Nicotine analysis in several non-tobacco plant materials

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Background

- Nicotine is found naturally in plants from *Solanaceae* family. The plants from *Nicotiana* genus contain large levels of nicotine. The plants from *Solanum* genus including potato, tomato, eggplant, and from *Capsicum* genus, which are used as food, also contain nicotine but at trace level.
- The results previously reported in the literature regarding the level of nicotine in such plant materials differ significantly from study to study, some as much as 300 times for the same plant material.
- Present study evaluated the level of nicotine in a number of plants (fruits, roots, leaves, tubers) from *Solanaceae* family (not including *Nicotiana* genus) and from several other vegetables commonly used as food, with the goal of finding the correct levels of nicotine.

Levels of nicotine in ng/g reported in the literature for several *Solanaceae* plants

Food source	Ref. [1]	Ref. [2]	Ref. [3]	Ref. [4]	Ref. [5]
Potato	Not reported	Not detected	15.3 ± 1.7	7.1 ± 5.9	4.5 ± 2.2
Potato skin	Not reported	14800	4.8 ± 0.8	Not reported	Not reported
Tomato	6.0 ± 2.4	2310	5.1 ± 0.8	4.1 ± 1.8	2.4 ± 1.2
Eggplant	100	2650	Not detected	Not reported	1.9 ± 0.7
Green pepper	5.7	3150	Not detected	Not detected	3.7 to 6.1
Red pepper	Not reported	Not reported	Not reported	Not reported	5.9

1. Castro, A., Monji, N.; *Biomed. Arch.*, 2 (1986) 91-97.
2. Sheen, S.J.; *J. Food Sci.*, 53 (1988) 1572-1573.
3. Davis, R.A., Stiles, M.F., Debethizy, J.D., Reynolds J.H.; *Food Chem. Toxicol.*, 29 (1991) 821-827.
4. Domino, E.F., Hornbach, E., Demena, T.; *Med. Sci. Res.,* 21 (1993) 571-572.
5. Siegmund, B., Leitner, E., Pfannhauser, W.; *J. Chromatogr. A,* 840 (1999) 249-260.

Method description (measurement of moisture)

- The first step before performing the nicotine analysis was the measurement of samples moisture.
- The plant materials were placed in aluminum pans (14.5 cm x 8.4 cm x 4.7 cm). For fruits, roots, and tubers, about 50 g of plant material were precisely weighed. For leaves, between 10 g and 20 g were precisely weighed.
- The plant materials were placed for 24 hours in a convection oven at 102 ^{o}C.
- The moisture (water content) was calculated from the weight difference (moist vs. dry).

Method description (sample extraction)

- Based on the water content, samples the equivalent to 300 mg dry material were placed in 50 mL screw-cap vials.
- Special precautions were taken not to contaminate the samples with external sources of nicotine, assuring the cleanliness of the utensils used to cut or handle the samples (knives, tweezers).
- To each vial were added 10 mL aqueous solution 5% NaOH.
- The samples were placed in a heating block for 30 min at 70 ^{o}C with occasional agitation.
- After the samples were cooled, 2 mL *tert*-butyl methyl ether (tBME) and 20 µL of an internal standard solution of d_3-nicotine in tBME were added.

Method description (sample extraction), cont.

- The internal standard solution contained 16 μg/mL d3-nicotine.
- The mixtures were agitated for 30 min on a wrist-action shaker.
- The sample solutions plus extractant were transferred in 50 mL polypropylene conical tubes and centrifuged for 5 min at 3000 rpm.
- The separation of the organic layer was good, and allowed the transfer of about 200 mL from each sample into vial inserts placed in 2 mL GC vials.
- These extracts were submitted for GC-MS/MS analysis.

Method description (GC separation)

- The column was a CAM 30 m x 0.25 mm i.d. with a film of 0.25 □m thick from Agilent (J&W) installed in a 7890B GC system from Agilent (Agilent Technologies Inc. Wilmington, DE, USA).

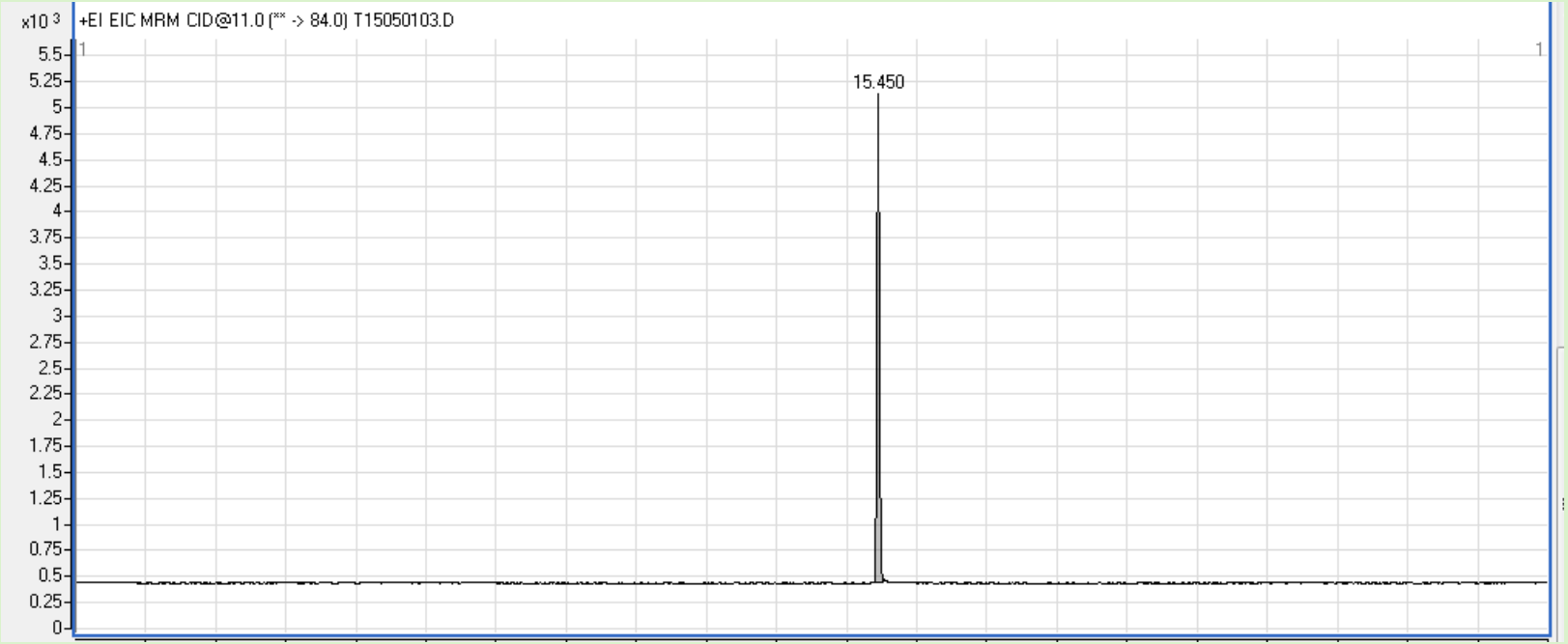
Parameter	Description	Parameter	Description
Initial oven temperature	50°C	Purge flow to split vent	15.0 mL/min
Initial time	1.0 min	Carrier gas	Helium
Oven ramp rate	10°C/min	Injection volume	1.0 mL
Oven temperature 1	240°C	Flow mode	Constant flow
Final time	5.0 min	Flow rate	1.0 mL/min
Total run time	25.0 min	Nominal initial pressure	12.05 psi
Inlet temperature	250°C	Average velocity	25.485 cm/sec
Inlet mode	Pulsed splitless	GC outlet	MS/MS
Pulse pressure	20 psi	Aux. temperature	240°C
Pulse time	0.75 min	Solvent delay	4.0 min

Method description (MS/MS detection)

- The analysis was performed using a 7890B/7000C GC-MS/MS system from Agilent.

Parameter	Description	Parameter	Description
Ion source	EI	MS1 resolution	Unit
Acquisition mode	MRM (positive)	MS2 resolution	Unit
Source temperature	230°C	Dwell time	120 ms
Collision cell He	2.25 mL/min	CE	11 V
N_2 collision gas	1.5 mL/min	Nicotine precursor ion	162.1
Gain factor	10	Nicotine product ion	84.1
Nicotine precursor ion	162.1	MS1 resolution	Unit
Nicotine product ion	84.1	MS2 resolution	Unit
d3-Nicotine precursor ion	165.1	Dwell time	120 ms
d3 Nicotine product ion	87.1	CE	11 V

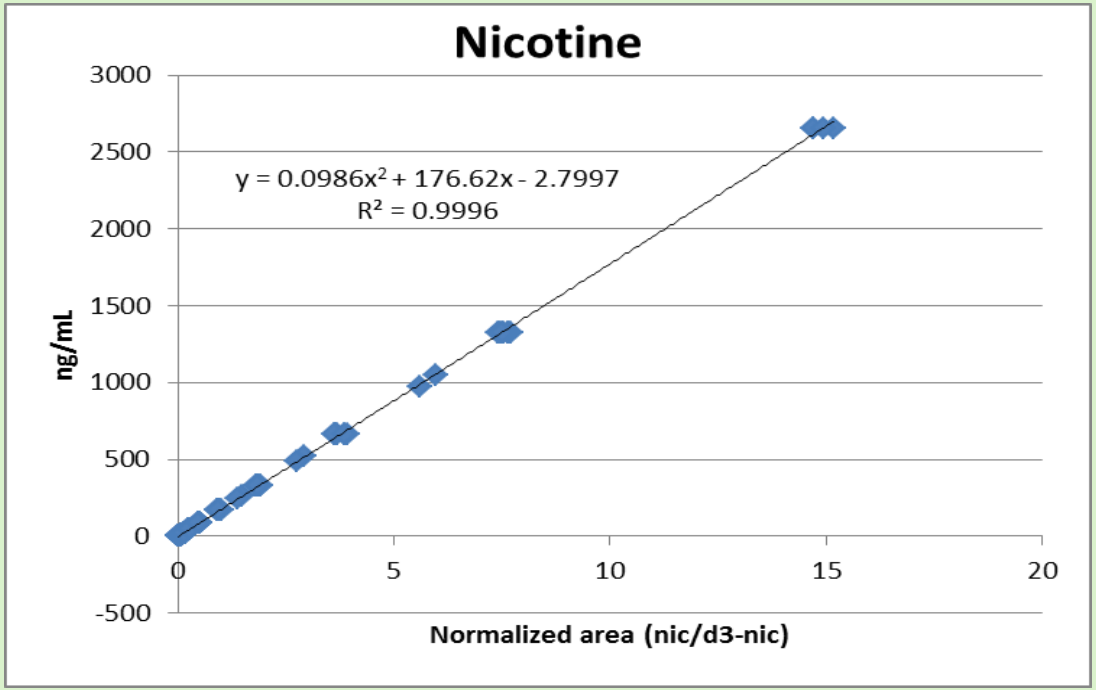
Example of extracted ion 84 from a nicotine standard containing 10.4 ng/mL.



Signal to noise S/N = 1379

Method description (quantitation)

- The quantitation for nicotine was performed using calibrations of (Amount nicotine) vs. (Peak area nicotine/Peak area I.S) with a set of 12 standards in the range 2655.0 ng/mL to 2.6 ng/mL nicotine, and I.S. 160 ng/mL d3-nicotine.

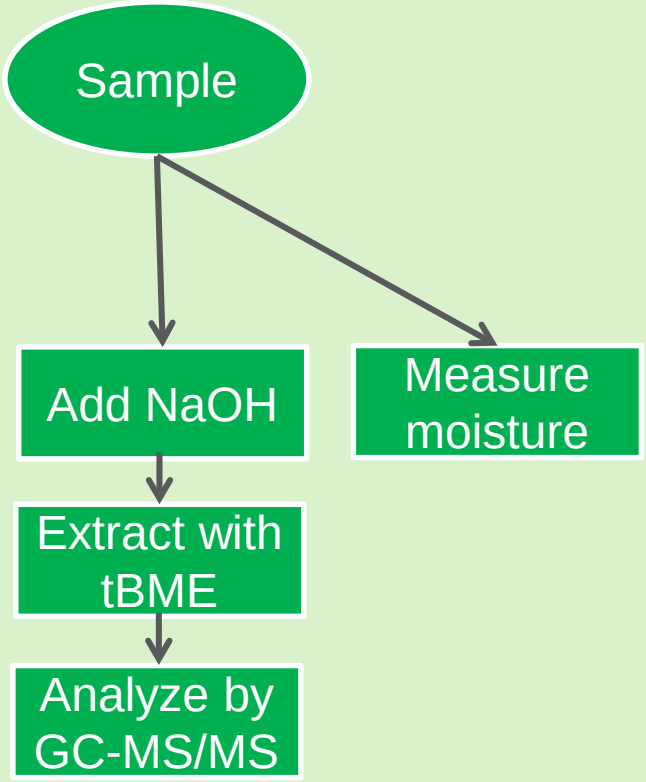


Method validation

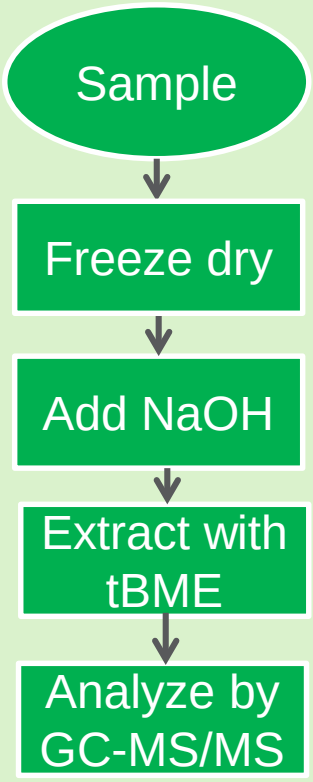
- Selectivity assured by the use of MS/MS detection with specific selection of the precursor and product ion in MRM operation mode.
- The precision evaluated from ten repeated analyses of a standard containing 10.4 ng/mL nicotine that generated a RSD% = 3.9%.
- Linearity generated by calibration with R^2 = 0.9996.
- LOQ very low, with a signal to noise ratio S/N = 1379 for the peak with 10.4 ng/mL.
- Recovery 98.6% for triplicate samples spiked with 435 ng nicotine.

Results

- Current procedure



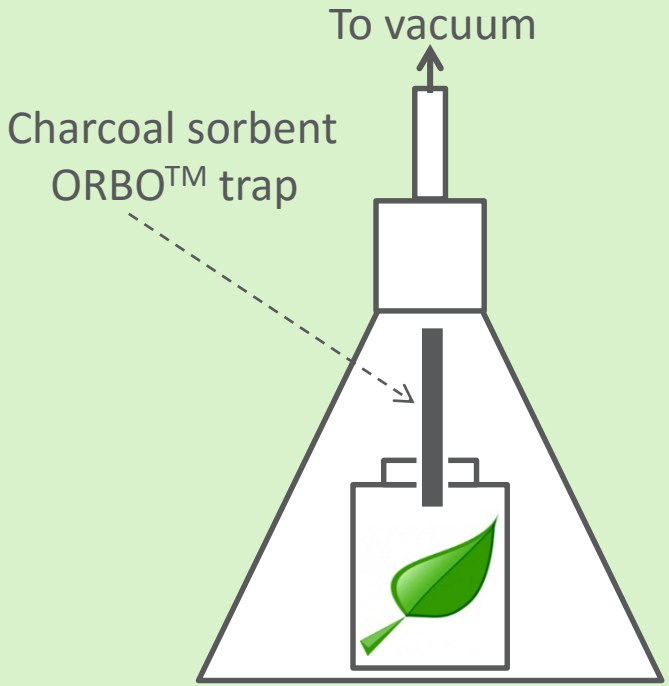
- Initial procedure



Initial WRONG results obtained using freeze drying of the sample

Sample	Average ng/g	Average µg/g	RSD%
Potato skin removed	4.0	0.004	~ 15.0
Potato with skin	4.1	0.004	~ 15.0
Eggplant	197.3	0.197	2.9
Tomato	549.8	0.550	3.8
Potato leaf	1162.8	1.163	0.2
Pepper leaf	618.2	0.618	0.3
Eggplant leaf	2129.6	2.130	0.3
Tomato leaf	795.8	0.796	0.6
Green apple	2295.3	2.295	0.4

Analysis of *Magnolia Virginiana* leaf



Nicotine results

Sample	Average ng/g	Average µg/g	RSD%
Magnolia leaf as is	5.1	0.005	3.4
Magnolia leaf ground	5.5	0.006	2.8
Magnolia leaf freeze dried	1255.1	1.255	0.3
Magnolia leaf freeze dried isolated through Orbo trap	9.5	0.010	2.4
Magnolia leaf spiked with 428 ng nic and freeze dried through Orbo trap	340.9	0.341	0.2

Results on water content

Sample	No. of samples	Average water %
Tomato	4	95.88
Tomato organic	2	95.44
Tomato leaf (young plant)	4	88.45
Eggplant	2	93.76
Baby eggplant	2	95.27
Potato (whole)	2	82.80
Green pepper	2	95.22
Carrot	2	88.55
Carrot leaf	2	86.64
Banana	2	79.95
Pear	2	86.76
Green apple	2	86.78
Blueberry	2	88.36
Strawberry	2	94.20

Nicotine levels in various plant material in ng/g dry material

Sample	No. of samples	Average ng/g	RSD%
Blank	5	6.4	19.9
Tomato	4	181.9	3.4
Tomato organic	2	82.3	2.8
Tomato leaf (young plant)	4	184.4	4.3
Eggplant	2	174.3	1.6
Baby eggplant	2	94.6	5.9
Potato (whole)	2	42.6	1.0
Green pepper	2	74.1	1.0
Carrot (root)	2	18.2	3.1
Carrot leaf	2	44.6	2.6
Banana	2	8.9	24.9
Pear	2	10.5	3.9
Green apple	2	1.9	10.8
Blueberry	2	8.9	2.7
Strawberry	2	23.5	8.6

Comparison with previously reported results in ng/g dry plant

Food source	Ref. [1]	Ref. [2]	Ref. [3]	Ref. [4]	Ref. [5]	This study
Potato	Not reported	Not detected	15.3 ± 1.7	7.1 ± 5.9	4.5 ± 2.2	42.6 ± 0.4
Potato skin	Not reported	14800	4.8 ± 0.8	Not reported	Not reported	Not reported
Tomato	6.0 ± 2.4	2310	5.1 ± 0.8	4.1 ± 1.8	2.4 ± 1.2	181.9 ± 6.1
Eggplant	100	2650	Not detected	Not reported	1.9 ± 0.7	174.3 ± 2.8
Green pepper	5.7	3150	Not detected	Not detected	3.7 to 6.1	74.1 ± 0.7
Red pepper	Not reported	Not reported	Not reported	Not reported	5.9	Not reported

Conclusions (on the method)

- A method for the analysis of nicotine at ng/mL level has been established.
- The method is based on “traditional” nicotine analysis procedures with addition of NaOH solution to the sample and extraction with tBME.
- The extraction uses heating at 70 ^{o}C to assure the decomposition of any molecular associations of nicotine with plant proteins.
- The detection is done using GC-MS/MS in MRM mode.
- Nicotine precursor ion is 162.1 and product ion 84.1.
- d3-Nicotine is used as internal standard.
- The method has been fully validated.

Conclusions (on the analysis results)

- Tomato, egg plant have levels of nicotine around 180 ng/g.
- The plant leaf shows similar levels of nicotine.
- Lower levels were measured for green pepper (74.1 ng/g), and even lower (42.6 ng/g) for potatoes.
- Other plant materials such as carrot, banana, pear, and blueberries showed a level around 10 ng/g of nicotine, which may be caused by contamination.
- Contamination may be the cause of high nicotine levels reported in one literature reference.
- Lack of sensitivity, incomplete extraction, and losses during extraction may be among the causes of low nicotine levels reported in other literature references.