Quantitative Analysis of Benzo(a)pyrene in Tobacco, Tobacco Products, Fiber-based Matrices and Tobacco-derived Products by (b) (4)

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Purpose

To determine the concentration of benzo(a)pyrene (B(a)P) (**Figure 1**) in tobacco, tobacco products, fiber-based matrices, and tobacco-derived products using (b) (4) .

Applies to

APS

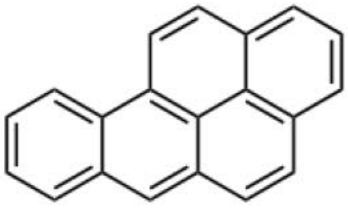
General information

Principle of the method

Internal standard (B(a)P-d_{12} (d_{12} refers to fully deuterated B(a)P) is added to the dry sample. B(a)P is extracted from the weighed out tobacco sample using methanol and a shaker. A part of the methanol phase is transferred to an evaporation tube and the extract evaporates in a parallel evaporator to enrich B(a)P. The sample is filtered into liquid chromatography vials. Separation and quantification are done with UPLC-FLD. Calculations are performed by (b) (4)

The laboratory's maximum capacity is about (b) (4) single samples/person/week.

Figure 1: Chemical Structure for Benzo(a)pyrene, B(a)P



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

The method is used for quantitative analysis of B(a)P in tobacco, tobacco products, fibre-based matrices, and tobacco derived products (Zyn).

The measurement range of the method is 0.6 to 150 ppb B(a)P for the sample matrix and (b) (4) (b) (4) ng/mL in the sample extract.

Measurement uncertainty

The combined relative uncertainty for a (b) (4) .

The contribution to measurement uncertainty is greatest from the accuracy and calibration curve.

Literature references

1. C.H. Risner. The determination of benzo(a)pyrene in the total particulate matter of cigarette smoke. J. Chrom. Sci. 26, p. 113-120, 1988
2. C.H. Risner. The determination of benzo(a)pyrene and benz(a)anthracene in mainstream and sidestream smoke of the Kentucky reference cigarette 1R4F and a cigarette which heats but does not burn tobacco: a comparison. Beitr. Tabakforsch. Inter. 15, p. 11-17, 1991
3. T. Oluseyi, K. Olayinka, B. Alo and R.M. Smith. Improved Analytical Extraction and Clean-up Techniques for the Determination of PAHs in Contaminated Soil Samples. Int. J. Environ. Res 5(3), p. 681-690, 2011

Internal reference documents (available upon request)

(b) (4)

Risk assessment and safety instructions

Summarised risk assessment

B(a)P is classified as a carcinogen.
All handling of B(a)P or solutions containing B(a)P must therefore be carried out with caution. Always wear protective gloves made of nitrile and work in a fume cupboard or draw bench with stock solutions of B(a)P.

Risk and safety phrases

(b) (4)

(b) (4)

Equipment

Apparatus

(b) (4). The system consists of the following modules:

(b) (4)

- Detector: (b) (4)
- Column oven: Column Manager
- Column (b) (4)
- Software: (b) (4) . Used for controlling pumps, autoinjector and fluorescence detector and for the collection of raw data and quantification.

Instrument parameters

- Mobile phase A: Milli-Q water
- Mobile phase B: 100% Acetonitrile
- Mobile phase C: 100% Isopropanol
- (b) (4)
- Hose labelled "Wash – Sample Manager Wash": strong wash; washes the outside of the needle: 100% acetonitrile (should be inserted in Mobile phase B)
- (b) (4)
- (b) (4)

Swedish Match

Quality and Environmental Management System

Document Title
Quantitative Analysis of Benzo(a)pyrene by (b) (4)

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(b) (6)

(b) (4)

Other equipment and laboratory utensils

- Orbital shaker, Gerhardt
- 100 mL Erlenmeyer flasks with ground glass stoppers Pyrex, Fisher Scientific, or equivalent
- Parallel evaporator Büchi Syncore 12 positions with Recirculating Chiller B-740, Vacuum Pump V-700 Vacuum Controller V-805, Büchi
- Evaporation tube for parallel evaporator
- Stainless steel evaporation tube stand
- Dispensette, Akkudrive 50 mL, Hirchmann Laborgeräte or equivalent
- Multipette, Multipette stream 1 mL tips, Eppendorf or equivalent
- Filtering option 1: Syringe filter, PTFE, 0.20 m, 15 mm diameter, Phenex or equivalent and disposable syringe, 2 mL BD Discardit II, BD or equivalent
- Filtering option 2: Filter vial: Whatman Mini-UniPrepTM PTFE filter 0.2 µm (or equivalent) with associated Whatman Mini-UniPrepTM Compressor
- Pasteur pipettes 230 mm or equivalent
- LC vials (amber coloured) with Teflon septa
- Pipette, 20-200 µL
- Pipette, 2-20 mL (or 1-10 mL)

Chemicals, reagents and solvents

- Acetonitrile (HPLC grade)
- Methanol (HPLC grade)
- Isopropanol (HPLC grade)
- Milli-Q water
- (b) (4)
-

The substances used for calibration of instruments, B(a)P, and as (b) (4) must have certificates of analysis. Standards should be stored in a refrigerator until preparation.

Check samples

The check sample must be stored in brown glass jars until use. One jar at a time can be brought out and used. Between weighing in operations, the jar can be stored at room temperature.
The check sample is an R19-tobacco flour, that is ground with a 0.5 mm grinding filter.

Preparation of stock and standard solutions

General information

The original template for traceability for prepared stock solutions, standard solutions, calibration standards, standard transitions, standard correlations and internal standards is in the form of an MS Excel document.
All documentation of standard handling is done in the same MS Excel document.
(b) (4) Enter new information regarding standard handling. (b) (4) .

- As a minimum requirement, comparisons between new and old standard curves are made when new stock solutions are prepared.
- Chemical solutions must, in addition to hazard symbols and associated text, also be labelled with chemical content, analytical method, date of manufacture, who made the solution (signature) and shelf life (or expiration date).
- The solutions containing (b) (4) are stored in a refrigerator. They are light sensitive and should be stored in brown flasks.

Stock standard solution B(a)P 1 ng/µL

The entire content of an ampoule certified B(a)P-standard (1 mL of 100 ng B(a)P/µL in acetonitrile) is transferred to a 100 mL volumetric flask and diluted to the mark with acetonitrile. The solution has a shelf life of two years in a refrigerator.
Hazard symbol: St. Andrew's cross, flame

B(a)P standards for spiking: 0.02, 0.06, 0.20, and 0.50 ng/µl

Four standards (A-D) are diluted from the stock solution with acetonitrile as shown in **Table 3** below. The solutions have a shelf life of one year in a refrigerator.

Hazard symbol: St. Andrew's cross, flame

Table 3. Dilution of B(a)P standard solutions

(b) (4)

Stock solution internal standard (B(a)P-d_{12}), 100 ng/µL

(b) (4) is weighed out into a (b) (4) mL volumetric flask (if (b) (4) mg is not exactly weighed out, record the weight). Dilute to (b) (4) mL with (b) (4) min. Once (b) (4) has dissolved, dilute to the mark with acetonitrile. Calculate the final concentration. The solution has a shelf life of (b) (4) in a refrigerator.
Hazard symbol: St. Andrew's cross, flame

Internal standard solution (B(a)P-d12), 0.5 ng/µL

Pipette a volume of (b) (4) stock solution that is equivalent to (b) (4) when diluted to (b) (4) mL. Transfer to (b) (4) mL volumetric flask and dilute to the mark with acetonitrile. Then transfer to a (b) (4) mL amber flask. The solution has a shelf life of one year in a refrigerator.

(b) (4)

Hazard symbol: (b) (4)

Calibration standards

Add (b) (4) mL of methanol to seven Erlenmeyer flasks (b) (4) (b) (4) ng/µL) as shown in **Table 4**. Prepare the calibration standards in the same way as the samples (see (b) (4) from point 5). When the calibration standards have been prepared and filtered into vials, they have a shelf life at room temperature of two months.

Table 4. Spiking of calibration standards.

(b) (4)

Handling and preparation of other solutions

Chemical solutions must, in addition to hazard symbols and associated text, also be labelled with chemical content, analytical method, date of manufacture and who made the preparation (signature) and shelf life (where applicable).

- (b) (4) is prepared using a measuring cylinder to (b) (4) (b) (4) Used as system fluid (b) (4) The shelf life date does not need to be specified.
  Hazard symbol: (b) (4)
- (b) (4) is taken from the solvent store. Used as a mobile phase directly from the original flask (b) (4) it does not need to be labelled, but must be dated/signed when opened. The shelf life date does not need to be specified.
  Hazard symbol: S(b) (4)
- (b) (4) is taken from the solvent store. A dispensette is put on the flask to dispense the extraction solution to the samples. It does not need to be labelled, but must be dated/signed when opened. The shelf life date does not need to be specified.
  Hazard symbol: (b) (4)
- (b) (4) is taken from the solvent store and poured over to a smaller Duran flask. It is used as a washing fluid (channel C). The shelf life date does not need to be specified.
  Hazard symbol: (b) (4)
- (b) (4) is taken from the Milli-Q system to 1 L Duran flask. It is used as a mobile phase (channel A) and the shelf life seven days.
  Hazard symbol: (b) (4)

Sample handling

Sample storage and preparation

Samples are prepared in accordance with INS “Sample Preparation” (available upon request).

Sample amount

The minimum amount of sample needed for duplicate analyses is [redacted] g and [redacted] g for re-analysis.

Analysis

Calibration and verification of apparatus

At the beginning of each run:

- Make sure the pump pressure is stable.

  Start by injecting two blank samples (b) (4)). Check the system's performance by injecting calibration standard [redacted]. Check that the signal, analyte peak and retention time all look good (compared to previous runs). A standard curve is generated in each sequence by injecting all the calibration solutions first in the sequence.

- Also check that the instrument runs normally after 1-2 hours. Measures to be taken if instrument performance problems occur are to check (b) (4) (b) (4)

When evaluating:

Evaluate the chromatogram for standards and samples (see **Figure 2**).

- Check that the retention time is comparable (b) (4) with the previous run.
- Evaluate the peak width for B(a)P (automatic evaluation when processing and also printed out with the analysis report).
  - The peak width at the base should be (b) (4)
- Peak width [redacted] s at the base may mean that the integration is inaccurate or that the chromatography is poor. Evaluate if integration needs to be redone.
- Signal to noise (S/N) should be [redacted]

Figure 2. The evaluation window where the peak width at half height (Width @ 50%) and S/N (EP s/n) are calculated.

Sample stability

Samples are stable in a vial (applies to both UniPrep and glass vials) for two months if stored in the dark at room temperature.

Analytical procedure

Sample preparation

1. Weigh out "x" g samples in a 100 mL Erlenmeyer flask on an analytical scale.

Note: In case of black pouch paper, exclude the paper from extraction.

Check samples, Swedish snus and chewing tobacco
- (b) (4)

Tobacco flour, moist snuff:
- (b) (4)

Smoked dried tobacco flour:
- (b) (4)

(b) (4) (Tobacco derived products):
- (b) (4)

Pouches are to be cut in half along the long side.

2. Add (b) (4) and wait for five minutes.

3. Add (b) (4) mL of (b) (4) with a (b) (4) to all samples. Insert glass stoppers.
4. Shake on the orbital shaker at 130 osc/min for 30 minutes. After shaking, the samples can stand for three days before the next part in sample preparation is performed.

5. Leave the sample rest for a while, allowing the matrix time to settle. Take 20 mL to an evaporation tube by pipetting 2 x 10 mL by pipette. Evaporate to about 1 mL with a parallel evaporator (see Instructions: (b) (4) Set the shaker to a temperature of (b) (4). After the program is complete, the pressure is maintained until the pump is turned off manually.

6. Filtration of samples: (b) (4) (or disposable syringes with attached PTFE syringe filter. If this is the case, filter the extract to (b) (4) (b) (4) with vial caps). No filtering is necessary when preparing (b) (4)

7. Analyse the samples with (b) (4)

Special instructions

- Black pouch paper should be separated from the sample matrix before analysis. Black pouch paper adsorbs B(a)P strongely and decreases the concentration of B(a)P in the extraction solution.

Documentation

Write on the work (b) (4) who have weighed out and prepared the samples, the (b) (4) (if different from the batch name), (b) (4) and any comments on the analysis or control chart. Instrument-related comments are entered in the logbook in line with the logbook cover sheet.

Data

Collection and storage of data

The samples are weighed in directly to (b) (4). The raw data is collected using (b) (4) (b) (4). The samples are injected by an (b) (4). In order to control the injector, a (b) (4) is created in which the sample identity is specified along with the methods to be used. The instrument software includes templates for these lists.

Calculations

Identification of analyte and quantification

Quantification is performed by using an internal standard (b) (4) and the area ratios of (b) (4) are used for the calibration curve and the quantification of the samples. (b) (4) included in (b) (4) software, adjusts a straight-line calibration curve using the least-squares method with (b) (4) to the data from the calibration standards analysis. (b) (4) data is exported to a text file that is then imported into (b) (4) where concentration of the unknown samples is calculated in ppb.

Quality assurance

Standard curve criteria

For each quantification, check the linearity and accuracy (% deviation) for the standard curve. The correlation coefficient (R^2) should be (b) (4) and accuracy may deviate up to (b) (4) for S1 and (b) (4) for S2-S7, in order for the samples to be approved.

Calibration points that do not meet these requirements can be excluded. However, the calibration curve must be based on at least four calibration standard points covering the concentration range in the unknown samples. In addition, the calibration standard that defines the analyte LOQ, "lowest standard," should always be included. If the calibration curve does not meet the specified requirements, new calibration standards should be prepared and the entire run should be re-analysed.

Control chart

The check samples that are run immediately before and after the samples are inserted into the control chart in (b) (4) Two prepared check samples are analysed for each analysis timepoint; one is weighed in and prepared first in the series, and the other one last in the series. Both check samples are analysed in duplicate for each analysis timepoint, and at the beginning of the sequence and at the end of the sequence. The control chart is evaluated according to QEMS DESC "(b) (4) ".(available upon request).

Duplicate and triplicate samples

In cases where duplicate samples are analysed, the spread between duplicate samples should not be greater than (b) (4) If this is exceeded, both samples must be weighed out again, re-prepared and re-analysed. A (b) (4) applies to triplicate samples, i.e. (b) (4).

(b) (4)

Reporting of analysis results

- B(a)P concentrations (ng) can either be entered in (b) (4) manually or automatically from (b) (4) The analysis result is converted to dry content (if it is not to be reported "as is") and divided by the sample weight. When values are imported into (b) (4), also values below LOQ are imported. These are replaced with "n.d.".
- Values for (b) (4) are imported to (b) (4) but the results are only reported to the client to two significant digits (as ng/g dry sample).
- When reporting analysis results < LOQ or n.d. via (b) (4) to the client, (b) (4) is always used.

Revision history

Significant changes and the date of the introduction of these

- New method and method validation for B(a)P 04/05/2013
- Changed shelf-life to 3 years for stock solution for internal standard (b) (4), 100 ng/µl 11/08/2014
- Modified to suit the (b) (4) software and refers to the new instruction "Instruction for Analysis and Data Processing with (b) (4) as well as some minor updates. 02/05/2016
- Addition of new matrix, Tobacco derived products (b) (4) Additional validation was done (repeatability, accuracy, extraction yield and specificity). 12/01/2018

Person responsible

Director APS

Validation

Validation report

The validation results for B(a)P are based on ten types of samples from eight different matrices. **Table 1** presents matrices/samples, marked with an "X" under the tests to which they belong. Besides repeatability, precision, accuracy, the limit of detection (LOD), and the limit of quantification (LOQ), the following parameters have also been examined: method comparison, carry-over, reproducibility, linearity, robustness, extraction yield and measurement uncertainty.

The supporting documentation for the validation report and detailed results are available upon request.

Table 1. The trial plan for the validation of B(a)P in tobacco flour and smokeless tobacco products and fibre-based matrices

(b) (4)

^ CRP2 is Coresta Reference Products obtained from North Carolina State University, USA.

* Ksample's internal check samples (batch 0.5 100521).

Dark Fired is a tobacco flour from the United States.

** Not Applicable.

Method comparison and specificity

(b) (4)

.

Differences in preparation

- The new method uses (b) (4) as IS instead of (b) (4)
- IS is added to the new method in the beginning of the preparation (for dry sample). In the previous method, IS was added to the final extract at the end of the preparation.
- The matrix-matched calibration standards are removed in the new method.
- Extraction procedure (new method)
  i. (b) (4) mL of (b) (4) is added to the sample and shaken for (b) (4) on the shaker. Transfer (b) (4) mL of extract to the evaporation tube.

  Extraction procedure (previous method)
  i. (b) (4) mL (b) (4) of sodium pyrophosphate solution was added to the sample and shaken for (b) (4) on the shaker.
  ii. (b) (4) to the sample and shake for (b) (4) on the shaker.
  iii. Transfer all the extract to centrifuge tubes (and centrifuge the samples if needed).
  iv. Take (b) (4) mL of the upper phase (organic phase) to the evaporation tube.
  v. Add (b) (4) mL of acetonitrile to the evaporation tube.
- For the new method, the parallel evaporation phase takes (b) (4); in the previous program, it took (b) (4).
- In the previous method, (b) (4) was added to the final extract and vortexed. This step is removed for the new method.
- For the new method, a (b) (4) can be used as an alternative for samples, instead of only using glass vials and traditional filtering.

Differences in UPLC method

- UPLC method new: isocratic. UPLC method previous: gradient.
- Column new method: (b) (4)

Sample comparison new and previous methods

In order to compare the new and previous methods, three samples (duplicates) were prepared using both method, and two different internal standards for each of the methods, (b) (4) and DFA. The results are reported in **Table 2**.

Concentrations between the new and previous methods match well for simpler sample matrices (b) (4) For more complex sample matrices (e.g., (b) (4) however (b) (4) were about 25% lower with the new method. The assessment

was made that it probably was not due to the extraction method as such, but rather the choice of the internal standard.

(b) (4) is more nonpolar than (b) (4)$_2$ (also less like (b) (4) compared with (b) (4) . The risk is that (b) (4) binds stronger to the matrix than (b) (4) which means that the internal standard does not behave in the same way as for the analyte. The results therefore imply false high levels of (b) (4) in complex matrices.

Further evidence for the above line of reasoning is obtained when comparing the results for the new method with (b) (4) as IS. Here the concentrations are 15-45% higher for all three samples, compared to when (b) (4) is used as IS. Methanol is more polar than isohexane and it is therefore quite possible that the (b) (4) is poorer with methanol, which gives false high levels of (b) (4) in all matrices. When (b) (4) is used as IS in both the new and previous methods, however, no differences were observed.

From this we can conclude that the new extraction method (with methanol) works as good as the old method (more complex). However, (b) (4) works much better as an internal standard compared to (b) (4) With the previous method, we probably obtained false values that were too high in the analysis of complex matrices. It was decided to proceed and validate (b) (4) using the new method.

Table 2. Sample comparison new and old B(a)P method.

(b) (4)

[1]The sample values were obtained by using the new B(a)P method.
[2]The sample values were obtained by using the previous B(a)P method.

Sample comparison external laboratories

Reproducibility for (b) (4) has been investigated in a proficiency test between (b) (4) and (b) (4) , where six different matrices were included. Both laboratories use the same method for (b) (4) analysis in their routine operation (i.e. according to the new method). The comparison looked good and the pooled spread between the laboratories was (b) (4)

A larger proficiency test is scheduled to take place within the (b) (4)

Chromatogram

Representative chromatograms for standards and various matrices are presented in **Figure 3**.

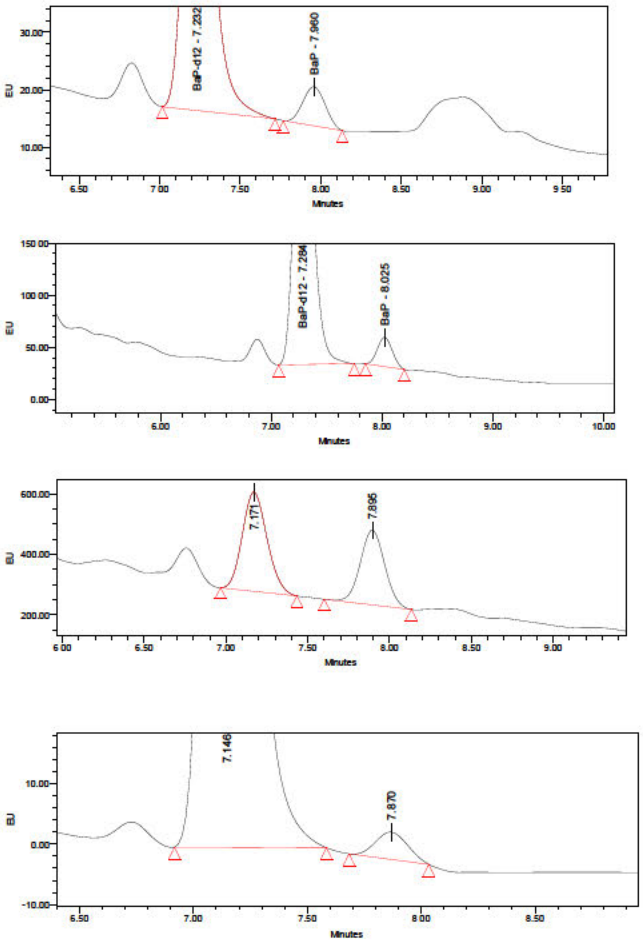

Figure 3. Chromatograms from top to bottom:
1) check sample, 5 ppb (5 ng/mL in extract), 2) Red Man, 2 ppb (4 ng/mL in extract), 3) Longhorn Natural LC 40 ppb (40 ng/mL in extract), 4) Onico enbär, 0.6 ppb (1.2 ng/mL in extract)

Repeatability

Repeatability was determined by analysing the same matrix six times in constant conditions on eight different products (eight matrices) (see matrices and concentrations in **Table 1**). The RSD % for the investigated matrices for (b) (4) ranged from (b) (4) (**Table 3**). The combined (b) (4) This should be considered as a good result.

Table 3. Repeatability within the laboratory for various Tobacco matrices.

(b) (4)

Precision within the laboratory

Precision within the laboratory has been determined and summarized for a (b) (4) at six different timepoints with three different laboratory analysts (**Table 4**). The results show good precision regardless of the matrix analyzed.

Table 4. Precision within the laboratory for various tobacco matrices.

(b) (4)

Accuracy (trueness)

One (b) (4) have been spiked using B(a)P at each of three concentration levels. Six determinations at each level were done and six unspiked replicates were also analysed. **Table 5** summarises the accuracy which was considered satisfactory.

Table 5. Precision (%) within the laboratory for various tobacco matrices

(b) (4)

Limit of detection and Limit of quantification for tobacco matrices

LOD and LOQ for B(a)P were estimated in different sample matrices with low concentrations of B(a)P (**Table 2**). This was done by measuring S/N and by analyzing the concentration of B(a)P in the different matrices.
LOD is calculated as: 3 × concentration divided by S/N
LOQ is calculated as: 10 × concentration divided by S/N

Table 6 shows the determined LOD and LOQ values specified partly as concentration in the matrix (ppb) and as concentration in the extract (ng/mL). Reporting to the client is done using the concentration unit (ppb). When reporting analysis results <LOQ via (b) (4) to the client, "< 0.6" is always used.

Table 6. LOD and LOQ for B(a)P in a matrix

(b) (4)

Additional validation of Tobacco derived products (Zyn) shows that LOQ in Zyn is lower than the set LOQ value of 0.6 ng/g.

Linearity

Linearity was examined by analysing seven calibration solutions (b) (4) in three series with concentrations from (b) (4) ng/mL for B(a)P. Each standard curve is weighted by 1/x.

The linearity for B(a)P is well within the standard curve concentration range with a correlation coefficient, (b) (4) (lowest standard).

Measurement range

The measurement range for the method is between (b) (4) for B(a)P. If the analysis result for a sample is higher than the measurement range, the sample preparation and analysis is redone using a lower amount of sample.

Robustness

Extraction times

The extraction time significance on the B(a)P measurement results have been evaluated for (b) (4) (duplicate) by extracting for 20, 30 and 40 minutes. No significant differences were observed.

Stability

The stock standard of (b) (4) and the interim standard of B(a)P (dissolved in acetonitrile) have a shelf life in a refrigerator of at least two years.

The prepared calibration and sample extract (in (b) (4)) stored in amber-coloured glass vials showed stability for at least eight weeks when stored in the dark at room temperature in trials conducted in 2012.

The prepared calibration and sample extracts (in 100% methanol) stored in filter vial (b) (4) (polyproylene vials) and amber-coloured glass vials in 2013 have also been shown to be stable for at least eight weeks when stored in the dark at room temperature.

Carry-over

Carry-over was checked by injecting the strongest standard (b) (4) ppb) followed by two blank injections. No carry-over was observed.

Measurement uncertainty

The combined relative measurement uncertainty for B(a)P is shown in **Table 7** with a (b) (4) for single and duplicate samples. The results are based on a seven points calibration curve (b) (4). By basing the calibration curve on only 5 points (b) (4) the measurement uncertainty is reduced to (b) (4) for duplicate samples.

Table 7. The combined relative measurement uncertainty for single, duplicate and triplicate samples with a coverage factor of 2.

Analyte	*Single sample (%)*	*Duplicate samples (%)*	*Triplicate samples (%)*
B(a)P	40	39	38

Bias from accuracy for (b) (4) is not included in the measurement uncertainty calculation, however, measurement uncertainty would have been lower, if Zyn had been included. Zyn is therefore covered by the stated measurement uncertainty.

Extraction yield (Recovery)

In order to investigate the extraction yield, (b) (4) and pure solvent (100% methanol) were spiked with B(a)P, before and after the preparation. In total, six samples were spiked for each level, but only two samples for Zyn.
The absolute yield is the ratio of the response for a spiked sample (in the matrix) which is treated according to the sample preparation procedure, in relation to a spiked "pure" solution without any sample preparation.

The relative extraction yield (or process efficiency) is the ratio of the response for a spiked sample (in the matrix), which is treated according to the sample preparation procedure, in relation to a spiked "pure" solution that is treated according to the sample preparation procedure. The extraction yield and relative extraction yield are reported in **Table 8**.

Table 8. Absolute yield for B(a)P in three different matrices

(b) (4)

(b) (4)

The relative extraction yield can be used together with the absolute yield to investigate if the losses in the sample preparation are due to matrix effects or poor extraction. The mean value for absolute and relative extraction yield is about the same (~80%), suggesting that losses in sample preparation can be primarily explained by incomplete extraction. The value of recovery (~80%) is in the same range as accuracy (~80), which also indicates incomplete extraction as the main reason.

Conclusion

The validation results show that the B(a)P method works very satisfactorily. It is a reliable and robust method. Compared to previous B(a)P methods (validated and accredited 2012), this is simpler and has fewer work stages, which thereby increases the capacity of sample throughput. It is also more environmentally and labour friendly. The comparison between the methods shows good compliance, except for complex matrices where this method is considered more reliable and accurate.

12/01/2018: The method is suitable for its purpose and designed to analyse the BaP of tobacco, tobacco products, fiber-based matrices, and tobacco-derived products. The validation requirements for the tobacco-derived products action plan are met.