

QUANTITATIVE ANALYSIS OF AMMONIUM IN TOBACCO, TOBACCO PRODUCTS, FIBRE-BASED MATRICES AND TOBACCO DERIVED PRODUCTS WITH A (b) (4)

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Purpose

To determine the level of ammonium (NH_4^+) in tobacco, tobacco products, fibre-based matrices and Tobacco derived products also called purified products) with a Discrete Photometric Analyzer (DFA).

Applies to

APS

General information

A sample solution prepared for the analysis of ammonium can also be used in the analysis of chloride and ethanol simultaneously using the same instrument.

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.

Principle of the method

DFA performs a quick analysis by creating chemical reactions at a microscale during incubation by using spectrophotometric detection.

(b) (4)

This reacts with salicylate ions in the presence of sodium nitroprusside at pH 12.6 to generate a blue compound. (b) (4)

Number of analyses/person/week is about (b) (4).

Method scope, measurement range and measurement uncertainty

The method is used for the quantitative analysis of Ammonium in tobacco, tobacco products and fibre-based matrices and tobacco derived products.

The measurement range of the method is (b) (4) mg/g as is in (b) (4)

Measurement uncertainty

(b) (4)

The greatest contribution to the measurement uncertainty for ammonium originates from bias from the accuracy determination, followed by the precision and uncertainty of the calibration curve. No clear largest contribution is evident regarding precision.

(b) (4)

. The measurement uncertainty would have been lower if Zyn had been included in the calculations. (b) (4)

Literature references

(b) (4)

Internal reference documents (available upon request)

(b) (4)

Risk assessment and safety instructions

Summarised risk assessment of the method:

The appropriate protective clothing must be worn for all handling. The greatest risk occurs when handling (b) (4) and (b) (4); both are corrosive (b) (4) which means it must be processed in the fume cabinet.

Hazard and precautionary statements

(b) (4)

Document Title
Quantitative analysis of ammonium with (b) (4)
Part of Process
Contract Analysis APS
Document Type
Method Description

Valid From
27/02/2018

Page
5(18)

Quality and Environmental
Management System

Approved By
(b) (6)
Document Publisher
(b) (6)

(b) (4)

(b) (4)

Tubing Maintenance Solution

EUH208 – contains isothiazolone compounds. May cause an allergic reaction.

P280 – Wear protective gloves/protective clothing/eye protection/face protection.

Equipment

Apparatus and laboratory utensils

Analytical instruments: Gallery Plus, Discrete Photometric Analyzer (DFA), Thermo Fischer Scientific

Data system: Gallery Plus, for managing analytical instruments and for the collection of raw data and quantification, Thermo Fischer Scientific

Instrument parameters

A paper copy of the method (test parameter) is filed in the binder labelled “Method binder” next to the instrument.

Other equipment

(b) (4)

Equipment for manual preparation

Plastic Pasteur pipette 2 ml

Dispensette for 50 ml with flask

Magnetic stirrer

Shaker

Chemicals, reagents and solvents

Water

MilliQ quality

The solutions are stored in a refrigerator.

(b) (4)

(b) (4)

This kit is used to check the repeatability and linearity of the instrument.

Reagent solutions Ammonium R1 and Ammonium R2, Tubing Maintenance Solution and Washing solution 4.5% are purchased ready-made from (b) (4) (Microgenics). Hydrochloric acid 0.1 M cleaning solution for simultaneous analysis of ammonium and ethanol, are purchased ready-made from (b) (4)

Check samples and reference materials

A certain type of loose snus is analysed as a check sample for ammonium, (specially prepared with ethanol added) each time an analysis is performed. The check sample is stored in a freezer, a new can is taken out for each batch and brought to room temperature before weighing.

Preparation of standards

(b) (4)

Preparation of other solutions

Washing solution robot

(b) (4)

(b) (4)

Shelf life of four months at room temperature.

Hazard symbol: Harmful (b) (4) (Warning)

Systemic Health Hazards (b) (4) (Warning)

Sample handling

Sample storage and preparation

The sample is stored and prepared in accordance with (b) (4)
(available upon request).

Sample amount

The minimum amount of sample required to perform an analysis is 7 grams for three replicates.

Analysis

Calibration and verification of apparatus

Before starting the calibration process or the sample sequence, the system's water blank is checked by making ten absorbance measurements on water. If the absorbency varies > 2 mAbs, it may be due to air in the system. For a list of measures, see (b) (4)
(b) (4) (available upon request).

A calibration of the instrument is made once a week, the calibration is valid for 7 days. See the Quality Assurance heading for inspection of the standard curve.

Sample stability

The prepared sample solution has a shelf life of 4 days in refrigerator.

Analytical procedure

When ethanol (volatile) is analysed on the same extract, weighing is to be carried out without interruption, and the stopper must be inserted immediately once the weight has been read. Water must then be added to all samples immediately after weighing in.

- Weigh out 0.7 – 1.3 g of sample in a 100 ml Erlenmeyer flask. For (b) (4) see Special instructions. Record the weight manually to 4 decimal places or automatically to (b) (4) by using a computer connected scale.
- Add 50 mL of water to all the samples.
- Carry out sample preparation with robot or:
 - Shake the flask without a stopper for 20 minutes with the shaker at 130 rpm or with a magnetic stirrer at a speed of 400 rpm.
 - Allow the sample to settle for 10 minutes.
 - Filter through the (b) (4) into a 5 ml sample tube or a 4 ml plastic cup placed in the sample rack used in the instrument for analysis.
- Analyse the sample solutions using the (b) (4)

Special instructions

When weighing pouch snus, hold the bag with tweezers and cut in two lengthwise with scissors directly into an Erlenmeyer flask, a half pouch of snus can be used.

Documentation

Raw data binder

A paper copy of the results report with batch name is signed and dated and filed in a binder.

Log book

The list in the first page of the log book details the information to be entered.

- The QC-batch name
- Name of the results file has been imported into (b) (4)
- Performer
- Name of used calibration solution (date/sign)
- Any departures from the method description or instruction
- Updating the method and instruction
- New or upgraded software

Instrument binder

Complete the protocol under the "Preventive maintenance" tab if you have performed any maintenance work.

Data

Collection and storage of data

For an in-depth description of registering a sample identity, see (b) (4)

The collection of data and the calculation of results are performed using the (b) (4) software.

The method (Test parameter) is called Ammonium and is stored under Test parameter in (b) (4)

All raw data is saved in Archive in (b) (4) under the date of analysis, see (b) (4)

The results files and import files are named with (b) (4) and saved.

A backup is performed as described in the APS quality manual.

Calculations

The calibration curve is calculated by the program (b) (4) using the least square method for linear equation. The method includes a calibration table with seven levels where the concentrations are given in mg/ml. The raw data obtained is corrected in the (b) (4)

Quality assurance

Standard curve criteria

A standard curve for (b) (4) is run once a week. The correlation coefficient is to be > 0.998.

The standard curve is to be rerun if the above criteria are not met. If the problem persists, prepare a new master standard solution and rerun the standard curve.

Control chart

For each analytical occasion, two check samples are analysed for each preparation with robots.

The check samples are analysed, evaluated and documented according to (b) (4)

(b) (4)

The results from the check samples are imported into the applicable control chart in (b) (4) as Replicate 1 and Replicate 2.

The mean value is calculated in an X chart, and the percentage difference between Replicate 1 and Replicate 2 is calculated in an R chart.

Evaluation of X chart and R chart

See the (b) (4) description

Proposals for measures if the results from the assessment of the control charts are not approved:

- Run a new calibration and re-run the batch.
- Run a new calibration with new reagent solutions and re-run the batch.

Duplicate and triplicate samples

When analysing duplicate samples, the permitted difference is 26% and for triplicate samples 28%.

If the response for a sample is higher than the measurement range

The instrument dilutes the sample to a response within the measurement range.

Reporting of analysis results

The results that are obtained (mg) are imported into LIMS. The value is corrected for the weighed out sample (mg/g sample) and dry content to mg/g dry sample. The values are automatically rounded to three significant digits. The mean values per sample are rounded to two significant digits.

Specify the level to at least two decimal places for manual entry in LIMS.

Concentrations < 0.5 mg/g is shown as 0.

Revision history

- 2018 February: 4-year revision, replacement of document publisher, addition of sample comparison.
- 2017 October: Addition of new matrix, Tobacco derived products (b) (4) and (b) (4) (b) (4) Additional validation is performed with Repeatability, Accuracy, Extraction yield, and Specificity and updated method.
- 2016: New check sample
- 2016: Additional validation of cigars/cigarillos with new measurement uncertainty.
- 2015 December: Extended shelf life on the stock standard solution.
- 2015 December: Cleaning solution for simultaneous analysis of (b) (4) and ethanol.

Person responsible

Director APS

Validation

Validation report

The validation examined ten samples distributed in ten different matrices. The validated samples were three snus varieties (b) (4) one tobacco flour (b) (4) one moist snuff (b) (4), one fibre-based product (b) (4) and one chewing tobacco (b) (4) (b) (4). An additional validation was made in Q1 2016 on (b) (4) (b) (4). An additional validation for tobacco derived products was made in Q3 2017 on (b) (4)

Table 1 summarises the type of validation conducted for each sample and the approximate concentration they had. The linearity is estimated based on the analysis of standard solutions. Blank samples and low standard solutions have been analysed to determine the LOD and LOQ.

Calculations and all data used for the calculations are available upon request.

Table 1. Examined samples in the validation of ammonium.



(b) (4)

(b) (4)

Specificity

Interferences of magnesium may occur but are counteracted by trisodium citrate that is present in (b) (4). In the event of simultaneous analysis of (b) (4) and ethanol, concentrations of (b) (4) that are too high may be obtained. This is counteracted by a (b) (4) which is run after using the ethanol reagent.

Repeatability

Repeatability was determined by analysing the same matrix six times under constant conditions for all ten samples in **Table 1**.

The relative standard deviation in percentage (RSD %) for the investigated samples is reported in **Table 2**. Repeatability is good for all matrices. As the tobacco derived product Zyn has concentrations < LOQ, repeatability has been estimated based on accuracy data level 1. All nine samples were pooled together to RSD pool 8.04%.

Table 2. RSD for repeatability for various matrices.

(b) (4)

Precision within the laboratory

Precision within the laboratory was determined by analysing triplicates at six different timepoints for chewing tobacco, moist snuff, snus and cigar. For the various analytical timepoints, the laboratory technician, calibration, reagent and standard solutions were all varied.

See **Table 3** for a summary. The RSD pool is 7.0% for the four matrices.

Table 3. RSD for Precision within the laboratory for different matrices.



Reproducibility/Interlaboratory comparison

A proficiency test with FAPAS was performed in 2013 on an aqueous solution. The results demonstrate comparable concentration levels. In 2016, (b) (4) analysis results for (b) (4) (b) (4) were compared with CORESTA's proficiency test. The method yielded comparable concentrations to CORESTA's results. In 2017, the APS analysis results for (b) (4) Cigar Filler, (b) (4) were compared with CORESTA's proficiency test results. The method yielded comparable results to CORESTA's results.

Accuracy (trueness)

One chewing tobacco, one moist snuff, one snus, one cigar and Tobacco derived products (b) (4) was spiked with (b) (4) at three levels of concentration at one timepont. Six determinations at each spiked concentration level, and six unspiked replicates were quantified.

See **Table 4** for a summary. Accuracy is satisfactory for all five matrices.

Table 4. Accuracy for spiked samples in five different tobacco matrices.

Spiked Conc. mg/g	Red Man LL %	CRP2, moist snuff %	CRP1, PS %	Cigar, SKU 1439	Zyn Peppermint
1	n/a	n/a	n/a	n/a	87.5
1.5	85	73	118	131	n/a
3	116	105	121	123	94.5
6	102	103	111	111	97.5

Bias from accuracy data

The estimated error in the method in relation to the true value in % (bias) is calculated as the square root of the sum of the yield-100 from "accuracy", and the uncertainty in the addition of the amount of analyte. Bias from "accuracy" is used for calculating measurement uncertainty. Bias for ammonium is calculated at 17.8%.

(b) (4)

Extraction yield (Recovery)

To calculate the relative and total extraction yields, one chewing tobacco (b) (4) one moist snuff (b) (4) and one snus (b) (4) were used as well as an extraction solution with ammonium at one timepoint. An additional validation of only Absolute Extraction Exchange was performed in the autumn of 2017 on Tobacco derived products (b) (4)

Procedure:

Spiked tobacco (sample C) were prepared and analysed to determine the relative and absolute recoveries, see Table 5. Additionally, unspiked samples were analysed in order to remove the original concentration when calculating the yields.

Extraction solutions were furthermore spiked (at the same spiking level) and prepared prior to analysis (sample B) to determine the relative extraction yield. Extraction solutions were also analysed as unprepared (sample A) to estimate the absolute extraction yield.

All levels were performed using 6 replicates. Table 5 presents the mean values.

Table 5. (b) (4)

(b) (4)

Limit of detection (LOD) and limit of quantification (LOQ)

Calculations were made based on the signal/noise ratios.

10 individual measurements were made of the absorbency on blank samples and standard solution 0.01mg/ml. See Table 6.

Table 6. Calculate LOD and LOQ. The method uses 0.5 mg/g as the lowest standard.

	LOD	LOQ
(b) (4)		

For Tobacco derived products (b) (4), LOQ is estimated to be lower than the lowest standard which is (b) (4) mg/g.

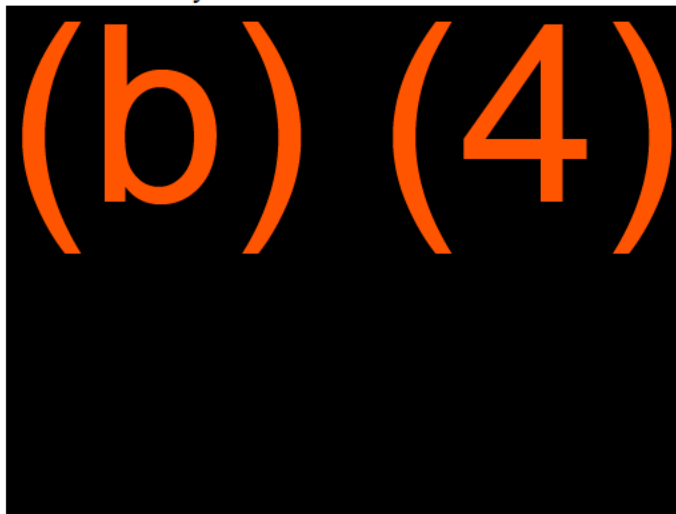
Linearity

Linearity is determined at 7 levels of concentration between (b) (4) mg/ml as in the sample solution equivalent to (b) (4) mg/g sample.

The determination of linearity was repeated twice using the same standard solutions on the same day.

Linearity is deemed to be good. No weighting of the curve is required due to the residuals in percentage being good across the measurement range. See charts 1 and 2.

Chart 1. Linearity



Absorbans = absorbancy

Mv = average value

Chart 2. Relative residuals (accuracy)



Robustness

Robustness for the method for the determination of (b) (4) is assessed using data from the control charts in (b) (4). Laboratory technicians, reagents and standard solutions are varied for the different analysis days.

The entire weighing in range of samples ((b) (4)g) has been used for repeatability.

Measurement range and measurement uncertainty

Measurement range

(b) (4) mg/g as is in sample.

Measurement uncertainty

The combined relative measurement uncertainty for (b) (4) is indicated with a coverage factor of 2.

The largest contribution to measurement uncertainty for (b) (4) comes from bias when determining accuracy. After this, in order of size, precision and uncertainty in the calibration curve contribute in particular. No clear largest contribution is evident in precision.

Combined relative measurement uncertainty

(b) (4)

(b) (4) (Tobacco derived product)

Bias from Accuracy from (b) (4) is not included in the measurement uncertainty calculation.

Measurement uncertainty would have been lower if (b) (4) had been included.

Zyn is therefore covered by the stated measurement uncertainty.

Conclusion

Validation results demonstrate that the method of analysis for (b) (4) is satisfactory. It is a reliable and robust method.

The method meets the requirements set out in the (b) (4)

The method is fit-for-purpose for the analysis of ammonium in tobacco, tobacco products, fibre-based matrices and tobacco derived products.