FOOD COMPOSITION AND ADDITIVES

Liquid Chromatographic Determination of the Glycoalkaloids α-Solanine and α-Chaconine in Potato Tubers: NMKL [1] Interlaboratory Study

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Twelve laboratories participated in a collaborative study to evaluate precision parameters of a liquid chromatographic method for analysis of the glycoalkaloids α-solanine and α-chaconine in potato tubers. Samples consisted of frozen potato tuber homogenates distributed as 3 blind duplicates and 3 split-level pairs. The analytical method included aqueous extraction, workup on disposable solid-phase extraction cartridges, and reversed-phase chromatography with photometric detection at 202 nm. Results for α-solanine and α-chaconine were received from 10 and 9 laboratories, respectively. Relative standard deviations for reproducibility for α-solanine and α-chaconine were similar, ranging from 8 to 13% in the applied concentration range of 12 to 260 mg/kg fresh weight.

The glycoalkaloids α-solanine and α-chaconine are potentially toxic and bitter-tasting naturally occurring constituents of the common potato, *Solanum tuberosum*. The difference between normal and toxic levels of glycoalkaloids in potato tubers is small, and many poisonings have been reported (1) in the past century. Glycoalkaloid concentration in the tuber is a genetic characteristic of the potato variety but is affected by environmental factors during growth, harvest, and storage. Consequently, glycoalkaloid levels in both new and old varieties must be monitored.

Despite the vast number of analytical methods for glycoalkaloids, methods that combine high sample throughput, laboratory safety, and measurement reliability (2, 3) are lacking. Progress has been made through liquid chromatographic (LC) methods. By combining LC with solid-phase extraction (SPE) for cleanup, many drawbacks of older methods have been eliminated. Since the mid-1980s, several procedures using SPE and LC have been reported (4–6).

The present method gave good results in intralaboratory validation (7). An early version compared well with methods based on other techniques (8). The present method has been used in this and other laboratories for several years for analysis of thousands of potato samples for research and regulatory purposes. The method was selected by the Nordic Committee on Food Analysis (NMKL) to be evaluated through a collaborative trial. This paper presents the results of that trial.

Interlaboratory Study

Study Design and Evaluation

The purpose of the study was to estimate the analytical method's precision parameters—reproducibility and repeatability. The study was designed and evaluated according to NMKL guidelines, based on recommendations of the IUPAC Committee on Interlaboratory Studies (9). Grubbs and Cochran tests were used to identify outliers.

Twelve food analysis laboratories in Nordic countries and Switzerland participated. Each laboratory received 12 randomly labeled test samples, including 3 blind duplicates and 3 split-level pairs, each to be analyzed in a single determination. Two training materials also were submitted, along with corresponding chromatograms and information on glycoalkaloid concentrations as obtained with the present method. Each sample consisted of ca 15 g frozen homogenate of raw potato tubers. Samples were shipped on dry ice, and participants were instructed to store them at −18°C, or below, until analysis.

Preparation of Test Samples

Test materials had a natural glycoalkaloid content. The material with the highest content was obtained from cut-injured tubers left in daylight for a few days; the rest were from normal healthy tubers. The 6 materials were from different household potato varieties. Split-level pairs were obtained by sorting tubers from the same lot in subsamples according to tuber size or by mixing different homogenates. The frozen homogenates

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were prepared as described below. Samples were collected in 35 mL polypropylene containers and stored at −20°C until distributed to participants.

Three of the materials (P, O, and R) were investigated for homogeneity. For each of these, 8 test samples were split into 2 subsamples that were extracted and analyzed. Sampling standard deviations (s_s; 10), reflecting sample inhomogeneity, were <1.6% of the average glycoalkaloid content for the 3 materials. Only the highest value, obtained for material R, was statistically significant ($p = 0.046$). The corresponding inhomogeneity value (i.e., the sampling standard deviation divided by the target value for the standard variation of the analytical method, in this case 10% relative standard deviation) was 0.16, which is less than the highest recommended value, 0.3.

METHOD

A. Principle

Glycoalkaloids are extracted from fresh tuber tissue with dilute acetic acid. The extract is concentrated and cleaned up on a disposable SPE cartridge. Separation and quantitation of α-solanine and α-chaconine are done by reversed-phase LC with UV detection at 202 nm.

B. Apparatus

(**a**) *Homogenisers.*—(*1*) *Ultra-Turrax T45.*—With shaft 45-N (Teflon bearing), generator TP45/26, and speed controller for disintegrating potato tissue in liquid nitrogen. A laboratory-made sliding Plexiglass lid was mounted on the shaft to protect the operator from splashes. (*2*) *Ultra-Turrax TP 18-10.*—With shaft 18-N and speed controller (Janke & Kunkel IKA-werk, Staufen i. Breisgau, Germany) for sample extraction.

(**b**) *SPE columns.*—Sep-Pak C_{18} solid-phase disposable extraction cartridges with 360 mg packing material (Waters Associates, Milford, MA).

(**c**) *SPE manifold.*—Vacuum manifold for multiple SPEs.

(**d**) *LC system.*—High-pressure pump for isocratic use, loop injection valve, column thermostat, variable-wavelength detector, electronic integrator, and recorder. Stainless steel column, 250 × 4.6 mm id, packed with Hypersil ODS (Shandon Southern Products Ltd, Astmoor, UK), 5 μm particle size, C_{18} phase, or equivalent. Operating conditions: flow rate, 1.5 mL/min; injection volume, 20 μL; column temperature, 40°C; detector set at 202 nm.

C. Reagents

Reagents should be of recognized analytical grade unless otherwise stated. Water should be ASTM type I, as obtained for example with the Milli-Q system from Millipore (Bedford, MA).

(**a**) *Acetonitrile.*—LC grade with ≥80% transmission at 200 nm; for example, "far UV grade" from Fisons Scientific Equipment, Loughborough, UK.

(**b**) *Extraction solution.*—Water–acetic acid–sodium hydrogen sulfite, 100 + 5 + 0.5. Mix 1.0 L water with 50 mL glacial acetic acid and dissolve 5.0 g sodium hydrogen sulfite ($NaHSO_3$).

(**c**) *SPE wash solution.*—15% acetonitrile. Mix 150 mL acetonitrile, *C*(**a**), with 850 mL water.

(**d**) *LC mobile phase.*—60% acetonitrile in 0.01M phosphate buffer. Mix 100 mL 0.1M potassium phosphate buffer, pH 7.60 ± 0.01, with 300 mL water and add 600 mL acetonitrile, *C*(**a**). Degas.

(**e**) *LC flush solution.*—60% acetonitrile. Mix 600 mL acetonitrile, *C*(**a**), and 400 mL water. Degas.

(**f**) *Standard solutions.*—(*1*) *Stock solution.*—Weigh to the nearest 0.05 mg ca 25 mg α-solanine and α-chaconine (e.g., from Sigma Chemical Company, St. Louis, MO), respectively. Dissolve in 0.1M potassium dihydrogen phosphate and dilute with the same to 100 mL. Solutions can be stored at 4°C for at least 3 months. (*2*) *LC injection solutions.*—Dilute aliquots from stock solution, *C*(**f**)(*1*), with 0.1M potassium dihydrogen phosphate to give 5.0, 10.0, 25.0, 50.0, 100, and 150 μg/mL, respectively. Solutions can be stored at 4°C for at least 3 months.

D. Sample Preparation

Shred 10–20 potato tubers with a food processor. Mix well and transfer a subsample of ca 200 g to a 2 L stainless steel beaker with liquid nitrogen. Add potato shreds in smaller portions and stir to prevent them from sticking together. While keeping the potato immersed in liquid nitrogen, disintegrate into fine particles with an Ultra-Turrax homogenizer *B*(**a**)(*1*). Immediately transfer homogenate to plastic containers and place in a cooler to allow nitrogen to evaporate. Before the potato tissue starts to thaw, cap the containers airtight and store at −18°C or below. (*Warning*: Overpressure can arise if nitrogen has not been allowed to evaporate before containers are capped.) Samples can be stored for at least 6 months before further processing.

Caution: Handle liquid nitrogen carefully. At its extremely low temperature (−196°C), it can cause frost injury on the skin similar to a burn. Leather gloves and safety goggles with side shield or face shield should be worn. Boiling and splashing always occur when a warm container is filled or when objects are inserted into the liquid. Always perform these operations slowly to minimize boiling and splashing. Store and use liquid nitrogen only in a well-ventilated place; owing to evaporation of nitrogen gas and condensation of oxygen gas, the percentage of oxygen in a confined space can become dangerously low.

E. Extraction

Remove and discard the top layer of the frozen potato homogenate (because it may contain condensed water). Weigh to the nearest 0.01 g ca 10 g frozen sample homogenate and immediately add 40 ± 0.1 mL extraction solution, *C*(**b**). Mix carefully (control speed to avoid foaming) with homogenizer *B*(**a**)(*2*) for ca 2 min. Clarify by centrifugation at ≥4000 × *g* for 30 min. Collect supernatant. The extract can be stored at 4°C for at least 1 week.

F. Cleaning of Extract

Place SPE columns *B*(**b**) on the vacuum manifold *B*(**c**) and condition each column with 5 mL acetonitrile *C*(**a**) followed by 5 mL extraction solution *C*(**b**). Pass 10.0 ± 0.05 mL of the sample extracts through columns. Wash with 4 mL SPE wash solu-

tion *C*(**c**). Elute with ca 4 mL LC mobile phase *C*(**d**) at a rate of 1–2 drops/s and adjust volume to 5.0 ± 0.05 mL with LC mobile phase *C*(**d**). Eluates can be stored at 4°C or below for at least 1 week.

G. Chromatography

Establish LC operating conditions—*B*(**d**), *C*(**d**)—and allow system to equilibrate. Inject standard solutions *C*(**f**)(2) and sample extracts. A representative chromatogram is shown in Figure 1.

After chromatography, rinse pump and column for at least 30 min with LC flush solution *C*(**e**). Before leaving column for longer periods, rinse it additionally with pure acetonitrile.

H. Calculation of Results

Construct calibration curves for α-solanine and α-chaconine by linear regression of peak area (variable *y*) on standard concentration expressed as μg/mL (variable *x*). Calculate amounts of α-solanine and α-chaconine, in sample from equation 1:

$$C_s = \frac{(A_s - \alpha) \times F}{\beta} \quad (1)$$

where C_s = concentration in sample (mg/kg), A_s = sample peak area (area units), α = intercept of calibration curve (area units), β = slope of calibration curve [area units/(μg/mL)], and F = dilution factor (mL/g) calculated from equation 2:

$$F = \frac{[V_1 + (C_w \times W_p)] \times V_3}{V_2 \times W_p} \quad (2)$$

where W_p = amount of potato tissue (normally 10 g), V_1 = volume of extraction solution (normally 40 mL), V_2 = part volume of sample extract applied onto SPE cartridge column (normally 10 mL), V_3 = final volume of dilute eluate from SPE cartridge column (normally 5 mL), and C_w = water content of potatoes (ca 0.8 mL/g if unknown).

Starting with 10.0 g potato tissue and all other conditions as specified, the dilution factor, F, will be 2.4 mL/g.

I. Notes

(a) This procedure was developed for fresh potato tuber tissue. It has also been used, but not collaboratively studied, on dry products after careful rehydration prior to extraction, for example, by soaking with extraction solvent, sometimes in combination with heating, and on boiled tubers. It might not be directly applicable to potato products with a high fat content, such as potato chips.

(b) The collaborative study did not include sample preparation step *D*, because samples were distributed as frozen tuber homogenates. Homogenization in liquid nitrogen produces a fairly homogenous powder of fine particles, from which subsamples easily can be drawn and from which glycoalkaloids easily are extracted. If other sample preparation procedures are preferred for convenience, such as grinding fresh tubers in a food processor or freeze-drying, homogeneity tests should be performed. It must also be checked that glycoalkaloid breakdown does not occur during sample preparation (*see* next paragraph).

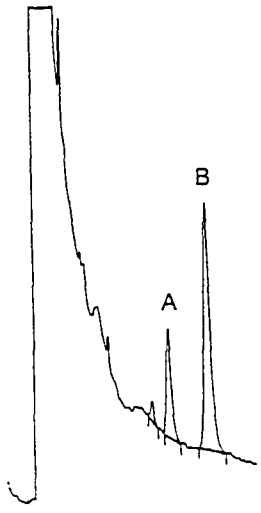

Figure 1. Chromatogram of a potato tuber extract (var. Bintje). Peaks: A = α-solanine (retention time, R_t = 5.9 min) and B = α-chaconine (R_t = 7.3 min), corresponding to tuber tissue concentrations at 22 and 46 mg/kg, respectively. Recorder setting, 0.01 absorbance units at full scale output; column, Shandon ODS (C_{18}) Hypersil 5 μm, 250 × 4.6 mm; column temperature, 40°C; mobile phase, 60% acetonitrile in 0.01M potassium phosphate buffer (pH 7.6); flow rate, 1.5 mL/min.

(c) Mono- and diglycosides, but not the aglycone, of α-solanine and α-chaconine can be detected if the run time is extended to about 20 min. Interfering peaks from matrix might appear with some samples. Appearance of β-chaconines can indicate sample deterioration, because they are formed rapidly in damaged tuber tissue as a result of enzymatic hydrolysis of α-chaconine. Under the present chromatographic conditions, retention times were ca 10, 13, and 18 min, respectively, for β_1-chaconine, β_2-chaconine, and γ-chaconine and 9 and 17 min, respectively for β_2-solanine and γ-solanine. The aglycone solanidine was not eluted.

Results and Discussion

The concentration ranges covered in the study were 12–218 mg/kg for α-solanine and 17–261 mg/kg for α-chaconine. The total glycoalkaloid content in household potatoes is usually 20–150 mg/kg (11), with a typical ratio of α-solanine to α-chaconine of 0.6–0.9 (12). The upper level for safe consumption is 200 mg/kg (13).

Results are presented in Tables 1 and 2. No outliers were detected. Statistical evaluation gave similar precision estimates for α-solanine and α-chaconine. Reproducibility relative standard deviations (RSD_R; reflecting variation for identical samples analyzed by different persons, at different times, in different laboratories) were between 8 and 13%. According to the

IUPAC harmonized protocol (14), these values are acceptable for the concentration range. IUPAC recommends that RSD_R should be within 0.5–2 times the figures calculated by Horwitz from a large number of studies. The ratios between the precision values obtained in the present study for α-solanine and α-chaconine at different concentration levels and the corresponding RSD_R values predicted by Horwitz are given at the bottom of Tables 1 and 2 (HORRAT values).

Repeatability relative standard deviations (RSD_r; reflecting variation for a sample analyzed repeatedly by the same person, within a short period of time, within the same laboratory) were 0.62 and 0.51 times the corresponding RSD_R values for α-solanine and α-chaconine, respectively, both within the ratio range 0.5–0.7 considered normal by Horwitz.

Two of 12 laboratories (1 and 8) failed to produce relevant results for α-solanine and α-chaconine. In the first case, useful calibration curves could not be obtained because of interference from a large negative peak appearing in the standard chromatograms. The problems could be linked to use of an LC column other than that recommended. Another collaborator using the same phase solved the problems by dissolving calibration standards in mobile phase instead of phosphate buffer. In the second case, results were reported but the collaborator commented on the bad appearance of the chromatograms. Large broad peaks appeared at random in standard and sample chromatograms, severely impairing peak area measurements.

In one other case (laboratory 13), the collaborator using another column different from that recommended reported that α-chaconine could not be separated from another peak of unknown origin in sample chromatograms. Because the concentrations of α-chaconine reported by this laboratory were consistently high compared with those of other collaborators, only results for α-solanine were accepted from this collaborator.

Chromatograms of some potato varieties contain matrix peaks with retention times close to those of the glycoalkaloids (Figure 1). Different stationary phases retain glycoalkaloids differently (15), and even with the same column, differences in retention may be due to differences in the efficiencies of column thermostats. It is therefore advisable to check for interfering peaks when the method is being set up by analyzing samples with a negligible glycoalkaloid content, such as from the inner tissues of large tubers. Additionally, glycoalkaloid-containing extracts from potatoes of different varieties can be chromatographed under different conditions. If the peak shapes are maintained, i.e., no double peaks appear when the column temperature or mobile phase pH is gradually changed, this indicates that the glycoalkaloid peaks are pure. Temperature has a pronounced effect on the selectivity between glycoalkaloid and matrix peaks. Raising the temperature increases glycoalkaloid retention while decreasing retention for matrix peaks. High pH has a similar effect on glycoalkaloid retention, but neutral and most acidic compounds are not affected.

Table 1. Raw data and summary statistics for α-solanine (mg/kg fresh tuber tissue)

	Duplicates 1		Split level 1		Duplicates 2		Split level 2		Split level 3		Duplicates 3	
Laboratory	F1	F2	K	P	I1	I2	O	E	G	H	R1	R2
2	13.4	11.8	9.5	16.8	31.0	29.5	30.9	39.2	99.2	108.7	205.9	221.6
3	12.0	12.6	15.4	18.6	40.8	37.8	39.7	41.5	109.6	126.7	251.8	255.2
4	11.1	11.4	14.9	16.3	33.2	34.9	37.0	40.5	96.0	115.2	225.4	225.3
5	12.6	9.2	12.2	16.5	27.4	22.1	26.8	35.6	77.4	106.1	195.1	157.5
6	13.8	12.6	—[a]	—[a]	28.3	28.8	31.8	34.3	91.0	111.0	223.8	216.2
7	10.6	10.2	13.0	16.1	32.8	32.4	33.9	37.8	94.3	112.1	222.7	219.2
10	10.2	14.6	16.2	18.2	32.3	28.3	35.6	36.5	90.6	109.1	205.2	216.6
11	11.0	12.3	12.9	16.6	31.6	33.1	33.0	42.2	91.5	109.3	194.5	215.6
12	10.4	9.6	13.6	14.9	34.8	34.0	31.1	35.3	82.6	107.2	210.9	227.9
13	10.8	9.4	15.6	20.3	36.0	32.2	35.5	39.4	101.9	122.8	235.4	236.6
Mean	11.6	11.4	13.7	17.2	32.8	31.3	33.5	38.2	93.4	112.8	217.1	219.2
Grand mean	11.5		15.4		32.1		35.9		103.1		218.1	
s_r[b]	1.4		1.3		1.9		2.1		3.5		11.4	
s_R[b]	1.5		1.9		4.2		3.2		8.1		21.6	
RSD_r, %[b]	12.3		8.5		6.1		5.9		3.4		5.2	
RSD_R, %[b]	13.1		12.1		13.0		9.0		7.9		9.9	
r[b]	4.0		3.7		5.5		5.9		9.9		32.0	
R[b]	4.2		5.2		11.6		9.1		22.8		60.6	
HORRAT value[b]	1.2		1.1		1.4		1.0		1.0		1.4	

[a] One of the samples in the pair was not analyzed.

[b] s_r and s_R are repeatability and reproducibility standard deviations, respectively. RSD_r and RSD_R are repeatability and reproducibility relative standard deviations, respectively. r and R are repeatability ($s_r \times 2.8$) and reproducibility ($s_R \times 2.8$) values, respectively. HORRAT values are ratios between the RSD_R obtained for α-solanine and RSD_R predicted by the Horwitz equation.

Table 2. Raw data and summary statistics for α-chaconine (mg/kg fresh tuber tissue)

	Duplicates 1		Split level 1		Duplicates 2		Split level 2		Split level 3		Duplicates 3	
Laboratory	F1	F2	K	P	I1	I2	O	E	G	H	R1	R2
2	16.8	16.7	32.9	38.8	46.5	48.1	58.9	67.0	165.9	177.9	257.2	271.6
3	18.3	19.2	41.1	44.5	55.9	54.9	76.0	76.1	179.6	194.2	305.1	307.5
4	17.3	16.8	37.4	40.0	50.7	52.6	69.1	73.9	157.1	176.4	277.1	274.4
5	15.9	14.1	27.2	37.1	41.1	30.7	49.7	59.9	122.6	160.3	228.0	184.1
6	19.5	19.7	—[a]	—[a]	47.4	52.3	67.7	72.1	160.6	175.4	274.2	265.9
7	16.7	14.9	34.5	40.4	49.2	48.9	65.1	71.3	152.5	168.8	263.8	263.8
10	14.6	17.2	33.0	38.1	44.7	45.8	66.7	64.3	150.4	162.2	242.1	254.0
11	17.2	17.3	33.5	39.1	48.8	50.6	65.6	77.8	147.3	166.7	232.8	259.9
12	19.3	17.2	38.4	39.5	57.8	52.7	61.9	67.2	135.4	168.9	257.3	276.2
Mean	17.3	17.0	34.8	39.7	49.1	48.5	64.5	70.0	152.4	172.3	259.7	261.9
Grand mean	17.2		37.2		48.8		67.2		162.3		260.8	
s_r[b]	1.0		1.9		3.1		3.3		6.6		13.8	
s_R[b]	1.7		3.4		6.3		6.6		13.8		28.7	
RSD_r, %[b]	6.0		5.1		6.3		4.8		4.1		5.3	
RSD_R, %[b]	9.7		9.1		12.8		9.8		8.5		11.0	
r[b]	2.9		5.3		8.6		9.1		18.5		38.8	
R[b]	4.6		9.5		17.5		18.5		38.7		80.2	
HORRAT value[b]	0.93		0.98		1.4		1.2		1.1		1.6	

[a] One of the samples in the pair was not analyzed.

[b] s_r and s_R are repeatability and reproducibility standard deviations, respectively. RSD_r and RSD_R are repeatability and reproducibility relative standard deviations, respectively. r and R are repeatability ($s_r \times 2.8$) and reproducibility ($s_R \times 2.8$) values, respectively. HORRAT values are ratios between the RSD_R obtained for α-chaconine and RSD_R predicted by the Horwitz equation.

The method prescribed a column temperature of 30°C. Because some collaborators experienced problems with interfering peaks, the temperature was changed to 40°C in the present procedure. To compensate for the resulting increase in glycoalkaloid retention times, the acetonitrile content of the mobile phase was changed from 55 to 60%.

Estimating the trueness (reflecting the average difference between results obtained and the true value) of the method was not part of the present trial. Certified reference materials were not available, and efforts to obtain spiked tissue material of sufficient homogeneity were not successful. Recovery for the workup procedure was estimated in this laboratory to be >90% (7). It is recommended that recovery be checked during setup of the method and during routine quality control, especially if SPE cartridges other than those recommended are used. Spiked extracts must be used, because pure standard solutions can give low recoveries from SepPak SPE cartridges, possibly because of strong absorption of the alkaloid bases to the stationary phase. Absorption might be blocked by other compounds in tuber extracts.

Several collaborators reported problems with excessive foaming during extraction of samples. These problems could be reduced by mixing the whole volume of extraction solution with the sample before extraction. The method has been amended accordingly.

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