Routine Analytical Chemistry Sub-Group

Adapted to the RAC template (Aug. 2010)

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Technical Report

Review of CORESTA Recommended Method N°9

Period of activities: 2003 to 2007

Co-ordinator: Linda Drake, British American Tobacco, Southampton, UK

Author: Hiromoto Yamazaki, Japan Tobacco Inc., Tokyo, Japan

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1 Introduction

The CORESTA Recommended Method Number 9 (CRM9) “Determination of Nicotine in Cigarette Filters by Gas Chromatographic Analysis” was first issued in October 1989. Its field of application is the determination of nicotine retention of cigarette filters made from paper or cellulose acetate plasticized with triacetin or triethyleneglycol-diacetate (TEGDA).

The applicability of this method to filters containing adsorbents or chemical additives was uncertain at the time of issue.

In 2003 the CORESTA Scientific Commission asked the Routine Analytical Chemistry (RAC) sub-group to review and update CRM9 to include:

- Recent advances in GC analysis techniques (i.e. use of megabore and capillary columns)
- r and R statistics

In addition, the RAC proposed that the method be reviewed to extend the scope to include filters containing carbon.

This work was initiated in order to evaluate these more advanced techniques and also to provide statistical data in support of CRM9 when applied to filters containing adsorbents or additives, specifically charcoal.

The following aspects are investigated in this report:-

- Extraction solvent
- Internal Standard (ISTD)
- Extraction process
- Applicability to filters with high levels of carbon loading

2 Summary

Two collaborative studies were conducted by the RAC following investigations to find a suitable extraction solvent by a volunteer laboratory (Japan Tobacco Inc.). These were:-

- Validation check of “the determination of nicotine in acetate filter” CRM9
- Determination of the repeatability and reproducibility of “the determination of nicotine in carbon filter”

In addition, a study was carried out by another volunteer laboratory (Filtrona Technology Centre, Jarrow, UK) to confirm the applicability of the methods to highly loaded carbon filters (> 50mg/tip carbon).

2.1 Validation check of “the determination of nicotine in acetate filter” CRM9

11 laboratories participated in a study to determine Sr & SR values using three extraction solvents, methanol, acidic methanol and basic methanol. CM4 and a test cigarette with an acetate filter, Sample A, were used in this study. The results are in TABLE 1

TABLE 1: Sr and SR Values for Nicotine in Acetate Filters.

CM4				
	# of Labs	Mean	Sr	SR
Method A	11	0.790	0.030	0.080
Method B	11	0.835	0.024	0.095
Method C	10	0.827	0.038	0.084

Sample A				
	# of Labs	Mean	Sr	SR
Method A	12	0.39	0.027	0.058
Method B	11	0.40	0.021	0.055
Method C	10	0.41	0.033	0.054

This validation check proved that there was no significant difference between the Sr and SR values when all three extraction solvents were compared using acetate filters.

2.2 Determination of the repeatability SD (Sr) and reproducibility SD (SR) of "the determination of nicotine in carbon filter"

Additional work in this report showed that the most effective extraction method for the determination of nicotine in carbon-containing filters was using acidic methanol with glycerol triproprionate as internal standard. The attempt to determine Sr and SR used this approach along with CM4 and five other test cigarettes, some containing carbon with loadings of 25 and 50 mg/cig. The results are shown in TABLE 2.

TABLE 2: CM4 Sr and SR Values for Nicotine in Acetate and Carbon Filters

Sample	Carbon Level	# of Labs	Mean	Sr	SR
CM4	None	10	0.778	0.031	0.057
A	None	11	0.389	0.025	0.058
B	~25mg/filter	12	0.458	0.032	0.061
C	~25mg/filter	12	0.341	0.022	0.049
D	~50mg/filter	11	0.374	0.018	0.049
E	~50mg/filter	12	0.275	0.017	0.040

2.3 Investigation of applicability of the methods to highly loaded carbon filters (>50mg/filter).

Further work was carried out using carbon filters with up to 100 mg carbon per filter and showed that the direct extraction procedure is not applicable for highly loaded carbon filtered cigarettes (>50 mg/filter). In addition, filter age has an effect on extraction efficiency and therefore the use of CRM9 for analysis of cigarettes containing carbon is not advised.

3 Investigation of a suitable extraction technique

3.1 Acetate filters

The first stage of the evaluation involved an international collaborative study to investigate a suitable extraction solvent for acetate filters only. This was performed in 2003 between 13 laboratories using the CORESTA Monitor 4 (CM4). The samples consisted of four batches of five filters which had been removed from CM4 smoked according to ISO4387.

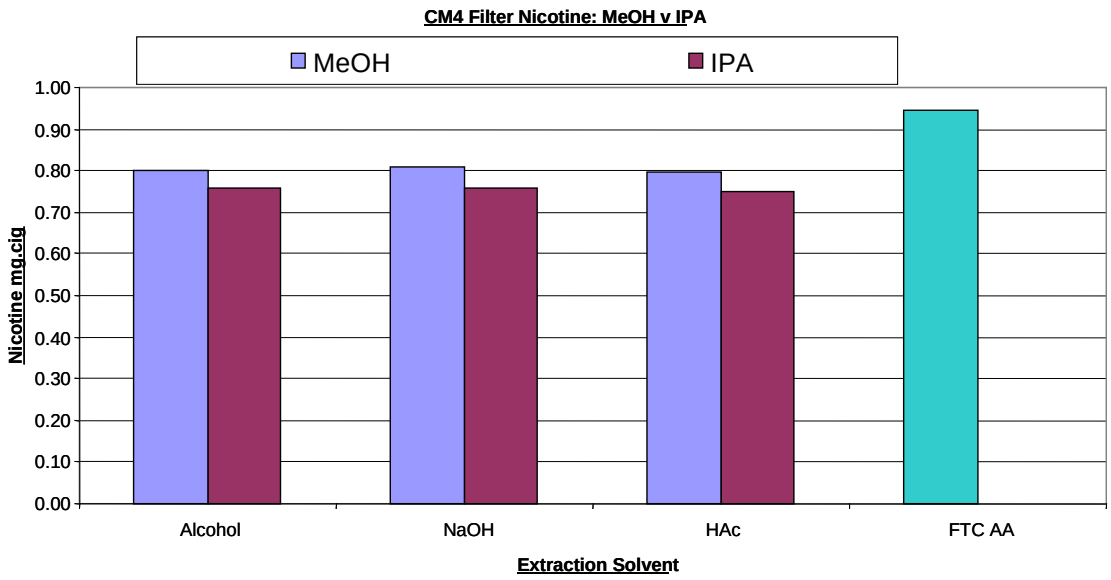
The extraction efficiencies of methanol (CRM9) and isopropyl alcohol under acidic (HAc), neutral and basic (NaOH) conditions were examined. As a comparison, the nicotine content, as total nicotine alkaloids (FTC AA), was also measured using continuous flow technology, the reference method. The results are shown in the following table:-

TABLE 3 Acetate Filters - Comparison of Extraction Solvents.

	# of Labs	Mean	SD
MeOH	13	38.90	4.13
IPA	13	37.29	3.39
MeOH + NaOH	13	38.55	3.35
IPA + NaOH	13	37.54	4.08
MeOH + HAc	13	38.57	3.95
IPA + HAc	13	36.91	3.86
FTC AA	1	39.72	N/A

In addition, these results are graphically depicted in Figure 1.

Figure 1: CM4 Filter Nicotine: MeOH v IPA



3.1.1 Conclusions

This study showed that methanol was more efficient at extracting nicotine from acetate filters than isopropyl alcohol and acidic or basic conditions did not improve extraction efficiency. Therefore the extraction solvent recommended in CRM9 is the most appropriate for extraction of nicotine in acetate filters

3.2 Carbon filters

During a period from September 2003 to April 2005, method optimisation for the determination of nicotine in both carbon impregnated and acetate filters was undertaken at a volunteer laboratory (Japan Tobacco Inc.). Summaries of these studies were reported to the CORESTA RAC and are summarized in this section.

3.2.1 Nicotine recovery test

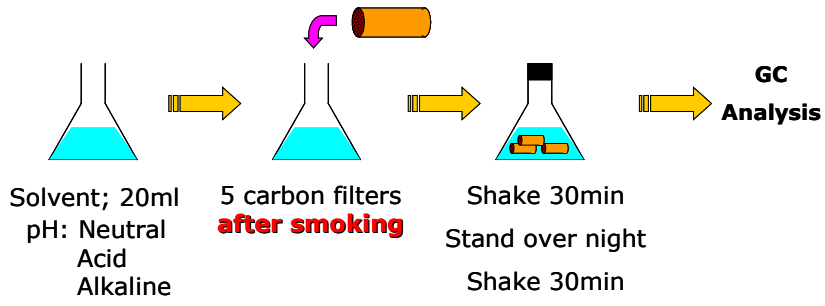
3.2.1.1 Objective

To confirm influences of the solvent selection and pH conditions when extracting nicotine from carbon filters. Please note that the ISTD approach was not used during this experiment because of the potential of the carbon to adsorb the ISTD.

3.2.1.2 Test design

Test cigarettes, containing approximately 35 mg carbon per filter, were smoked according to ISO 4387. The filters were removed from the cigarette butts. 20ml of solvent (both methanol and isopropyl alcohol under acidic, normal and basic conditions as in section 3.1 above) was added to separate batches of 5 filters according to the Figure 2 illustration below.

Figure 2: Test Design Graphic



Four batches of 5 filters were extracted for each condition.

3.2.1.3 Results

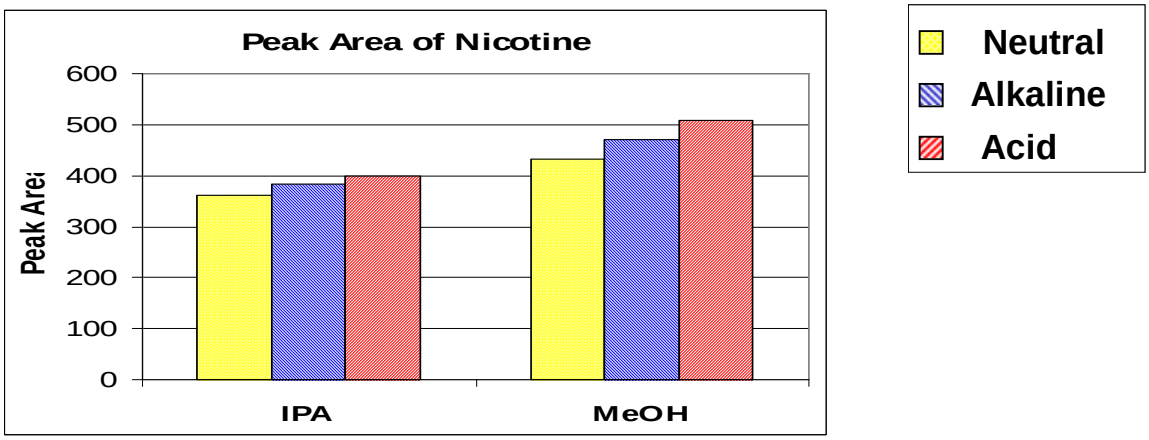
The results from this experiment are to be found in TABLE 4.

TABLE 4: Carbon Filters – Comparison of Extraction Solvents

		GC - Peak Area Nicotine				Mean	SD
IPA	Neutral	323	395	355	373	361	31
	Alkaline	366	393	378	401	384	16
	Acid	406	406	384	401	399	10
MeOH	Neutral	468	451	432	383	433	37
	Alkaline	486	475	500	424	471	33
	Acid	496	517	522	501	509	13

The results are graphically depicted in the following Figure 3:-

Figure 3: Carbon Filters – Comparison of Extraction Solvents



3.2.1.4 Conclusion

This experiment has shown that acidic methanol removed nicotine from the carbon the most efficiently.

3.2.2 Investigation of a suitable internal standard (ISTD)

3.2.2.1 Objective

To research an optimal ISTD by carrying out an adsorption test using carbon filters before smoking. Potential ISTD candidates are:

- n-heptadecane
- anethole
- 1,3-butanediol
- Quinaldine
- Glycerol tripropionate (CAS#;139-45-7 Tripropionin)

3.2.2.2 Test design

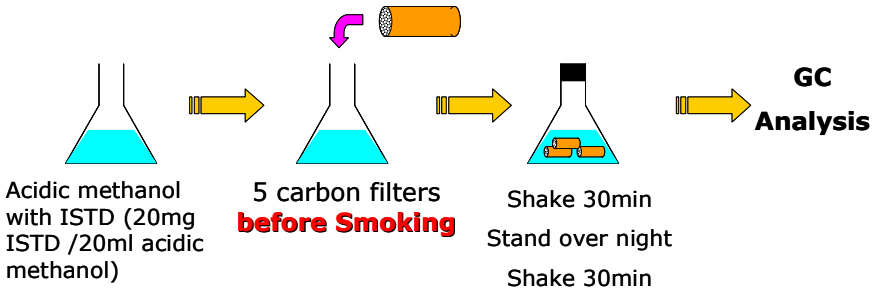
Three different test filters were used containing 3 levels of carbon according to TABLE 5 below:-

TABLE 5: Carbon Content of the Filters

Carbon Filter	Carbon Content (mg/filter)
Test Filter – 1	35 mg level
Test Filter – 2	50 mg level
Test Filter – 3	75mg level

Acidic methanol, which was successfully used in section 3.2.1, was used to extract 5 unsmoked test filters 1 and 2 as shown in Figure 4 below:-

Figure 4: Acidic Methanol Extraction of Carbon FIlters



Acid methanol: 10cm^3/L Acetic acid

With the exception of glycerol triproprionate, the higher levels of carbon found in test filter 2 appeared to adsorb the prospective internal standards. Therefore only test filter 3 with glycerol triproprionate as the internal standard was extracted.

3.2.2.3 Results

The results are shown in TABLE 6.

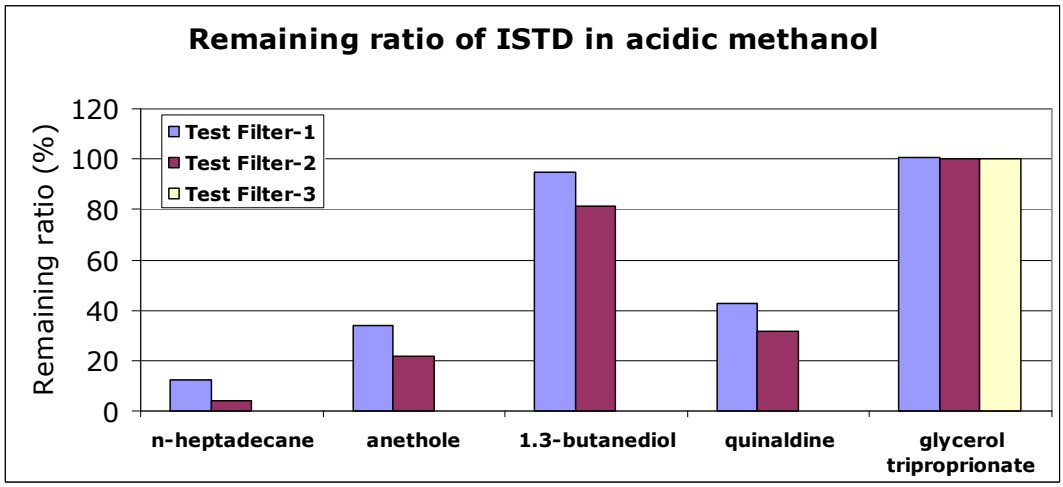
TABLE 6 Comparison of ISTD Adsorption

	Test Filter - 1	Test Filter -2	Test Filter -3
n-heptadecane	12.4	4.1	-
anethole	34.1	21.5	-
1,3-butanediol	94.9	81.1	-
quinaldine	42.5	31.6	-
glycerol tripropionate	100.7	100.1	99.9

This experiment showed that glycerol triproprionate was the most suitable ISTD for the extraction of carbon filters under acidic methanol conditions.

A graphical illustration is found below:-

Figure 5: ISTD Adsorption in Acidic Methanol



3.2.2.4 Conclusion

Glycerol triproprionate can be used as the ISTD when carbon filters are analysed for nicotine.

3.2.3 Optimisation of extraction time using a shaker

3.2.3.1 Objective

To optimise the shaking time necessary for removing nicotine from carbon filters

3.2.3.2 Test design

4 batches of 5 unsmoked carbon filters were spiked with nicotine (1.5mg/filter) and allowed to stand overnight in an airtight vessel to allow the nicotine to equilibrate into the filter. The filters were then extracted with acidic methanol containing glycerol triproprionate under the following four conditions;-

1) 60 minutes using a flat bed shaker at 200 throws per minute.

2) 120 minutes using a flat bed shaker at 200 throws per minute

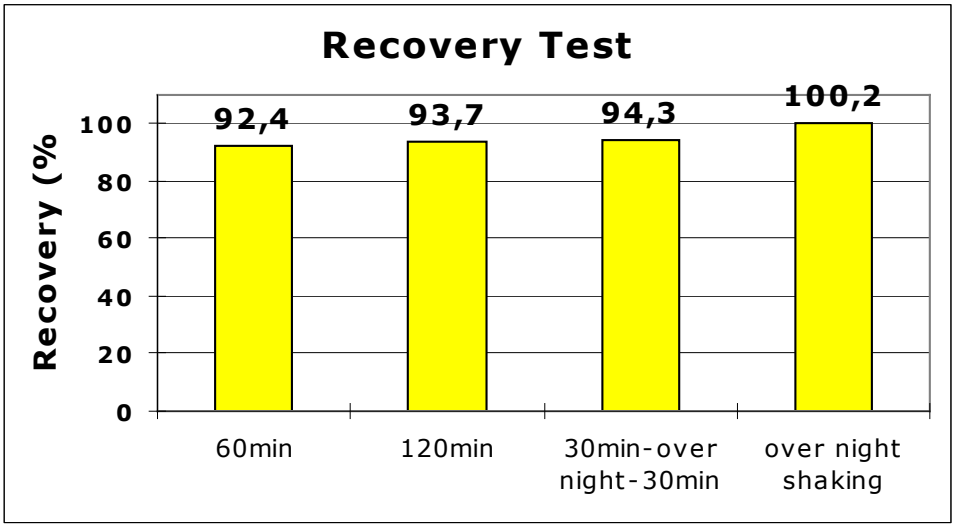
3) 30 minutes using a flat bed shaker at 200 throws per minute, stood over-night, and then 30 minutes using a flat bed shaker at 200 throws per minute

4) Over night shaking using a flat bed shaker at 200 throws per minute

3.2.3.3 Results

The results are depicted in Figure 6 below.

Figure 6: Recovery Test



3.2.3.4 Conclusion

Nicotine in carbon filters is extracted completely by overnight shaking (16 hours) on a linear shaker.

3.2.4 Comparison of available extraction techniques for nicotine in carbon filters

3.2.4.1 Objective

Methods for the determination of nicotine in carbon filter developed at the volunteer laboratory were evaluated by comparing with a Soxhlet extraction method because Soxhlet extraction has been shown to be very effective. Furthermore, a back extraction method was also evaluated as this method has been used for the analysis of nicotine in tobacco.

3.2.4.2 Test design

Four batches of five filters were smoked according to ISO4387 and extracted according to one of the five conditions detailed in TABLE 7 below.

The five conditions included three of the developed methods already discussed in this report along with soxhlet and back extraction methods.

TABLE 7 Extraction Conditions – Carbon Filters

	Developed method 1	Developed method 2	Developed method 3	Soxhlet extraction	Back extraction method
Smoking Method	ISO 4387				
Number of Filters	5 filters * 4 times (total 20 filters)				
Solvent	Acidic MeOH (10mL/L acetic acid)				water 20mL Hexane 40mL Conc. NaOH 10mL
	20mL	20mL	20mL	80mL	
Extraction Condition	30 min. shake stand overnight 30 min. shake	Overnight shaking (16 hours)	Overnight shaking (16 hours)	Reflux for 16 hours with oil bath temp. at 90°C	1 hour shaking
ISTD	Glycerol triprionate	Glycerol triprionate	None	Add 25mg of n-heptadecane after reflux	None

3.2.4.3 Results

The results are shown in TABLE 8.

TABLE 8 Results of the Extraction Conditions - Nicotine (mg/filter)

Method	1	2	3	4	Average	Standard Deviation
Developed Method 1	0.522	0.523	0.495	0.533	0.52	0.016
Developed Method 2	0.589	0.606	0.602	0.582	0.59	0.011
Developed Method 3	0.600	0.615	0.593	0.560	0.59	0.023
Soxhlet	0.592	0.568	0.600	0.594	0.59	0.014
Back Extraction Method	0.302	0.351	0.414	0.336	0.35	0.047

3.2.4.4 Conclusions

Nicotine could not be extracted completely with the back extraction method. The most efficient extraction condition was overnight shaking with glycerol triproprionate as ISTD. This method, depicted as developed method 2 in the table above also gave the lowest SD.

3.2.5 Comparison of available extraction techniques for nicotine in acetate filters

3.2.5.1 Objective

To confirm if the developed methods are suitable for the application of nicotine in acetate filter

3.2.5.2 Test design

The experiment carried out in section 5 was repeated for acetate filters with the exception of the back extraction method which was replaced by the original collaborative study method using methanol as the extraction solvent and heptadecane as the ISTD. See TABLE 9 for the different conditions undertaken.

TABLE 9: Extraction Conditions – Acetate Filters

	Developed method 1	Developed method 2	Developed method 3	Soxhlet extraction	Previous collaborative GC
Smoking Method	ISO 4387				
Test Article	CM4				
Number of Filters	5 filters * 4 times (total 20 filters)				
Solvent	Acidic MeOH (10mL/L acetic acid)				MeOH
	20mL	20mL	20mL	80mL	
Extraction Condition	30 min. shake stand overnight 30 min. shake	Overnight shaking (16 hours)	Overnight shaking (16 hours)	Reflux for 16 hours with oil bath temp. at 90°C	1 hour shaking
ISTD	Glycerol triproprionate	Glycerol triproprionate	None	Add 25mg of n-hexadecane after reflux	None

3.2.5.3 Results

The results from this study are shown in TABLE 10.

TABLE 10: Results of Extraction of Acetate Filters - Nicotine (mg/filter)

Method	1	2	3	4	Average	Standard Deviation
Developed Method 1	0.822	0.787	0.795	0.834	0.81	0.022
Developed Method 2	0.811	0.834	0.814	0.825	0.82	0.011
Developed Method 3	0.794	0.840	0.794	0.795	0.81	0.021
Soxhlet	0.813	0.792	0.791	0.835	0.81	0.021
Previous Collaborative Study GC Method	0.789	0.819	0.837	0.839	0.82	0.023

3.2.5.4 Conclusions

For nicotine in acetate filters, any of these methods would be suitable because all methods show same average results on CM4

4 Additional Collaborative Studies by the RAC

4.1 Acetate Filters - Validation check of "the determination of nicotine in acetate filter"

As CRM9 does not have R & r values associated with it, in order to determine these values, this study was agreed in 2005 at an RAC meeting and conducted in 2006. 11 laboratories participated in the study.

Results from the laboratories were analyzed according to ISO 5725-2 after checking for outliers, and the repeatability (Sr) and reproducibility (SR) of each

method were estimated. Furthermore, results from the methods were compared with those from the Soxhlet extraction method (as reference method).

4.1.1 Samples

Two samples were used for this study, neither containing carbon but having low and high NFDPM yields.

TABLE 11: Sample Information

Sample Name	CM4	Sample-A
Filter Type	Acetate filter	Acetate filter
Tar level	~14.0mg/cig	~4.0mg/cig
Filter Ventilation	No	Yes
Sample Type	CORESTA Monitor	Test cigarette

4.1.2 Methods

The different methods used are listed in the following table:-

TABLE 12: Method Information

	Method A (JT method developed for acetate filters)	**Method B** (Previous collaborative study)	**Method C** (CORESTA Recommended Method No. 9)	**Soxhlet Extraction** (Reference method)
Smoking Method	ISO4387			
Number of filters	5 filters / extraction			
Extraction Solvent	4 extractions / sample (Total 20 test articles)			
	Acidic MeOH	MeOH	Alkali MeOH	Acidic MeOH
	10 mL/L acetic acid			10 mL/L acetic acid
	20 mL	20 mL	20 mL	80 mL
Extraction Condition	shake 30 min. & stand overnight	shake 30 min. & stand overnight	shake 30 min.	Reflux for 16 hours
				Oil bath temp. at 90℃
ISTD	Glycerol triproprionate or n-heptadecane	n-heptadecane	n-heptadecane	Add 25mg n-heptadecane after reflux
GC Measurement	2 replicates per extraction for a total of 8 GC measurements per sample			

Note: The solvent was alkaline MeOH in CRM9 (1989 version).

4.1.3 Results

The results from this experiment are listed in the following table.

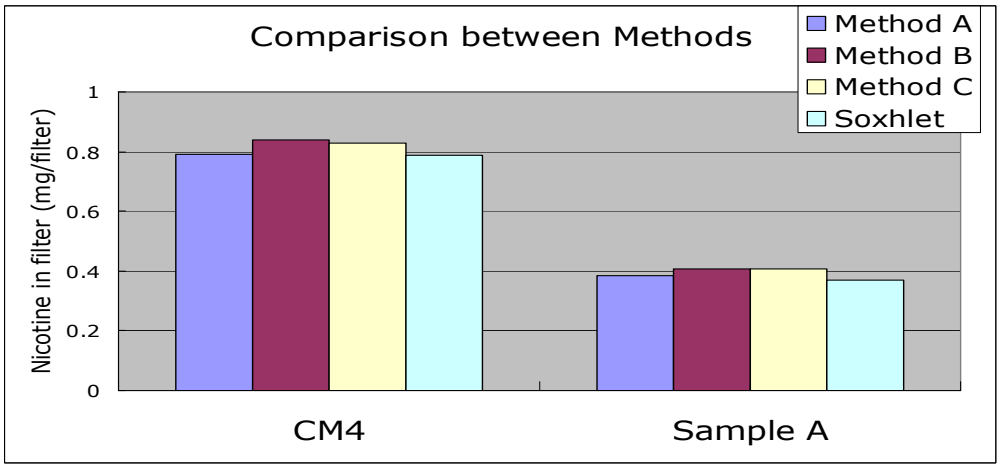
TABLE 13: Results. Nicotine (mg/filter)

CM4				
	# of Labs	Mean	Sr	SR
Method A	11	0.790	0.030	0.080
Method B	11	0.835	0.024	0.095
Method C	10	0.827	0.038	0.084

Sample A				
	# of Labs	Mean	Sr	SR
Method A	12	0.39	0.027	0.058
Method B	11	0.40	0.021	0.055
Method C	10	0.41	0.033	0.054

These are also graphically depicted in Figure 7 below:-

Figure 7: Comparison between Solvent Extraction Methods



4.1.4 Conclusion

Results from the method developed for acetate filter by the volunteer laboratory (method A), the method used at the collaborative study on suitable extraction solvent for nicotine in acetate filter (method B) and the CRM9 (method C) did not exhibit a significant difference in the results from the 'reference' Soxhlet extraction method. Therefore, any one of the methods A, B or C would be suitable for determining nicotine in acetate filter.

4.2 Carbon Filters - Determination of the repeatability and reproducibility of "the determination of nicotine in carbon filter"

This collaborative study for the repeatability and reproducibility of "the determination of nicotine in carbon filters (Developed method 2 from TABLE 9 = Method 1 in this collaborative study) was also proposed at the CORESTA RAC meeting in 2005 and conducted in 2006.

Results from the laboratories were analyzed according to ISO 5725-2 after checking for outliers, and the repeatability (S_r) and reproducibility (S_R) of Method 1 were estimated.

4.2.1 Samples

TABLE 14 Sample Information

Sample Name	CM4	Sample A	Sample B	Sample C	Sample D	Sample E
Carbon Level	None		Low (~ 25mg/filter)		High (~ 50mg/filter)	
Tar Level	High	Low	High	Low	High	Low
Filter Ventilation	No	Yes	No	Yes	No	Yes

4.2.2 Methods

As in previous experiments, samples were smoked according to ISO4387 before the filters were detached. 5 filters were extracted per replicate and 4 replicates per sample were prepared according to the conditions specified in the following table:-

TABLE 15: Extraction Information. Carbon Filters.

	Method - 1	**Soxhlet Extraction**
	(JT method developed for carbon filters)	(Reference method)
Smoking Method	ISO4387	
Number of filters	5 filters / extraction	
Extraction	4 extractions / sample (Total 20 test articles)	
Solvent	Acidic MeOH (10 mL/L acetic acid)	
	10 mL/L acetic acid	10 mL/L acetic acid
	20 mL	80 mL
Extraction Condition	Overnight shaking	Reflux for 16 hours
		Oil bath temp. at 90℃
ISTD	Glycerol triproprionate	Add 25mg n-heptadecane after reflux
GC Measurement	2 replicates per extraction for a total of 8 GC measurements per sample	

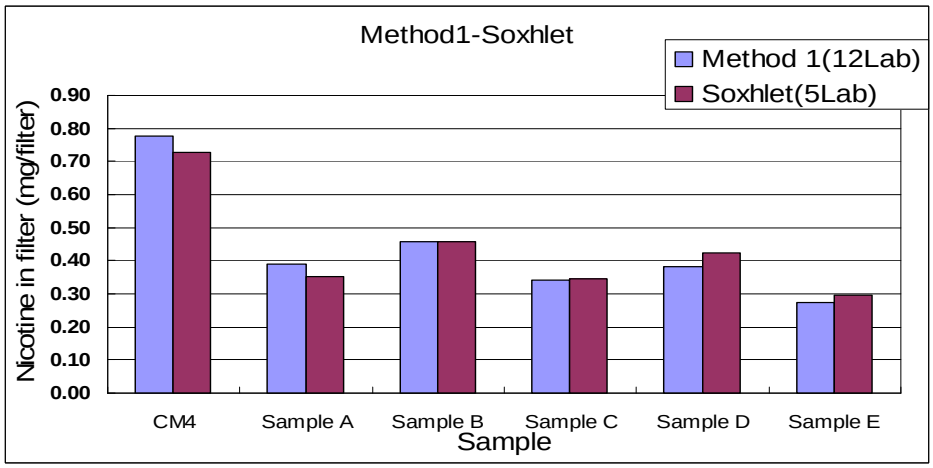
4.2.3 Results

TABLE 16: Nicotine Results (mg/filter) for Method 1

	# of Labs	Mean	Sr	SR
CM4	10	0.778	0.031	0.057
Sample A	11	0.389	0.025	0.058
Sample B	12	0.458	0.032	0.061
Sample C	12	0.341	0.022	0.049
Sample D	11	0.374	0.018	0.049
Sample E	12	0.275	0.017	0.040

The results are graphically depicted in Figure 8.

Figure 8: Method 1 v Soxhlet Extraction



4.2.4 Conclusion

Results from Method 1 did not show a significant difference to the results from Soxhlet extraction method. Therefore Method 1 would be suitable for analysis of nicotine in smoked carbon filters should the Soxhlet reference method not be available.

5 Additional work by a volunteer laboratory -Filtrona Technology Centre, U.K. (FTC)

At the CORESTA RAC meeting in October 2007, some concerns were raised on the applicability of applying a liquid solvent extraction technique such as in CRM9 or the collaborative experiment method to carbon filters. In particular the possible incomplete recovery of nicotine from highly loaded (> 50mg/tip carbon) filters. FTC volunteered to carry out some work to investigate this.

5.1 Direct and Indirect Measurement of Nicotine Retention in Charcoal Filters

The collaborative study with filters containing up to 50 mg of carbon per filter has shown some promising results for the measurement of nicotine filter retention using methanol extraction of the filter followed by GC analysis of nicotine. However, a general global trend has been towards more carbon usage in cigarette filters and commercial brands are available with much higher carbon loadings than 50 mg. One of the highest loaded carbon filters is on the BF family of brands (Greece and Southern Europe) that uses about 150 mg of carbon per filter. To investigate the efficiency of the methanol extraction procedure for the measurement of nicotine filter retention, further work has been carried out using carbon filters with up to 100 mg carbon per filter.

5.1.1 Samples

A series of three carbon filters were produced all of which were dual dalmation type filters with a 7 mm acetate segment at the mouth end and a 20 mm length dalmation segment at the tobacco column end of the filter. These filters were made with low medium and high carbon loadings as shown in the table below and were designed to have about the same overall retention as the acetate filter on the current (CM5) CORESTA monitor cigarette.

TABLE 17: List of Samples

Filter	Carbon Loading (mg/tip)
Low Carbon Load	24.4
Medium Carbon Load	48.6
High Carbon Load	100.4

Cigarettes were produced with these filters using tobacco columns cut from CORESTA monitor cigarettes (which were then aged for at least three weeks before testing).

5.1.2 Method

The nicotine retention of the filter was measured using two procedures:-

1) Indirect – Unfiltered tobacco columns and the cigarettes smoked in the same run. Nicotine retention calculated as [(tobacco rod yield – cigarette yield)/tobacco rod yield] x 100.

2) Direct – The nicotine content of the CF pads and the filters were measured by extraction with methanol (overnight shaking) and determined by GC analysis. To remove any possible complications from adsorption of internal standard the internal standard was not used in the calculation of nicotine content. The nicotine retention was calculated as [measured nicotine in filter/ (measured nicotine in filter + measured nicotine yield)] x 100.

5.1.3 Results

TABLE 18: Results from Direct and Indirect Measurement Techniques

Sample	Average Nicotine Retention %	
	Indirect Measure	Direct Measure
CM5	33.5	38.4
Low Carbon Load	36.4	37.1
Medium Carbon Load	38.9	29.6
High Carbon Load	37.3	24.8

5.1.4 Conclusion

The decrease in measured value for the direct method with increasing carbon loading strongly suggests that for highly loaded (or carbons of higher activity than currently used) carbon filters some problems of extraction of nicotine still exist even when using overnight extraction with methanol as the solvent. As these cigarettes were quite fresh (relatively little carbon deactivation during storage) when tested this probably represents the worst case and higher extraction efficiencies would be expected for older filters. Nevertheless the data does show that the direct extraction procedure is not applicable for highly loaded carbon filtered cigarettes. In addition, filter age has an effect on extraction efficiency and therefore the use of CRM9 for analysis of cigarettes containing carbon is not advised.