Quantitative Analysis of Polyaromatic Hydrocarbons, including Benzo[a]Pyrene and Naphthalene, in Tobacco, Tobacco Products, Fiber-based Matrices, and Tobacco Derived Products using Gas Chromatography-Mass Spectrometry

Purpose **4**

Applies to **4**

General information **4**
Principle of the method 4
Method scope, measurement range, and measurement uncertainty 6
Literature references 8
Internal reference documents (available upon request) 8

Risk assessment and safety instructions **8**
Summarized risk assessment 8
Substances hazardous to the environment: 8
Flammable substances: 8

Equipment **9**
Apparatus and laboratory utensils 9
GC system: 9
(b) (4) 9
..... 9
..... 10
..... 10
..... 12
..... 12
..... 13
Other equipment: 14
Chemicals, reagents and solvents 14
Check samples and reference materials 15
Preparation of standards 15

General information 15
Preparations 15
Comparison of old and new standards 20
(b) (4) 21
..... 21
..... 21
..... 21
Sample handling 21
Sample storage and preparation 21
Sample amount 21
Analysis 21
Calibration and verification of apparatus 21
Sample stability 23
Analytical procedure 23
General information 23
Sample preparation 23
Solid phase extraction with Gilson SPE robot 24
Special instructions 24
Documentation 26
Data 26
Collection and storage of data 26
How to find results and raw data 26
Calculations 26
Quality assurance 27
Control chart 27
Standard curve criteria 28
Duplicate and triplicate samples 28
Confirmation 28
Concentration above calibration curve 29
Reporting of analysis results 29
Revision history 29
Person responsible 29
Validation report 30
Supporting documentation for validation 30
Specificity 30
Repeatability 34
Precision within the laboratory 35
Accuracy 37
Limit of quantification (LOQ) 37
Linearity 38

Robustness 39
Measurement range and measurement uncertainty 39
Conclusion 40

Purpose

To determine the concentration of 18 polyaromatic hydrocarbons (PAHs) including benzo[a]pyrene (B[a]P) and naphthalene in tobacco, tobacco products, fiber-based matrices, and tobacco derived products with (b) (4) and head space (b) (4), and to determine the concentration for B[a]P with (b) (4).

Applies to

APRS

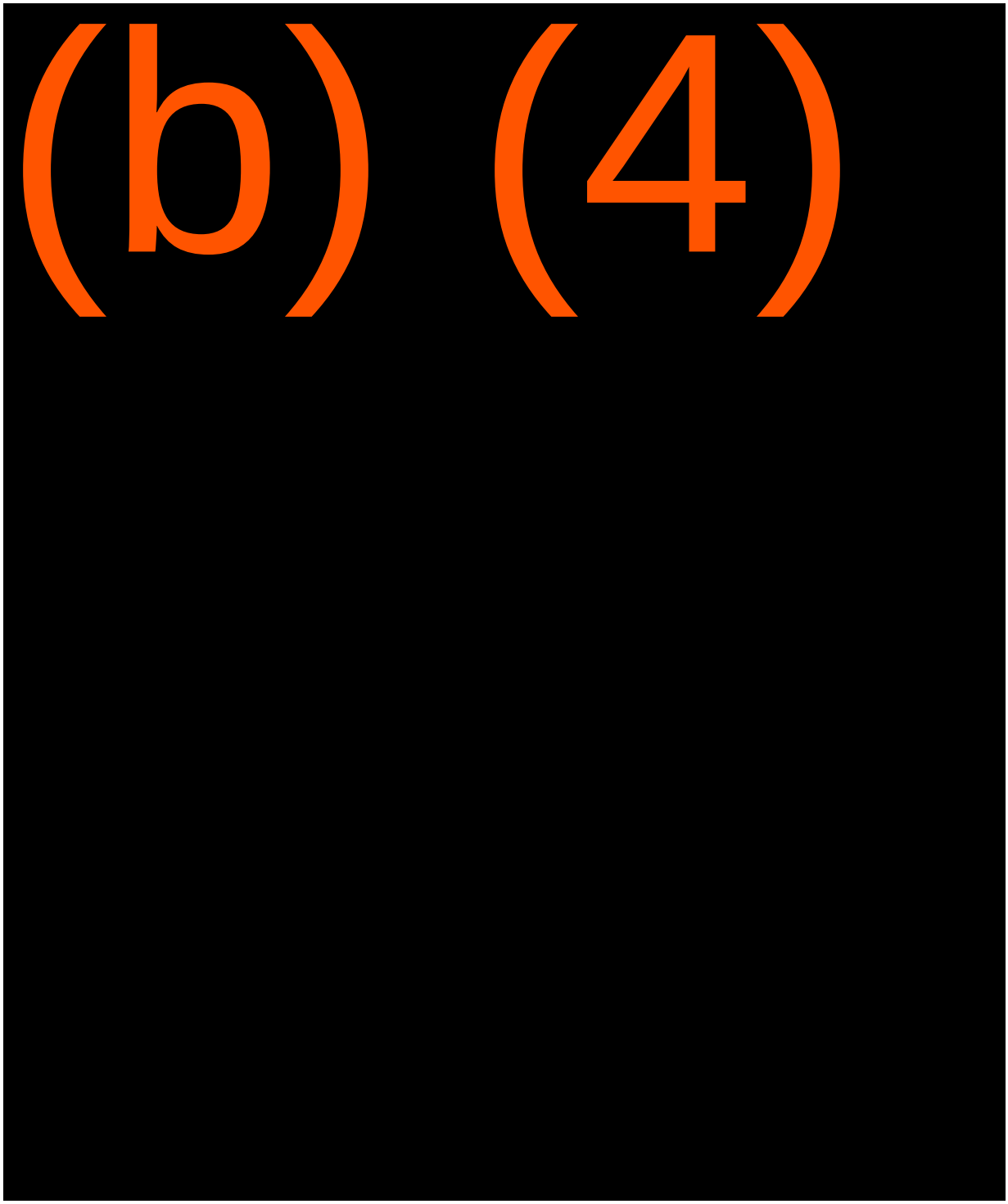
General information

Principle of the method

(b) (4)

The method is a modified CORESTA method (b) (4). The modifications are:

(b) (4)



Note: All reference documents and additional information stated “available upon request” are in Swedish. They are available upon request but need to be translated into English first.

Method scope, measurement range, and measurement uncertainty

(b) (4)

Table 1. (b) (4)

(b) (4)

Table 2. Demarcations for GC-MS analysis of B[a]P.

Analyte	*Calibration range (ng/ml)*	*Measurement range (ng/g as is)*	*LOQ GC-MS (ng/g as is)*	*Measurement uncertainty GC-MS (%) Duplicate samples*
(b) (4)				

(b) (4)

Literature references

(b) (4)

(b) (4)

Risk assessment and safety instructions

(b) (4)

Summarized risk assessment

(b) (4) Wear suitable protective clothing, protective gloves and goggles.
All work when preparing solutions for PAHs and internal standards are to be carried out in a fume cabinet, on a draw bench or places with spot extractor.
Protective gloves should be worn, along with protective goggles.

Substances hazardous to the environment:

(b) (4)

Flammable substances:

(b) (4)

Equipment

Apparatus and laboratory utensils

(b) (4)

(b) (4)

Table 3. (b) (4)

(b) (4)

GC parameters for headspace (naphthalene analysis)

(b) (4)

Table 4. Naphthalene: Quantifier and qualifier (SIM).

Name	Abbreviation	Time Segment [min]	Quantifier	Qualifier
(b) (4)				

(b) (4)

(b) (4)

(b) (4)

Check samples and reference materials

(b) (4)

Preparation of standards

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Comparison of old and new standards

When preparing standards from a new primary standard, the difference in absolute peak area should not differ by more than 10%. Evaluate by analyzing calibration standard level 4 in triplicate and alternate the new and old standards when analyzing and performing the assessment based on the mean value. This is checked by setting the mean value of the old and new peak areas in Equation 1 below.

$$ABS|\bar{x}_{new}-\bar{x}^{(b)\ (4)} \quad 1$$

Where;

$\bar{x}_{new} = average\ absolute\ peak\ area\ of\ new\ standard\ solution$

$\bar{x}_{old} = average\ absolute\ peak\ area\ of\ old\ standard\ solution$

Preparation of other solutions

(b) (4)

Sample handling

Sample storage and preparation

(b) (4)

Sample amount

The minimum sample amount is (b)(4) g

Analysis

Calibration and verification of apparatus

Before a sample series can be run, prepare the instrument.

It is important that the instrument is checked before starting a sample sequence.

1. Before testing a sample series, the retention times must be checked using calibration standard 5. If the retention times have been changed resulting in the peaks falling outside their segment or close to the start and end, the data collection time segments must then be updated in the collection method.
2. Also check that area units for IS for calibration standard 5 is consistent with the area for IS at the last calibration. The area for IS must not have reduced by more than 50%. If the reduction is greater, investigate and correct as below:
    1. Replace liner
    2. Search for leaks with a leak detector
    3. Perform new autotune
    4. Cut about a 5 cm column on the injector side
    5. Contact the person responsible for the method

6. Replace column
7. Clean/replace the ion source

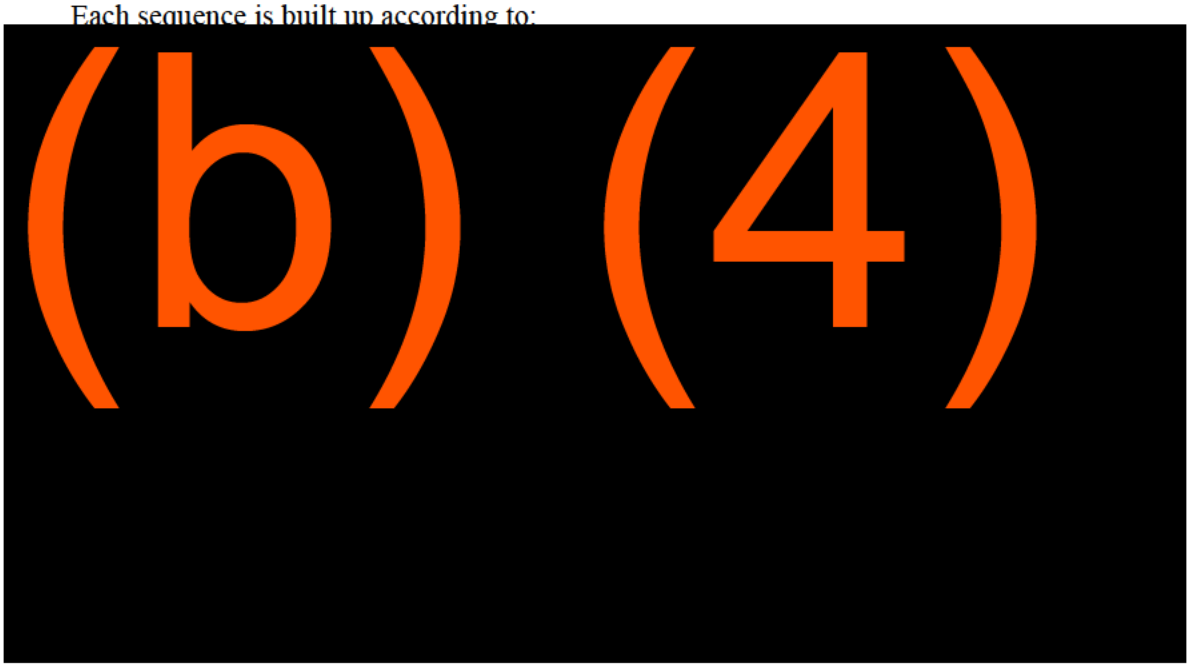
Inject calibration standard 5 again and evaluate if more measures need to be performed.

System performance is also checked when evaluating a batch. At start-up and at the end of each sample sequence, all calibration standards are also injected, which is evaluated as follows:

- (b) (4)
- Replace liner
- Perform new autotune
- Cut about a 5 cm column on the injector side
- Contact the person responsible for the method
- Replace column
- Clean/replace the ion source

A standard curve is generated in each sequence. Before samples are analyzed in a sample sequence (following equilibration of the system), the sample sequence is injected with all calibration standards, followed by a blank and two check samples that are evaluated as set out in DESC "Control Charts and Check Samples". Once all the samples have been analyzed, the check samples are injected again, and finally all calibration standards are also injected, (also applies when analyzing naphthalene).

Each sequence is built up according to:



Sample stability

The shelf life of prepared samples in vials with whole septum stored in the refrigerator is one week.

Analytical procedure

General information

(b) (4)

Sample preparation

(b) (4)

(b) (4)

(b) (4)

Documentation

Data

Collection and storage of data

(b) (4)

How to find results and raw data

(b) (4)

Calculations

Quantification is performed by using internal standards, which means it is the area ratios of analyte and internal standard that are used as a response in the preparation of the calibration curve and the quantification of the samples.
The method includes a calibration table with linear calibration curves for each analyte. The calibration table has concentrations given in ng/ml. (b) (4)

(b) (4)

(b) (4)

Quality assurance

Control chart

For the internal quality control one check sample is injected, both before and after the unknown samples in the sequence, on every analysis occasion (b) (4) . The analysis of the check sample after the unknown samples is used where troubleshooting/investigations are required.

(b) (4) The reason that only one check sample is used is that the largest contribution to the drift is probably derived from the instrument and not from the sample preparation. It is therefore important that the criteria for the calibration residuals are met.

A remark is entered as a comment, for example, when new stock solutions are prepared, and when replacing the column.

Measures when verification is not approved:

- Prepare new internal standard solution
- Replace septa
- Replace liner
- Cut the column at the inlet
- Recalibrate using existing calibration solutions
- Prepare new stock and/or calibration solutions and calibrate.

Standard curve criteria

Calibration of GC instrument with calibration standards is made for each sequence. Concentrations are given in ng/ml. (b) (4)

If these criteria are not met, the measures below are taken in the following order:

- Higher calibration levels in the batch can and should be excluded, if there are no samples in the sequence that end up above the highest remaining calibration level. At least three calibration levels must be used.
- Re-analyze the sequence following a review/troubleshooting of the cause of the failure. Based on needs:
    1. Replace septa
    2. Replace liner
    3. Cut the column at the inlet
    4. Run an autotune on MS
- Re-prepare and re-analyze the sample
- Prepare new stock and/or calibration and internal standard solutions and calibrate.

Duplicate and triplicate samples

When analyzing with duplicate samples, the difference between the samples should not be greater than 3 standard deviations of what was measured when determining repeatability in the matrix in question. (b) (4)

When analyzing with triplicate samples, this difference between samples should not exceed 3.3 standard deviations.

Confirmation

(b) (4)

Concentration above calibration curve

Samples with concentrations above the calibration curve are mainly prepared using less sample weight. (b) (4) . Dilute enough to ensure that the response area of the analyte is estimated to fall within the upper half of the calibration range. The internal standard makes a correction for the dilution and there is therefore no need for an adjustment to the calculations.

Reporting of analysis results

(b) (4)

Revision history

Version	Amendments
(b) (4)	

Person responsible

Director APS

Validation report

Supporting documentation for validation

Calculations and all raw data used are in MS Excel are available upon request.

The results are calculated as ng/g as is.

Sample	Description	Validation
(b) (4)		

(b) (4)
(b) (4) . The measurement range is applied to the different types of matrices in the validation such as tobacco flour, cigar and non-smoking products. An expanded relative measurement uncertainty has been calculated from the combined relative errors.

(b) (4)

Specificity

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Repeatability

(b) (4)

(b) (4)

Table 11. Repeatability for GC-MS analysis.

	Pooled RSD%	**Max difference in % for duplicate samples**
(b) (4)		

Precision within the laboratory

Precision within the laboratory has been determined by three individuals who have analyzed three replicates of three tobacco matrices at six different timepoints where the extraction solution and standard curves have been varied. CRP1.1, CRP2.1 and CRP4.1 have been analyzed. In Table 12 you can see the pooled RSD % for analysis with GC-MS/MS, after compilation, and corresponding data for the GC-MS analysis are found in Table 13.

Table 12. Pooled RSD % for GC-MS/MS analysis.

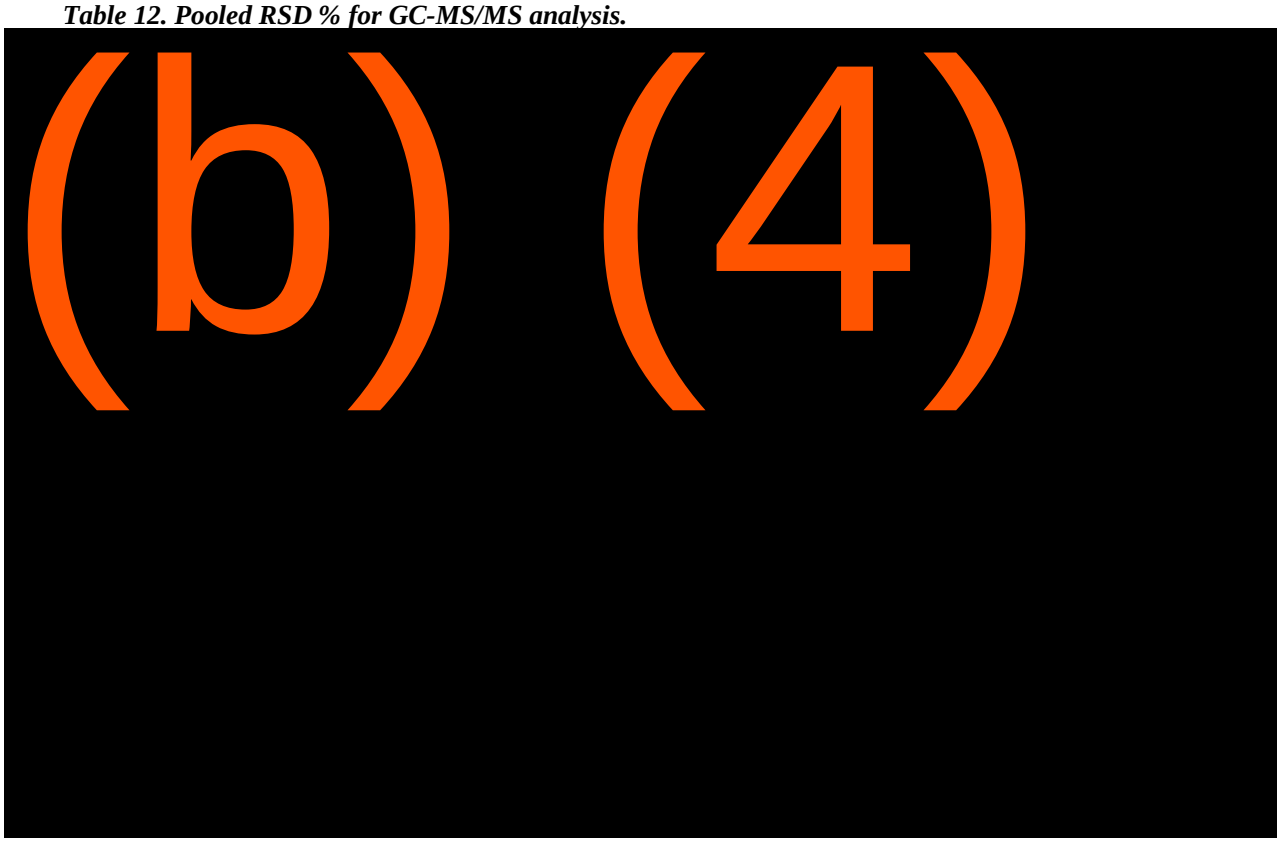


Table 13. (b) (4)

	RSD % (pooled)
(b) (4)	

Accuracy

(b) (4)

Table 14. Accuracy data for GC-MS/MS analysis.

(b) (4)

Table 15. Accuracy data for GC-MS analysis.

(b) (4)

Limit of quantification (LOQ)

LOQ is calculated according to (10 x the concentration found)/S/N ratio and the root mean square (RMS) algorithm was used in the (b) (4) Data from repeatability was used as supporting documentation, in the case of absence of substances in the tested matrices, LOQ has been calculated based on accuracy data (spiked levels). For most analytes, the LOQ/reporting limit has been set slightly higher than the estimated LOQ.

In Table 16, the limits of quantification for analysis with GC-MS/MS are listed and in Table 17 for analysis with GC-MS.

Table 16. Limit of quantification for GC-MS/MS analysis.

(b) (4)

Table 17. Limit of quantification for GC-MS analysis.

	LOQ GC-MS (ng/g as is)
[redacted]	[redacted]

The LOD for the method is not calculated as it is not used for reporting.

Linearity

(b) (4) The pooled values for the relative standard uncertainty, when using a weighted (1/x) calibration curve, are included in the determination of the measurement uncertainty and are listed in the table below.

Robustness

(b) (4)

Measurement range and measurement uncertainty

(b) (4)

Table 18. Measurement uncertainty when analyzed with GC-MS/MS in % of the detected concentration.

	Single sample	Duplicate samples	Triplicate samples
(b) (4)			

Table 19. Measurement uncertainty when analyzed with GC-MS in % of the detected concentration.

	Single sample	Duplicate samples	Triplicate samples
[redacted]			

(b) (4)

Conclusion

The method is deemed fit-for-purpose.