ST 46 - Taschner - Determination of menthol in cigarette components by GC

Author: TASCHNER P.

Determination of menthol in cigarette components by GC

Petra Taschner
H.F. & Ph.F. Reemtsma GmbH, Research & Development, Albert-Einstein-Ring 7, 22761 Hamburg, Germany

Summary

Peppermint, botanically known as *Mentha piperita* has a long history of being an excellent culinary spice and its oil is also one of the most widely used flavourings in cigarettes. The major flavouring characteristic of peppermint oil derives from its main constituent menthol.

The flavouring formula is predominantly applied to the filter or the cigarette paper. Due to its volatility, certain amounts of the flavouring migrates to the different parts of the cigarette. Depending on its use, methods for the determination of menthol should be suitable for controlling target values for filter rods (production control) and for fulfilling scientific research requirements (e.g. migration process).

The presentation will compare 2 GC methods currently used for menthol determination within the laboratories of the REEMTSMA Group. Several years ago, a method was developped with the purpose of covering these objectives. Its scope includes filter rods, filter tips as well as cut rag tobacco with ranges of menthol of 2 - 20 mg (filter rod), 0.2 - 2.0 mg (filter tip) and 0.4 - 4 mg (tobacco rod). This method is also suitable for other flavouring substances used at lower concentration levels. Sample preparation consists of an extraction with a mixture of dichloromethane/hexane, followed directly by GC/FID analysis on a 60 meter capillary column (DB 1701), with extraction times of 2 h for filter rods and 72 h for filter tips and cut rag tobacco. In the light of an increasing need for a simple and rapid method to control target values for filter rods, advance have been made in order to modify the original GC method by replacing the extraction solvent with iso-propanol and changes in the GC system, such as the column, to shorten the total GC run time. The modified method has shown sufficient efficiency to analyze filter rods for their menthol content and its scope can also be extended to filter tips and cut rag tobacco by adequately prolonging the extraction time for these sample types.

Experimental

One of the basic features of the original GC method (*GC 1*) developed by the Reemtsma R&D lab in the early 1990's is the wide scope of application regarding sample types and analytes covered: it is used to check target values of menthol and 2 other flavour ingredients of similar chemical structure for different cigarette components.

A mixture of hexane/dichloromethane has been choosen for extraction as is shows sufficient extraction capacity for the analytes, without giving interferring signals in the chromatogram

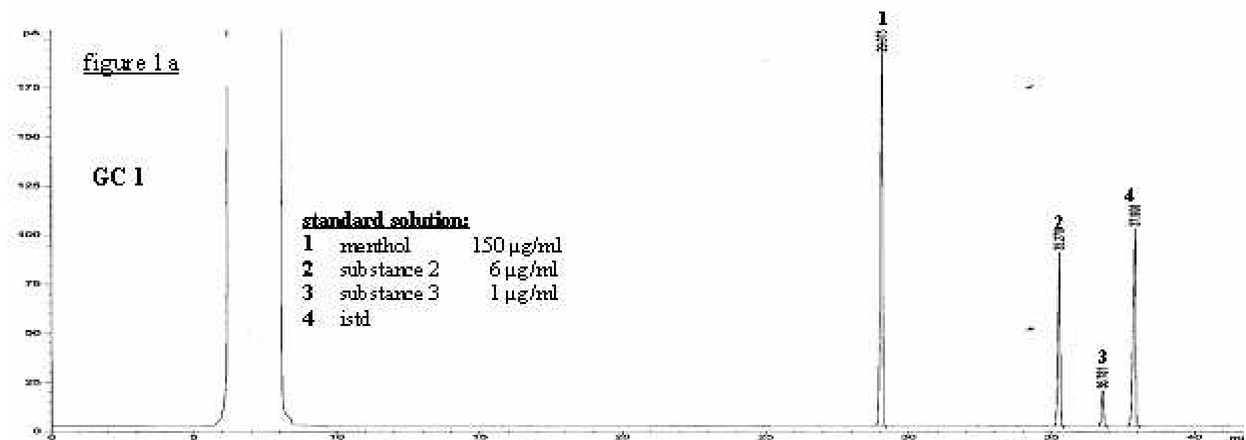
which may arise from the tobacco matrice. Table 1 outlines the sample preparation for method 1.

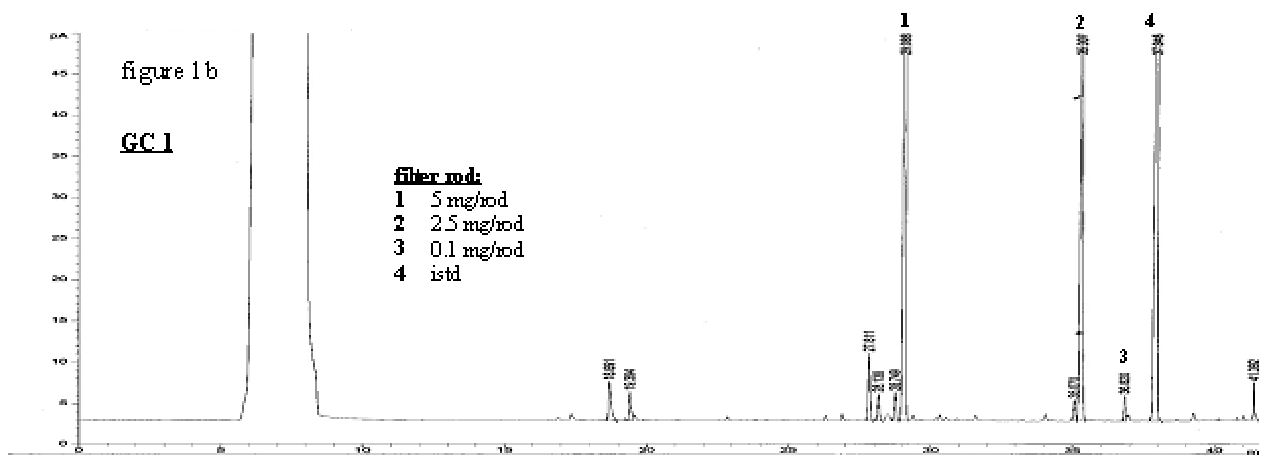
GC analysis is carried out on a capillary column of medium polarity with flame ionization detection, using an internal standard (tetradecane) for quantification. Figure 1 shows typical chromatograms and outlines suitable GC conditions for this method.

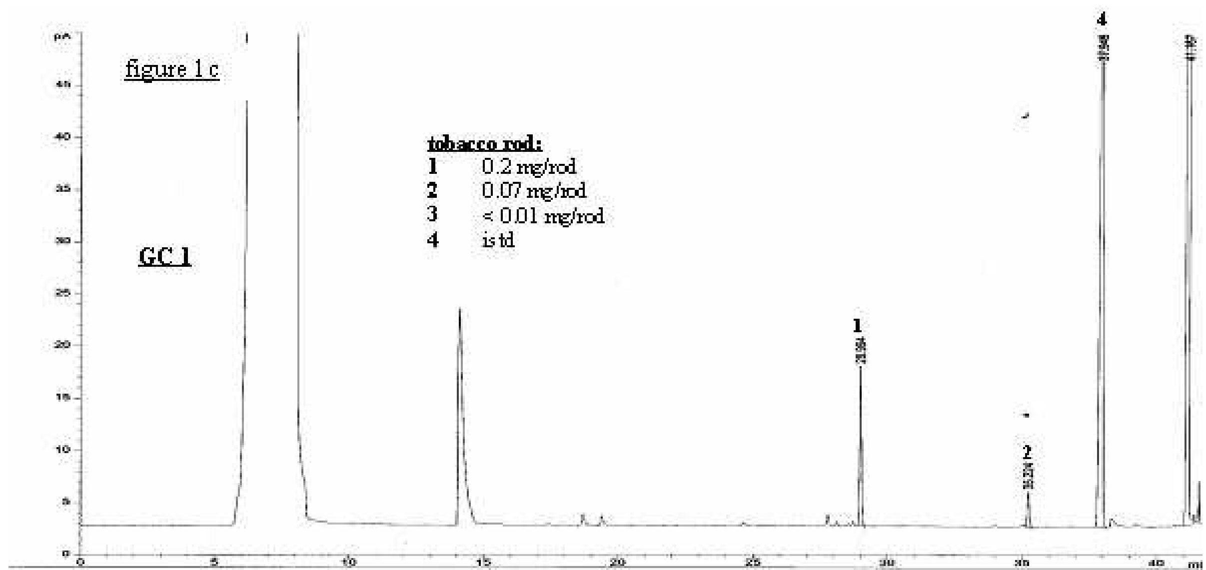
Table 1: Sample preparation

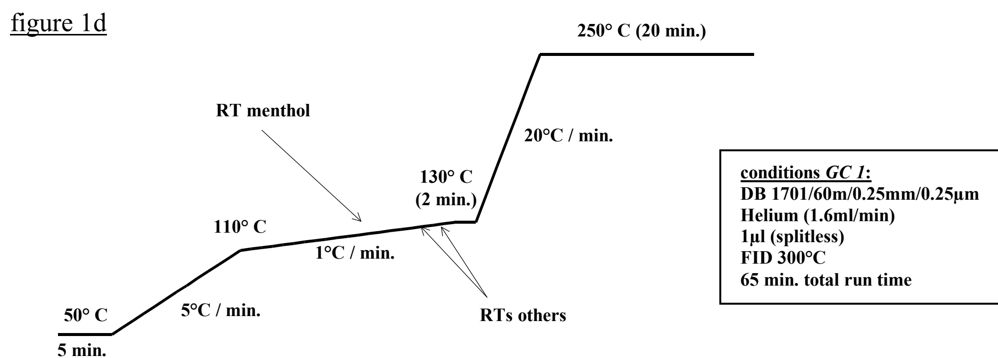
	filter rods	**cigarettes**
sample size (2 replicates)	20 rods	10 cigarettes
preparation	- discard a segment of 3 cm from each rod - cut off a segment representing 1/10 of the total length from each rod (=> 20 segments) - divide 20 segments into 2 portions and transfer each portion into extraction vial - add 50 ml extraction solvent extract by shaking for 2 hours (150 rpm)	- separate tobacco rod and filter tip - cut each tip with scissors and tra into extraction vial - cut each rod into 2 pieces, cı cigarette paper and transfer ma another extraction vial - add 25 ml extraction solvent to the tips - add 50 ml extraction solvent to the the tobacco rod - extract by shaking for 72 hours (15
further clean up	none	none

Figures 1a to d: Chromatograms GC 1









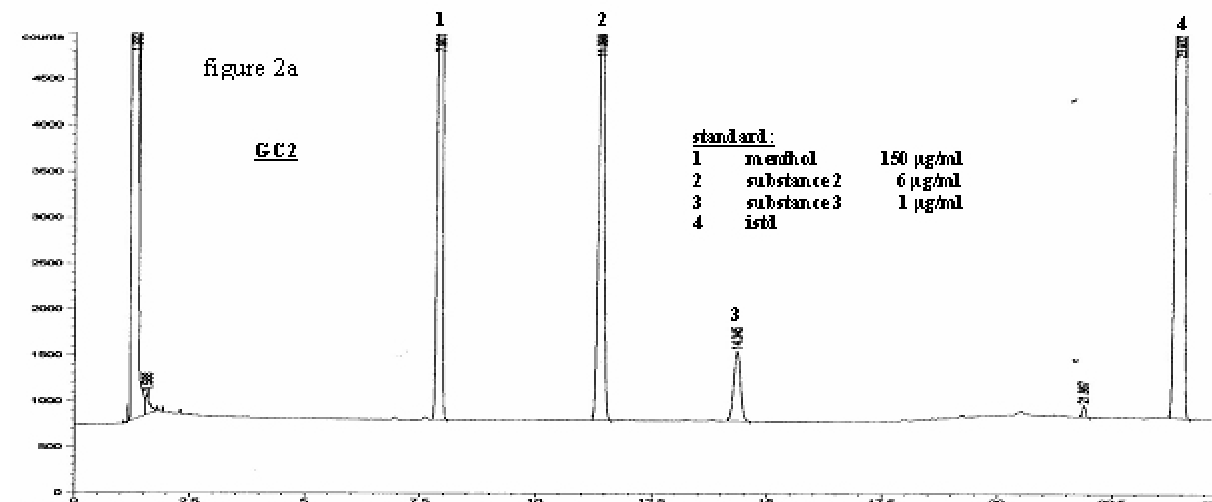
Using conditions as mentioned in figure 1d, all analytes of interest elute between 29 and 37

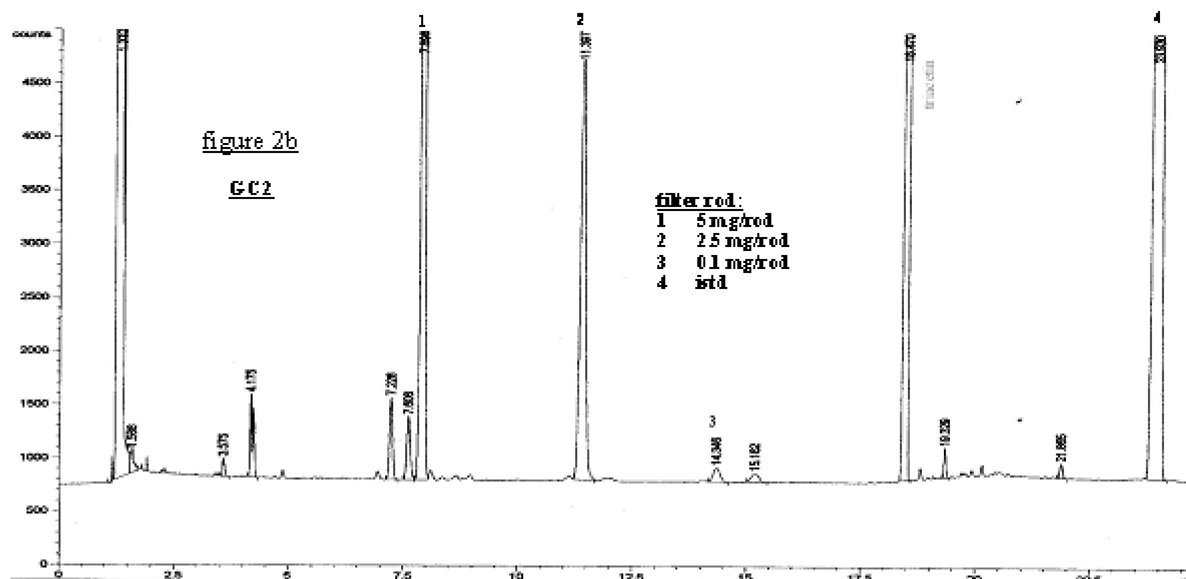
minutes. The temperature programme starts with a moderate ramp of 5°C/min to accelerate elution. It has to be lowered to 1°C/min for a proper separation of various smaller peak which elute close to the analytes of interest (RT 28.7 and 35.0 min) as well as another interfering peak close to substance 3. Another major ingredient of filter rods, triacetin, elutes with a RT of more than 45 minutes. For filter rods the total run can be set to 50 minutes, it has to prolonged to 65 minutes for tobacco samples.

In the light of an increasing need for a simple and rapid method to control target values for filter rods, experiments started in 2001 with the aim to modify the original GC method *GC 1*. The basic objective for modifying *GC 1* was to replace the extraction solvent, as the use of dichloromethane gives rise to concern from the safety-at-work perspective. Another performance detail to address was the modification of the GC system to allow shorter total run times. In case of positive results in a validation process, this method would then be qualified as an in-house standard method for filter rods, to be used in all Reemtsma laboratories where menthol analysis is requested.

First trials performed by the Reemtsma laboratory in Debrecen/Hungary replacing the extraction mixture with i-propanol and using a less polar column showed promising results. Sample preparation remains unchanged as described in table 1, followed by GC analysis on a HP-5 column, using n-heptadecane as an internal standard. The idea behind this modification was driven by a quite simple and practical consideration: the system iso-propanol/n-heptadecane is used in smoke analysis to extract nicotine. Figure 2 shows typical chromatograms and outlines suitable GC conditions for the method *GC 2*.

Figures 2a to d: Chromatograms GC 2





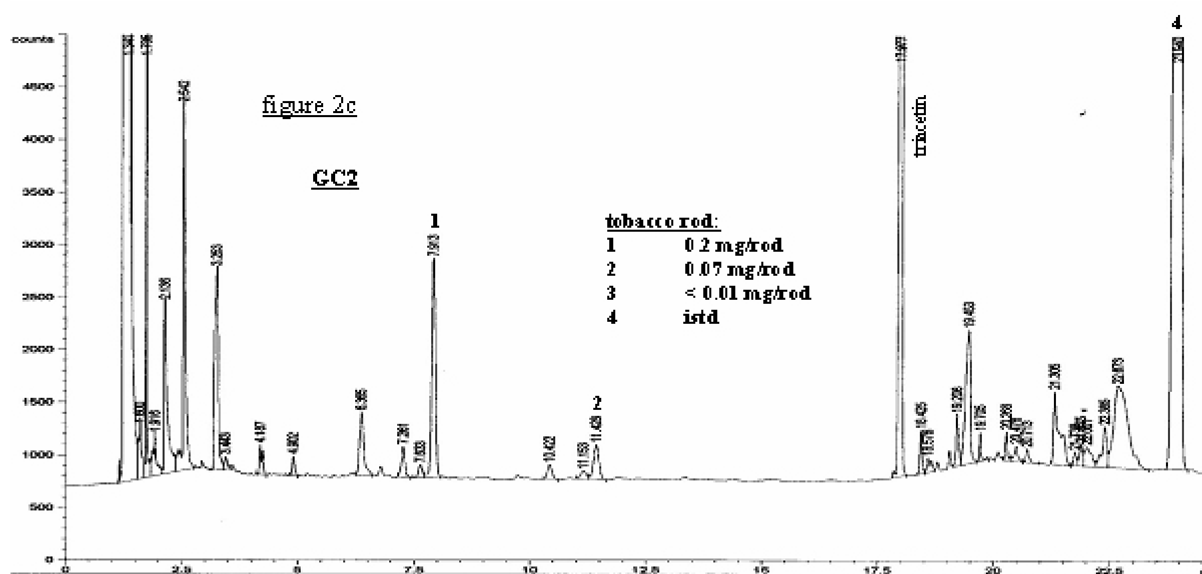
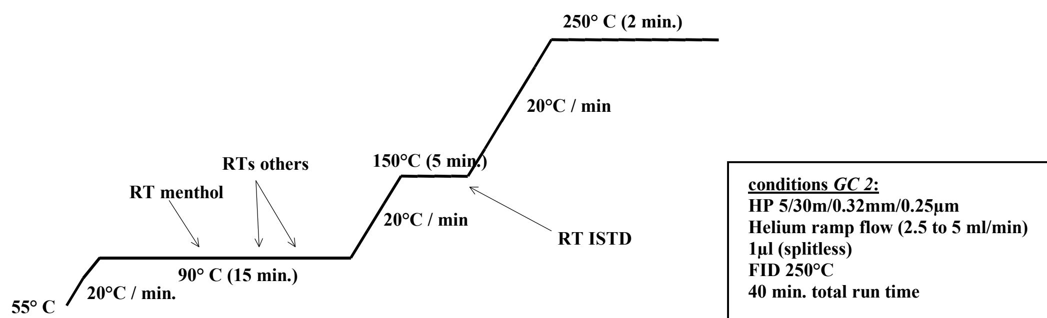


Figure 2d: Temperature ramp / GC conditions for GC 2



With RTs between 7 and 14 minutes, elution of the analytes of interest is accelerated due to the shorter and the less polar column used for GC 2 and the higher flow rate. For filter rods the total run time can be set to 30 minutes, for tobacco samples it has to extended to 40 minutes. Several signals arising from the matrice can be observed in the chromatograms, which are separated sufficiently from the peaks to be quantified. The largest signal from the matrice relates to triacetin (RT 18 min.) which should be quantitatively extracted with iso-propanol.

After fixing all relevant method details, it was decided to undergo a validation process with this modification (*GC 2*).

Validation process

Based on the first promising results obtained from *GC 2* it was decided to include different sample types (filter rods, filter tips and tobacco rod) into the validation process.

Before starting a validation process, its purpose should be clearly defined to agree on a validation concept which is suitable for the specific decision to be taken. Regarding *GC 1* and *GC 2* the purpose of validation was to compare the performance of both methods for different sample types, as it has to be decided whether to replace *GC 1* by *GC 2* for filter rods or even for all matrices.

The validation process used here addresses in a first phase the accuracy and precision of the methods within one lab. For this purpose *GC 1* and *GC 2* were implemented in the Reemtsma central lab on GC systems of identical type and all tests were performed parallel with both methods. Beside standard solutions, filter rods and cigarette samples with different menthol contents were used as well as manually spiked samples and the respective blanks. Almost all test samples were freshly produced. To cover effects that may be related to the sample age, some tests were repeated after a certain period of time to check, whether the sample age has an impact on the method performance, mainly regarding extraction.

Table 2 summarizes the experiments agreed to carry out in phase 1 of the validation.

In total, phase 1 requires approximately 200 injections. After evaluation of the results of phase 1 it will be decided, if an interlaboratory test should be initiated as the 2nd phase of the validation process. Phase 2 would then comprise of an interlaboratory test between Reemtsma laboratories.

Table 2: Validation experiments, phase 1

experiment	test material / injections	data analysis
1. linearity of calibration curve	standard solutions 6 levels n = 6 injections levels 2 to 5 n = 10 injections levels 1 and 6	- calibration functions mean, std. dev. per level
2. working range	blank samples (filter rods, tobacco) spiked with standard solutions (level 1 plus level 0) n = 10 replicates per level/matrice	- mean, std. dev.per level/ matrice - estimation for LOQ (3s / 10s) for 2 matrices
3. accuracy	blank samples (filter rods, tobacco) spiked with standard solutions at 4 independent concentrations n = 10 replicates per level/matrice	- recovery rates per level/matrice repeatability per level/matrice
4. precision (1)	3 cigarette samples representing different menthol levels (test to be repeated after 2 weeks to check influence of sample age) n = 10 replicates per sample	- repeatability per level/matrice

Results and discussion

A summary of the results obtained during phase 1 of the validation is given in table 3.

linearity of calibration

A 6-level-calibration was performed on both GC systems, whereas an identical concentration range for the 3 analytes was covered. For menthol, the calibration range represents 1.8 to 15 mg per filter rod, 0.35 to 3.0 mg per tobacco rod or 0.18 to 1.5 mg per filter tip, using the sample preparation as described in table 1.

Using a linear function, the correlation coefficient r^2 for *GC 1* was calculated to 0.99998 for all 3 substances, *GC 2* showed slightly lower r^2 values of 0.9996. With multiple injections for each level the variability of the detector signal was checked as well. As expected, both systems showed consistent variability for the concentration range covered. The coefficient of variation for the lowest level (level 1) tested came out as 0.1% (GC 1) and 0.2% (GC 2).

practical working range

A practical approximation which is often used to calculate the limit of detection (LOD) or the limit of quantification (LOQ) is based on the standard deviation, where 10 times the standard deviation s is considered to represent an estimate for LOQ.

For this method comparison, 2 blank samples - 1 filter rod and 1 cigarette - were spiked with standard solutions on 2 levels (ten replicates), with level 1 representing the lowest calibration point and with level 0 set to a level below the calibration range. The menthol amount spiked at level 1 was 1.9 mg per filter rod or 0.38 mg per tobacco rod, whereas level 0 was set to 0.75 mg (filter) and 0.15 mg (tobacco).

The filter rod was spiked by injecting respective aliquotes into the rod using a syringe. The tobacco sample was spiked by adding aliquotes directly on the tobacco in the extraction vial. For both sample types, extraction was carried out after a certain period of time to allow the spiking solutions to penetrate through the sample material.

Based on 10 replicates for level 1, *GC 1* and *GC 2* give quite similar standard deviations of 0.02 / 0.01 / 0.002 for the 3 analytes for filter rods. For menthol, the LOQ could be theorectically lowered by a factor of 10 for this matrice. The same could be applied to the LOQ of substance 2, whereas the LOQ calculated for substance 3 represents almost the lowest calibration point established.

For tobacco a more distinctive influence from the matrice could be expected, based on a lower analyte/matrice ratio. However, the LOQ values follow the same pattern as mentioned for filter rods for both methods.

accuracy and precision

The same sample material and spiking procedure as mentioned above was used to evaluate the accuracy of the methods. Four spiking levels, covering the whole calibration range, were established: for menthol 2.2 to 13.5 mg (filter rod) and 0.45 to 2.7 mg (tobacco rod). Recovery rates were calculated based on 10 replicates per level and matrice. For each matrice/level combination repeatability r was calculated as well.

For one standard Reemtsma filter rod material, repeatability r was calculated as well, based on routine samples (2 replicates) analysed over a period of 2 months.

Generally, recovery rates for both sample types were in line with target values stated in the literature. Nevertheless, there is some evidence that *GC 2* gives lower recovery rates compared to *GC 1* for higher levels of the analytes when analyzing filter rods. This is may be related to the polarity of the extraction solvent, as such an effect was not observed for the tobacco sample containing lower contents of the analytes. The topic of extraction sufficiency was added to the validation phase 1. For this experiment a filter rod sample as well as a tobacco sample underwent an extraction procedure, where aliquotes of the same extract were injected over a period of 2 to 72 hours (data not yet available).

Repeatability values r (in %) for filter rods for *GC 1* are consistently low for all analytes (1 to 2%), except level C that came out with 4%. For *GC 2* repeatability ranges consistently between 3 and 4%, with lower values for level B.

Recovery rates for the tobacco sample are as well in line with recommended values, with *GC 1* showing a slight decrease for menthol at higher concentrations (level D). *GC 2* gives consistent repeatability values, that do not significantly exceed the values obtained for filter rods. In general, no significant difference regarding recovery and repeatability can be observed between the 2 methods.

Regarding the precision of both methods, 3 filter rods produced under normal manufacturing conditions and representing different analyte levels will be analyzed, as well 3 finished products (tobacco rod and filter tip). Suitable amounts of sample material for ten replicates per sample will be available in August.

Up to now, data for precision can be calculation based on routine filter rod samples, analyzed

using both methods (2 replicates). Almost all samples represent a menthol content of approximately 5 mg/rod and were analyzed 1 to 3 weeks after production.

The data base for calculating the value for repeatability - based on 2 replicates - consists of 18 samples (*GC 1*) and 17 samples (*GC 2*). All values obtained were quite low: for menthol r is calculated to 0.19 (*GC 1*) and 0.13 (*GC 2*).

Depending on the results of tests which are not yet finished, there is evidence that the performance of GC 2 corresponds quite well with GC 1. Some minor adaptations for method GC 2 will also be considered, such as to search for another internal standard, or to lower its concentration.

Table 3: Summary data phase 1

		substance 1		**substance 2**	
		GC 1	**GC 2**	**GC 1**	**G**
calibration	range covered (µg/ml) level 1 to level 6	35 - 300		15 - 140	
	range covered (mg/filter rod)	1.8 - 15		0.8 - 7.0	
	range covered (mg/tobacco rod)	0.35 - 3.00		0.15 - 1.40	
	range covered (mg/filter tip)	0.18 - 1.50		0.08 to 0.70	
linearity	r	0.99999	0.99978	0.99999	0.9
	r^2	0.99998	0.99956	0.99998	0.9
working range	number of replicates	10	10	10	1
	spiking level 1 *(filter rod)* mg/rod	1.90	1.87	0.86	0.
	spiking level 0 *(filter rod)* mg/rod	0.76	0.75	0.35	0.
	spiking level 1 *(tobacco rod)* mg/rod	0.38	0.37	0.17	0.
	spiking level 0 *(tobacco rod)* mg/rod	0.15	0.15	0.07	0.
	level 1 *filter rod* mean (mg/rod)	1.90	1.84	0.87	0.
	level 0 *filter rod* mean (mg/rod)	0.76	0.75	0.35	0.
	level 1 *filter rod* std. dev.	0.021	0.015	0.009	0.0
	level 0 *filter rod* std. dev.	0.012	0.012	0.006	0.0
	LOQ *filter rods* (estimated) (10*std. dev.) mg/rod	0.21	0.15	0.09	0.
	level 1 *tobacco rod* mean (mg/rod)	0.38	0.38	0.17	0.
	level 0 *tobacco* rod mean (mg/rod)	0.14	0.15	0.07	0.
	level 1 *tobacco rod* std. dev.	0.002	0.004	0.001	0.0
	level 0 *tobacco rod* std. dev.	0.002	0.002	0.001	0.0
	LOQ *tobacco rods* (estimated) (10*std. dev.) mg/rod	0.02	0.04	0.01	0.
accuracy	spiking levels *filter rods* (mg/rod)	2.2 - 13.5		1.0 - 6.2	
	spiking levels *tobacco rods* (mg/rod)	0.45 - 2.7		0.2 - 1.2	
	number of replicates			10	
	recovery rates filter rods: in %				
	level A	103.6	99.3	104.4	9
	level B	100.1	98.8	100.7	9

	level C	101.0	96.0	101.5	9
	level D	100.6	96.5	101.1	9
	repeatability filter rods (%)				
	level A	2.2	4.2	2.2	4
	level B	0.8	1.3	0.8	1
	level C	4.1	3.4	4.1	3
	level D	1.7	3.9	1.8	3
	recovery rates tobacco rods: in %				
	level A	98.1	100.0	99.1	10
	level B	97.1	100.3	97.9	10
	level C	95.8	101.3	96.5	10
	level D	96.6	100.3	97.2	10
	repeatability tobacco rods (%)				
	level A	6.8	2.6	2.7	3
	level B	1.6	2.1	1.6	2
	level C	2.3	4.2	2.4	4
	level D	1.7	2.2	1.5	2
precision	material	filter rod			
	level tested (mg/rod)	5.2 (equals appr. level B)			
	number of samples	18	17	18	1
	number of replicates	2 for each sample			
	repeatability (based on n=2)	0.190	0.129	0.093	0.0
	repeatability in %, (based on n=2)	3.6	2.5	3.4	2