aokinmycontrolCAF

Order No.: MY-15

Sample preparation

*aokin*mycontrol*CAF*

Analytical-kit for rapid and quantitative determination of Caffeine (CAF) in solid and liquid samples.

Materials

aokin*mycontrol****CAF*** (Order No.: MY-15-100)

Package content

A) *Materials for analytical measurement:*
aokin*ReactionBuffer,* Reaction buffer
aokin*mycontrol****CAF,*** Reagent 1 (yellow cap), F-CAF, (for 5 analyses each)
aokin*mycontrol****CAF,*** Reagent 2 (white cap), A-CAF, (for 5 analyses each)

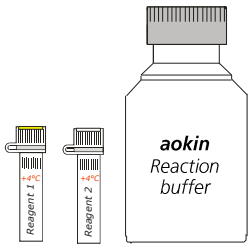


Figure 1:
Reagent 1 (yellow cap),
Reagent 2 (white cap),
Reaction buffer (1 L bottle)

B) *Materials for internal quality control:*
aokin*mycontrol* ***CAF,*** negative control CAF (transparent), (for zero value measurements)
aokin*mycontrol****CAF,*** Reagent 1 (yellow cap), F-CAF, (for 5 analyses each)
aokin*mycontrol****CAF,*** Reagent 2 (white cap), A-CAF, (for 5 analyses each)

Note: All substances provided are precisely weighed and calibrated. Control of the volume and concentration of the individual solutions are essential for the precision of the analysis.
Storage Conditions: Reagents 1 and 2 must be stored at temperature of +4°C. All other components may be stored at room temperature.
Quality Control: All materials and reagents are prepared according to strict quality control protocols. Exchanging reagents between kits having different Lot-numbers will lead to erroneous results and is not permitted.

Order Information:
aokin*mycontrol****CAF*** (Order No.: MY-15-100)

Introduction

aokin*mycontrol****CAF*** is a rapid and precise quantitative method for analyzing Caffeine (CAF). It has been specifically designed and calibrated for the analysis of caffeine in coffee, tea, caffeinated soft drinks and water.

aokin*mycontrol****CAF*** is available with a calibration, which has been validated for Caffeine in solid and liquid samples. Please use professional care and check the accuracy by regularly analyzing reference materials (e.g. ***aokin****ReferenceMatrixMaterials*) and/or standards. Participation in proficiency tests is recommended.
aokin will gladly assist you customising the test for your specific sample type and application. Please do not hesitate to contact us.

Sample	coffee, tea, caffeinated soft drinks and water
Time required for sample preparation	5-15 minutes
Time required for measurement	3 minutes

Caffeine

Caffeine (CAF) is found in varying quantities in the seeds, leaves, and fruits of some plants, as coffee, tea, guarana or cola nut, where it acts as a natural pesticide. Caffeine is a white crystalline xanthine alkaloid and stimulates heart activity, respiration and metabolism.

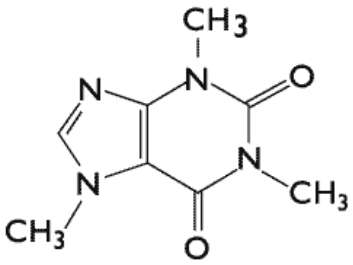


Figure 2: Chemical formula for caffeine $C_8H_{10}N_4O_2$. Molecular weight: 194,19 g/mol

Recommended Accessories

All required materials are available from ***aokin***.
Tel.: +49 30 9489 2160

	Order No.:
aokin*extractor* (food blender)	EX-07-06
Weighing scale, d = 0,01 g	LB-03-04
Variable pipette (1000 µl)	LB-04-05-1000
Pipette tips (1000 µl)	LB-04-08-1000
Funnel	LB-05-04
aokin*ReferenceMatrixMaterial*	RMM-15

Sample preparation

The following protocol is an example. The quantification ranges are dependent on dilutions. Actual volume settings in the software may vary.

Note: It is of critical importance to use the correct sample preparation protocol for each determination. Use volumes displayed in the ***aokin*** software.

1. Sample collection, grinding and mixing

The analysis sample is collected, ground, and mixed (homogenised) according to an approved procedure. Small sample volumes may be ground and mixed using the ***aokin****extractor*.

2. Weighing and extraction

A. Preparation of solid samples (such as coffee)

Extraction:

Determine the weight of the heat-resistant vessel. Add 0,2 g of the ground and mixed sample, and 200 ml of hot water (95°C) in the heat-resistant vessel (Figure 3). Heat the sample for 10 minutes on a hot plate (100°C) and cover the lid.

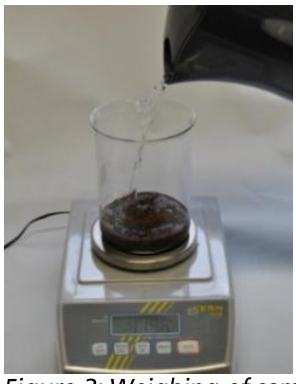


Figure 3: Weighing of sample and addition of hot water

Take the vessel from the hot plate, place it on the scale, and refill with water to a total of 400 g of sample mixture, as a compensate to the vaporized liquid.

Filtration:

Place the filter on a suitable funnel and the funnel onto a collection container. Pour the extract onto the filter (Figure 4) and collect the filtrate. Discard the filter paper and filter cake. Shake/stir the filtrate to ensure homogeneity. Transfer 1 ml of the filtrate into a clean tube. Continue with the analysis (step 3).

Figure 4: Filtration of the extracted sample

B. Preparation of liquid samples

Weigh 0,2 g of your liquid sample and 400 g of water in a vessel. Transfer 1 ml of the prepared sample into a clean tube. Continue with the analysis (step 3).

4. Analyzing

Use the prepared sample for the analysis in the ***aokin****spectrometer****FP470***.

Please follow detailed instructions for spectrometer use (***aokin****spectrometer****FP470*** & ***aokin****LHW03* Instructions for use).

This includes:

1) Open and place ***Reagents 1*** and ***2*** into position A6 and B6 of the sample rack of your spectrometer.
2) Fill up the ***Clean1*** solution and place a clean 2 mL vial in position A1.
3) Place an empty waste bottle in the holder. Check presence of ***Reaction buffer*** and check if tubing is below the surface.
4) Place a new cuvette with a clean stirrer into the spectrometer.

5. Quality control

Included in the analytical kit there are following additional materials for your internal quality control: ***Reagent 1***, ***Reagent 2***, and ***negative control*** samples (corresponding to samples free of Caffeine) for measurements of zero values.

Please perform measurements of blank samples regularly, this ensures the accuracy of your analyses.

If you notice increased values for a blank sample, then change cuvette and repeat measurement. If blank sample results remain high, contact the ***aokin*** team.

6. Conversion factor µg/kg → mass percent caffeine in dry coffee

Example:

0.1 % by weight of caffeine (in decaffeinated coffee)

0.1% caffeine in your (coffee) sample are =
0.001 kg caffeine / kg coffee =
1 g caffeine / kg coffee =
1000 mg caffeine / kg coffee =
1 000 000 µg caffeine / kg coffee

→ 0.1% caffeine = 1000 mg/kg = 1000 000 µg/kg

Conversion factor: analyte concentration in cuvette (nM) to amount in sample (µg/kg)

aokinmycontrol CAF
Standard

Step 1: Extraction

- *Sample mass:* m_{Sample} = 0,2 g
- *Volume extraction solvent:* V_{Water} = 400 ml
- *Molar mass Caffeine:* $MW_{Caf} = 194{,}19\ \left[\frac{g}{mol}\right]$

Caffeine concentration in the sample extract:

$$\boldsymbol{c}\left[\frac{\mu\boldsymbol{mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Extract}} = \frac{m_{Sample}[kg]}{V_{Solvent}[l] * MW_{Mykotoxin}\left[\frac{g}{mol}\right]} * c\left[\frac{\mu g}{kg}\right]_{Sample} = \frac{0.0002}{0.4 * 194{,}19} * c\left[\frac{\mu g}{kg}\right]_{Sample} = 0.00000257 * c\left[\frac{\mu g}{kg}\right]_{Sample}$$

Step 2: Measurement

aokin FP 470 / *LHW 03*

- *Sample volume:* $V_{Column\ eluate} = V_{Sample} = 200\ \mu l$
- *Total volume in the cuvette:* $V_{Cuvette} = 2600\ \mu l$

Caffeine concentration in the cuvette:

$$\boldsymbol{c}\left[\frac{\mu\boldsymbol{mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Cuvette}} = \frac{V_{Sample}\,[\mu l]}{V_{cuvette}\,[\mu l]} * c\left[\frac{\mu mol}{l}\right]_{Eluate} = \frac{200}{2600} * c\left[\frac{\mu mol}{l}\right]_{Eluate} = 0.0769 * \left[\frac{\mu mol}{l}\right]_{Eluate}$$

Conversion factor: Extraction, Dilution and Measurement

It follows the conversion factor from 1 to 2 above:

$$\boldsymbol{c}\left[\frac{\mu\,\boldsymbol{mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Cuvette}} = 0.00000257 * 0.0769 * c\left[\frac{\mu g}{kg}\right]_{Sample} = 0.00000019763 * c\left[\frac{\mu g}{kg}\right]_{Sample} \text{ or}$$

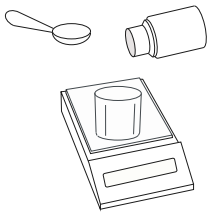
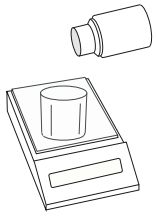
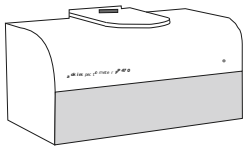
$$\boldsymbol{c}\left[\frac{\mu\,\boldsymbol{g}}{\boldsymbol{kg}}\right]_{\boldsymbol{Sample}} = \frac{1}{0{,}00000019763} * c\left[\frac{nmol}{l}\right]_{Cuvette} = \boldsymbol{5.06 * 10^6 * c\left[\frac{nmol}{l}\right]_{Cuvette}}$$

Caffeine / standard samples:

- Recommended for coffee, tea, caffeinated soft drinks, water

Procedure:

***aokin**mycontrol CAF*
Standard

Extraction		**Weighing:** 0,2 g sample 200 g water
		**Heating and weighing:** 10 min heat on a hot plate fill to total sample weight of 400 g with water
		**Filtration:** collect filtrate (discard filter cake) transfer filtrate into clean 2 mL reaction tube
Measurement		**Automatic Analyse (FP470 / LHW03)** place the 2ml reaction tube in the sample holder of the *LHW03* 2200 µl ***aokin** Reaction buffer* 200 µl sample (diluted filtrate) *(diluted 1:1 - RANGE 1)* *(diluted 1:2 - RANGE 2)* *(diluted 1:4 - RANGE 3)* 100 µl ***aokin**mycontrol **CAF** Reagent 1* 100 µl ***aokin**mycontrol **CAF** Reagent 2*

CAF = Caffeine, Conversion factor: 1 nmol CAF/l in cuvette = 5060000 µg/kg in sample