EURLMP-method_014 (version 1)
Determination of glycoalkaloids in potatoes by LC-MS/MS

Analyte group:	**Plant toxins** - glycoalkaloids
Analyte(s):	Alpha-chaconine Alpha-solanine Gamma-chaconine Solanidine
Commodity group:	Potatoes
Commodities validated:	Unprocessed potato tubers, processed potatoes
Technique:	Liquid Chromatography / Tandem Mass Spectrometry (LC-MS/MS)

Modifications compared to previous version:
Not applicable

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Contents

1 Introduction

Glycoalkaloids (GAs) are secondary metabolites found in the *Solanaceae* family that function as natural toxins to insects and other herbivores. From a chemical point of view, glycoalkaloids are steroidal glycosides that are toxic to both humans and livestock. They can be found in foods such as potatoes and to a lower extent in aubergines (eggplant) and tomatoes. The GAs that are most abundant in potatoes include α-solanine and α-chaconine. They occur in different parts of the potato, including the tubers, sprouts, leaves and blossoms. Concentrations may differ as well, for instance the concentration of GAs in the peel is about 3-10 times higher than in the tuber. Furthermore, there is variation in GA content among potato cultivars. Damage of the tubers or certain storage conditions such as light and temperature may cause an increase in GAs. When potatoes with high levels of GAs are consumed, a bitter or burning sensation may be experienced in the mouth. This may be followed by nausea, vomiting and diarrhoea and in very exceptional cases to death. Currently there is no European legislation for GAs, but this may be introduced in the future. Recently, a recommendation to monitor the glycoalkaloids α-solanine and α-chaconine in potatoes and potato products has been issued by the European Commission [1]. If possible, also the degradation products β- and γ-solanine and β- and γ-chaconine and the aglycon solanidine should be analysed.

2 Scope

This method describes the quantitative determination of three glycoalkaloids: α-solanine, α-chaconine, γ-chaconine and the aglycone solanidine in raw and processed potatoes. The method is suited for the range of 0 to 250 mg/kg in potato tubers and from 0 to 50 mg/kg in processed potatoes.

3 Principle

Samples are prepared by addition of stabilisation solution (1% formic acid in methanol) to the roughly cut product followed by homogenisation in a food blender. The glycoalkaloids are extracted from the homogenised suspension by using a mixture of methanol/water/formic acid (60/40/0.4, v/v/v).
An aliquot of the supernatant is diluted with extraction solvent. The samples are analysed by LC-MS/MS using a reversed-phase column to separate the analytes, followed by MS/MS detection. Quantification is performed by external calibration in blank surrogate matrix extract.

4 Reagents

All reagents and solvents shall be of quality for LC analysis, unless otherwise specified.

4.1 Analytical standards

4.1.1 Alpha-chaconine

4.1.2 Alpha-solanine

4.1.3 Gamma-chaconine

4.1.4 Solanidine

4.2 Chemicals

4.2.1 Methanol, 98-100%, LC-MS grade

4.2.2 Formic acid, 99%

4.2.3 Water, LC-MS grade
Water purified by a Milli-Q purification system with a minimal resistance of 18.2 MΩ/cm can be used as well.

4.3 Solutions and reagents

4.3.1 Extraction solvent: 0.4% formic acid in methanol/water (60/40) (v/v)
Mix 600 ml of methanol (4.2.1), 400 ml of water (4.2.3) and 4 ml of formic acid (4.2.2) in a bottle of 1000 ml. This solution is stored at room temperature and can be used for 3 months.

4.3.2 Stabilisation solvent: 1% formic acid in methanol
Mix 10 ml of formic acid (4.2.2) with 1000 ml methanol (4.2.1). This solution is stored at room temperature and can be used for 3 months.

4.3.3 Mobile phase A: 0.1% formic acid in water
Mix 1 ml of formic acid (4.2.2) with 1000 ml water (4.2.3). This solution is stored at room temperature and can be used for 1 month.

4.3.4 Mobile phase B: methanol (4.2.1)

4.4 Standard solutions

4.4.1 Stock solutions (200 µg/ml)
Accurately weigh (± 0.02 mg) into separate amber-coloured glass bottles (5.1.3) between 3 and 5 mg (5.1.1) of the standards (4.1.1 to 4.1.4). However, when the standard is only available in a quantity of 3 mg or less, the entire content of the container is used. In that case the weight reported by the supplier is used. Flush the contents of the bottle three times with methanol (4.2.1) to dissolve and collect all material. Add a volume of methanol (4.2.1) in such a way that the concentration of the solution is 200 µg/ml. Take into account the weight, the purity and the appearance form of the standard. The solutions can be used for 24 months when stored at -20°C.

4.4.2 Mixed standard solution (10/2 µg/ml)
Pipette 1000 µl of stock solutions 200 µg/ml of α-chaconine (4.1.1), α-solanine (4.1.2) and 200 µl of stock solutions 200 µg/ml of γ-chaconine (4.1.3) and solanidine (4.1.4) into a calibrated volumetric flask of 20 ml. Make up the volume with methanol (4.2.1) and mix. The solution can be used for 12 months when stored at -20°C.

4.4.3 Mixed standard solution (1000/200 ng/ml)
Pipette 1 ml of mixed standard solution 10/2 µg/ml (4.4.2) in a calibrated volumetric flask of 10 ml and make up the volume with methanol (4.2.1) and mix. The solution can be used for 12 months when stored at -20°C.

4.4.4 Mixed standard solution (100/20 ng/ml)
Pipette 1 ml of mixed standard solution 1000/200 ng/ml (4.4.3) in a calibrated volumetric flask of 10 ml and make up the volume with methanol (4.2.1) and mix. The solution can be used for 12 months when stored at -20°C.

4.4.5 Working standard (100/20 ng/ml) in extraction solvent

Pipette 100 µl of mixed standard solution 1000/200 ng/ml (4.4.3) in a vial and add 900 µl extraction solvent (4.3.1) and mix. Prepare a fresh solution every new day of analysis.

5 Materials & equipment

Any reference to type and/or product is only to inform the user and to identify the equipment and does not imply exclusion of similar equipment.
Usual laboratory glassware and equipment, in particular the following, can be used.

5.1 Materials

5.1.1 Analytical balance, accuracy ±0.02 mg

5.1.2 Analytical balance, accuracy ±0.02 g

5.1.3 Glass bottle, 4, 20, 30 or 60 ml, amber-coloured, with screw cap

5.1.4 Overhead or **horizontal shaker**

5.1.5 Vortex mixer

5.1.6 Blender, 2 l

5.1.7 Centrifuge tubes, 50 ml, polypropylene, with screw cap

5.1.8 Centrifuge, suitable for 50 ml centrifuge tubes

5.1.9 Mini-UniprepTM PTFE filter vial, 0.45 µm, 500 µl

5.1.10 Compressor for filter vials

5.1.11 Various pipettes (use positive displacement pipettes for solutions prepared in methanol)

5.1.12 Heating plate

5.1.13 Potato peeler

5.2 LC-MS/MS system with the following components:

5.2.1 LC pump, capable of delivering a binary gradient at flow rates appropriate for the analytical column in use with sufficient accuracy.

5.2.2 Injection system, capable of injecting an appropriate volume of injection solution with sufficient accuracy, and cross-contamination below 0.1%.

5.2.3 Analytical column, capable of retaining the target glycoalkaloids and capable of baseline separation of the analytes.

5.2.4 Column oven, capable of maintaining a constant temperature of 50 °C

5.2.5 Tandem mass spectrometer (MS/MS), capable of ionisation of the compounds in positive mode, performing Multiple Reaction Monitoring (MRM), with a sufficiently wide dynamic range and capable of unit mass separation and equipped with a computer-based data processing system. Any ionisation source giving sufficient yield may be employed.

6 Procedures

This document describes the quantification of glycoalkaloids in unprocessed potatoes, such as raw, unpeeled, potato tubers, and in processed potatoes, such as peeled and cooked potatoes, based on external matrix matched calibration in blank extract.
In Annex A 1 to 4 the steps described in section 6.1 to 6.6 are shown in the format of checklists.

6.1 Preparation of the test sample

To obtain homogeneous samples, the material is weighed and cut into parts or wedges. Then, a sub-sample is weighed and blended to a slurry in the presence of a stabilisation solvent. Samples are stored at -20°C.

6.1.1 Blank materials

For unprocessed potato: cut 200-300 g of unprocessed sweet potato into parts or wedges.
For processed potato: peel (5.1.13) 300 g of sweet potatoes, cut these into parts or wedges and boil (5.1.12) 250 g of material with 500 ml of water for 25 minutes.
Transfer 200 ± 5 g of the wedges to a blender (5.1.6) and add 100 ml of stabilisation solvent (4.3.2). Homogenise in the blender and store aliquots of 6 g slurry in test tubes at -20°C for future use or continue with the sample preparation procedure (6.3.1-6.4.1).

6.1.2 Unprocessed potatoes

Select a number of potatoes (approximately 500 g) that are representative of the sample and cut these into parts or wedges. Transfer 200 ± 5 g to a blender (5.1.6) and add 100 ml of stabilisation solvent (4.3.2). Homogenise in the blender and store the sample slurry at -20°C or continue with the sample preparation procedure (6.3.3).

6.1.3 Processed potatoes

Select a number of unprocessed potatoes (approximately 500 g) that are representative of the sample, peel the potatoes using a peeler (5.1.13), and cut these into parts or wedges. Add 250 g of the wedges to 500 ml of boiling water (5.1.2) and cook for 20 minutes (floury varieties) or 25 minutes (waxy varieties).
Transfer 200 ± 5 g of the boiled potatoes or potato product to a blender (5.1.6) and add 100 ml of stabilisation solvent (4.3.2). Homogenise in the blender and store the sample slurry at -20°C or continue with the sample preparation procedure (6.4.3).

6.2 Test portion

The amount of homogenised sample slurry examined is 6.0 ± 0.1 g. Mix the sample slurry well before taking the test portion.

6.3 Unprocessed potato

6.3.1 Calibration standards in blank matrix

The calibration standards are prepared by addition of standard solution to blank sample extract according to Table 1 for unprocessed potatoes. Use sweet potato as blank material. Sweet potato does not contain GAs analysed in this method.
Weigh a test portion of 6.0 g sweet potato slurry (6.1.1) in a PP tube of 50 ml (5.1.7). Add 35 ml extraction solvent (4.3.1) to the tube and extract for 30 min on a rotary tumbling machine (5.1.4). Centrifuge the sample for 15 min at 3000 g (5.1.8) and transfer 8 aliquots of 10 µl supernatant to 500 µl filter vials (5.1.9). Add mixed standard solutions 4.4.2, 4.4.3 and 4.4.4 and extraction solvent (4.3.1) according to Table 1. Mix and close the vials with the help of a compressor (5.1.10).

Table 1: Preparation of matrix matched calibration standards for unprocessed potato (P)

Calibration level	Concentration in extract	Concentration in sample	Mixed standard 100/20 ng/ml (4.4.4)	Mixed standard 1000/200 ng/ml (4.4.3)	Mixed standard 10/2 µg/ml (4.4.2)	Blank extract (6.3.1)	Extraction solvent (4.3.1)
	(ng/ml)	(mg/kg)	(µl)	(µl)	(µl)	(µl)	(µl)
P1	0/0	0/0	-	-	-	10	490
P2	2.5/0.5	1.25/0.25	12.5	-	-	10	477.5
P3	5/1	2.5/0.5	25	-	-	10	465
P4	10/2	5/1	50	-	-	10	440
P5	25/5	12.5/2.5	-	12.5	-	10	477.5
P6	50/10	25/5	-	25	-	10	465
P7	100/20	50/10	-	50	-	10	440
P8	250/50	125/25	-	-	12.5	10	477.5

6.3.2 Quality control samples for unprocessed potatoes

6.3.2.1 Quality control sample limit of quantification (QC_{LOQ} 1/0.2 mg/kg)

Transfer an aliquot of 6.0 g of sweet potato slurry (6.1.1) to a 50 ml PP tube (5.1.7). Add 400 µl of mixed standard solution 10/2 µg/ml (4.4.2) and shake vigorously. Add 35 ml of extraction solvent (4.3.1), shake vigorously and extract for 30 minutes on a shaking machine (5.1.4). Centrifuge the sample for 15 min at 3000 g (5.1.8) and transfer 10 µl supernatant to 500 µl filter vials (5.1.9). Add 490 µl extraction solvent (4.3.1). Mix and close the vials with the help of a compressor (5.1.10).

6.3.2.2 Quality control sample recovery (QC_{Rec} 10/2 mg/kg)

Transfer an aliquot of 6.0 g of sweet potato slurry (6.1.1) to a 50 ml PP tube (5.1.7). Add 4000 µl of mixed standard solution 10/2 µg/ml (4.4.2) and shake vigorously. Add 31 ml of extraction solvent (4.3.1), shake vigorously and extract for 30 minutes on a shaking machine (5.1.4). Centrifuge the sample for 15 min at 3000 g (5.1.8) and transfer 10 µl supernatant to 500 µl filter vials (5.1.9). Add 490 µl extraction solvent (4.3.1). Mix and close the vials with the help of a compressor (5.1.10).

6.3.3 Unprocessed potato – sample preparation procedure

Transfer 2 aliquots of 6.0 g potato slurry (6.1.2) to 50 ml PP tubes (5.1.7). Add 35 ml of extraction solvent (4.3.1), shake vigorously and extract for 30 minutes on a shaking machine (5.1.4). Centrifuge the samples for 15 min at 3000 g (5.1.8) and transfer 10 µl supernatant to 500 µl filter vials (5.1.9). Add 490 µl extraction solvent (4.3.1). Mix and close the vials with the help of a compressor (5.1.10). The dilution factor is 500 (see note).

Note: Samples that contain one or more GAs exceeding the range of the calibration curve are 5 times diluted with blank sweet potato extract (6.3.1) and extraction solvent (4.3.1). Transfer 200 µl of the sample supernatant (6.3.3) to a test tube of 10 ml and add 800 µl of blank sweet potato supernatant (6.3.1) and mix. Transfer 10 µl of the mixed supernatant to a filter vial (5.1.9) and add 490 µl extraction solvent (4.3.1). Mix and close the vial with the help of a compressor (5.1.10). The dilution factor is 2500.

6.4 Processed potato

Potatoes that are peeled but not cooked are also considered processed potatoes and can be analysed using the sample preparation procedure described in this section.

6.4.1 Calibration standards in blank matrix

The calibration standards are prepared by addition of standard solution to blank sample extract according to Table 2 for processed potatoes. Use cooked sweet potato as blank material. Sweet potato does not contain GAs analysed in this method.
Weigh a test portion of 6.0 g cooked sweet potato slurry (6.1.1) in a PP tube of 50 ml (5.1.7). Add 35 ml extraction solvent (4.3.1) to the tube and extract for 30 min on a rotary tumbling machine (5.1.4). Centrifuge the sample for 15 min at 3000 g (5.1.8) and transfer 8 aliquots of 25 µl supernatant to 500 µl filter vials (5.1.9). Add mixed standard solutions 4.4.2, 4.4.3 and 4.4.4, and extraction solvent (4.3.1) according to Table 2. Mix and close the vials with the help of a compressor (5.1.10).

Table 2: Preparation of matrix matched calibration standards for processed potato (PP)

Calibration level	Concentration in extract	Concentration in sample	Mixed standard 100/20 ng/ml (4.4.4)	Mixed standard 1000/200 ng/ml (4.4.3)	Mixed standard 10/2 µg/ml (4.4.2)	Blank extract (7.4.6)	Extraction solvent (5.3.1)
	(ng/ml)	(mg/kg)	(µl)	(µl)	(µl)	(µl)	(µl)
PP1	0/0	0/0	-	-	-	25	475
PP2	2.5/0.5	0.5/0.1	12.5	-	-	25	462.5
PP3	5/1	1/0.2	25	-	-	25	450
PP4	10/2	2/0.4	50	-	-	25	425
PP5	25/5	5/1	-	12.5	-	25	462.5
PP6	50/10	10/2	-	25	-	25	450
PP7	100/20	20/4	-	50	-	25	425
PP8	250/50	50/10	-	-	12.5	25	462.5

6.4.2 Quality control samples for processed potatoes

6.4.2.1 Quality control sample limit of quantification (QC_{LOQ} 0.5/0.1 mg/kg)

Transfer an aliquot of 6.0 g of boiled sweet potato slurry (6.1.1) to a 50 ml PP tube (5.1.7). Add 200 µl of mixed standard solution 10/2 µg/ml (4.4.2) and shake vigorously. Add 35 ml of extraction solvent (4.3.1), shake vigorously and extract for 30 minutes on a shaking machine (5.1.4). Centrifuge the sample for 15 min at 3000 g (5.1.8) and transfer 25 µl supernatant to 500 µl filter vials (5.1.9). Add 475 µl extraction solvent (4.3.1). Mix and close the vials with the help of a compressor (5.1.10).

6.4.2.2 Quality control sample recovery (QC_{Rec} 10/2 mg/kg)

Transfer an aliquot of 6.0 g of boiled sweet potato slurry (6.1.1) to a 50 ml PP tube (5.1.7). Add 4000 µl of mixed standard solution 10/2 µg/ml (4.4.2) and shake vigorously. Add 31 ml of extraction solvent (4.3.1), shake vigorously and extract for 30 minutes on a shaking machine (5.1.4). Centrifuge

the sample for 15 min at 3000 g (5.1.8) and transfer 25 µl supernatant to 500 µl filter vials (5.1.9). Add 475 µl extraction solvent (4.3.1). Mix and close the vials with the help of a compressor (5.1.10).

6.4.3 Processed potato – sample preparation procedure

Transfer 2 aliquots of 6.0 g processed potato slurry (6.1.3) to 50 ml PP tubes (5.1.7). Add 35 ml of extraction solvent (4.3.1), shake vigorously and extract for 30 minutes on a shaking machine (5.1.4). Centrifuge the samples for 15 min at 3000 g (5.1.8) and transfer 25 µl supernatant to 500 µl filter vials (5.1.9) and add 475 µl extraction solvent (5.3.1). Mix and close the vials with the help of a compressor (5.1.10). The dilution factor is 200 (see note).

Note: Samples that contain one or more GAs exceeding the range of the calibration curve are 5 times diluted with boiled blank sweet potato extract (6.5.1) and extraction solvent (4.3.1). Transfer 200 µl of the sample supernatant (6.4.3) to a test tube of 10 ml and add 800 µl of blank boiled sweet potato supernatant (6.5.1) and mix. Transfer 25 µl of the mixed supernatant to a filter vial (5.1.9) and add 475 µl extraction solvent (4.3.1). Mix and close the vial with the help of a compressor (5.1.10). The dilution factor is 1000.

7 LC-MS/MS analysis

Chromatographic and mass spectrometric conditions may be chosen freely. The optimal measurement conditions strongly depend on the instrumentation used.

The chosen column dimensions and chromatographic conditions should be appropriate to obtain base line separation of glycoalkaloids from compounds with the same mass-to-charge ratio. A complicating factor is that glycoalkaloids may show in-source fragmentation (loss of one or more glycosidic groups) when introduced in the mass spectrometer. This may result in additional peaks in the chromatograms of glycoalkaloids of lower molecular mass (i.e. β- and γ-forms). The chosen chromatographic conditions should therefore be appropriate to obtain base line separation between the α, β and γ-forms of the GAs that can produce precursor ions or in-source fragments of the same molecular mass. The injection volume should be optimised for the column dimension and the sensitivity of the mass spectrometric system.

The chosen mass spectrometric conditions should be appropriate to measure the analytes with sufficient sensitivity and specificity. Preferably, the protonated molecular parent ion should be selected as precursor ion and the product ions should be specific for the compound. Preferably, product ions that are formed by the loss of water from the protonated molecular parent ion should not be selected. Select at least two precursor-to-product ion combinations to be included in the multiple reaction monitoring (MRM) method. Each chromatographic peak should be composed of at least 10 data points.

The LC-MS system is conditioned by injecting the working standard solution of 100/20 ng/ml (4.4.5). at least 6 times or until stable retention times and sensitivity is obtained. The system should be able to detect the product ion with the lowest intensity with a S/N ratio of 250 for the most critical component.

These injections should meet the following criteria:

- Retention times and peak areas should be stable.
- Sensitivity should be sufficient and fit-for-purpose. Sensitivity is sufficient if the GAs can be measured at the reporting limit. The limit of quantification for the determination of each GA in

potatoes and potato-derived products should preferably be 1 mg/kg or lower and not higher than 5 mg/kg.
- Check for carry-over effects, by injecting the highest calibration standard, followed by a solvent injection. If significant carry-over is observed, the system should be cleaned before starting the analysis series.

Example LC-MS/MS conditions and example LC-MS/MS chromatograms are given in Annex B.

7.1 Injection sequence
Analyse the calibration samples and the sample extracts in the order as given below:

- Standard working solution 100/20 ng/ml (4.4.5), injected at least 6 times
- Extraction solvent (4.3.1)
- Calibration standards in blank matrix extract (6.3.1 or 6.4.1)
- Extraction solvent (4.3.1)
- Quality control sample limit of quantification (6.3.2.1 or 6.4.2.1)
- Quality control sample recovery (6.3.2.2 or 6.4.2.2)
- Extraction solvent (4.3.1)
- Samples in duplicate (6.3.3 or 6.4.3)
- Extraction solvent (4.3.1)
- Calibration standards in blank matrix extract (6.3.1 or 6.4.1)

8 Evaluation and calculations
Peak areas are used for all subsequent calculations. For each injection, check peak assignment and integration for all measured transitions and adjust if needed.

8.1 Verification of linearity of LC-MS/MS measurement
The calibration standards (6.3.1, 6.4.1) are used to determine the linearity of the LC-MS/MS system. For calibration standards the sum of the area of the product ions is plotted as function of the added concentration to the sample extract (ng/ml) (see note). Apply linear regression using the least squares method.
Linearity is demonstrated and the calibration curve is fit-for-purpose when the deviation of the back-calculated concentrations of the calibration standards from the true concentrations, using the calibration equation, do not exceed 20%. The correlation coefficient of the lines should be ≥0.990.

Note: two calibration curves are constructed, one using calibration standards 1 to 6 (low range calibration curve) and one using calibration standards 1 to 8 (full range calibration curve). Analytes for which the peak area falls within the low range calibration curve are calculated with the low range calibration curve. Analytes for which the peak area falls outside the low range calibration curve but within the full range calibration curve are calculated with the full range calibration curve. Analytes for which the peak area falls outside the full range calibration curve are diluted and reanalysed.

8.2 Identification of GAs in the samples
Identify the presence of GAs in the samples by comparing retention time and ion ratio with that of the calibration standards (6.3.1, 6.4.1) according to SANTE/11312/2021 [2].

Glycoalkaloids are considered present and identified when:

a) the retention time (RT) of the peak observed in the individual calibration standards (6.3.1, 6.4.1), individual samples (6.3.3, 6.4.3) and fortified samples (6.3.2.1, 6.3.2.2, 6.4.2.1, 6.4.2.2) differs not more than 0.1 min from the average retention time as calculated (**Equation I)** from the calibration standards (6.3.1, 6.4.1)
b) the relative deviation of the ion ratio (D) in the individual calibration standards (6.3.1, 6.4.1), individual samples (6.3.3, 6.4.3) and fortified samples (6.3.2.1, 6.3.2.2, 6.4.2.1, 6.4.2.2) differs not more than 30% from the average ion ratio in the calibration standards (6.3.1 or 6.4.1) by using **Equation II** and **Equation III**.
c) in the blank QC sample (6.1.1, 6.1.2), no peak at the retention time of the GA is present that exceeds 30% of the LOQ.

Equation I: Deviation of the retention time (ΔRT)

$$\Delta RT = RT_{sample} - RT_{avg}$$

where:

ΔRT	is the deviation of the retention time of the analyte in the individual sample extract or fortified sample extract, compared to the average retention time in the calibration standards (min)
RT_{sample}	is the retention time of the analyte in the sample extract (min)
RT_{avg}	is the average retention time of the analyte in the calibration standards (min)

Equation II: Calculation of ion ratio (IR)

$$IR = \left(\frac{A_{low}}{A_{high}}\right) \text{ x } 100\%$$

where:

IR	is the ion ratio (%)
A_{low}	is the area of the product ion with the lowest intensity
A_{high}	is the area of the product ion with the highest intensity

Equation III: Relative deviation of the ion ratio (D)

$$D = \left(\frac{IR_{sample} - IR_{avg}}{IR_{avg}}\right) \text{ x } 100\%$$

where:

D	is the relative deviation of the ion ratio of the analyte in the sample, compared to the average ion ratio of the analyte in the calibration standards (%)
IR_{sample}	is the ion ratio of the analyte in the individual sample (%) (**Equation II**)
IR_{avg}	is the average ion ratio of the analyte in the calibration standards (%) (**Equation II**)

Note: for calculation of the reference ion ratio use only responses with an S/N > 10. For the higher concentrations, exclude peak areas exceeding the linear range from calculation of the reference ion ratio.

8.3 Quantification of GAs in the samples

8.3.1 Recovery

Calculate the recovery (Rec) of the recovery QC samples (6.3.2.2, 6.4.2.2) with **Equation IV**. The recovery should be between 70-120%.

Equation IV Recovery for GAs:

$$Rec = \left(\frac{A_{Rec} - b}{a}\right) x \frac{DF}{C_{spike}} x\ 100\%$$

Where:

Rec	is the recovery of the analyte in the QC_{Rec} sample (%)
A_{Rec}	is the sum of the area of the product ions for the analyte in the recovery sample QC_{Rec}
b	is the intercept of the calibration standards curve
a	is the slope of the calibration standards curve
DF	is the dilution factor of the extract
C_{spike}	is the concentration spiked to the recovery sample QC_{Rec}

8.3.2 Quantification

The concentration of glycoalkaloids in the sample (6.3.1, 6.4.1) is calculated using **Equation V**.

Equation V: Calculation of the concentration of each GA

$$C_{sample} = \left(\frac{A_{sample} - b}{a}\right) x\ DF\ x\ \frac{1}{Rec}$$

Where:

C_{sample}	is the concentration of the glycoalkaloid in the sample in mg/kg
A_{sample}	is the sum of the area of the product ions for the analyte in the sample
b	intercept of the calibration standards curve
a	is the slope of the calibration standards curve
DF	is the dilution factor of the extract
Rec	is the recovery of the QC_{Rec} sample (**Equation IV**)

Note: When the recovery is between 90 and 110%, correction for recovery is not mandatory.

8.4 Final result

The concentrations of glycoalkaloids in the sample are expressed in mg/kg. Quantification is based on the sum of the areas of the two product ions. When the recovery is between 90 and 110%, it is not necessary to correct the concentration for recovery. When the recovery is less than 90% or more than 110%, the concentration of the analyte is corrected for recovery.

9 References

[1] COMMISSION RECOMMENDATION (EU) 2022/561 of 6 April 2022 on monitoring the presence of glycoalkaloids in potatoes and potato-derived products. O. J. EU, L 108/66-67. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022H0561

[2] DG_SANTE, Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed SANTE/11312/2021. https://ec.europa.eu/food/system/files/2022-02/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf

Annex A.1 Checklist for sample preparation of unprocessed potatoes

Technician:	
Date:	
Lab. journal / page:	

A.1.1. Sample pre-treatment (6.1)

Blank material (6.1.1)	☐	Cut 200-300 g of blank material (sweet potato) into parts or wedges. Transfer 200 ± 5 g to a blender and add 100 ml of stabilisation solvent. Homogenise in the blender and store aliquots of 6 g slurry in test tubes at -20°C for future use or continue with the sample preparation procedure (6.3)
Potatoes (6.1.2)	☐	Select a number of potatoes (approximately 500 g) that are representative of the sample and cut these into parts or wedges. Transfer 200 ± 5 g to a blender and add 100 ml of stabilisation solvent. Homogenise in the blender and continue with the sample preparation procedure (6.3)

A.1.2. Sample preparation and extraction (6.3)

MMS (6.3.1)	QC LOQ 1/0.2 mg/kg (6.3.2.1)	QC Rec 10/2 mg/kg (6.3.2.2)	Samples (6.3.3)	
☐	☐	☐		Weigh 3 portions of 6.0 ± 0.1 g of sweet potato slurry in 50-ml PP tubes
			☐	Weigh 2 portions of 6.0 ± 0.1 g of sample slurry in 50-ml PP tubes
	☐			Spike with 400 µl of mix 10/2 µg/ml
		☐		Spike with 4000 µl of mix 10/2 µg/ml
☐	☐		☐	Add 35 ml of extraction solvent and shake vigorously
		☐		Add 31 ml of extraction solvent and shake vigorously
☐	☐	☐	☐	Extract for 30 minutes on a shaking machine
☐	☐	☐	☐	Centrifuge the sample for 15 min at 3000 g
☐				Transfer 10 µl of supernatant to filter vials. Spike and add extraction solvent according to Table A.1.3
	☐	☐	☐	Transfer 10 µl of supernatant to filter vials and add 490 µl extraction solvent. In case of samples containing GAs that exceed the calibration range: dilute 5x according to A.1.4
☐	☐	☐	☐	Mix and close the vials with the help of a compressor

Table A.1.3. Spike procedure MMS unprocessed potato (P) (6.3.1)

	Name	Conc. extract (ng/ml)	Conc. sample (mg/kg)	Mix 100/20 ng/ml (4.4.4) (µl)	Mix 1000/200 ng/ml (4.4.3) (µl)	Mix 10/2 µg/ml (4.4.2) (µl)	Blank extract (6.3.1) (µl)	Extraction solvent (4.3.1) (µl)
☐	P1	0/0	0/0	-	-	-	10	490
☐	P2	2.5/0.5	1.25/0.25	12.5	-	-	10	487.5
☐	P3	5/1	2.5/0.5	25	-	-	10	475
☐	P4	10/2	5/1	50	-	-	10	450
☐	P5	25/5	12.5/2.5	-	12.5	-	10	487.5
☐	P6	50/10	25/5	-	25	-	10	475
☐	P7	100/20	50/10	-	50	-	10	450
☐	P8	250/50	125/25	-	-	12.5	10	487.5

A.1.4. Sample extract dilutions (6.3.3)

☐	Transfer 200 µl of potato supernatant to a 10-ml PP tube
☐	Add 800 µl of blank sweet potato supernatant and mix
☐	Transfer 10 µl of the mixed supernatant to a filter vial
☐	Add 490 µl extraction solvent

Annex A.2 Checklist for sample preparation of processed potatoes

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A.2.1. Sample pre-treatment (6.1)

Blank material (6.1.1)	☐	Peel 300 g of sweet potatoes, cut these into parts or wedges and boil 250 g of material with 500 ml of water for 25 minutes. Transfer 200 ± 5 g to a blender and add 100 ml of stabilisation solvent. Homogenise in the blender and store aliquots of 6 g slurry in test tubes at -20°C for future use or continue with the sample preparation procedure (6.4).
Potatoes (6.1.3)	☐	Select a number of potatoes (approximately 500 g) that are representative of the sample, peel and cut these into parts or wedges. Boil 500 ml of water and cook 250 g of the wedges for 20 minutes (floury varieties) or 25 minutes (waxy varieties). Transfer 200 ± 5 g of boiled potatoes to a blender and add 100 ml of stabilisation solvent. Homogenise in the blender and continue with the sample preparation procedure (6.4).

A.2.2. Sample preparation and extraction (6.4)

MMS (6.4.1)	QC LOQ 0.5/0.1 mg/kg (6.4.2.1)	QC Rec 10/2 mg/kg (6.4.2.2)	Samples (6.4.3)	
☐	☐	☐		Weigh 3 portions of 6.0 ± 0.1 g of boiled sweet potato slurry in 50-ml PP tubes
			☐	Weigh 2 portions of 6.0 ± 0.1 g of sample slurry in 50-ml PP tubes
	☐			Spike with 200 µl of mix 10/2 µg/ml
		☐		Spike with 4000 µl of mix 10/2 µg/ml
☐	☐		☐	Add 35 ml of extraction solvent and shake vigorously
		☐		Add 31 ml of extraction solvent and shake vigorously
☐	☐	☐	☐	Extract for 30 minutes on a shaking machine
☐	☐	☐	☐	Centrifuge the sample for 15 min at 3000 g
☐				Transfer 25 µl of supernatant to filter vials. Spike and add extraction solvent according to Table A.2.3
	☐	☐	☐	Transfer 25 µl of supernatant to filter vials, add 475 µl extraction solvent. In case of samples containing GAs that exceed the calibration range: dilute 5x according to A.2.4
☐	☐	☐	☐	Mix and close the vials with the help of a compressor

A.2.3. Spike procedure MMS processed potato (PP) (6.4.1)

	Name	Conc. in extract	Conc. in sample	Mixed standard 100/20 ng/ml (4.4.4)	Mixed standard 1000/200 ng/ml (4.4.3)	Mixed standard 10/2 µg/ml (4.4.2)	Blank extract (7.4.6)	Extraction solvent (5.3.1)
		(ng/ml)	(mg/kg)	(µl)	(µl)	(µl)	(µl)	(µl)
◻	PP1	0/0	0/0	-	-	-	25	475
◻	PP2	2.5/0.5	0.5/0.1	12.5	-	-	25	462.5
◻	PP3	5/1	1/0.2	25	-	-	25	450
◻	PP4	10/2	2/0.4	50	-	-	25	425
◻	PP5	25/5	5/1	-	12.5	-	25	462.5
◻	PP6	50/10	10/2	-	25	-	25	450
◻	PP7	100/20	20/4	-	50	-	25	425
◻	PP8	250/50	50/10	-	-	12.5	25	462.5

A.2.4. Sample extract dilutions (6.4.3)

☐	Transfer 200 µl of potato supernatant to a 10-ml PP tube
☐	Add 800 µl of blank cooked sweet potato supernatant and mix
☐	Transfer 25 µl of the mixed supernatant to a filter vial
☐	Add 475 µl extraction solvent

Annex B Example of LC-MS/MS conditions

B.1 LC conditions

The equipment and measuring conditions shown here are provided as an example. Other analytical equipment, columns, mobile phases and gradient conditions may work equally well.

Example conditions for the UPLC system

UPLC system:	Xevo TQ-S or TQ-XS
Analytical column:	Waters BEH C18 1.7 µm, 100 x 2.1 mm
Column temperature:	50°C
Mobile phase solvent A:	0.1% formic acid in water
Mobile phase solvent B:	Methanol
Flow rate:	0.4 ml/min
Injection volume:	2-5 µl
Vial tray temperature	10°C
Gradient program:	Table B.1

Table B.1 Gradient for the UPLC system

Time (min)	Mobile phase A (4.3.3) %	Mobile phase B (4.3.4) %
0.0	65	35
10	40	60
10.1	65	35
12.0	65	35

See Annex B.3 for an example LC-MS/MS chromatogram.

B.2 MS conditions

The conditions given in Table B.2.1 are guidelines; in practice adjusted settings may be required to obtain an optimal performance of the LC-MS/MS system.

Example for MS conditions Xevo TQ-S or TQ-XS

Ionisation mode	ESI positive
Capillary voltage	2.50 kV
Cone voltage	30.0 V
Source temperature	150°C
Desolvation temperature	600°C
Cone gas flow	150 L/hr
Desolvation gas flow	1000 L/hr
CID gas, pressure	Argon; 4,3 10-3 mbar
Solvent discard	0-1.5 and 10-12 min

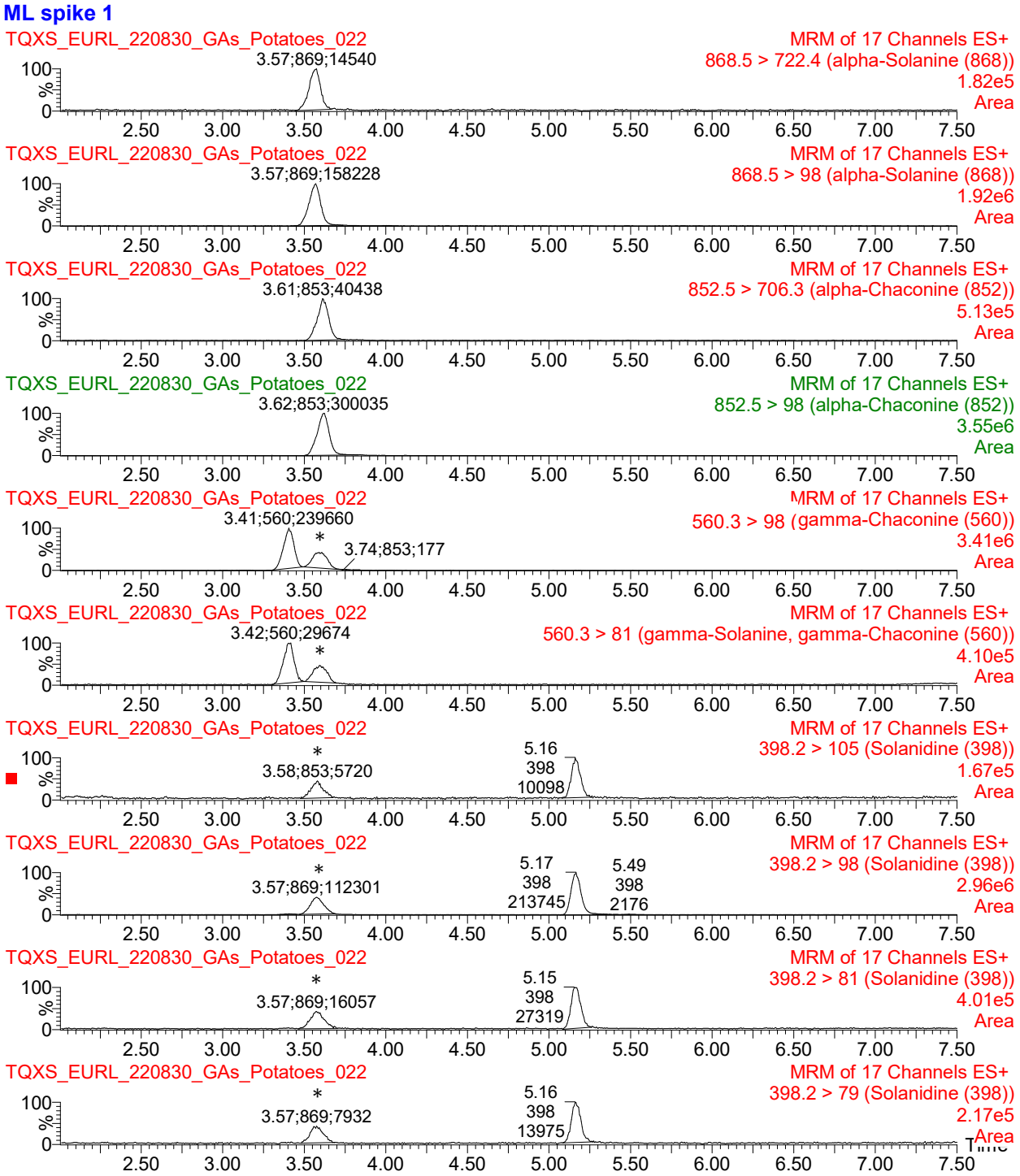
The precursor ions fragment to structurally related products ions. In Table B.2 the theoretical masses of the precursor ion and corresponding product ions are shown. Depending on the instrument, a deviation of ± 0.3 Da is allowed. All transitions shown in Table B.2 are included in the MS method installed on the LC-MS/MS. The retention times can differ from column to column and between UPLC systems. The retention times shown in Table B.2 are therefore indicative.

Table B.2 Example for MS conditions

Component	Indicative RT (min)	Precursor ion (m/z)	Cone voltage (V)	Product ion 1 (m/z)	Collision energy 1 (eV)	Product ion 2 (m/z)	Collision energy 2 (eV)	Product ion 3 (m/z)	Collision energy 3 (eV)	Product ion 4 (m/z)	Collision energy 4 (eV)
Solanidine	5.15	398.2	20	98.0	40	81.0	50	79.0	60	105.0	50
γ-Chaconine	3.40	560.3	20	98.0	50	81.0	60				
β2-Chaconine*	3.30	706.4	20	98.0	70	560.3	50	81.0	70		
α-Chaconine	3.60	852.5	20	98.0	70	706.3	60				
α-Solanine	3.55	868.5	20	98.0	70	722.4	70				

*: No analytical standard available, included for informative purposes

B.3 LC-MS/MS example chromatogram of cooked potatoes fortified at 2.5/0.5 mg/kg



***: in source fragment ions of α-solanine and/or α-chaconine**