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Development of a simple sample preparation technique for gas chromatographic–mass spectrometric determination of nicotine in edible nightshades (Solanaceae)

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Abstract

The purpose of this study was to develop a rapid analytical method for the reliable determination of low concentrations of nicotine in foods for large numbers of samples. Food material was extracted using a simple liquid–liquid extraction method. For processed foods, further clean-up steps had to be employed to eliminate interfering compounds. The determination of nicotine was performed by gas chromatography–mass spectrometry. Quantitative analysis was accomplished using deuterium labeled nicotine as an internal standard. Recoveries of over 95% were obtained for a single step extraction, as well as for a multiple-stage extraction procedure, respectively. The method has been applied to the determination of nicotine in edible nightshades (i.e. tomatoes, potatoes and aubergines) and their processed products. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Food analysis; Fruits; Sample handling; Nicotine

1. Introduction

Within the last few years there has been increased interest in the determination of nicotine in food. This is in part a result of suggestions that dietary nicotine intake could impact the level of nicotine metabolites otherwise attributed to exposure to environmental tobacco smoke (ETS). Presence of nicotine in flora is known. Leete, 1983 [1] reported its presence in 12 families and 24 genera, including the nightshade-family (Solanaceae), among them are some common vegetables such as potatoes, tomatoes or eggplants (aubergines). The function of nicotine in these plants is still not fully known. However, it is assumed that nicotine serves as a natural defense against fungi, bacteria, insects and animals [2,3]. Additionally, it is widely known that nicotine is used as an insecticide in some parts of the world and could, therefore, contaminate food [5]. Even though the influence of dietary nicotine is not considered to be very important [4–9] discussion about its significance continues. Only a small number of papers has been published dealing with the determination of nicotine in food material [3,4,7,8]. The methods used in previous publications have either not been validated fully or are not applicable to large numbers of samples.

In 1986, Castro and Monji [7] were the first to

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provide reliable data on nicotine-concentrations of edible nightshades using radioimmunoassay (RIA). Although the procedure has been validated, the method is analytically selective but not specific. There are possibilities for cross-reactivity with the antibodies as well as non-consistent specificity based on differences of the antiserum used and the hapten analog which is employed for the preparation of the nicotine conjugate. In addition, because of the manipulations required, the method is not well suited for application to large numbers of samples. Nevertheless, Castro and Monji [7] show some interesting results.

Sheen [8] investigated nightshades for their nicotine concentrations using gas–liquid chromatography. The results that are reported generate questions concerning the analytical approach. No attempts appear to have been made to check possible nicotine background contamination. For example, potato peel was reported to show large nicotine concentrations, whereas in potato flesh no nicotine was detected. These large differences were interpreted as a defense mechanism by the plant against fungi or bacteria. A possible surface contamination of the fruit was not taken into consideration. Additionally, the analytical techniques used were not validated.

Davis et al. [3] presented an interesting study of nicotine in food using a sample preparation technique, which has been fully validated for the extraction and detection of nicotine and cotinine in plasma [9] that was applied for the analysis of diverse vegetables. Once again, no attempts were made to determine whether the detected nicotine concentrations were genuinely from the bulk food samples or whether they were derived in part from possible surface contamination.

In a more recently published study concerning the occurrence of nicotine in common vegetables [4] only rough descriptions were provided about the analytical techniques used. No detailed descriptions of the method, its validation, recoveries or attempts to avoid contamination by environmental tobacco smoke were discussed.

The aim of the present study was to develop, validate and apply an analytical method for routine analysis of nicotine in large numbers of samples. The method should be quick, but nevertheless rugged, reliable and sufficiently sensitive for the detection of small concentrations of nicotine. Special emphasis was put on the analytical performance and sample preparation as well as on avoidance of possible contamination from any source. Gas chromatography–mass spectrometry (GC–MS) was employed as detection system for the quantitative determinations using a deuterated isotope of nicotine as internal standard. The use of an appropriate internal standard is required to compensate for lack of fully quantitative extraction of the analytes. The selection of suitable compounds as internal standards for nicotine analysis has been discussed widely [9–11]. Deuterated isomers of the analytes such as $[^2H_3]$methylnicotine provide the best compounds as internal standards.

2. Experimental conditions

2.1. Solvents and chemicals

Toluene, ethyl acetate, methanol, *n*-hexane, Na_2SO_4 granular (all for residue analysis quality) were purchased from Promochem, Wesel, Germany. Butyl acetate (HPLC quality, 99.7%), NH_4OH (purum, $[NH_3]$ 28.1%), 4-vinylpyridine (purum, 98%), quinoline (purum, 98%), triethylamine (purum, 99%) and 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 99.9%) were purchased from Sigma–Aldrich, Steinheim, Germany. NaOH (analytical-reagent grade), HCl (analytical-reagent grade, fuming) and NaCl (analytical-reagent grade) were purchased from Merck, Darmstadt, Germany, and (−)-nicotine (purum, >99%) was purchased from Fluka, Buchs, Switzerland. Deuterated nicotine ($[^2H_3]$methylnicotine, purum, 98%) was obtained from Cambridge Isotope Labs., Andover, USA. Deactivated glass wool (pesticide grade glass wool) was purchased from Supelco, Bellefonte, PA, USA. XAD-4 sampling tubes (macroreticular polystyrene–divinylbenzene copolymer beads) were obtained from SKC, Eighty Four, PA, USA.

All chemicals were checked for their nicotine concentrations to avoid possible contamination from this source.

Standard solutions for the compounds (−)-nicotine, $[^2H_3]$methylnicotine, 4-vinylpyridine and

quinoline were prepared in 0.01% triethylamine in butyl acetate in concentrations of about 100 g/l. These standard solutions were kept in the deep freezer at −18°C in the dark to avoid photodegradation of the compounds. Dilutions of the analytes were prepared using 0.01% triethylamine in ethyl acetate in brown vials just before use.

2.2. Glassware

Glassware was cleaned carefully using the following steps: washing with detergent, rinsing with tap water and double-distilled water, then rinsed with acetone for 10 min in an ultrasonic bath. Afterwards, the glassware was heated at 300°C for at least 4 h in a heating oven which was only used for this purpose. The glassware was kept in the oven until use to avoid adsorption of airborne nicotine.

2.3. Gas chromatography–mass spectrometry

The gas chromatographic analyses were performed on a Hewlett-Packard HP 5890 II plus gas chromatograph equipped with a Hewlett-Packard mass-selective detector (HP MSD 5972). The capillary column employed was a HP 5 MS (30×0.25 mm, 0.25 μm). Helium (quality 5.0) was used as the carrier gas. The operating conditions were as follows: a split/splitless injector was used in the splitless mode with an injection volume of 2 μl; the injector temperature was 235°C. The following pressure program using a pressure pulse at the beginning of the chromatographic run was used: 150 kPa were held for 0.5 min, then the pressure was decreased instantly with a rate of 500 kPa/min–50 kPa; constant flow was used for the remaining time (0.87 ml/min or 24.1 cm/s, respectively). The following temperature program was used: 70°C for 1 min; then a temperature ramp of 25°C/min was carried out to a final temperature of 280°C, which was held for 3 min. The detector temperature was 280°C. For the performance of mass spectrometry electron impact ionization (70 eV) was performed. The data were acquired in the selected ion mode. The following characteristic ions were used for the selective detection of the compounds: nicotine: *m/z* 84, *m/z* 133, *m/z* 162; [$^{2}H_3$]methylnicotine: *m/z* 87, *m/z* 136, *m/z* 165; 4-vinylpyridine: *m/z* 78, *m/z* 105; quinoline: *m/z* 102, *m/z* 129.

Special attention was given to the condition of the liner of the injector to obtain good peak shapes for the analytes. The liners were cleaned using Caro's acid (H_2SO_4–H_2O_2), rinsed thoroughly with tap water, double-distilled water and acetone, then heated at 300°C for at least 4 h. After cooling to room temperature, the liner was deactivated by using 1,1,1,3,3,3-hexamethyldisilazane and dried at 100°C for 15 min. The single-taper liners were filled with deactivated glass wool (0.5–1 cm high in the bottom quarter of the liner).

2.4. Calibration and quantification

For the quantitative determination of nicotine, calibration curves were prepared using 1 ml of 0.01% triethylamine in ethyl acetate containing variable amounts of nicotine (0, 12, 60, 120, 240 ng) and a constant concentration of the internal standard [$^{2}H_3$]methylnicotine (82 ng). Linear regression was used for all calibrations; linearity tests were performed according to Mandel; the suitability of the chosen models were checked by analyses of the residuals [14]. The variances of the measured values were examined for their homogeneity using an F-test [14]. The determination of the values for the limit of detection (LOD) and limit of quantification (LOQ) were based on the calibration curves and calculated according to DIN 32645 [12,14]. All quantitative analyses were computed considering the response ratios between analytes and internal standard.

Calibration curves were prepared before each set of sample measurements. After each 10th sample measurement, a standard solution containing 60 ng/ml of nicotine and 82 ng/ml of the internal standard was measured to check the stability of the system. If necessary the observed drift was taken into account in the final calculation. In addition, possibly carry-over effects were investigated by analysing blank samples after each fifth chromatographic run.

Accuracy of the method was tested by standard addition experiments. For these investigations nicotine was added to the sample in increasing amounts (0, 24, 36, 60, 120 ng). The extraction was performed as described in Sections 2.8 and 2.9, respec-

tively, and the quantification was performed by extrapolation.

Ruggedness (robustness) of the method was checked by performance of experiments with variations of sample amounts, solvent amounts and concentration of the internal standard. These investigations were also used to show that the detected nicotine concentrations were genuine from the investigated substrate and not introduced by contamination or by any chemicals or equipment used.

2.5. Nicotine air contamination

To ensure that the observed nicotine was not introduced from environmental tobacco smoke, air nicotine measurements were performed according to the ASTM Method D-5075-96 [13]. The airborne concentrations of nicotine as well as 3-vinylpyridine were investigated. For the calibration of 3-vinylpyridine, the commercially available isomer 4-vinylpyridine was used. Quinoline was used as internal standard. Nicotine was sampled through XAD-4 sampling tubes for 4 h either continuously or spread uniformly over 24 h using a sampling rate of 1 l/h. Elution of the analytes was performed with 1.25 ml 0.01% triethylamine in ethyl acetate. Determination of the compounds was performed using the GC–MS parameters described above.

2.6. Instrument stability

Instrument stability of the analytes was checked prior to the measurement of calibration curves or sample extracts. Especially after liner clean-up procedures a series of injections (in average 10–15 injections) of a standard solution containing 120 μg/l nicotine and 82 μg/l internal standard was necessary to obtain stable signals. Instrument stability was checked by plotting the injection number versus peak area (m/z 84 for nicotine and m/z 87 for $[^2H_3]$methylnicotine) to observe possible signal drift as well as by calculating the mean areas and standard deviations of at least ten successive measurements. A standard deviation of maximally 5% was accepted.

2.7. Sample pre-treatment

Two matrices were used for the validation of methods – fresh ripe tomatoes and commercially available tomato ketchup. The applicability of the method was also checked for other Solanaceae than tomatoes (i.e. aubergines and potatoes) that were treated similarly.

Fresh ripe tomatoes bought in a local supermarket were washed thoroughly with hot water and double-distilled water, dried and transferred into a glass vessel of a Büchi homogenizer (Mixer B400, Büchi, Flawil, Switzerland). The homogenized tomatoes were stored in carefully cleaned polyethylene boxes (cleaned with detergent, tap water, double-distilled water and acetone) in the deep freezer at −18°C until use.

Potential surface contamination by nicotine was examined as follows. The surfaces of four average tomatoes (about 100 g each) from the batch were rinsed thoroughly with methanol–ammonia (2%). Two ml of toluene were added to the rinse solution to ensure complete evaporation of methanol. The solution was reduced gently to 0.5 ml using a Zymark rotary evaporator (Turbo Vap 500, Zymark, Hopkinton, MA, USA). The resulting solution was analyzed by GC–MS as described.

For the development of methodology for processed foods commercially available tomato ketchup was used. The content of one bottle (400 g) was homogenized and aliquots were used for the investigations.

2.8. Extraction procedure for fresh fruits

A 3–5 g amount of homogenized tomatoes were weighed into a 10 ml screw cap vial (Wheaton, Millville, NJ, USA). The samples were spiked with the internal standard (82 ng of $[^2H_3]$methylnicotine) and shaken vigorously to distribute the internal standard homogeneously within the sample. One ml of NH_4OH was added to achieve pH 12; the pH was checked regularly. Three ml of toluene were added. The samples were mounted onto an automatic shaking-device in horizontal position to provide optimal shaking conditions. The samples were shaken overnight (15 h, 250 rpm). Then the samples were centrifuged (15 min, 4200 rpm) to obtain good phase separation. For samples that did not show good phase separation, a spatula tip of NaCl was added, shaken intensely and centrifuged again. Two

ml of the organic extract were separated and reduced gently to 0.5 ml using the rotary evaporator. This final extract was injected directly into the GC–MS system.

2.9. Optimised liquid–liquid extraction as clean-up procedure

The organic layer which was obtained after the extraction and centrifugation described in 2.8 was not reduced to 0.5 ml, but was further processed. An aliquot of 2 ml of the organic extract was separated and transferred in a new 10 ml screw cap vial. One ml of 0.05 *M* HCl was added and shaken on the Vortex (Ika, Janke & Kunkel, Staufen, Germany) for 1 min. If necessary to achieve good phase separation, the sample was centrifuged shortly. An aliquot of the aqueous sample was separated (0.7–0.8 ml) and transferred into a third screw cap vial. Three drops of 5 *M* NaOH and 0.5 ml of toluene were added. The samples were shaken again for 1 min using the Vortex and centrifuged shortly. The organic layer was used directly for GC–MS measurements. The amounts used have to be considered in the final calculations.

3. Results and discussion

3.1. Analytical performance

The use of gas chromatography-mass spectrometry as an analytical technique provides a very selective and sensitive method for nicotine analysis. With the described experimental conditions, all investigated compounds can be analyzed within 8 min.

The use of the selected ion mode significantly increases the selectivity as well as the sensitivity of the analytical method. The identification of the compounds is based on retention times, as well as on the ratio of the selected ions to one another, which eliminates the danger of false positive results.

Linear calibration can be used for all compounds. Linear regression curves were obtained from five different concentrations (in duplicate or fourfold, respectively, for the lowest and highest concentrations used) versus the peak areas and yielded correlation coefficients $R^2 \geq 0.98$. LODs and LOQs, as well as the standard deviation of regression (SDR) are shown in Table 1. Calculations of LODs and LOQs are based on the calibration function[1]. The linearity of the calibration curves was checked by linearity tests according to Mandel [14]; the suitability of the linear model was verified by analysis of the residuals [14]. Furthermore, for validation of the methods the variances were tested for their homogeneity based on the 95% and 99% confidence interval. Linearity was found from the detection limit up to 500 μg/l. [$^2H_3$]methylnicotine provided an excellent compound as internal standard. For all quantitative measurements – besides standard addition experiments – the response ratio between nicotine and [$^2H_3$]methylnicotine was considered. Especially when measuring high sample numbers special emphasis was put on signal drift as well as on possible carry-over effects. The performance is described in detail in Section 2.4.

Due to the polarity of the analytes, the performance of the GC–MS measurements is highly dependent on the condition of the injector system and the analytical column with special attention needed for the liner. Best results were obtained using a split/splitless injector and liners of single-taper geometry packed with deactivated glass wool in the splitless

[1] LOD is defined as the lowest concentration differing significantly from zero; LOQ the lowest concentration of the analyte that can be determined with a standard deviation ≤5%.

Table 1
Limits of detection (LOD) and quantification (LOQ) for the investigated compounds

Compound	LOD (pg)	LOQ (pg)	LOD (μg/kg) wm[a]	LOQ (μg/kg) wm[a]	SDR[b] (%)
Nicotine	3.0	10.8	0.8	2.7	4.3
[$^2H_3$]Methylnicotine	4.2	14.8	1.1	3.7	4.6

[a] LOD/LOQ is calculated under consideration of 3g tomatoes; LOD/LOQ are referred to wet mass (wm).
[b] SDR=Standard deviation of regression.

mode. The surface purity of the liner plays a significant role for the sensitivity of the system. Fig. 1 shows a comparison of the selected ion chromatograms of a nicotine standard solution of the same concentration before and after cleaning and deactivating the liner. The cleaning procedure described results in an improvement of the peak shape as well as in an increase of the signal-to-noise ratio by a factor six. In addition to the condition of the liner, the chosen pressure pulse at the beginning of the chromatographic run increased the peakshape significantly.

It was found that high instrument stability for the analytes could not be guaranteed immediately after cleaning and deactivating the liner. A number of injections of standard solutions had to be performed to achieve high stability, which is considered to be necessary for deactivating possible active sites in the freshly treated liner. Under optimum conditions the instrument stability was very high (s_{max} ±4%).

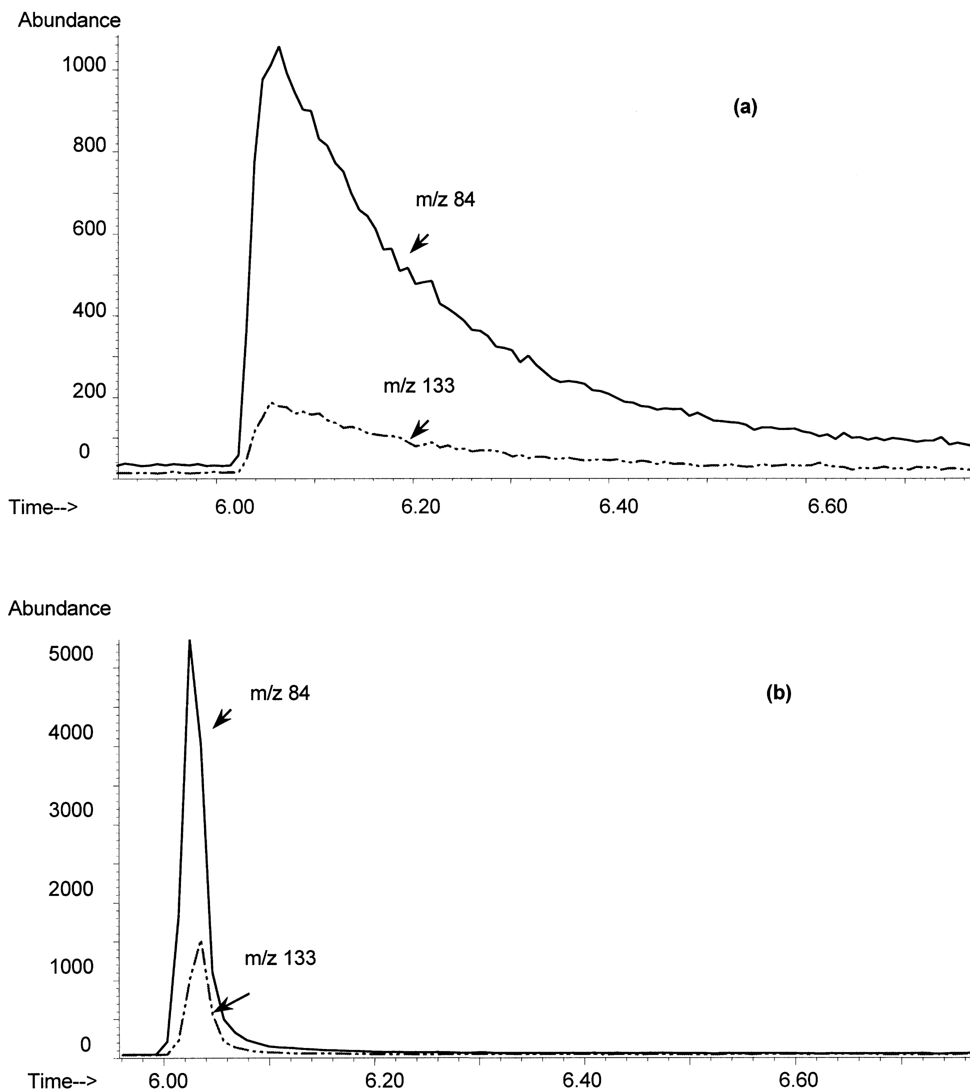

Fig. 1. Comparison of selected ion chromatograms of nicotine standard solution of the same concentration (120 μg/l): (a) before cleaning the liner and (b) after cleaning the liner; (a) peak-to-peak signal to noise: 128; (b) peak-to-peak signal to noise: 758. Time scale in min.

3.2. Possible sources of contamination

In this work special emphasis was put on the possibility of nicotine contamination from the chemicals and equipment used, contamination from environmental tobacco smoke (ETS), as well as from surface contamination of the fruit samples. All chemicals is were checked for their nicotine concentrations prior to use. The only chemical showing a slight nicotine blank concentration was the solvent mixture of 0.01% triethylamine in ethyl acetate which was used for the standard solutions. This signal was equivalent to about 0.5 μg/l, which is 10% of the lowest concentration used for the calibrations, this blank concentration was taken into consideration in the calibration curves of nicotine.

The determination of airborne nicotine and 3-vinylpyridine in the laboratory and adjacent environments was performed according to an ASTM method [13]. The determination of 3-vinylpyridine, in addition to nicotine, was performed as this compound represents an excellent tracer for nicotine in ETS; usually the concentrations of 3-vinylpyridine are lower than the observed nicotine concentrations [13,15,16]. Under the sampling conditions employed, the LOQs and LOQs that were achieved are for nicotine 4.4 ng/m^3 and 15.9 ng/m^3, respectively, and for 3-vinylpyridine 3.8 ng/m^3 and 13.8 ng/m^3, respectively. In none of the relevant locations was nicotine observed in a concentration range higher than 0.35 $\mu g/m^3$–0.60 $\mu g/m^3$. The 3-vinylpyridine, which is normally less concentrated than nicotine in ETS, could not be detected in any location. Nicotine concentrations in various indoor environments is reported to range from non-detectable to 70 $\mu g/m^3$, with values usually near the lower end of the range [17]. Contamination by airborne nicotine did not appear to represent a major problem in the laboratory.

The history of possible exposure of samples to nicotine that had been purchased in local supermarkets is not known; consequently, surface contamination of the observed fresh fruits was studied very carefully. The examined fruits were rinsed with ammonical methanol (2%) and the rinsing solution was examined as described. The values for nicotine derived from surface contamination were recalculated assuming that the tomatoes behave like ideal spheres and potatoes and aubergines like ideal cylinders, respectively. The results are shown in Table 2. Considering some of the previous reports [3,7], nicotine concentrations in diverse foods are expected to be in the order of 5–100 μg/kg fresh fruits. Nicotine concentration estimated from surface contamination (0.01–0.09 μg/kg fruits) is two to four orders of magnitude lower than the expected concentrations in the bulk vegetable and, therefore, out of statistical relevance for the nicotine concentration of the whole fruit.

3.3. Extraction of the analytes: performance and discussion of the results

Foods represent difficult substrates for analysis because in most cases the matrix is very complex. Therefore, a carefully validated sample preparation was required for selective and efficient extraction of nicotine in food stuff. Method development was performed using ripe tomatoes purchased in a local supermarket and commercially available tomato ketchup. Subsequently, the method's applicability for the extraction of nicotine out of other edible nightshades (i.e. potatoes and aubergines) was investigated.

Table 2
Nicotine concentrations from surface contamination of the investigated fruits

Parameter	Tomato[a]	Potato[b]	Aubergine[c]
Estimated average surface area per fruit (cm^2)	113.1	75.4	1963.4
Approximate average weight per fruit (g)	100	100	400
Surface nicotine per fruit (ng)	4.7	3.7	9.0
Estimated nicotine per kg fruits, wet mass (μg/kg)	0.05	0.09	0.01

[a] Average diameter 6 cm.
[b] Average diameter 4 cm, average height 6 cm.
[c] Average diameter 10 cm, average height 25 cm.

For the development of methodology, a number of extraction techniques that are widely used for the extraction of organic compounds from plant or food material were investigated, but none of them showed satisfying results. Nevertheless, these methods are described briefly in the following paragraph to show the difficulties that arise when extracting nicotine out of food material, and to allow future researchers to avoid unsuccessful approaches.

(1) An AOAC method [18] designated for the determination of nicotine residues in apples, cabbage and spinach using spectrophotometric detection was adapted to the needs of gas chromatography. The main problem that arose was the formation of very stable emulsions that could not be broken with methods normally used for this purpose. In addition, the recoveries were very low ($\leq$50%) and not consistent.

(2) Simultaneous distillation-extraction (SDE) according to Likens and Nickerson [19,20] represents a very simple and in many cases very effective sample p reparation technique. It is widely used for the extraction of volatile organic compounds in aqueous systems [21]. As nicotine has a high affinity for the aqueous matrix, recoveries for the analyte were very poor.

(3) The use of a microextraction device according to DIN 38407 [22] often provides a simple extraction method for organic analytes from aqueous matrices with the use of very small amounts of organic solvents. This results in high enrichment factors. Again the phase separations as well as the achieved recoveries were very poor.

(4) The use of a rotation perforator permits a continuous extraction of aqueous matrices to be performed using an organic solvent heavier than water. Inconvenient manipulation of the apparatus opened opportunities for contamination. Carry-over effects from prior extractions made this technique unsuitable for nicotine residue analysis.

(5) A sample preparation method that is widely used for the extraction of pesticides from plant material [23] was adapted for the extraction of nicotine using acetone as the organic solvent. In addition to poor recoveries of the analyte, a classical aldol condensation took place in course of the procedure (acetone reacts to form 4-hydroxy-4-methyl-2-pentanone under basic conditions) that provided an interfering peak with m/z 84 which is used as target ion for the analyte nicotine.

A very simple sample preparation technique for the extraction of low concentrations of nicotine from plasma was described by Degen and Schneider [24]. In this method a liquid-liquid extraction of the plasma samples is performed at pH 10 with toluene–*n*-hexane (1:1) and the extract is injected directly into GC–MS. This simple sample preparation technique was adapted for the extraction of food samples (see Section 2.8 for detailed description). The main advantage of this method is its simplicity, which provides the possibility of processing large numbers of samples in very simple way. Furthermore, the glass vials used for the extraction can be shaken automatically using about 30 samples simultaneously. Another advantage is that the entire extraction procedure is performed in a single glass vial, which minimises the risk of contamination.

In the course of the establishment of the final experimental conditions, the influence of different parameters to gain optimum recoveries was investigated. First, the influence of the base used for pH adjustment was checked. Results from experiments using 5 *M* NaOH (as described in [24]) and using NH_4OH showed that the use of NH_4OH results in an increase in recovery of five percent in comparison to NaOH. Further experiments showed that the selection and amount of organic solvent used has the greatest influence on the recoveries. Degen and Schneider [24] describe the use 1 ml of toluene–*n*-hexane (1:1) for the extraction of serum. A series of experiments showed that for the extraction of nicotine in various food materials a larger volume of solvent is necessary. Furthermore, due to higher polarity, toluene was more effective than toluene–*n*-hexane. Fig. 2 shows that with 3 ml of toluene as an extraction solvent the highest recoveries could be achieved. No further attempts were made to increase the solvent amount to prevent further sample dilution. For the most important fruits the recoveries were as follows: tomatoes: 96.4$\pm$10.7%, potatoes 98.3$\pm$3.2% and aubergines 102.2$\pm$5.6%. The data compare well for all fruits investigated.

The performance of the method using [$^2H_3$]methylnicotine as in internal standard was investigated by comparison with standard addition experiments. No reference material as well as no

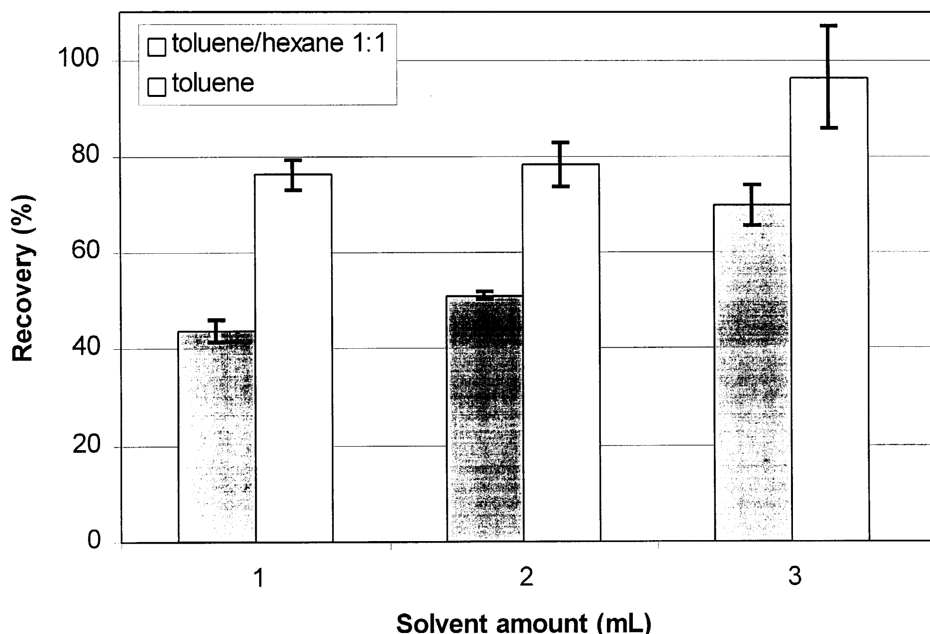

Fig. 2. Recoveries of nicotine from vegetables depending on the solvent used and the volume of the solvent.

alternative technique was available for the determination of nicotine in food material, therefore, standard addition experiments were also used to investigate the accuracy of the method [14]. In Table 3 the results from standard addition experiments are listed in comparison to those obtained by calculation via the internal standard for tomatoes and potatoes as well as aubergines. For all substrates the results compare well.

The ruggedness of the method was examined by a set of experiments using different amounts of internal standard as well as different amounts of sample. These experiments were also used to ensure that the detected amounts of nicotine were genuine from the substrate and not introduced into the system by any source of contamination. The results are shown in Table 4. With a mean concentration of 7.3 μg/kg and standard deviation of 13.7%, the measured

Table 4
Comparison of results obtained by experiments using different experimental approaches

Sample amount (g)	Conc. of the I.S. (μg/kg)	Measured nicotine conc. (μg/kg)
6.9±0.3	11.6±0.6	7.0±0.3
3.5±0.2	5.9±0.4	8.6±1.0
51.1±0.3[a]	24.1±0.3	6.2±0.2

[a] The extraction was done in a 250 ml separation funnel; solvent amounts were adapted.

Table 3
Nicotine concentrations from tomatoes obtained by quantification using the internal standard and standard addition methods

Substrate	Nicotine concentration via internal standard (μg/kg)	Nicotine concentration via standard addition (μg/kg)
Tomatoes	8.4±0.3	7.0±0.7
Potatoes	10.1±0.8	9.9±1.5
Aubergines	9.8±0.8	7.5±1.2

concentrations compare well. Considering a standard deviation of ±10% for the recoveries of nicotine, as shown above, the observed differences are out of statistical relevance.

3.4. Clean-up of the extracts

Investigations of tomato ketchup as an example of processed foods showed that further clean-up steps were necessary for products containing other ingredients in addition to vegetables, for example spices or flavourings. In these products compounds were found with similar *m/z* ratios to nicotine (*m/z* 84) or to the internal standard (*m/z* 87) which co-eluted with nicotine making identification and quantification impossible.

For a lot of analytical problems, solid-phase extraction (SPE) provides an excellent tool for simple and effective sample clean-up procedures. However, in this case we found that SPE using silica gel cartridges was not a suitable extraction technique mainly due to two reasons:

(1) Silica gel is a very good adsorbent for nicotine and, therefore, the blank concentration of nicotine on the silica material was high. Even using rather drastic eluting conditions like methanol–ammonia (2%) did not adequately reduce the blank found on the SPE cartridges.

(2) Experiments with nicotine and [$^2H_3$]methylnicotine solutions resulted in very low recoveries. The standard compounds were adsorbed by the SPE material very strongly and could not be eluted from the cartridge with recoveries greater than about 50%. In addition, the recoveries were not reproducible.

Finally, an easy liquid-liquid extraction procedure starting with the toluene extract was established making use of the polar and basic properties of nicotine and pH value; this extraction procedure is summarized in Fig. 3.

Fig. 4 shows the selected ion chromatograms of the extracts of commercially available tomato ketchup for nicotine (*m/z* 84) and [$^2H_3$]methylnicotine (*m/z* 87) before and after the clean-up procedure. It can be seen very clearly that the established procedure is highly effective, interference is eliminated and peak shape and signal-to-noise ratios are improved significantly. In spite of the relatively high number of extraction steps, the losses of the analytes are very low. For the whole sample preparation procedure including the clean-up steps, recoveries of 96.5±18.5% (n=14) could be achieved.

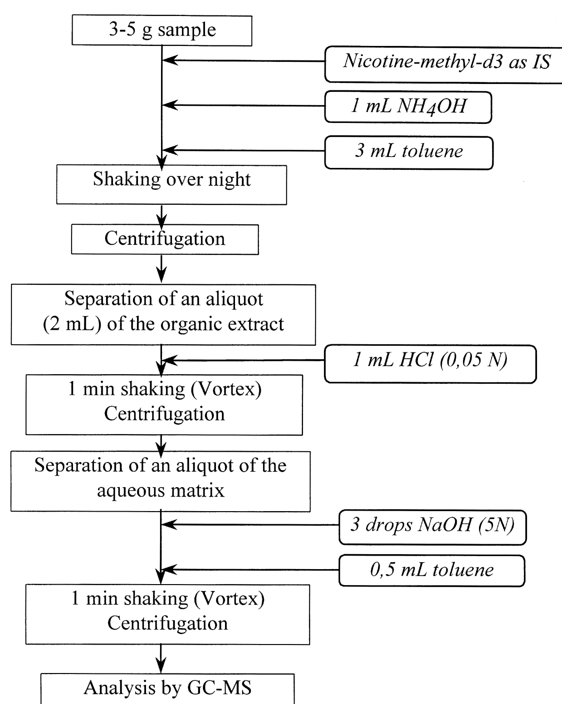

Fig. 3. Clean-up procedure for processed foods.

4. Conclusion

The methodology described in this report is suitable for the extraction of nicotine present at low concentrations in food material with high selectivity as well as high sensitivity. It was shown in detail that analytical performance can be improved for this specific analytical approach. Special emphasis was drawn on the examination of any possible contamination sources. For processed foods containing ingredients other than vegetables, single extraction is not sufficient because of interfering compounds. A clean-up procedure is described that is also simple and effective and fulfils the demands of the analytical technique. As a result of its simplicity, the

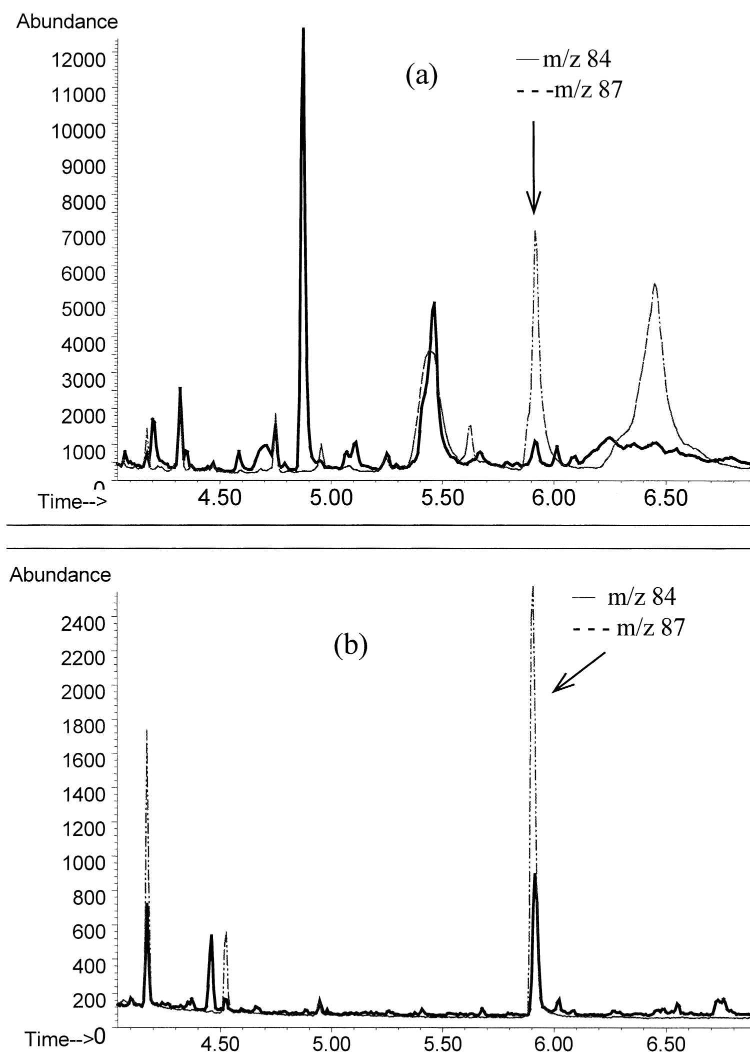

Fig. 4. Comparison of chromatograms of tomato ketchup (a) before and (b) after the clean-up procedure; the selected ion chromatograms for nicotine (m/z 84) and for $[^2H_3]$methylnicotine (m/z 87) are superimposed.

method is applicable to the examination of large numbers of food samples for their nicotine content.

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References

[1] E. Leete, in: S.W. Pelletier (Ed.), Alkaloids Chemical and Biological Perspectives, Vol. I, Wiley, New York, 1983, p. 86.

[2] B.N. Ames, Science 221 (1983) 1256.
[3] R.A. Davis, M.F. Stiles, J.D. de Bethizy, J.H. Reynolds, Food Chem. Toxicol. 29 (1991) 821.
[4] E.F. Domino, E. Hornbach, T. Demana, Med. Sci. Res. 21 (1993) 571.
[5] J. Repace, Brit. Med. J. 1 (1994) 61.
[6] M.J. Jarvis, Brit. Med J. 1 (1994) 62.
[7] A. Castro, N. Monji, Biomed Arch. 2 (1986) 91.
[8] S.J. Sheen, J. Food Sci. 53 (1988) 1572.
[9] R.A. Davis, J. Chromatogr. Sci. 24 (1986) 134.
[10] P. Jacob, M. Wilson, N.L. Benowitz, J. Chromatogr. 222 (1981) 61.
[11] P. Jacob III, L. Yu, M. Wilson, N.L. Benowitz, Biol. Mass Spec. 20 (1991) 247.
[12] DIN 32645, DIN Deutsches Institut für Normung e.V. Beuth Verlag, Berlin, 1994.
[13] ASTM Method 5075-96, American Society for Testing and Materials, Philadelphia, PA.
[14] W. Funk, V. Damman, G. Donnevert, Qualitätssicherung in der Analytischen Chemie, VCH, Weinheim, 1992.
[15] K. Phillips, D.A. Howard, M.C. Bentley, G. Alván, Int. Arch. Occup. Environ. Health 71 (1998) 201.
[16] K. Phillips, M.C. Bentley, D.A. Howard, G. Alván, Environ. Int. 24 (1998) 405.
[17] L.C. Holcomb, Environ. Int. 19 (1993) 9.
[18] Association of Official Analytical Chemists, AOAC Official Methods of Analysis, Vol. 1, 15th Ed., Association of Official Analytical Chemists, Arlington, VA, 15th ed., 1990, pp. 307–308, method 964.20.
[19] S.T. Likens, G.B. Nickerson, Am. Soc. Brew. Chem. Proc 5 (1964).
[20] G.B. Nickerson, S.T. Likens, J. Chromatogr. 21 (1966) 1.
[21] B. Siegmund, E. Leitner, I. Mayer, P. Farkaš, J. Sádecká, W. Pfannhauser, M. Kovác, Dtsch. Lebensm.-Rdsch. 92 (1996) 286.
[22] DIN 38407 (EN ISO 6468), Part 1, Normenauschuss Wasserwesen in DIN Deutsches Institut für Normung e.V. Beuth Verlag, Berlin, 38. Lieferung, 1997.
[23] W. Specht, M. Tilkes, Fresenius Z. Anal. Chem. 301 (1980) 300.
[24] P.H. Degen, W. Schneider, J Chromatogr. B 563 (1991) 193.