Swedish Match

Quality and Environmental Management System

Document Title
Quantitative Analysis of Coumarin by (b) (4)

Part of Process
Contract Analysis APS

Document Type
Method Description

Valid From
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Page
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Approved By
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QUANTITATIVE ANALYSIS OF COUMARIN IN TOBACCO, TOBACCO PRODUCTS, FIBER-BASED MATRICES, AND TOBACCO-DERIVED PRODUCTS BY (b) (4)

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Purpose

To determine the concentration of coumarin in tobacco, tobacco products, fiber-based matrices, and tobacco-derived products with (b) (4)

Applies to

APS

General information

Principle of the method

After weighing samples and adding the internal standard, coumarin is extracted into a mixture of water and methanol. The extract is then diluted and filtered into a vial. Separation and quantification are performed by (b) (4). The calculations are performed by (b) (4) and (b) (4). The capacity per instrument and analyst is (b) (4) single samples/week. The chemical structural formula for coumarin is shown in **Figure 1**.

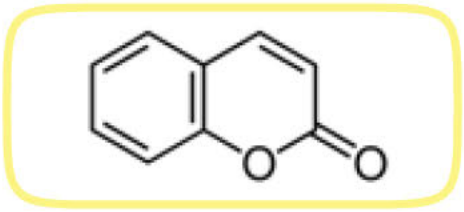


Figure 1. Chemical structural formula for coumarin.

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 the quantitative analysis of coumarin in (b) (4) and derivatives thereof, as well as fiber-based matrices and tobacco derived products. **Table 1** sets out the delimitations of the method.

Method's calibration range:
(b) (4) ng/mL

Method's measurement range:
(b) (4) µg/g

Measurement uncertainty:

The combined relative measurement uncertainty for coumarin is stated with a coverage factor of 2. **Table 1** shows the combined relative measurement uncertainty for single, duplicate and triplicate samples.

Table 1. The combined relative measurement uncertainty for single, duplicate, and triplicate samples with a coverage factor of 2

(b) (4)

The contribution to measurement uncertainty is greatest from precision within the lab and the bias from accuracy. In order to reduce the measurement uncertainty contribution from precision, careful addition of the internal standard is important.

Literature references

(b) (4)

Internal reference documents (available upon request)

(b) (4)

Risk assessment and safety instructions

(b) (4)

Summarised risk assessment

Exercise care when weighing pure coumarin. Wear suitable protective clothing, protective gloves and goggles. All work involving preparation of solutions containing acetonitrile, isopropanol, methanol, and formic acid are to be carried out in a fume cabinet, on a draw bench or using a point extractor. Protective gloves and goggles must also be worn when working with acetonitrile, isopropanol, methanol, and formic acid.

Substances hazardous to the environment

(b) (4)

Flammable substances

Methanol, isopropanol, acetonitrile, formic acid

Equipment

Apparatus and laboratory utensils

Apparatus

(b) (4)

Table 2. (b) (4)

(b) (4)

Run events: Disabled
Gradient start (relative to injection): At injection

(b) (4)

MS/MS-parameters

Polarity: ESI positive mode

NOTE: The parameters below may be changed if necessary, for example, following service and cleaning when technicians re-optimise certain parameters or if the sensitivity changes during operation and needs to be adjusted. The parameters should therefore be considered as approximate or as initial values.

(b) (4)

(b) (4)

Multiple Reaction Monitoring (MRM) Functions för (b) (4)

Function 1

(b) (4)

Ion Count Threshold: 25.0

Data processing:

SIR/MRM Chromatogram Spike Removal	ON
SIR/MRM Smoothing	OFF
Smoothing window size (scans)	3
Number of smooths	2

Table 3 and Table 4 summarize the substance-specific parameters for the quantification and confirmation of coumarin.

Table 3. Multiple Reaction Monitoring (MRM) parameters for detection

(b) (4)

(b) (4)

Table 4. General quantification parameters

(b) (4)

Other equipment

(b) (4)

Chemicals, reagents, and solvents

(b) (4)

Check samples and reference materials

(b) (4) is used as check sample. (b) (4) A bag is taken out and brought to room temperature for analysis prior to weighing. Only one bag at a time is used until they run out, although no longer than one month. (b) (4) Opened bags must be sealed properly and put back in the freezer.

Preparation of standards

General information

The solutions are stored in a refrigerator. All stock, standard and calibration solutions have a shelf life of two years.

Preparations of stock, standard and calibration standard solutions

(b) (4)

Toxic GHS06 (Danger)
Systemic Health Hazards GHS08 (Danger)

Calibration standards

(b) (4)

Comparison of new and old calibration standards

When new calibration standards have been prepared, both the new and old calibration standards are analysed. The slope of the new and the old standard curve may not vary more than ±10%. If it differs by more than that, contact the person responsible for the method to discuss measures to take.

Preparation of other solutions

(b) (4)

Sample handling

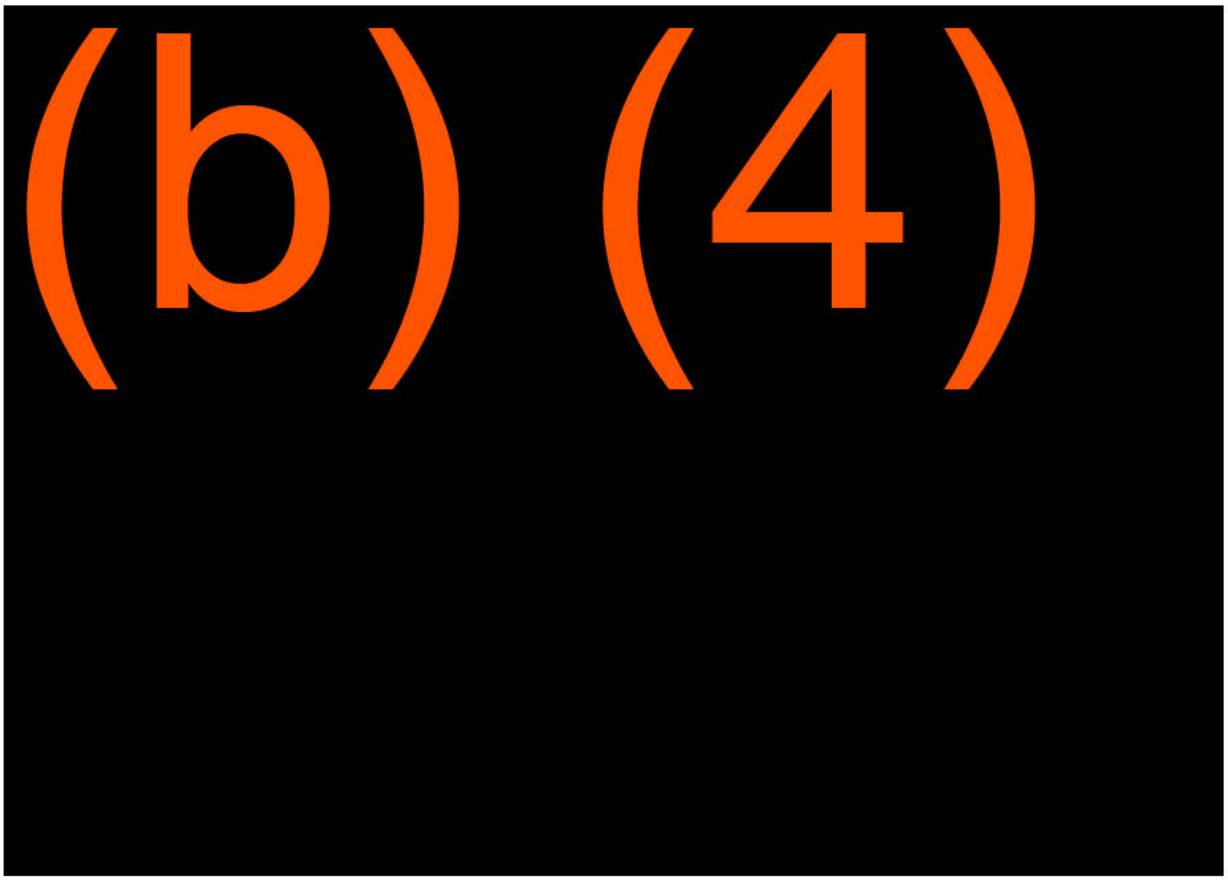
Sample storage and preparation

Tobacco, tobacco products, fiber-based matrices, and tobacco-derived products are stored and prepared according to the instruction for preparation of samples (b) (4).

Sample amount

The minimum required amount of sample for all matrices is (b) g in order to perform duplicate analysis and re-analyses. The minimum amount of sample for performing an analysis is around (b) g.

Analysis



Sample stability

The shelf life of prepared samples in vials that are stored in a refrigerator or sample manager is (b) (4)

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Analytical procedure

Sample Preparation for coumarin

(b) (4)

Special instructions

(b) (4) .

Documentation

(b) (4)

Data

Collection and storage of data

(b) (4)

Calculations

(b) (4)

Quality assurance

Control chart

(b) (4)

(b) (4)

• A new internal standard solution for spiking of samples is to be prepared

Comments regarding the problems are entered in the control chart and in the event of extended comments, refer to the work list.

Standard curve criteria

For each quantification, the adjustment of the calibration standards to suit the standard curve and its accuracy is checked. (b) (4)

Duplicate and triplicate samples

(b) (4)

If this is exceeded, all the samples must be weighed again, re-prepared, and re-analysed.

If a value <LOQ and a value >LOQ is obtained for duplicate samples, then a re-analysis should be done if the last mentioned value is greater than three standard deviations above LOQ (as it is calculated for repeatability).

Confirmation

In addition to the MRM signal which is used for the quantification of coumarin, another MRM signal is followed for confirmation (**Table 3**). In order for coumarin to be positively confirmed in the sample, (b) (4)

f the ion ratio in a sample for coumarin does not meet the requirements, the MRM signals in the sample are not solely derived from coumarin, which is why the sample has to be re-prepared and re-analysed. Where there is a need to check the ion ratio, the analyst responsible for the method can be contacted in order to evaluate the results.

If the response in a sample is higher than the highest standard

Sample extracts with levels higher than the highest calibration standard are re-analysed, but with a smaller sample amount. Only the results from the newly prepared sample are reported. The sample injected after the sample with high analyte concentration may, in the case of suspected carry-over, be re-analysed to exclude carry-over.

Reporting of analysis results

The values are reported to the (b) (4) in ng/mL where they are corrected for weight, moisture content, and dilution factors, and in addition the analysis results are automatically recalculated to µg/g (weight). The results are reported to the client in µg/g for dry sample to two significant digits.

(b) (4)

Revision history

(b) (4) .

Person responsible

Director APS

Validation report

The matrices and samples that are used for validation are listed in **Table 6**.

Supporting documentation for validation

Calculations and all raw data files used are available upon request.

Table 6. (b) (4)

(b) (4)

(b) (4)

(b) (4)

Specificity/selectivity

(b) (4)

Cross talk

(b) (4)

Carry-over

(b) (4)

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Repeatability

(b) (4)

Table 7. (b) (4)

(b) (4)

Precision within the laboratory

(b) (4)

Table 8. (b) (4)

(b) (4)

Accuracy

(b) (4)

Table 9. (b) (4)

(b) (4)

Bias from accuracy data

The estimated concentration error in the method to the true (spiked) concentration in % (bias) is calculated as the square root of the sum of squares of accuracy -100 from the accuracy and the uncertainty in the addition of the amount of analyte. Bias from accuracy is used for calculating measurement uncertainty. Bias for coumarin is calculated at (b) (4).

ME PE RE (Matrix effect, Process efficiency and Recovery)

(b) (4)

Matrix effect

The absolute matrix effect was determined by comparing the peak areas for samples of all matrices spiked with coumarin after the sample preparation with peak areas for coumarin spiked in pure extraction solution. (b) (4)

However, (b) (4) as an internal standard compensates well for this.

Absolute yield

The "true" yield (irrespective of matrix effects in the detector) for the extraction process was determined by comparing the peak areas for samples of all matrices spiked with coumarin before sample preparation with peak areas for samples spiked after sample preparation. (b) (4)

Process efficiency

The overall process efficiency of the coumarin quantification method was determined by comparing the peak areas for samples of all matrices spiked with coumarin before sample preparation with peak areas for coumarin spiked in pure extraction solution. Process efficiency is reported in **Table 10** and is good for most matrices; (b) (4)

Table 10. (b) (4)

(b) (4)

Limit of detection and limit of quantification

(b) (4)

Table 11. (b) (4)

(b) (4)

(b) (4).

Linearity

(b) (4)

(b) (4)

Robustness

Extraction times

(b) (4)

Weighing interval

(b) (4)

Stability of prepared samples

(b) (4)

Measurement range and measurement uncertainty

(b) (4)

The most significant contributions to measurement uncertainty come from precision within the laboratory and bias from accuracy.

Conclusion

(b) (4)