QUANTITATIVE ANALYSIS OF CHLORIDE IN TOBACCO, TOBACCO PRODUCTS, AND FIBER-BASED MATRICES WITH A (b) (4)

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Purpose

To determine the concentration of chloride in tobacco, tobacco products, and fiber-based matrices with Discrete Photometric analyzer (DFA).

Applies to

APS

General information

A sample solution prepared for the analysis of chloride can also be used in the analysis of ammonium 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 analysis is performed by creating chemical reactions at a microscale during incubation and then using spectrophotometric detection.
Chloride is extracted from samples using Milli-Q quality water. Chloride reacts with mercury (II) thiocyanate to form soluble unionized mercury (II) chloride and liberate free thiocyanate ions. The free thiocyanate ions react in an acidic solution with iron (III) nitrate to form a red/brown iron (III) thiocyanate complex. The absorbency of this complex is measured spectrophotometrically at the wavelength of 480 nm.

Number of analyses/person/week is about 300.

Method scope, measurement range, and measurement uncertainty

Scope

To determine the concentration of chloride in tobacco, tobacco products, and fiber-based matrices with Discrete Photometric analyzer.

Measurement range

(b) (4) mg/g) in sample

Measurement uncertainty

The combined relative measurement uncertainty for chloride is indicated with a coverage factor of 2.
The largest contribution to measurement uncertainty for chloride comes from bias when determining accuracy, as well as the uncertainty in the calibration curve.

Combined relative measurement uncertainty

(b) (4)

Literature references

1. Application Note; (b) (4)

Internal reference documents (available upon request)

(b) (4)

Risk assessment and safety instructions

Summarized risk assessment

Suitable protective clothing such as lab coat, gloves, and glasses must be worn in all instances involving handling of (b) (4), which is a systemic health hazard, or (b) (4) (b) (4) which is corrosive. Application of (b) (4) should suitably be done in a fume cabinet. (b) (4)

Hazard and precautionary statements

(b) (4)

(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

Analysis instruments: (b) (4)

Data system: (b) (4) for managing analysis instruments and for the collection of raw data and quantification.

Instrument parameters

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

Other equipment

Other equipment

(b) (4)

Extra equipment for manual preparation

Plastic Pasteur pipette 2 mL
Dispensette for 50 mL with flask
Magnetic stirrer
Shaker

Chemicals, reagents and solvents

(b) (4)

Check samples

(b) (4)

Preparation of standard solution

(b) (4)

Standard solution of chloride (0.05 g/dL)
(b) (4) is diluted to 100 mL with water in a volumetric flask.
Shelf life in a refrigerator is three months.
Hazard symbol: *Not subject to labelling*

Calibration solutions

Stock standard solution of chloride ((b) (4) g/dL) is diluted by the instrument to 6 concentration levels that correspond to (b) (4) g/dL, which corresponds to 0.4 to 6.25% in the sample.
Hazard symbol: *Not subject to labelling*

Reagent solutions

(b) (4) is purchased ready-made from (b) (4). Each package is marked with the expiration date by the supplier.
Hazard symbol: *Flammable* (b) (4)
Harmful GHS07 (Warning)
Systemic Health Hazards (b) (4)
Environmentally hazardous (b) (4)

Washing solutions

(b) (4) is purchased ready-made from (b) (4). Each package is marked with the expiration date by the supplier.
Hazard symbol: *Corrosive* (b) (4)

Tubing Maintenance Solution
Used for quarterly cleaning; is purchased ready-made from (b) (4). Each package is marked with the expiration date by the supplier.
Hazard symbol: *Not subject to labelling*

Washing solution for the robots
(b) (4) methanol is diluted with Milli-Q quality water to 2000 mL.
Hazard symbol: Harmful
Systemic Health Hazards (b) (4)

Sample handling

Sample storage and preparation

Tobacco flour and tobacco products are stored and pretreated according to (b) (4) (b) (4) (available upon request).

Sample amount

The minimum amount of sample for performing an analysis is 5 grams.

Analysis

Calibration and verification of apparatus

Before starting the calibration process or the sample sequence, check the system's water blank by making ten absorbance measurements on water. If the (b) (4), this may be due to air in the system. See (b) (4)
The instrument is calibrated once a week. The calibration is valid for 7 days. See under the heading Quality Assurance, "Criteria for standard curve".

Sample stability

The prepared sample solution remains stable for 4 days in refrigeration.

Analytical procedure

Weigh out all samples in a batch without interruption. Insert the stopper immediately after weighing. Afterwards, add water to all samples in a sequence.

- Weigh out 0.7 to1.3 g samples in a (b) (4) Record the weight manually to (b) (4) or automatically to the laboratory information and management system (b) (4) by using a computer connected scale.
- Add 50 mL of (b) (4) quality water with a dispensette (b) (4)

OR

- Add 50 mL of (b) (4) with a dispensette.
- Shake the flask without a stopper for 20 minutes with the shaker at (b) (4) or with a magnetic stirrer at a speed of (b) (4).
- 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.

Analyze the sample solutions using the DFA. See (b) (4) (b) (4) (available upon request).

Special instructions

When weighing pouch snus, the bag is held with tweezers and cut in two lengthwise with scissors directly into an Erlenmeyer flask. A half pouch of snus may be used.

Documentation

Logbook

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

- Name of the results file has been imported into (b) (4)
- (b) (4)
- Changes to method description
- New or upgraded software
- Services

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) (b) (4)
The collection of data and the calculation of results are performed using the (b) (4) (b) (4)
The method (Test parameter) is called Chloride and is stored under Test parameter in (b) (4) (b) (4)
All raw data is saved in Archive in (b) (4) under the day of the analysis date.

(b) (4)

Bring up old calibration

- Select F4 and 3 Calibration results, select Date for desired date

Calculations

Calculations are performed in the (b) (4). The method includes a calibration table calibration curve at six levels where the concentrations are given in g/dl (corresponds to weight-% in the sample). The concentration of the calibration solutions are plotted in relation to absorbency and the calculation is performed using the second degree equation.
The obtained quantified data based on the (b) (4), is corrected in the program for the (b) (4). A correction for weighed out amount of sample is made in (b) (4).

Quality assurance

Accuracy test of the instrument is performed at the annual service, see instrument binder.

Control chart

For each analytical occasion, two check samples are analyzed for each preparation with robots of 1-60 samples. See (b) (4)) and (b) (4) for sample preparation for (b) (4)" (QEMS). The check samples are analyzed, evaluated and documented as described in the "Control Charts and Check Samples" description (available upon request).
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 difference between Replicate 1 and Replicate 2 is calculated in an R chart.

Evaluation of X chart and R chart
See description "Control Charts and Check Samples" (available upon request).

Proposals for measures if the results from the assessment of the control charts are not approved:
Check and compare in relation to the previous calibration curve
Run a new calibration and re-run the batch.
Run a new calibration with new reagent solutions and re-run the batch.
Weigh out a new check sample and rerun the batch.
Make a new standard solution, calibrate and re-run the batch.

Standard curve criteria

The (b) (4) calculated as a second degree equation.

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

Duplicate and triplicate samples

When analyzing with duplicate samples, the difference between the samples should not be greater than (b) (4) of what was measured when determining repeatability.
The permissible difference in % between duplicate samples is (b) (4).

The spread between triplicate samples should not exceed (b) (4) the standard deviations as is calculated for the matrix in question.
The permissible difference between triplicate samples is (b) (4)

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

At a concentration above the measurement range, the instrument makes the sample dilution.

Reporting of analysis results

The values are imported into (b) (4) in % by weight with the default weight set to 1 g, see (b) (4) (b) (4) The value is automatically rounded down in (b) (4) to one decimal place following correction for the collected sample and dry sample calculation. Specify the level to at least two decimal places for manual entry in (b) (4) The analysis results are reported in weight %. Concentrations (b) (4) are specified as (b) (4)

Revision history

Person responsible

Director APS

Validation

Validation report

The validation examined seven samples distributed in five different matrices. The validated samples were (b) (4)

Table 1 summarizes 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 analyzed 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 chloride.

(b) (4)

Specificity

The known substances that might interfere with the analysis (eg, (b) (4)) are not expected to be present at high concentrations in any of the matrices; therefore, specificity of the method was not determined.

Repeatability

Repeatability was determined by spiking and analyzing six replicates of each matrix listed in **Table 1** under constant conditions.

The relative standard deviation in percentage (RSD %) for the spiked samples is reported in **Table 2**. Repeatability is good for all matrices. The RSD % for all samples pooled together (RSD pool) was (b) (4)

Table 2. RSD for repeatability for various matrices.

(b) (4)

Precision within the laboratory

Precision within the laboratory was determined by (b) (4) for one chewing tobacco, one moist snuff, and one snus. For the various analytical timepoints, (b) (4)

See **Table 3** for a summary. The RSD pool is (b) (4) for the three matrices.

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

(b) (4)

Reproducibility/Interlaboratory comparison

Not determined.

Accuracy (trueness)

One chewing tobacco, one moist snuff, and one snus were spiked with sodium chloride solution. (b) (4)

See **Table 4** for a summary. Accuracy is good for all three matrices.

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

(b) (4)

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 chloride is calculated at 10.4%.

Extraction yield (Recovery)

To calculate the relative and total extraction yields, one chewing tobacco, one moist snuff, and one snus were used as well as an extraction solution with chloride at one timepoint. The extraction solution was (b) (4) analysis (B) and also analyzed as unprepared (A). (b) (4) were also analyzed in order to remove the original concentration when calculating the yields. All levels were performed using 6 replicates.

C = spiked tobacco-original concentration. Table 5 presents the mean values.

Table 5. Extraction yield of chloride

(b) (4)

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

Due to (b) (4) is used between the 2 lowest standard points in order to estimate LOD/LOQ.
LOD is estimated to be 0.15% chloride (g/dL) which is about 2.5 times lower than reported (0.4% chloride (g/dL))
LOQ is estimated to be 0.22% chloride (g/dL) which is approximately 2 times lower than reported (0.4% chloride (g/dL))

Table 6. LOD and LOQ.

	LOD %	LOQ %
(b) (4)		

Linearity

Linearity is determined at 6 levels of concentration between 0.008 to 0.125 g/dL equivalent to 0.4 to 6.25% in the sample solution.
(b) (4) on the same day.
The relative residuals are high for low concentrations. The (b) (4) when quantitating samples. (b) (4) Linearity of the analytical method was deemed to be acceptable. See charts 1 and 2.

Chart 1. The calibration curve from the instrument

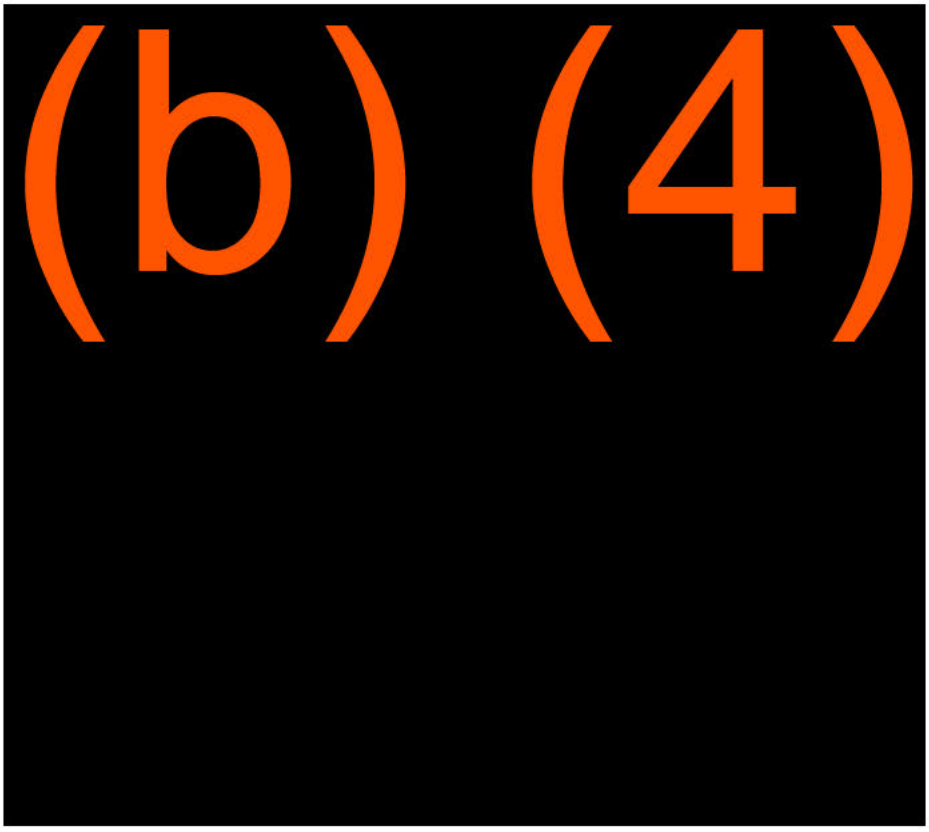
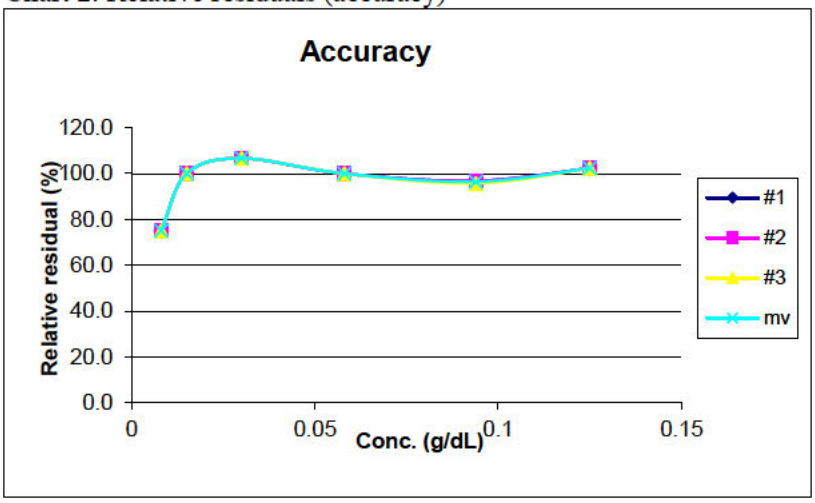


Chart 2. Relative residuals (accuracy)



Robustness

Robustness for the method for the determination of chloride is assessed using data from the (b) (4) for the different analysis days.
The entire (b) (4) has been used for repeatability. The method is considered to be robust.

Measurement range and measurement uncertainty

Measurement range
(b) (4) mg/g) in sample.

Measurement uncertainty
The combined relative measurement uncertainty for chloride is indicated with a (b) (4)

The largest contribution to measurement uncertainty for chloride 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)