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WP8 - D8.4 Report on the replication of laboratory measurements

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JOINT ACTION ON TOBACCO CONTROL

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Version	Date of creation	Title	Handling
1	20/11/2020	Initial document	WP8 IRFM partners
2	16/12/2020	First D 8.4 draft	WP8 Objective leaders.
3	17/12/2020	Final draft approved by WP8 objective leaders	WP8 Partners JATC Coordination Team

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Background

Specifically, for tobacco control, the work programme notes that "Reporting, assessing and regulating tobacco ingredients are important aspects of an effective and comprehensive tobacco control policy which contributes to the broad objective of promoting good health, reducing tobacco related disease and deliver real benefits to citizens".

Laboratory measurements are essential for effective application of various provisions of Directive 2014/40/EU of the European Parliament and of the Council of 3 April 2014 (the Tobacco Products Directive, TPD)

In Work Package 8 (WP8) -Laboratory verification, collaboration and analyses- in order to support the building up of appropriate laboratory capacity, to verify the submitted data and support the enforcement, we aimed to identify, organise and network the laboratory capacities across EU MS and work to develop homogenised approaches in product evaluation.

Original Objective WP8 Objective 3 -To develop collaborations and communication with other international activities on tobacco laboratory assessment- was designed to review laboratory activities routinely performed by MS competent authorities, critically evaluating the quality of these activities and the independence of the corresponding laboratories. Available analytical data was to be processed using pooled- or meta-analytical approaches with the purpose of making comparisons between the same products measured through different laboratories.

During internal meetings of WP8 partners, conferences and meeting of former GoToLab members, it appeared that collection of original data from EU labs was not feasible, due to different national rules within EU about data disclosure.

For this reason, during the 2nd JATC Interim Meeting that took place in Brussels (4-5/2/2020), and during the 2nd Internal meeting (switched to a TC meeting due to the COVID pandemic), we decided to move to a strategy to obtain original data instead of routine data. Since JATC was not providing funds to perform chemical analyses, we organized a collaborative project, among MS independent laboratories, in order to be able to review quality of laboratory activities, and to perform replicate measurements on data across laboratories.

At present, we obtained both these information from a large number of EU independent labs, that allowed to assess comparability across them (obj.2 for D 8.3), and we achieved collaboration and communication also with other international activities (GoToLab & TobLabNet) to enhance collaboration on the area of tobacco evaluation (obj.3, D8.4).

Methodology

During the 2nd JATC Interim Meeting which took the week 4-5/2/2020 in Brussels, attended by representatives of the majority of the Institutions that participate in JATC (beneficiaries & collaborating partners), we proposed the following strategy to acquire data from laboratories within EU, through a collaborative exercise, an Inter-laboratory Test, named "Am I doing It Right?".

The method conceived was a multi-step design based on:

1. developing an EU Standard Operating Procedure (SOP) for a simple and cheap analysis (nicotine in e-liquids);
2. involve independent laboratories, identified during previous work (see D8.2) on a voluntary basis;
3. acquire real data and analyse results in order to understand quality and inter-laboratory variability of EU MS data.

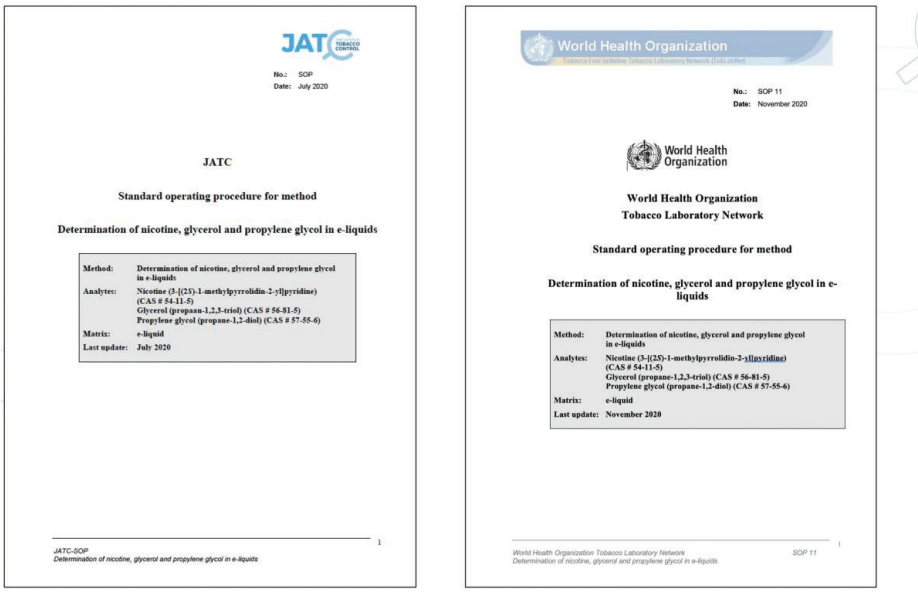
Nicotine has been chosen because of new method proposal. ISO NP 4352 was undergoing at the time, on nicotine, glycerol and propylene glycol by HPLC/MS, but since nicotine in e-liquids was

analysed by an ISO method designed for world-wide needs, while in EU we have nicotine limitation at 20 mg/mL, a new method was necessary in order to encounter EU needs. Moreover, nicotine is an analyte that laboratories perform already and it could be performed with minimal time and economical efforts by independent laboratories.

Another activity has been the basis for Objective 3: to develop collaborations and communication with other international activities on tobacco laboratory assessment, which is the main subject of this Deliverable 8.4.

A **main deviation** occurred from the original project: as already explained, in order to obtain information relative to laboratories performing analysis on tobacco and e-cigarettes, understand laboratory capacity and the quality of their activities, original data were needed. However, since it was not feasible, due to different national rules within EU about data disclosure, we decided to move to a new strategy and collect original data instead of routine data. This lead to the approach described in Deliverable 8.3 and to the development of a Standard Operating Procedure, which is now becoming an official WHO TobLabNet method (WHO TobLabNet SOP 1, Fig. 1)

Figure 1: Cover page of JATC-SOP developed and the draft of the WHO SOP 11 that is based on the results obtained in JATC-SOP



THIS ACTIVITY IS PART OF THE PROJECT / JOINT ACTION '761297 / JATC' WHICH HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HEALTH PROGRAMME (2014-2020).

Participants involved & equipment

As previously described in Deliverable 8.3, five test samples of e-liquids with different concentrations of nicotine, glycerol and propylene glycol were sent in replicates to the 31 laboratories participating to the study, 32 if we also consider Istituto di Ricerche Farmacologiche Mario Negri in Milan which prepared the samples. They were asked to analyse these e-liquids with the method, described in the provided SOP and study protocol, and to send back their results in order to evaluate repeatability and reproducibility of the different laboratories participating to the study and in this way obtain information on their capabilities, quality and defined capacity.

Laboratory involved were:

17 different EU independent laboratories

27 considering TobLabNet laboratories

32 considering academic participants

Countries involved were:

Germany, Belgium, The Netherlands, Spain, Greece, Ireland, Latvia, France, Luxembourg, Slovenia, Hungary, Italy for JATC and USA, Indonesia, Costa Rica, Burkina Faso, Japan, China, South Korea, Bulgaria, and Singapore.

In some cases, more than one laboratory for each country participated. They were asked to fill in a sheet with their results and to indicate the instrumentation used for the analysis.

The following table shows instrumentation used by each participant: most of them used GC-FID, which was the main approach prescribed by the SOP. However, it was suggested that different instrumentation could be used, as for example GC-MS. Some participant decided to use GC-MS or both GC-MS and GC-FID. One of the participants also used LC-MS for nicotine. This table was already presented in D8.3, but it has been updated, since new participants reported their results (Table 1).

All raw data is reported in Annex 1.

Statistical evaluation was conducted both on GC-FID and GC-MS results and showed that, even if results from mass spectrometry are less in number than those for flame ionization detector, they are comparable in reproducibility and repeatability, as shown in Deliverable 8.3, GC-MS can be considered a valid alternative to GC-FID. This is very important since in this way more laboratories with different equipment could be involved. Moreover, GC-MS can be used to easily analyse other components, such as flavours in e-liquids.

Table 1: Laboratories codes and equipment used in analytical method

Lab code	Detector	Type of GC column	Details
Lab 1	FID	Agilent DB-ALC1 (30 m x 0,32 mm, 1,8 µm) + Zebron Guardcolumn (0,320 mm x 10 m)	
Lab 2	FID	HP Innowax (30 m x 250 µm x 0.25 µm)	
Lab 3	FID	Agilent DB-ALC1 (30 m x 0.32 mm,1.8 µm)	
Lab 4	FID	Factor Four ,VF-17, 30 m x 0.53 mm ID DF=1.00	
Lab 5	FID	Agilent DB-ALC1 (30 m x 0,32 mm, 1,8 µm)	
Lab 6	FID	Capillary HP INNOWAX 30m x 0,250mm	
Lab 7	FID	Agilent DB-ALC1 (30 m x 0,32 mm, 1,8 µm)	
Lab 8	FID	DB-35 capillary	
Lab 9	FID	Agilent DB-ALC1 (30 m x 0,32 mm, 1,8 µm)	
Lab 10	FID	CP-Wax 57 CB 50 m x 0,32 mm	
Lab 11 / Lab MS1	MS	Capillary VF-35 Ms 30m x 0.25 mm x 0.25 µm	For nicotine
	FID	Capillary 5 Ms 60m x 0.25 mm x 0.25 µm	For glycerol and propylene glycol
Lab 12	FID	Agilent DB-ALC1 (30 m x 0.32 mm,1.8 µm)	
Lab 13	FID	ZB-BAC1 30m x 0.32mm x 1.80um	
Lab 14 / Lab MS2	FID	Restek GC Columns Rtx®VMS (30 m x 0,53 mm ID; 3 µm)	Pipetting e-liquids
			Weighting e-liquids
	MS	DB-1701 / Capillary Column / 60m x 0.25mm x 0.25µm	Pipetting e-liquids
			Weighting e-liquids
Lab 15	FID	HP-Innowax	
Lab MS3	MS	Agilent DB-ALC1 (30 m x 0.32 mm,1.8 µm)	
Lab MS4	MS	1. Agilent DB-ALC1 (30 m x 0.32mm, 1.8 µm) and 2. ZB-WAXplus column (30m x 0.25mm x 0.25um)	
Lab 16	FID	Agilent DB-ALC1 (30 m x 0.32 mm,1.8 µm)	

Lab MS5	MS	Restek Rtx-BAC1, 30 m, 0.32 mm, 1.8 m, 5 inch format (Cat No 18003-Rtx-BAC1)	
Lab 17	FID	HP-PLOT/Q 30m x 40 um x 0,530 mm	
Lab 18	FID	Agilent Capilllaire HP-5% phenyl methyle siloxane (30 m x 0,32 mm x 0.25 µm)	
Lab 19	FID	Agilent DB-ALC1 (30 m x 0.32 mm,1.8 µm)	
Lab 20 / Lab MS5	FID	Agilent DB-ALC1 (30 m x 0.32 mm,1.8 µm)	
	MSMS	Atlantis T3 3um, 2.1 x 150mm	Liquid chromatography (rather than GC) – only for nicotine
Lab 21	FID	Agilent DB-ALC1 (30 m x 0.32 mm,1.8 µm)	
Lab 22	FID	Capillary Innovax 30 m	
Lab 23 / Lab MS6	FID	Agilent DB-ALC1 (30 m x 0.32 mm,1.8 µm)	
	MS	DB-WAX 30m x 0.32mm, 0.25um	
Lab MS7	MS	Capillary column 5% Phenyl Polysilphenylene-siloxane	
Lab 24	FID	Agilent DB-ALC1 (30 m x 0.32 mm,1.8 µm)	
Lab MS8	MS	Agilent DB-UI 8270D (30 m × 0.250 mm, 0.25 µm)	
Lab MS11	MS	Restek Rtx- BAC PLUS 1 (30m, 0,32mmID, 1,8um df)	

Results

Overall data

Statistical analysis was conducted on results in order to exclude outliers and evaluate laboratory capacity and consistency of results. In Deliverable 8.3, reproducibility and repeatability were reported. This time, in order to evaluate replication of laboratory measurements, we reported z-scores, calculated for both GC-FID and GC-MS results (Table 2; Table 3; Table 4).

Briefly, z-score is $z = (x-\mu)/\sigma$, where x is the raw score, µ is the population mean, and σ is the population standard deviation.

The z-score is a description of the mean for each sample of each lab in comparison to the overall mean for the specific component of the sample. It evaluates how many standard deviations results are away from the mean. If a z-score is equal to 0, it is on the mean, if a z-score is equal to -2, participant's results are 2 standard deviations below the mean.

When a participant reports a z-score above 3,0 or below -3,0, the result should be considered as an "action signal" (unsatisfactory). Likewise, a z-score above 2,0 or below -2,0 should be considered as a "warning signal" (questionable). Figures show z-scores values for the different laboratories with reference to the warning and action signals (Figure 1; Figure 2; Figure 3).

The z-score table will help to determine if results reported using other measurement techniques than prescribed in the SOP can also be used for the determination of the components. This is useful since repeatability and reproducibility were calculated only for data reported using the equipment as prescribed in the SOP (GC-FID), and data reported using other equipment as prescribed in the SOP (e.g. GC-MS) were not included in the repeatability and reproducibility.

Table 2: z-scores for nicotine (in yellow questionable results and in red unsatisfactory results)

Nicotine					
Lab code	Sample A	Sample B	Sample C	Sample D	Sample E
	z-score	z-score	z-score	z-score	z-score
Lab 1	0,38	-0,19	-0,30	-0,35	-0,27
Lab 2	-0,24	-0,16	-0,23	-0,26	-0,31
Lab 3	-0,26	0,01	-0,40	-0,16	-0,45

Lab 4	-0,63	-0,67	-0,52	-0,40	-0,36
Lab 5	2,65	1,32	0,29	-0,06	0,09
Lab 6	-0,48	0,11	4,78	4,62	4,96
Lab 7	-0,52	-0,28	-0,42	-0,34	-0,48
Lab 8		-0,58	-0,40	-0,35	-0,31
Lab 9	-0,07	0,10	0,02	0,04	-0,06
Lab 10	-0,24	0,56	-0,15	-0,35	-0,31
Lab 12		-0,12	-0,29	0,35	-0,32
Lab 13	-0,35	-0,58	-0,58	-0,67	-0,54
Lab 14	-0,45	-0,69	-0,37	-0,58	-0,64
Lab 15	-0,47	-0,16	-0,29	-0,38	-0,31
Lab 24	-0,44	0,39	-0,21	0,33	0,01
Lab 16	-0,35	0,18	-0,08	0,01	-0,11
Lab 17	-0,10	0,95	0,27	0,48	0,15
Lab 18		-1,84	-1,23	-0,61	-0,72
Lab 19		-2,20	0,23	0,48	0,18
Lab 20	-0,59	-0,58	-0,37	-0,29	-0,40
Lab 21	0,12	0,08	-0,14	-0,15	-0,18
Lab 22		1,16	0,58	1,02	0,36
Lab 23		0,08	-0,19	-0,08	-0,16
Lab MS1	-0,68	-1,22	-0,86	-0,85	-0,71
Lab MS2	0,04	-0,60	-0,37	-0,05	-0,52
Lab MS4	0,17	1,68	1,43	0,79	1,12
Lab MS5	3,47	0,20	-0,37	-0,63	-0,31
Lab MS6	-1,15	0,27	0,00	0,20	-0,01
Lab MS7	0,50	0,29	0,95	0,22	0,72
Lab MS8		-0,44	-0,42	-0,70	-0,49
Lab MS3	0,54	-0,34	-0,36	0,53	-0,41
Lab MS11	-0,55	3,30	-0,01	-1,77	0,77

Figure 2: z-scores graph for nicotine (in yellow the limit for warning signal, in red the limit for action signal)

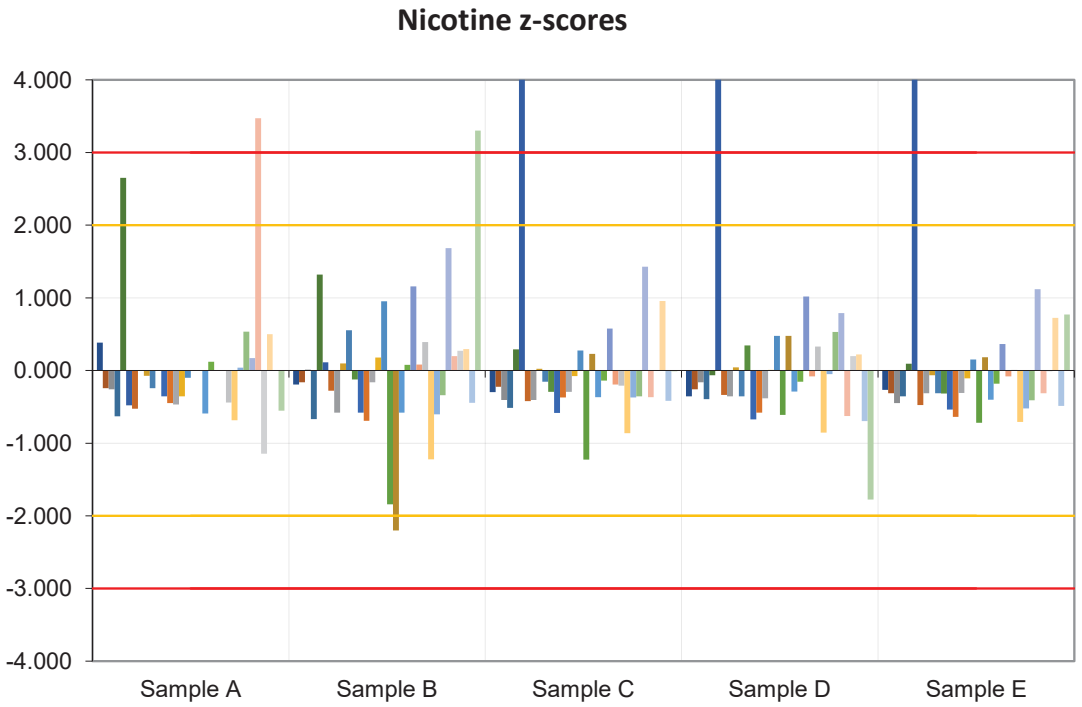


Table 3: z-scores for glycerol (in yellow questionable results and in red unsatisfactory results)

Glycerol					
Lab code	Sample A	Sample B	Sample C	Sample D	Sample E
	z-score	z-score	z-score	z-score	z-score
Lab 1	0,84	0,69	0,78	1,03	1,25
Lab 2	0,23	0,16	0,27	0,46	0,44
Lab 3	0,23	0,03	-0,01	0,25	-0,21
Lab 4	0,13	0,02	0,08	-0,12	0,13
Lab 5	0,33	0,24	0,37	0,40	0,67
Lab 6	1,43	2,64	1,02	0,35	-0,23
Lab 7	0,02	-1,14	0,16	-0,11	-0,47
Lab 8	0,26	-0,14	0,23	0,27	0,07
Lab 9	0,25	-0,77	0,35	0,13	0,13
Lab 10	0,17	0,00	-0,17	-0,07	-0,30
Lab 11	0,47	0,93	0,32	0,77	0,71
Lab 12	-3,61	-1,35	-3,50	-3,13	-2,54
Lab 13	0,20	0,07	0,26	0,12	0,26
Lab 14	0,37	-0,96	0,74	-0,38	-0,54
Lab 15	0,28	0,18	0,29	0,41	0,42
Lab 24	0,19	0,05	0,14	0,45	0,26
Lab 16	0,14	1,06	0,42	0,97	0,91
Lab 17	0,84	1,58	0,80	1,63	1,44
Lab 18	-0,36		0,23	-2,09	-1,04
Lab 19	0,06	-2,68	0,10	0,07	0,40
Lab 20	0,10	-0,59	0,13	-0,07	-0,07
Lab 21	0,21	0,23	0,29	0,47	0,42
Lab 22	-0,86	0,37	-1,35	0,12	-0,27
Lab 23	0,19	0,20	0,27	0,45	0,55
Lab MS2	0,34	0,49	0,13	0,48	0,22
Lab MS4	-3,16	-1,44	-3,15	-2,49	-2,87
Lab MS5	0,26	0,81	0,39	-0,42	0,76
Lab MS6	0,06	0,14	0,11	0,28	0,20
Lab MS7					
Lab MS8	0,62	0,34	0,62	0,90	0,65
Lab MS3	0,59	-1,16	0,69	0,08	-2,22
Lab MS11	-0,86	0,02	-1,00	-1,19	0,90

Figure 3: z-scores graph for glycerol (in yellow the limit for warning signal, in red the limit for action signal)

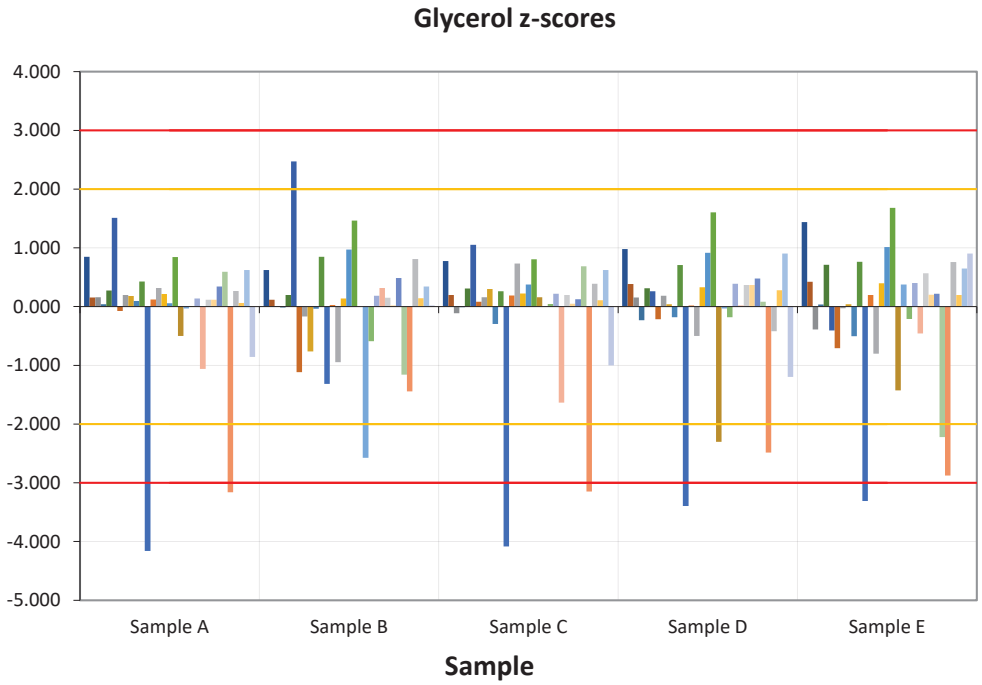
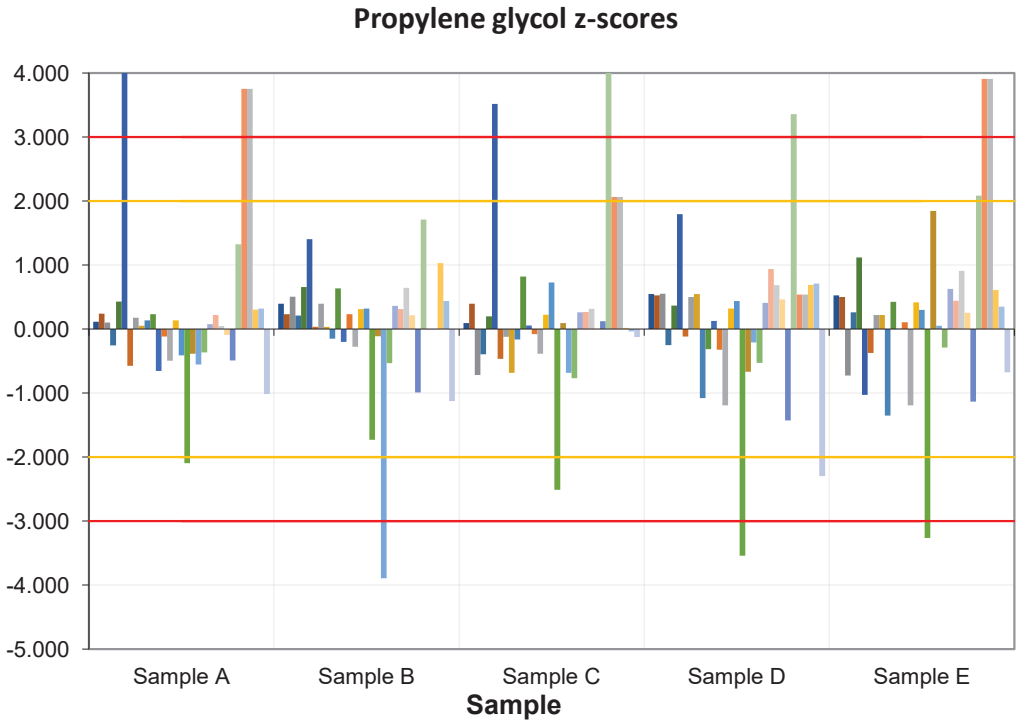


Table 4: z-scores for propylene glycol (in yellow questionable results and in red unsatisfactory results)

Propylene glycol					
Lab code	Sample A	Sample B	Sample C	Sample D	Sample E
	z-score	z-score	z-score	z-score	z-score
Lab 1	-0,08	0,30	-0,21	0,32	0,05
Lab 2	0,01	0,14	-0,06	0,31	0,03
Lab 3	-0,09	0,41	-0,59	0,32	-0,62
Lab 4	-0,35	0,12	-0,44	-0,19	-0,09
Lab 5	0,15	0,56	-0,16	0,20	0,37
Lab 6	2,75	1,29	1,41	1,12	-0,78
Lab 7	-0,58	-0,05	-0,47	-0,11	-0,43
Lab 8	-0,03	0,30	-0,31	0,29	-0,12
Lab 9	-0,13	-0,05	-0,57	0,32	-0,12
Lab 10	-0,06	-0,24	-0,33	-0,72	-0,96
Lab 11	0,00	0,54	0,14	-0,23	-0,01
Lab 12	-0,63	-0,29	-0,22	0,05	-0,23
Lab 13	-0,25	0,14	-0,29	-0,24	-0,18
Lab 14	-0,52	-0,36	-0,43	-0,80	-0,87
Lab 15	-0,06	0,22	-0,14	0,17	-0,01
Lab 24	-0,23	0,13	-0,25	0,27	-0,10
Lab 16	-0,46	0,23	0,09	0,25	-0,07
Lab 17	-1,68	-1,79	-1,43	-2,30	-1,98
Lab 18	-0,44	-0,20	-0,21	-0,46	0,76
Lab 19	-0,56	-3,92	-0,57	-0,16	-0,21
Lab 20	-0,43	-0,61	-0,61	-0,37	-0,39
Lab 21	-0,11	0,27	-0,13	0,23	0,10
Lab 22	0,00	0,22	-0,12	0,57	0,00
Lab 23	-0,13	0,55	-0,10	0,41	0,25
Lab MS2	-0,49	-0,99	0,12	-1,43	-1,13
Lab MS4	3,75	-0,01	2,06	0,54	3,91

Lab MS5	-0,29	1,04	-0,41	-0,83	0,46
Lab MS6	0,30	1,03	0,02	0,69	0,61
Lab MS7					
Lab MS8	0,32	0,44	-0,04	0,71	0,35
Lab MS3	1,33	1,71	4,35	3,36	2,09
Lab MS11	-1,02	-1,12	-0,12	-2,30	-0,67

Figure 4: z-scores graph for propylene glycol (in yellow the limit for warning signal, in red the limit for action signal)



Mass spectrometry extension of the SOP

During SOP development, as mentioned, we explored also the extension of the method for mass spectrometry detection.

This is strategic since laboratories that will analyse e-liquids will probably be equipped with mass spectrometers in order to be able to run also flavors and, hopefully, CMR compounds like aldehydes etc. that have been highlighted in **Recommendation G4** in D8.2 for electronic cigarettes:

ID	Recommendation (IRFMN proposal)	Inspiration	Response: Inherent to WP8?	ResponseTOT score average (1-5)
G3	ICOTINE EMISSIONS MS should develop, implement and enforce verification programs for nicotine emissions using both AFNOR, CEN draft or peer-reviewed independent publications	TPD [ART.19.1] [ART. 20.3.f]	8 x Yes 1 x No	4.7
G4	ANALYTES OTHER THAN NICOTINE CMR properties of the tobacco product TPD [ART.7.13] VOCs, aldehydes and TSNAs measurements should be strongly recommended.	TPD [ART.7.13] [ART. 20.3.e]	7 x Yes 1 x No	4.0

We received results from nine laboratories using mass spectrometry for detection of the method described in the SOP.

Calculating z-scores within MS detection, we obtain very homogeneous results. More, they show that z-scores are all within the “action level” of 3 SD and are almost all within the 2 SD “warning level” (Figure 5; Figure 6; Figure 7)

Figure 5: z-scores graph for nicotine (in yellow the limit for warning signal, in red the limit for action signal)

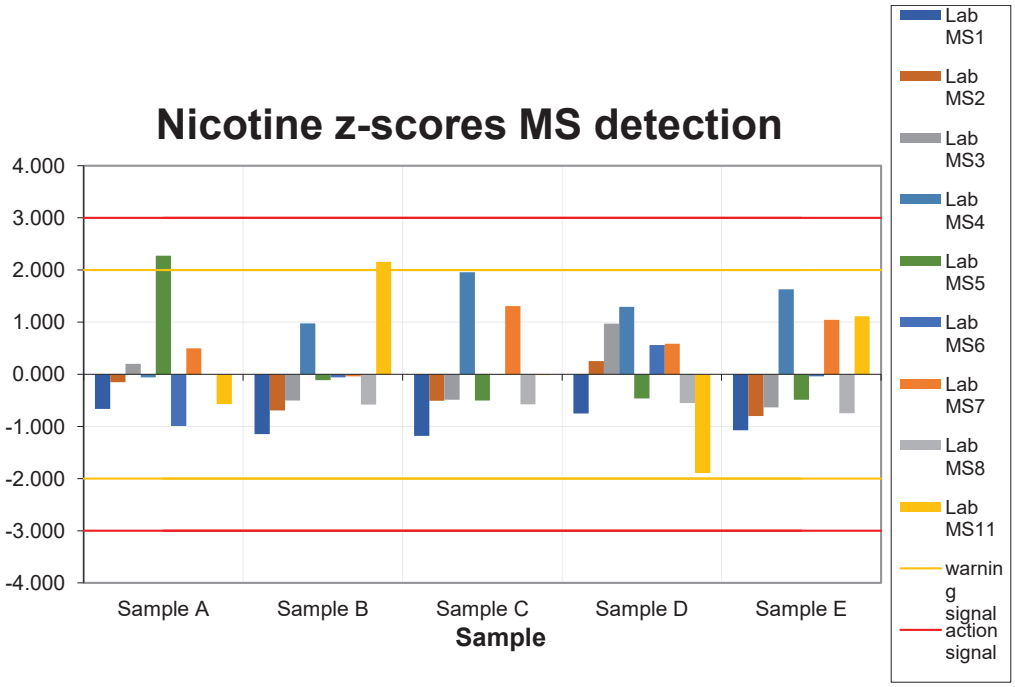


Figure 6: z-scores graph for glycerol (in yellow the limit for warning signal, in red the limit for action signal)

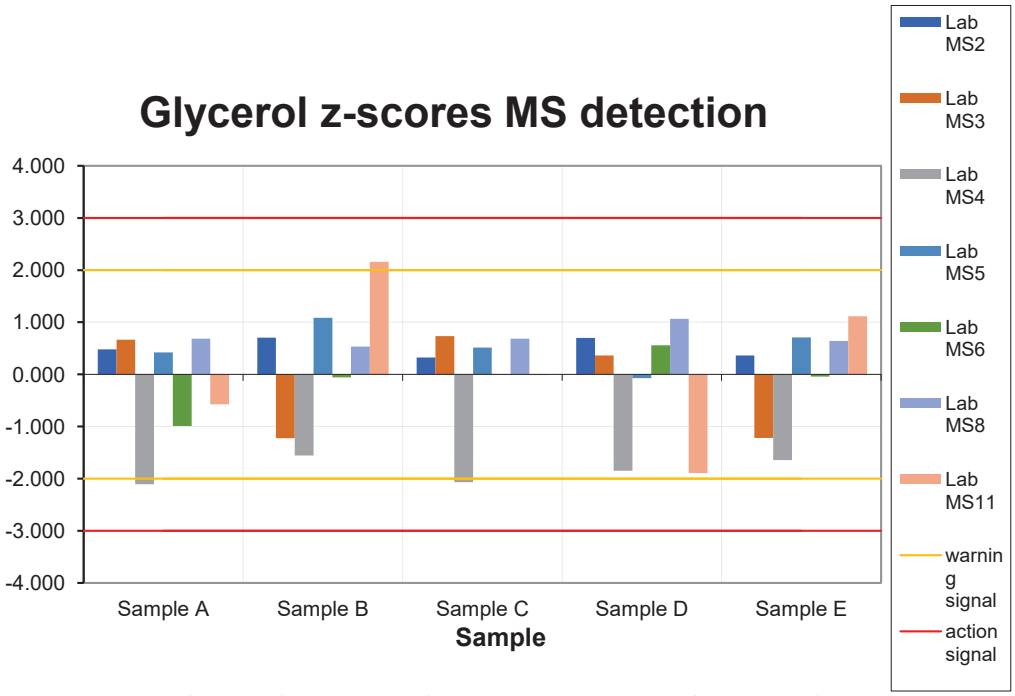
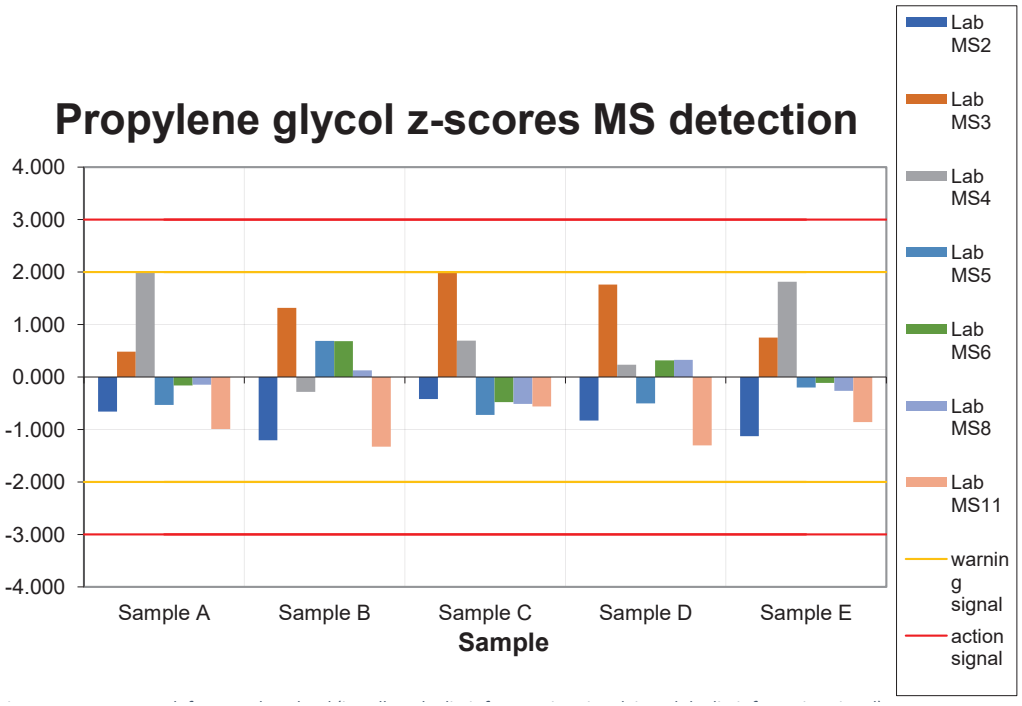


Figure 7: z-scores graph for propylen glycol (in yellow the limit for warning signal, in red the limit for action signal)



Besides providing an high level of homogeneous results, also recovery from"true" values was in general within +/- 10% (Table 5: Mean values obtained by GC/LC-MS analysis and recovery results). Sample A, nicotine was below the lowest calibration point, so results are extrapolated from the calibration line and this might be the reason for being the lowest recovery value (134,8%) obtained.

Table 5: Mean values obtained by GC/LC-MS analysis and recovery results

Sample	*Mean values (mg/ml)*		
	Nicotine	Glycerol	Propylene Glycol
A	0,34	510,8	588,0
B	5,03	202,9	835,5
C	8,24	669,4	303,7
D	22,74	300,8	742,9
E	11,89	332,0	604,9
Sample	**Recovery GC/LC-MS results (%)**		
	Nicotine	Glycerol	Propylene Glycol
A	134,8%	89,9%	103,4%
B	99,2%	94,9%	97,7%
C	102,6%	86,7%	109,1%
D	106,9%	93,6%	99,1%

Looking at all results obtained using MS instead of FID detection, it appears that MS can be considered a valid alternative to GC-FID. This is much important since in this way more laboratories with different equipment could be involved. Moreover, GC-MS can be used to easily analyse other components, such as CMR compounds and flavours in e-liquids, like discussed before.

Conclusions

This Deliverable is the conclusive part for both Objective 2 and 3 of WP8 for JATC, whose aims were "To review laboratory analysis activities performed by MS and to assess comparability across laboratories" and "To develop collaborations and communication with other international activities

on tobacco laboratory assessment".

Our SOP developed, gave us the possibility to evaluate both laboratory quality and capacity and to develop collaborations: thanks to z-scores and reproducibility/repeatability data, we managed to prove that our network of independent laboratories have excellent analytical capacity, high quality data management and willingness to participate to new analytical challenges, where possible.

The SOP has been fully validated as a GC-FID method, but results from a proficiency test, using mass spectrometry as detection system, proved that methods are equivalent in term of z-scores results.

The network of laboratories we created includes laboratories from both Europe and TobLabNet members from WHO. We also managed to include some academic laboratories.

All of the participants were glad and proud in participating this study. They have always answered in time and respected deadlines when possible (due to difficulties caused by the pandemic).

For this reason, we think we fully succeed our objectives and we think that laboratories part of this reconstituted network will be pleased and adequate to participate also in the following JATC 2.

Annex 1

Raw data from the SOP validation results

EA - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,56	606	566
B	2	4,99	225	852
C	3	0,56	606	570
D	4	7,84	786	270
E	5	21,3	339	748
F	6	11,3	390	577
G	7	5,01	226	848
H	8	7,81	777	270
I	9	21,2	338	746
L	10	11,3	393	577

EX - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,26	562	562
B	2	5,29	211	837
C	3	0,28	565	564
D	4	7,89	722	268
E	5	23,32	321	743
F	6	11,87	365	573
G	7	5,31	213	845
H	8	7,96	727	270
I	9	23,46	323	747
L	10	11,95	363	571

EB - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,34	567	573
B	2	5,04	215	848
C	3	0,34	564	569
D	4	7,91	734	274
E	5	21,50	320	744
F	6	11,20	369	577
G	7	4,99	213	836
H	8	7,89	738	273
I	9	21,60	324	749
L	10	11,20	369	576

WA - MS

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,50	339	736
B	2	6,20	189	872
C	3	0,50	345	654
D	4	10,20	449	248
E	5	27,10	253	808
F	6	12,50	251	620
G	7	5,70	171	798
H	8	10,20	414	404
I	9	22,60	220	701
L	10	16,30	304	790

EC - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,34	566	568
B	2	5,12	211	855
C	3	0,32	565	568

WB - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,27	558	556
B	2	5,15	232	846
C	3	0,28	561	555

D	4	7,50	700	256
E	5	21,89	316	749
F	6	11,27	365	575
G	7	5,09	211	855
H	8	7,76	724	265
I	9	21,79	315	746
L	10	10,53	337	534

D	4	8,11	751	278
E	5	22,30	334	747
F	6	11,66	381	575
G	7	5,23	235	847
H	8	8,11	749	277
I	9	22,46	339	743
L	10	11,65	383	571

ED - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,20	565	559
B	2	4,76	211	843
C	3	0,21	553	559
D	4	7,51	729	266
E	5	21,18	306	727
F	6	11,11	358	572
G	7	4,71	211	839
H	8	7,48	711	263
I	9	21,06	304	732
L	10	11,09	362	573

WC - MS

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	1,7	586	583
B	2	5,3	230	893
C	3	1,6	550	539
D	4	7,8	759	263
E	5	22,3	321	768
F	6	11,4	380	596
G	7	5,1	226	879
H	8	7,6	735	267
I	9	18,5	272	648
L	10	11,0	376	585

EE - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	1,40	571	575
B	2	5,73	216	865
C	3	1,33	574	576
D	4	8,60	746	271
E	5	22,23	321	743
F	6	12,19	376	588
G	7	5,83	215	860
H	8	8,65	744	271
I	9	22,09	319	743
L	10	12,01	375	587

WD - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,42	599	528
B	2	5,55	246	747
C	3	0,36	613	503
D	4	8,61	782	240
E	5	23,98	358	661
F	6	12,16	395	495
G	7	5,63	243	748
H	8	8,59	785	239
I	9	23,74	354	655
L	10	12,30	399	524

EF - FID

WE - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,36	705	711
B	2	4,59	279	911
C	3	0,15	585	613
D	4	12,39	734	280
E	5	33,15	345	743
F	6	16,96	341	513
G	7	5,73	256	887
H	8	17,40	872	340
I	9	40,54	293	805
L	10	28,85	361	586

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	ND	531	559
B	2	4,2	ND	825
C	3	ND	523	553
D	4	6,6	767	280
E	5	20,3	249	721
F	6	10,1	299	560
G	7	4,1	ND	826
H	8	6,4	699	260
I	9	20,6	247	720
L	10	10,5	357	641

EG- FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,24	525	555
B	2	4,95	185	836
C	3	0,24	579	548
D	4	7,46	711	261
E	5	22,10	313	737
F	6	10,90	344	559
G	7	4,96	187	829
H	8	7,79	743	266
I	9	20,50	298	728
L	10	10,80	344	563

WF - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,00	556	551
B	2	5,29	202	840
C	3	0,00	553	553
D	4	8,56	721	262
E	5	24,10	316	734
F	6	12,50	371	574
G	7	2,64	104	445
H	8	8,51	721	260
I	9	23,60	305	727
L	10	12,10	365	563

EH - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,00	571	571
B	2	4,80	209	853
C	3	0,00	565	568
D	4	7,70	735	268
E	5	21,50	320	751
F	6	11,10	359	569
G	7	4,80	206	847
H	8	7,60	731	267
I	9	21,00	313	741

WG - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,22	556	558
B	2	4,83	197	803
C	3	0,22	558	555
D	4	7,67	711	262
E	5	21,70	299	727
F	6	11,00	352	554
G	7	4,79	199	807
H	8	7,67	737	258
I	9	21,20	314	720

L	10	11,30	359	574

L	10	11,00	358	571

EI - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,42	565	566
B	2	5,22	196	851
C	3	0,41	569	567
D	4	8,19	741	260
E	5	22,60	314	748
F	6	11,70	360	566
G	7	5,10	192	814
H	8	8,31	747	262
I	9	22,40	311	746
L	10	11,80	361	577

WG - LCMSMS

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,23		
B	2	4,96		
C	3	0,23		
D	4	7,71		
E	5	21,37		
F	6	11,00		
G	7	4,99		
H	8	7,49		
I	9	21,67		
L	10	11,03		

EL - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,28	559	563
B	2	5,51	216	847
C	3	0,40	565	574
D	4	8,28	734	279
E	5	21,40	310	718
F	6	11,40	351	556
G	7	5,26	205	800
H	8	7,75	661	255
I	9	21,10	303	705
L	10	11,00	346	531

WH - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,47	565	569
B	2	5,12	215	848
C	3	0,47	564	566
D	4	8,00	733	271
E	5	21,90	323	745
F	6	11,50	368	577
G	7	5,16	216	849
H	8	8,05	743	273
I	9	21,80	322	743
L	10	11,50	369	580

EM - FID(gly,pg) - MS(nic)

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,18	580	570
B	2	4,44	231	873
C	3	0,18	583	572
D	4	6,96	739	277
E	5	19,59	322	717

WI - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1		516	574
B	2	5,57	218	859
C	3		472	567
D	4	9,09	598	274
E	5	25,39	314	764

F	6	10,27	378	578
G	7	4,50	230	850
H	8	7,06	743	280
I	9	19,78	340	740
L	10	10,37	376	572

F	6	13,47	353	563
G	7	5,83	219	833
H	8	8,91	586	270
I	9	25,76	310	747
L	10	12,78	346	588

EN - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	ND	351	541
B	2	5,08	178	818
C	3	ND	274	558
D	4	7,90	404	270
E	5	23,32	223	733
F	6	12,25	288	571
G	7	5,16	186	824
H	8	7,85	396	269
I	9	23,55	213	743
L	10	12,17	285	565

WL- FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,00	557	562
B	2	5,12	216	866
C	3	0,00	569	571
D	4	7,96	731	271
E	5	21,74	318	740
F	6	11,57	374	586
G	7	5,16	213	858
H	8	7,94	742	274
I	9	22,48	326	760
L	10	11,51	371	582

EO - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,33	564	563
B	2	4,83	213	845
C	3	0,34	563	562
D	4	7,44	738	269
E	5	20,60	318	741
F	6	10,80	366	572
G	7	4,82	211	839
H	8	7,42	733	267
I	9	19,90	306	715
L	10	10,60	362	567

WL- MS

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,02	549	576
B	2	5,25	215	891
C	3	0,02	561	586
D	4	8,17	716	273
E	5	22,59	313	748
F	6	11,89	368	603
G	7	5,22	213	881
H	8	8,27	728	278
I	9	23,38	320	771
L	10	11,86	357	588

EP - FID (pipetted)

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,25	458	436

WM - MS

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,50	N.A	N.A

B	2	4,56	180	784
C	3	0,27	598	565
D	4	7,89	744	247
E	5	21,39	283	692
F	6	11,73	356	552
G	7	4,88	181	775
H	8	7,93	747	248
I	9	21,79	293	692
L	10	11,86	353	553

B	2	5,20	N.A	N.A
C	3	0,50	N.A	N.A
D	4	8,70	N.A	N.A
E	5	22,60	N.A	N.A
F	6	13,20	N.A	N.A
G	7	5,30	N.A	N.A
H	8	10,40	N.A	N.A
I	9	23,50	N.A	N.A
L	10	13,80	N.A	N.A

EP - MS (pipetted)

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,36	444	431
B	2	4,59	217	762
C	3	0,41	545	560
D	4	7,87	699	261
E	5	23,41	314	659
F	6	12,47	354	544
G	7	4,71	213	740
H	8	7,91	694	253
I	9	23,79	321	666
L	10	12,11	370	540

UA - MS

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	n.q.	591,6	581,3
B	2	4,87	217,9	856,6
D	4	7,63	767,7	274,1
E	5	20,18	334,8	760,3
F	6	10,81	374,9	586,9

EP - FID (weighed)

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,24	583	562
B	2	4,67	184	796
C	3	0,30	568	545
D	4	7,77	777	271
E	5	20,49	290	710
F	6	9,64	318	513
G	7	4,82	195	839
H	8	7,62	779	258
I	9	20,61	305	708
L	10	11,31	366	579

UD - MS

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0.23	494	537
B	2	16.8	211	780
D	4	8.2	623	272
E	5	6.8	274	658
F	6	13.6	382	553

EP - MS (weighed)

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,45	568	564
B	2	4,81	223	757
C	3	0,43	578	545
D	4	8,14	724	283
E	5	21,86	322	709
F	6	10,08	341	506
G	7	4,76	219	816
H	8	7,26	724	273
I	9	22,55	322	666
L	10	11,38	385	570

UE - MS

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,64	611	637
B	2	5,08	192	948
C	3	0,59	568	592
D	4	7,76	778	384
E	5	22,69	294	803
F	6	11,17	300	656
G	7	4,77	180	890
H	8	7,68	770	380
I	9	25,35	328	897
L	10	10,79	290	634

EQ - FID

		Nicotine (mg/mL)	Glycerol (mg/mL)	Propylene glycol (mg/mL)
A	1	0,25	569	569
B	2	5,03	215	851
C	3	0,27	569	568
D	4	7,80	736	272
E	5	21,20	321	743
F	6	11,18	368	573
G	7	5,00	214	841
H	8	7,81	740	271
I	9	21,12	320	741
L	10	11,23	369	577