Characterization of e-cigarettes liquid contents and transformation products by LC-MS, GC-MS and ICP-MS techniques

Characterization of e-cigarettes liquid contents and transformation products by LC-MS, GC-MS and ICP-MS techniques.

*Claudio Medana, Cecilia Sala, Raffaele Pellegrino, Riccardo Aigotti, Federica Dal Bello, Giancarlo Bianchi and Enrico Davoli**

**Department of Molecular Biotechnology and Health Sciences, University of Turin, via P. Giuria 5 , 10125 Torino – Italy*

#Department of Environmental Health Sciences, IRCCS Mario Negri Institute, via La Masa 19, 20156 Milano – Italy

E-mail: claudio.medana@unito.it – enrico.davoli@marionegri.it

OVERWIEW

- A fast and sensitive LC-MS² method to detect nicotine and analogues in e-cigarette refill liquids was developed and validated
- We searched for volatile organic and metal inorganic contaminants in polyalcoholic e-liquids solvents
- The formation of toxic degradation compounds from polyalcoholic e-liquids solvents was evaluated using a modified smoking machine

INTRODUCTION

While it is stated that e-cigarettes are less toxic than regular cigarettes, there are still safety and health issues that need to be clarified before they can be said to be safe and clean.

The purpose of this study was to develop analytical MS methods to investigate both on **chemical composition of e-liquids**, characterize their quality and monitoring the aerosol composition during operation of e-cigarette. Final objective was the evaluation of **toxic compounds**, present in liquids and formed, as transformation products.

Low-quality nicotine, glycerol, propylene glycol or flavors could greatly increase the toxicity. Quantitative determination involved nicotine contaminants as pro-carcinogen NOR-nicotine, toxic ethylene glycol and residues of volatile toxicants and metals present in e-liquids. Pyrolysis-originated aldehydes were also measured.

DISCUSSION

The nicotine concentration in samples analyzed strongly disagree from the stated quantity.

By analyzing VOC fraction we could observe significant organic solvents contamination in a number of cases.

Finally, we observed the formation of new products, like acetaldehyde and acrolein, during the vaporization. Glycols undergo pyrolysis during vaporization, and this process seems to be specific for liquids with different composition and for different vaporizer designs, and needs to be characterized.

This brings us to the observation that inhaled “vapors” are not just vaporized e-liquids, but mixtures of new compounds that will necessarily have to be investigated and defined[3].

CONCLUSIONS

Different MS approaches were developed to characterize e-liquids main components and contaminants. Pyrolysis formation of aldehydes was demonstrated with a smoking machine.

A new regulation and quality control of e-liquids and e-cigarettes are important issues in smoking and tobacco related medical research.

Reproduction of a Maya priest smoking from the Temple at Palenque, Mexico , *Wikimedia Commons*

METHODS

LC-MS/MS OF ALKALOIDS

Evaluation of alkaloids content in e-liquids

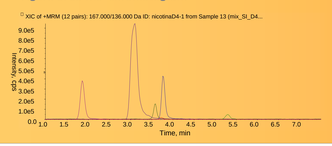
We optimized the LC-MS separation of highly hydrophilic nicotine related compounds (**nicotine, cotinine, anabasine, myosmine, nornicotine and N-nitroso-nornicotine**) on an ion pair RP-HPLC chromatographic system. A full validation study was then completed to make possible quantitative determination on commercial e-liquid samples. Internal standard: nicotine-*D4*

Sample preparation: water-ACN dilution.

LC: Shimadzu Nexera, Kinetex 1.7 µm C18 100 x 2.1mm column, HFBA 2.5 mM – ACN gradient , 0.5 mL min^{-1} Injection volume : 5 µL.

MS: AB Sciex 5500 Q-trap, ESI source, MRM acquisition.

Figure 1: LC-MS² chromatogram



Analite	Q1 (*m/z*)	Q3 (*m/z*)	Retention time SD (min)
anabasine	163	146, 120	5.44 0,02
cotinine	177	98, 80	1.88 0,02
myosmine	147	130, 118	3.66 0,25
nicotine	163	132, 106	3.81 0,07
nicotine-*D4*	167	136, 110	3.82 ± 0,06
NNN	178	148, 120	3.11 0,04
Nor-nicotine	149	130, 80	3.62 0,07

GC-MS VOCs – ICP-MS METALS

Evaluation of VOCs content in e-liquids

In order to identify and quantify volatile organic contaminants we apply the GC-MS methodology normally employed for drinking water analysis (EPA8260). Purge and Trap analysis was performed with chlorobenzene-*D5*, 1,4-dichlorobenzene-*D4* and fluorobenzene as internal standards.

Sample preparation: water-dilution and P&T extraction.

GC: Varian Saturn 4000, equipped with a Tekmar Purge and Trap concentrator and a PTV Varian 1177 injector. Varian VF624 30 m, i.d. 0.25 mm column. T 35 - 250 C. Injector temperature 170 C / splitless. Helium gas. Injection volume : 2 µL

MS: Varian Saturn 2100 T ion trap analyzer.

Figure 2: VOCs GC-MS chromatogram evidencing benzene contamination

Evaluation of heavy metal content in e-liquids

Heavy metal content was determined after water dilution by **ICP-MS** on an Agilent 7700 instrument with quadrupole analyzer. Plasma: Argon 15 mL min^{-1}; nebulizing gas: 1 L min^{-1}. RF power 1.55 kW.

SPME GC-MS OF ALDEHYDES

Evaluation of aldehyde formation in e-cigarettes smoking

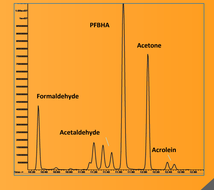
Smoking machine procedure: A modified Borgwaldt RM 1/G-R58.02 smoking machine was used. Two different commercial e-cigarettes were tested, producing 70 mL of aerosol, with 20 s interval time. The generated aerosol was collected in glass vials with septa allowing to expose SPME fiber. Internal standard: acetaldehyde-*D4*.

Sample preparation: aerosol samples from smoking machine were extracted using SPME, i.e., exposing a 2-cm triphasic divinylbenzene / carboxen /polydimethylsiloxane fibre saturated with o-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) into the headspace .

GC: Agilent GC5890. Varian CP-Select 624 CB 60 m, i.d. 0.32 mm column. T 80 - 220 C. Injector temperature 250 C / splitless. Helium gas. Injection volume : 2 µL

MS: Agilent MSD5975C quadrupole analyzer.

Figure 3: derivatized aldehydes GC-MS chromatogram evidencing acrolein presence



RESULTS

LC-MS/MS OF ALKALOIDS

Alkaloid LC-MS quantitation: main results:

LC-MS/MS method validation: LOQ 0.5-20 ng mL^{-1}; selectivity < 21%, intra-run repeatability 3-14%; inter-run repeatability 5-20%; precision (RSD% of ACC%) < 13%. Room temperature stability 24 h. Recovery 98%. Total analysis time 15’.

We analysed several e-liquids, from different producers, produced in Italy, China, Poland and Germany. The nicotine concentration in analyzed samples is not compliant with declared values. We found differences between declared and actual concentrations ranging from -70% to +20%. This has been observed by other authors too[1,2], indicating that it is a common problem in the e-cigarette market.

No significant contamination by N-Nitroso-Nicotine (NNN) or minor alkaloids was detected (> 0.02% of nicotine)

Scheme 1: nicotine ESI MS/MS main fragmentation pathways

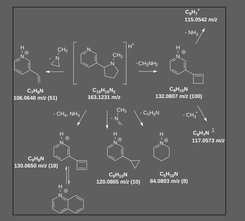


Table 1: nicotine quantitation in selected e-liquid samples

Brand A; different flavors and nicotine content	Measured **NICOTINE (mg/mL)**
TOBACCO, NICOTINE 9 MG/ML	8.18 ± 0.52
MINT, NICOTINE 9 mg/mL	9.40 ± 0.61
CIGAR, NICOTINE 9 mg/mL	8.70 ± 0.94
SWEET, NICOTINE 18 mg/mL	13.26 ± 1.11
MENTOL, NICOTINE 9 mg/mL	8.18 ± 0.72
MINT, ZERO NICOTINE	0.015 ± 0.0006
TOBACCO, ZERO NICOTINE	0.012 ± 0.0005
MENTOL, ZERO NICOTINE	0.011± 0.0005
Medium content verified	*91%*

Brand B; different flavors and nicotine content	Measured **NICOTINE (mg/mL)**
TOBACCO 1, NICOTINE 18 mg/mL	3.73 (21% than the declared)
TOBACCO 2, NICOTINE 18 mg/mL	4.48 (25% than the declared)
MINT, NICOTINE 18 mg/mL	9.6 (53% than the declared)
TOBACCO 1, NICOTINE 18 mg/mL	12.6 (70% than the declared)
CARAMEL, NICOTINE 9 mg/mL	8.48 (91% than the declared)
Medium content verified	*52%*

GC-MS VOCs – ICP-MS METALS

VOCs GC-MS quantitation: results:

By analyzing VOC fraction we could observe benzene, styrene and ethanol significant contamination in a number of cases.

Solvent impurities are related to low-quality nicotine raw material used.

VOCs ICP-MS quantitation: results:

Heavy metal are not significant pollutants of e-liquids, with the exception of strong contaminated outlier samples (*Table 3).

Table 2: main VOCs contaminants quantitation in selected e-liquid samples

Brand A, B, different flavors *and* nicotine content	BENZENE (µg/g)	TOLUENE (µg/g)	ALKYL-BENZENES (µg/g)	ETHANOL (%)
KIWI, NICOTINE 11 mg/mL	10.6	< 0.05	20.2	1.8
TOBACCO, NICOTINE 18 mg/mL	29.3	0.17	3.4	1.8
TOBACCO 2, NICOTINE 11 mg/mL	17.6	0.31	0.40	2.0
TOBACCO, NICOTINE 0 mg/mL	0.42	< 0.05	<0.05	2.5
TOBACCO 2, NICOTINE 0 mg/mL	0.27	0.08	<0.05	1.9
GREEN, NICOTINE 18 mg/mL	13.5	0.29	0.51	0.70
SWEET, NICOTINE 11 mg/mL	3.3	0.11	42.3	3.6
TOBACCO 3, NICOTINE 16 mg/mL	0.21	0.38	1.36	< 0.05
TOBACCO 4, NICOTINE 16 mg/mL	0.23	0.42	0.78	< 0.05
ORANGE, 16 mg/mL	< 0.05	0.12	20.2	1.2
PEACH, 6 mg/mL	< 0.05	< 0.05	1,6	2.5
MINT, 11 mg/mL	< 0.05	1.1	23.8	2.0
BEER, 11 mg/mL	< 0.05	0.12	3.6	0.31

Table 3: main metal contaminants quantitation in selected e-liquid samples

Sample	CHROMIUM (µg/L)	NICKEL (µg/L)	ARSENIC (µg/L)	LEAD (µg/L)
1	36.84	2.90	17.50	0.50
2	36.30	0.60	3.50	0.40
3	38.10	0.60	12.70	0.10
4	43.50	2.30	2.90	0.30
5	49.40	10.00	9.80	3.40
6	45.60	0.70	13.40	0.60
7	50.70	2.40	1.10	0.50
8	49.20	6.60	16.20	8.30
9	52.10	1.90	27.50	0.40
*10**	412.62	307.65	35000	89.43

SPME GC-MS OF ALDEHYDES

Generated aldehydes GC-MS quantitation: results:

By analyzing e-cigarette generated aerosol (5 puffs) we could observe acetaldehyde and acrolein significant formation.

Aldehydes formation depends on puff time and e-cigarette model (different operating temperature).

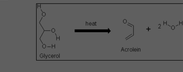
The studied reaction is not influenced by the presence or absence of nicotine.

Figure 4: Smoking machine generating aerosol from e-cigarette

Table 4: Aldehydes formation evaluated in e-cigarette smoking

	ACETALDEHYDE (peak area/ I.S peak area)				ACROLEIN (peak area/ I.S peak area)			
Puff time	*2 s*	*5 s*	*7 s*	*10 s*	*2 s*	*5 s*	*7 s*	*10 s*
E-CIGARETTE A	29.7	14,7	3.1	85.8	0.2	0.3	0.04	0.6
E-CIGARETTE B	3.8	3.9	12.0	6,7	<LLOD	<LLOD	0.2	0.1

Scheme 2: acrolein pyrolysis formation from glycerol



References: 1) M.L. Goniewicz, T. Kuma, M. Gawron, J. Knysak, L. Kosmider. Nicotine levels in electronic cigarettes. *Nicotine & Tobacco Research* **15**, 158-166 (2013)

2) P. Kubica, A. Kot-Wasik, A. Wasik, J. Namieśnik. “Dilute & Shoot” approach for rapid determination of trace amounts of nicotine in zero-level e-liquids by RPLC and HILIC coupled with tandem MS-ESI. *J. Chrom. A*. **1289**, 13-18 (2013)

3) E. Davoli, C. Medana, S. Garattini. E-cigarette regulation needs: production, sale and use. *British Medical Journal*, rapid response to **346**:f3418 (2013) . http://www.bmj.com/content/346/bmj.f3418/rr/661922