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## Nicotine determination in mushrooms by LC-MS/MS and early studies on the impact of drying on nicotine formation

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### Abstract

Recently a problem concerning significant amounts of nicotine in dried wild mushrooms (mainly *Boletus edulis* from China) was reported to the European Commission. As a consequence the European Food Safety Authority (EFSA) proposed temporary maximum residue levels (MRLs) of 0.036 mg kg<sup>-1</sup> for fresh wild mushrooms and 1.17 mg kg<sup>-1</sup> for dried wild mushrooms (2.3 mg kg<sup>-1</sup> just for dried ceps). The EFSA also has highlighted the necessity for a monitoring and testing programme that will be launched by food business operators at the start of the forthcoming 2009 harvest season. In the present work a quick and sensitive analytical method for the routine analysis of nicotine in fresh and dried mushrooms was developed and validated by a single laboratory procedure. The method, that employs an LC-MS-MS system and (±)-nicotine-d<sub>4</sub> as internal standard, has a limit of quantification of 6 µg kg<sup>-1</sup> and 60 µg kg<sup>-1</sup> for fresh and dried product respectively. Analyses of spiked samples with different quantities of nicotine showed recovery ranges from 107 to 122% with relative standard deviation ranging from 2.9 to 10.1 %, depending on the spiking level. The combined uncertainty, calculated at low level for frozen (0.015 mg kg<sup>-1</sup>) and at high level for dried (2 mg kg<sup>-1</sup>) matrix was 13% and 10%, respectively. The application of the method to some real samples of mushrooms purchased on the market or obtained from local producers shows ranges of nicotine content of 0.01-0.04 mg kg<sup>-1</sup> and 0.1-4.5 mg kg<sup>-1</sup> in fresh/frozen and dried matrixes, respectively. To understand the reasons for this unexpected high content of the nicotine in dried matrixes some experiments involving drying mushrooms were performed in laboratory in different conditions.

**Keywords:** nicotine; mushrooms; LC-MS-MS; method validation.

## Introduction

Nicotine is the main alkaloid in tobacco (*Nicotiana tabacum*) and other tobacco species where it occurs at concentrations ranging from 2% to 8%; it is synthesised in the tobacco root from ornithine and/or arginine by way of putrescine (Hibi et al. 1994). Low concentrations were also measured in other plants belonging to the family of Solanaceae, such as tomatoes, aubergines, peppers and potatoes (Andersson et al. 2003). Mushrooms are usually not reported as a significant source of nicotine, although both amino acids, ornithine and arginine have been identified in them (Ribeiro et al., 2008). Nevertheless, recently significant amounts of nicotine were detected in Germany in dried wild mushrooms (ceps) from China (BfR, 2009), raising questions about the origin of nicotine in these products and the possible concerns for human health. Recently, the European Commission has been informed by food business operators that dried wild mushrooms (mainly *Boletus edulis*, but also truffles and chanterelles) may contain levels of nicotine higher than 0.01 mg/kg on a fresh weight basis. This is the default maximum residue level (MRL) set by Article 18.1.b of Regulation (EC) No 396/2005. According to the information received, 99% of samples tested from 2008 production did not comply with the current MRL, irrespective of the wild mushrooms' origin, although most of them originated from China. No clear reason has thus far been established for this unexpected presence of nicotine that could be of various origin, but also results from a combination of different factors: 1) pesticide use; in organic farming nicotine is not allowed to be used as pesticide (Regulation EC No 889/2008) and in general in European countries the use of plant protection products containing nicotine will phase out by the latest in June 2010, but its use in Third Countries may continue and may lead to residues of nicotine in food; 2) inherent presence of nicotine in wild mushrooms and its eventually arise due to an increase of its biosynthesis (*via* Putrescine, Hibi *et al.*, 1994) caused by some not properly controlled conditions of storage during the drying phase; 3) cross contamination due to bad practices in the storage/drying and packaging process (smoking people handling the mushrooms, storage in rooms that have been disinfected with nicotine, simultaneous drying of tobacco and mushrooms in the same room). As a consequence of this alert the European Food Safety Authority (EFSA) received an urgent request on 27 April 2009 from the European Commission for a scientific opinion in relation to the risks for human health due to the presence of nicotine in wild mushrooms at concentrations up to 0.5 mg kg<sup>-1</sup>. In reply to this request, the EFSA issued on 7 May 2009 a statement, concluding that a level of 0.5 mg kg<sup>-1</sup> is not safe, because it would lead an exceedence of the acute reference dose (ARfD). The EFSA has proposed alternative MRLs to be set on a temporary basis both for fresh (0.036 mg kg<sup>-1</sup>) and for dried (1.17 mg kg<sup>-1</sup>) wild mushrooms. Nevertheless, as regards dried ceps it would be more appropriate to restrict the evaluation of a safe level of nicotine to the consumption data available for

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2 the adult population because of the poor data as regards consumption by children. As a  
3 consequence, for dried cepts, an acceptable level of  $2.3 \text{ mg kg}^{-1}$  could be derived. The EFSA also  
4 highlighted that the evaluation performed was affected by a number of uncertainties and limitations  
5 on the data available (consumption of wild mushrooms in Europe and, in particular on dried ones,  
6 contamination levels, actual unit-to-unit variability in fresh mushrooms). Consequently, EFSA  
7 recommends that the proposed MRLs should be considered on a temporary basis only (Annex III of  
8 Regulation 396/2005). Finally, it is noted that a more robust basis for the exposure assessment and  
9 MRLs setting will derive from the data provided by the monitoring and testing programme  
10 recommended by the European Commission that will be launched by food business operators at the  
11 start of the forthcoming 2009 harvest season. A variety of methods for isolation, identification and  
12 quantification of nicotine and related compounds in plants and biological tissues have been  
13 published. These methods range from early paper chromatographic procedures through to GC/MS,  
14 thin-layer and HPLC procedures; also several radioimmunoassay have been developed for nicotine  
15 (Castro and Monji, 1986). Sheen (Sheen, 1988) investigated the nicotine content of Solanaceous  
16 food plants using a GC technique using alkali flame detector with a LOD of around  $1 \text{ mg kg}^{-1}$  in  
17 dry material. Davis et al. (Davis, 1991), Domino et al. (1993), and Siegmund et al. (1999) used  
18 GC/MS with LODs of around  $1 \mu\text{g/ kg}^{-1}$ . Nevertheless, no methods are published yet concerning  
19 the determination of nicotine in wild mushrooms. The aim of the present study was to develop,  
20 validate and apply an analytical method for routine analysis of nicotine in fresh and dried  
21 mushrooms. The method should be quick and sufficiently sensitive for the detection of small  
22 concentrations of nicotine. An LC-MS-MS spectrometer was employed as detection system for the  
23 quantitative determination using a deuterated isotope of nicotine as internal standard .  
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## 43 **Materials and methods**

### 44 *Chemicals and samples*

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46 Nicotine and ( $\pm$ )-Nicotine-D<sub>4</sub> standards were purchased from Ehrenstorfer-Schafers ( Augsburg,  
47 Germany) and from Cerilliant (Round Rock, TX), respectively. Nicotine and ( $\pm$ )-Nicotine-D<sub>4</sub>  
48 standard stock solutions at  $49192 \text{ mg kg}^{-1}$  and  $10 \text{ mg kg}^{-1}$ , respectively, and nicotine working  
49 solutions in the range  $2 - 500 \mu\text{g kg}^{-1}$  were made with acetonitrile and stored at  $-25^\circ\text{C}$ . For  
50 extraction and clean-up, bidistilled water, pesticide-quality acetonitrile (from VWR) and ammonia  
51 solution 30% (from Carlo Erba Reagenti) were used without further purification. QuEChERS kit  
52 were purchased from Waters (U.S.A.). Dried and frozen wild mushrooms were obtained from local  
53 producers.  
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### ***Sample preparation***

Extraction and clean-up of samples were done using QuEChERS kit. 0.5 g of dried or 5 g of fresh/frozen homogenized mushrooms were added to a 50 ml DisQuE extraction tube containing 1.5 g of sodium acetate and 6 g of magnesium sulphate. 14.5 g (for dried sample) or 10 g (for fresh/frozen sample) of  $\text{NH}_4\text{OH}$  0.1 M and the internal standard (75  $\mu\text{l}$  of 10 mg/Kg ( $\pm$ )-Nicotine- $\text{D}_4$  solution in acetonitrile) were added. The sample was vigorously hand shaken and put into an ultrasonic bath for 30 min, than was left for other 30 min at room temperature without any shaking. 15 ml of acetonitrile was added and the sample was vigorously shaken by hand for 1-2 min, then was centrifuged at about 2000 rpm for 2-4 min. 1 ml of the acetonitrile extract was transferred into a 2 ml DisQuE extraction tube containing 50 mg of PSA and 150 mg of magnesium sulphate. The tube was shaken for 1-2 min using a vortex agitator than was centrifuged at about 2000 rpm for 2-4 min. The supernatant organic phase was injected to an LC-MS-MS spectrometer.

### ***Drying of fresh/frozen mushrooms***

10 g. of fresh or frozen homogenized mushrooms were weighted in a porcelain capsule and dried in an oven at  $70^\circ\text{C}$  under vacuum for about 2 hours, then sample was weighted again and the concentration ratio due to loss of water was calculated. A same experiment was done laying the sample exposed at the direct sun light until loss of water corresponding to 8-10, as normally done by dried mushrooms producers. Nicotine in the resultant final dried material was extracted following the procedure described in the previous paragraph.

### ***LC-MS-MS analysis***

The HPLC analyses were performed on an Agilent 1100 Series liquid chromatograph equipped with an Applied Biosystems API 2000 triple quadrupole mass detector. The column employed has been a Sinergy Polar-RP 80A 4  $\mu\text{m}$ , 150x2 mm (Phenomenex USA). The operating conditions have been as follows: injection volume 2  $\mu\text{l}$ ; mobile phase of 30% component A (0.2% formic acid aqueous solution) and 70% component B (acetonitrile); total flow rate of 0.250 ml/min; total running time for each analysis of 7 min. The following characteristic transitions were monitored:  $m/z$  163>80, 163>84, 163>106, 163>130, 163>132 for the nicotine and 167>84, 167>110, 167>121, 167>134 and 167>136 for the internal standard ( $\pm$ )-Nicotine- $\text{D}_4$ . For the quantitative determination of nicotine the following transitions were used:  $m/z$  163>130, 163>132, 167>134 and 167>136 (where the last two are referred to the internal standard). The calibration curve was prepared using acetonitrile solutions containing variable amounts of nicotine (2, 5, 20, 100, 200, 500  $\mu\text{g kg}^{-1}$ ) and a constant concentration of the internal standard (50  $\mu\text{g kg}^{-1}$ ); a linear regression was applied. Calibration

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2 curve was prepared before each set of sample measurements, using fresh, or properly stored (-  
3 18°C), standard solutions.  
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### 5 6 7 ***Method validation***

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9 The performance characteristics of the method were established by single-laboratory validation  
10 employing analyses with naturally nicotine-contaminated as well as spiked frozen and dried wild  
11 mushrooms. Repeatability, within and between days, as well as test of recoveries were evaluated.  
12 For within-day and between-day studies one naturally nicotine-contaminated sample was analysed  
13 ten times on the same day (two samples at a time, five times, within five hours) and on ten different  
14 days, respectively, both for frozen and dried mushrooms. For the determination of recoveries,  
15 nicotine-spiked mushrooms samples were analysed. This last study was performed at two different  
16 levels for the dried matrix ( 0.2 and 2 mg kg<sup>-1</sup>) and at one level for the frozen material ( 50 µg kg<sup>-1</sup>).  
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## 25 **Results and discussion**

### 26 27 28 ***Sample preparation***

29 Because of nicotine is a tertiary base with a pK<sub>a1</sub>=6.16 and pK<sub>a2</sub>=10.96 (Siegmund et. al. 1999) it is  
30 bound to the acid matrixes, as vegetables, as a salt. For this reason a particular attention must be  
31 done to the pH conditions of extraction solutions. Different aqueous solutions were tested:  
32 bidistilled water, NaOH 0.05 M, NaOH 0.1, NH<sub>4</sub>OH 0.1, 0.2 and 0.5 M. Extraction times for each  
33 of these solutions were tested: 10-30-45-60 minutes (half time in ultrasonic bath). Also two  
34 different organic phases were tested for the extraction of the nicotine from the aqueous solution:  
35 acetonitrile and acetonitrile containing 0.1% of acetic acid. From the results of these experiments it  
36 seems clear that a strong basic environment is absolutely necessary to completely extract nicotine  
37 from the matrix and the best results were obtained using NH<sub>4</sub>OH 0.1 M for a total of 60 minutes  
38 and acetonitrile as final organic solvent.  
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### 52 ***Development and validation of the method***

53 The use of HPLC coupled with tandem mass-spectrometry provides a very selective and sensitive  
54 method for the nicotine analysis. The use of (±)-Nicotine-D<sub>4</sub> as internal standard has been suitable to  
55 compensate both for lack of fully quantitative extraction of the analyte and for the LC-MS-MS  
56 response suppression caused by the presence of sample matrix. In the described experimental  
57 conditions the retention time of the investigated compound and the deuterated internal standard is  
58 around 2.2 minutes. In these conditions to obtain repeatable retention times and good peak shapes  
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2 for the analyte and the internal standard too a small injection volume has been required (2  $\mu\text{l}$ ) and  
3 special attention was also given to the equilibration of the column. All the characteristic transitions  
4 of the nicotine and the ( $\pm$ )-Nicotine-D<sub>4</sub> were monitored during all the instrumental analyses. For the  
5 quantification the ratio between the sums of the two most abundant transitions of the analyte (m/z  
6 163>130,163>132) and the deuterated internal standard (m/z 167>134 and 167>136) was  
7 considered (Fig. 1). Standard solutions of nicotine in acetonitrile were analysed by HPLC with a  
8 triple quadrupole mass detector establishing a minimum detection limit of 1  $\mu\text{g kg}^{-1}$  (S/N = 3) and a  
9 limit of quantification of 2  $\mu\text{g kg}^{-1}$  (S/N =10); the concentration of the internal standard, ( $\pm$ )-  
10 Nicotine-D<sub>4</sub>, was 50  $\mu\text{g kg}^{-1}$  for each solution (as in the final sample acetonitrile extract). The  
11 standard curve for nicotine was linear from 2 to 500  $\mu\text{g kg}^{-1}$  ( $R^2=0.999$ ), equivalent to 0.06-15 mg  
12  $\text{kg}^{-1}$  and 6-1500  $\mu\text{g kg}^{-1}$  in dried and fresh/frozen samples, respectively. To test the repeatability of  
13 the method, naturally nicotine-contaminated samples of dried and frozen mushrooms were analysed  
14 ten times during the same days (within-day repeatability) and ten times in ten different days  
15 (between-day repeatability). Table 1 shows the calculated average data and the relative standard  
16 deviations (RSDs). For the recovery test, nicotine-free of dried and nicotine-low contaminated (10-  
17 15 mg  $\text{kg}^{-1}$ ) of frozen mushrooms samples were spiked with standard nicotine solutions at different  
18 levels. The tested levels were been 0.2 and 2 mg  $\text{kg}^{-1}$  for dried samples and 50 mg  $\text{kg}^{-1}$  for the  
19 frozen material; test was performed 8 times for the two different matrixes. As shown in Table 1  
20 average recoveries of nicotine was ranged from 107 to 122% and RSDs was ranged from 2.9 to  
21 10.1%. The combined uncertainty of the quantitative result of a sample has been calculated by using  
22 the bottom-up approach, taking account of the following contributes: uncertainty of repeatability;  
23 uncertainty of recovery; uncertainty of calibration curve; uncertainty of standard solutions;  
24 uncertainty of volumes and uncertainty of weight. Two different naturally nicotine-contaminated  
25 samples were analysed ten times each one: a dried mushrooms containing nicotine at level of 2 mg  
26  $\text{kg}^{-1}$  and a frozen matrix with a contamination level of 0.015 mg  $\text{kg}^{-1}$ . The average results and the  
27 relative calculated uncertainties were been:  $2.0 \pm 0.2$  mg  $\text{kg}^{-1}$  for the dried sample and  $0.015 \pm$   
28  $0.002$  mg  $\text{kg}^{-1}$  for the frozen one, both of them considering a coverage factor of 2, which gives a  
29 level of confidence of approximately 95%.

### 54 *Naturally nicotine-contaminated mushrooms*

55 After method development some samples of dried, frozen and also fresh mushrooms were analysed.  
56 Results of the analyses of different kind (whole, sliced and diced) of frozen mushrooms purchased  
57 on the market show a range of nicotine from 0.01 to 0.03 mg  $\text{kg}^{-1}$ . This natural occurrence of  
58 nicotine is confirmed from the result of the analysis on a fresh matrix obtained from a local  
59 mushroom harvester that shows a nicotine-contamination at level of 0.04 mg  $\text{kg}^{-1}$ . Dried  
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2 mushrooms from different origin (Europe, Asia and South Africa) were also analysed and in these  
3 samples nicotine is present in the range of concentration from  $< 0.1$  ( $< \text{L.Q.}$ ) to  $4.5 \text{ mg kg}^{-1}$ . From  
4 these data it seems that, in general, dried matrixes could contain an higher level of nicotine respect  
5 those that should be on the base of the results obtained on the fresh (or frozen) samples and  
6 considering a concentration factor of about 8 from fresh to dried material. To understand this fact,  
7 samples of fresh and frozen mushrooms were dried in laboratory and their nicotine content were  
8 measured before and after the drying treatment. In this experiment 10 g. of fresh/frozen  
9 homogenized mushrooms were dried in two different way: in an oven at  $70^{\circ}\text{C}$  after vacuum for  
10 about 2 hours and by exposition to the direct sun light for some days. For each experiment samples  
11 were weighted before and after the drying treatment and concentration factors due to loss of water  
12 were calculated. As it is evident from data show in table 2 the nicotine content in final dried  
13 matrixes has been not higher than those that were expected. These results, even still partially, don't  
14 get any confirm to the hypothesis that nicotine could be biosynthetized in mushrooms at higher  
15 level during drying phase in not properly conditions.  
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## 28 **Conclusions**

29  
30 This work describes the development and the application of an analytical method to determine  
31 nicotine content in fresh and dried mushroom samples. The procedure for extraction and clean-up of  
32 nicotine from fresh, frozen and dried mushrooms by using QuEChERS kit is easy and fast. The  
33 final acetonitrile extract was analysed by LC-MS-MS and nicotine was determined with a limit of  
34 quantification limit (LOQ) of  $2 \mu\text{g kg}^{-1}$  corresponding to 6 and  $60 \mu\text{g kg}^{-1}$  in fresh/frozen and dried  
35 matrix, respectively. The method was validated by single-laboratory tests of repeatability (within  
36 and between days) and tests of recoveries. The combined uncertainty calculated at low level for  
37 frozen ( $0.015 \text{ mg kg}^{-1}$ ) and high level for dried ( $2 \text{ mg kg}^{-1}$ ) material was 13% and 10%, respectively  
38 (both of them considering a coverage factor of 2). Application of this method to analyse some real  
39 samples of fresh, frozen and dried mushrooms shows as results ranges of nicotine content of  $0.01$ -  
40  $0.04 \text{ mg kg}^{-1}$  and  $0.1$ - $4.5 \text{ mg kg}^{-1}$  in fresh/frozen and dried matrixes, respectively. Results of some  
41 experiments performed in laboratory to dry fresh and frozen mushrooms show that conditions of  
42 drying process seem not influence the nicotine content in final dried samples.  
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## 57 **References**

58  
59 Hibi, N., Higashiguchi, S., Hashimoto, T. and Yamada, Y. (1994). Gene expression in tobacco low-  
60 nicotine mutants. *Plant Cell* 6:723-735.

1  
2  
3  
4 Andersson, C., Wennstrom, P. and Gry, J. (2003). Nicotine alkaloids in Solanaceous food plants.  
5 TemaNord 2003:531.  
6  
7

8  
9 Ribeiro, B., Andrade, P.B., Silvia, B.M., Bapatista, P., Seabra, R.M., Valentao, P. (2008).  
10 Comparative study of free amino acid composition of wild edible mushroom species. J. Agric. Food  
11 Chem. 56:10973-10979.  
12  
13

14  
15  
16 BfR (2009). Nikotin in getrockneten Steinpilzen: Ursache der Belastung muss geklärt werden.  
17 Stellungnahme 009/2009 des BfR vom 28. Februar 2009.  
18  
19

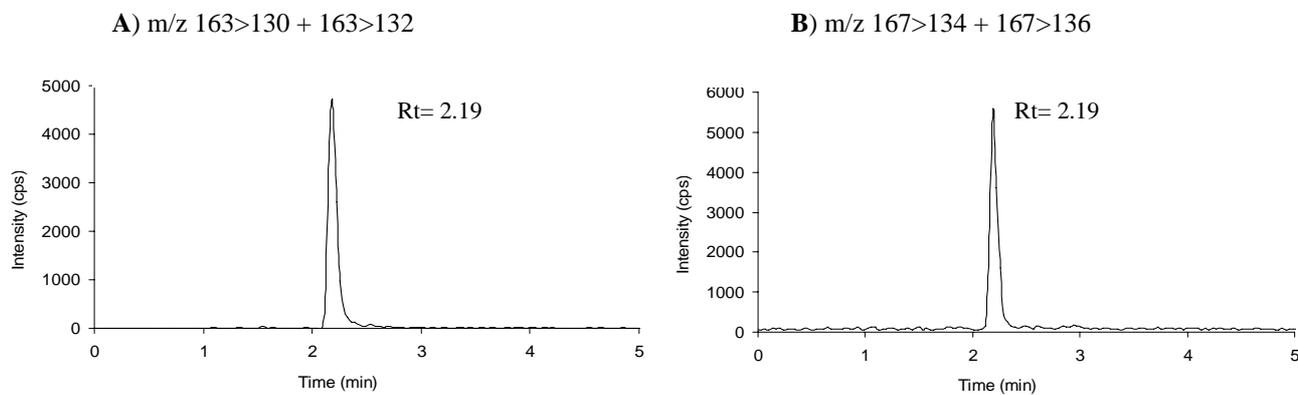
20  
21 Castro, A. and Monji, N. (1986). Dietary nicotine and its significance in studies on tobacco  
22 smoking. Biomed. Arch., 2:91-97.  
23  
24

25  
26  
27 Sheen, S.J. (1988). Detection of nicotine in foods and plant materials. J. Food Sci., 53:1572-1573.  
28  
29

30 Siegmund, B., Leither, E. and Pfannhauser, W. (1999). Determination of the nicotine content of  
31 various edible nightshades (Solanaceae) and their products and estimation of the associated dietary  
32 nicotine intake. J. Agric. Food Chem., 47:3113-3120.  
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## Figure

Fig. 1 . Sums of the two most abundant masses transitions for nicotine (**A**) and ( $\pm$ )-Nicotine-D<sub>4</sub> internal standard (**B**) peaks in a dried contaminated mushrooms sample. Nicotine concentration 1.73 mg kg<sup>-1</sup>; . I.S. 1.50 mg kg<sup>-1</sup>



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## Tables

**Table 1.** Average data and relative standard deviations of nicotine determination in recoveries, within-day and between-day repeatability tests

Type of matrix (mushrooms)	Repeatability				Recovery		
	within day		between day		Spiking level (mg kg <sup>-1</sup> )	Recovery (%)	RSD (%)
	nicotine (mg kg <sup>-1</sup> )	RSD (mg kg <sup>-1</sup> )	nicotine (mg kg <sup>-1</sup> )	RSD (mg kg <sup>-1</sup> )			
Dried	2.0	0.1	2.0	0.1	0.2	122	6.5
					2	107	2.9
Frozen	0.02	0.002	0.02	0.002	0.05	108	10.1

**Table 2.** Data of the drying experiments on some fresh and frozen mushroom samples

Experiment	Sample *	Drying conditions	Dried weight (Dw)	Fw/Dw	Nicotine in fresh/frozen sample (Nf) (µg kg <sup>-1</sup> )	Nicotine in dried sample (Nd) (µg kg <sup>-1</sup> )	Nd/Nf
1	fresh	70°C, vacuum, 2 h	1.27	8	45.4	319	7
2	fresh	70°C, vacuum, 2 h	1.28	8	45.4	321	7
3	frozen	70°C, vacuum, 2 h	0.92	11	20.1	309	15
4	frozen	70°C, vacuum, 2 h	0.91	11	20.1	252	12
5	frozen	70°C, vacuum, 2 h	0.88	11	20.2	239	12
6	frozen	70°C, vacuum, 2 h	0.88	11	20.2	213	11
7	fresh	sun light	1.3	8	45.4	271	6
8	fresh	sun light	1.3	8	45.4	283	6
9	frozen	sun light	0.91	11	20.2	151	7
10	frozen	sun light	0.90	11	20.2	162	8

\* The weight of fresh/frozen sample was 10 g for each experiment