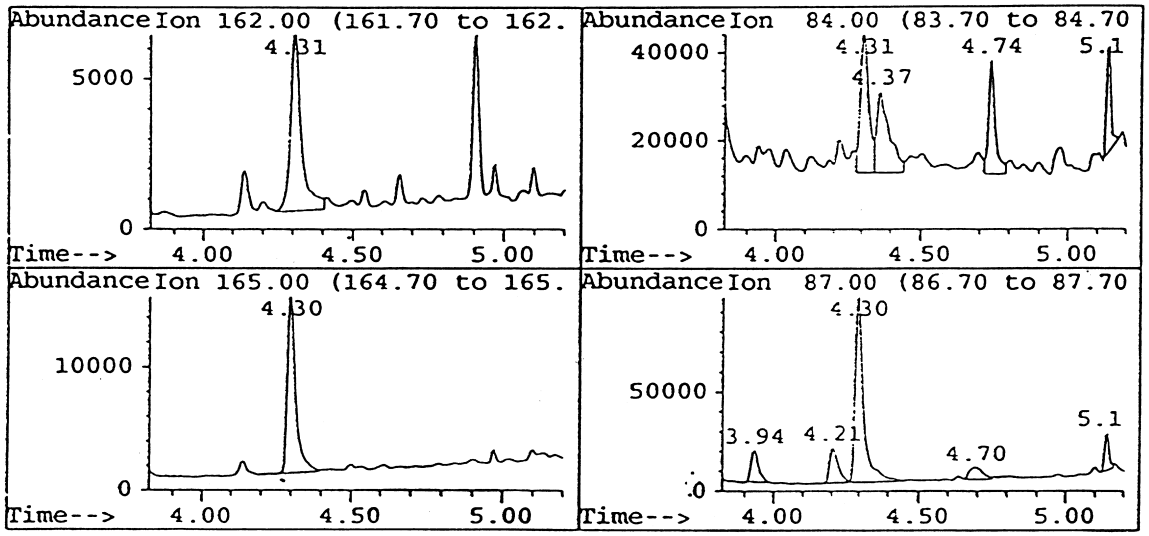
Added (ng ml^{-1})	Found (ng ml^{-1})	Absolute error	Deviation (%)
1	1.002	0.002	0.2
1	0.81	−0.19	19.0
1	1.05	0.05	5.0
Mean	0.95		
R.S.D.	13.30		
5	5.27	0.27	5.4
5	4.90	−0.10	2.0
5	4.85	−0.15	3.0
Mean	5.01		
R.S.D.	4.57		
10	9.99	−0.01	0.1
10	9.09	−0.91	9.1
10	9.64	−0.36	3.6
Mean	9.57		
R.S.D.	4.74		
20	20.60	0.60	3.0
20	20.13	0.13	−0.6
20	19.96	−0.04	0.2
Mean	20.23		
R.S.D.	1.64		
		Mean	4.16

phase. Samples have been loaded on Extrelut-1 diatomaceous earth columns, used as received from the manufacturer. After 10 min, the columns were eluted under gravity with 5 ml of methylene chloride. Here the flow was 2 ml min^{-1}. We added 60 μl of isooctane to the eluted organic phase and gently evaporated it under nitrogen at room temperature to a final approximate volume of 60 μl, then transferring it to the automatic injector micro vials fitted with 100-μl microvolume inserts.

A calibration curve was prepared in 1 ml of plasma, containing variable amount of nicotine (0, 0.5, 1, 2, 5,10, and 25 ng in plasma) and a fixed amount of nicotine-d_3 (5 ng). All quantitative analysis were computed with reference to calibration curves prepared fresh every time.

2.5. Pharmacokinetics

Nicotine plasma kinetics were studied in three healthy male volunteers aged 30–35 years who habitually smoked 5–25 cigarettes per day. Nicotine pharmacokinetics was determined in plasma after chewing a single strip, per volunteer, of chewing gum containing 0.4, 1 or 4.0 mg of the drug [9]. The volunteers were asked to refrain from smoking 48 h before the experiment. The strips were chewed for 10 min and 10-ml blood samples were collected in heparinized test tubes at time 0, 5, 10, 15, 30, 45, 60 120, 180 and 360 min. Plasma was obtained by centrifugation and kept at −80°C until analysis. The area under the curve was calculated according to the trapezoid method.



ion	ret. time	area	
162	4.31002	147977	nicotine d0
84	4.31002	641669	nicotine d0
84	4.36893	549317	
84	4.74033	400123	
84	5.14335	264642	
165	4.30095	277290	nicotine d3
87	3.93863	288049	
87	4.20587	384965	
87	4.29643	1.95472e+006	nicotine d3
87	4.69505	194736	
87	5.14335	208899	

Fig. 1. SIM chromatogram of a 1-ml plasma extract containing 2 ng ml^{-1} of nicotine. Although the nicotine peak area at m/z 84 is more than four times the area at m/z 162, its signal-to-noise ratio is worst.

3. Results and discussion

Nicotine is a relatively volatile drug and we observed large loss during the concentration step. Even though it was done carefully under a gentle nitrogen stream, the recovery from 5 ml of eluate was less than 30% when samples were concentrated to an approximate volume of 50 μl. The addition of a non-volatile solvent (isooctane) considerably improved recoveries. Then elution solvent (ethyl acetate) and volume (5 ml) were chosen to achieve the highest recovery, with the lowest elution volume: with ethyl acetate the maximum recovery was 86%, after 4 ml elution volume, while dichloromethane gave a maximum recovery of 79% with an elution volume of 8 ml. In Table 1 accuracy and precision of the method are shown.

The linear regression coefficient for calibration curve in plasma, obtained under the experimental conditions described above in the range 0.5–25 ng ml^{-1}, was better than 0.99 and the relative standard error was 2.6%. The line y intercept was 0.12 and the slope 0.14x. Analysis were all carried out monitoring the molecular ions and the base peaks both of nicotine and of nicotine-d_3. While the molecular ions (m/z 162 and 165) have only a 20% relative abundance of the base peak (m/z 84 and 87), the signal-to-noise ratio of samples was higher in the molecular ion SIM trace because of the greater interference at m/z 84. Therefore for quantitative computations the molecular ions areas were used. Fig. 1 shows a SIM chromatogram of a 2 ng ml^{-1} plasma extract. Although the nicotine peak area at m/z 84 is more than four times that at m/z 162, its signal-to-noise ratio is the worst. The lowest detectable level (with a signal-to-noise ratio >3) in plasma was 0.5 ng ml^{-1}.

Fig. 2 reports the time course of nicotine levels. The plasma areas under the curve (AUC) were proportionally higher in the volunteers chewing the strips containing the lowest dose: the AUC for the 0.4 mg strip was 1668.8 ng ml^{-1} min^{-1}, for the 1.0 mg strip 1993.6 ng ml^{-1} min^{-1} and for the 4.0 mg strip 9622.0 ng ml^{-1} min^{-1}). The elimination half-life (T-HALF) for the 0.4 mg strip was 301.03 min, for the 1.0 mg strip 150.50 min and for the 4.0 mg strip 60.20 min. It was not possible to calculate the volume of distribution because the biodisposition of

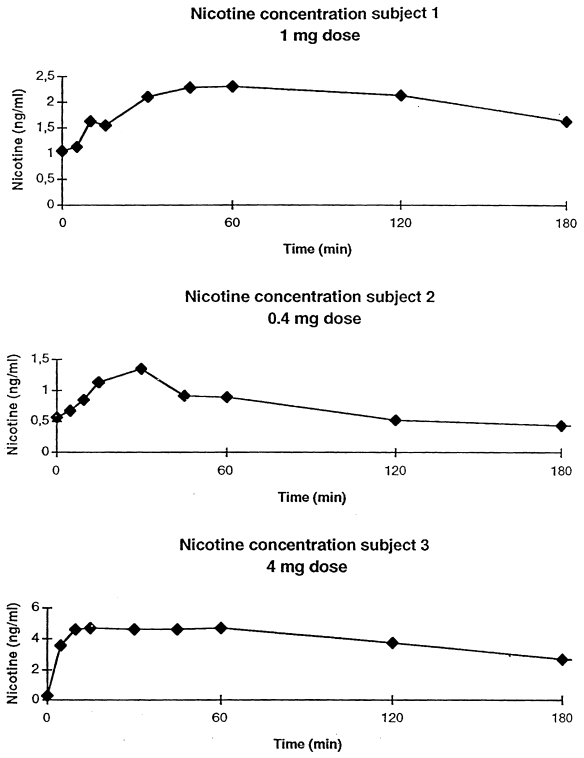


Fig. 2. Plasma nicotine concentration in human volunteers after chewing a new, low-dosage, nicotine gum.

nicotine in the chewing gums could not be adequately characterized. The plasma nicotine peak was reached 30 min after starting chewing the 0.4 mg strip, and 60 min with the 4.0 mg strip. Plasma peak concentrations were 1.32 ng ml^{-1} and 4.7 ng ml^{-1}. These results show that this solid-phase extraction with Extrelut-1 glass columns is a technique capable of isolating very low nicotine levels from biological samples (in this application levels were 2 order of magnitude less than levels in active cigarettes smokers). Sample preparations steps are reduced to a minimum and the final extract quality is compatible with automated GC–MS analysis.

Acknowledgements

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