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Comparison of rapid liquid chromatography-electrospray ionization-tandem mass spectrometry methods for determination of glycoalkaloids in transgenic field-grown potatoes

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Abstract

Two rapid methods for highly selective detection and quantification of the two major glycoalkaloids in potatoes, α-chaconine and α-solanine, were compared for robustness in high-throughput operations for over 1000 analytical runs using potato tuber samples from field trials. Glycoalkaloids were analyzed using liquid chromatography coupled to tandem mass spectrometry in multiple reaction monitoring mode. An electrospray interface was used in the detection of glycoalkaloids in positive ion mode. Classical reversed phase (RP) and hydrophilic interaction (HILIC) columns were investigated for chromatographic separation, ruggedness, recovery, precision, and accuracy. During the validation procedure both methods proved to be precise and accurate enough in relation to the high degree of endogenous biological variability found for field-grown potato tubers. However, the RP method was found to be more precise, more accurate, and, more importantly, more rugged than the HILIC method for maintaining the analytes' peak shape symmetry in high-throughput operation. When applied to the comparison of six classically bred potato cultivars to six genetically modified (GM) lines engineered to synthesize health beneficial inulins, the glycoalkaloid content in potato peels of all GM lines was found within the range of the six cultivars. We suggest complementing current unbiased metabolomic strategies by validating quantitative analytical methods for important target analytes such as the toxic glycoalkaloids in potato plants.

Keywords: Metabolite profiling; Liquid chromatography; Mass spectrometry; Reversed phase; Hydrophilic interaction; Method validation; High-throughput screening; Substantial equivalence; Fructans

Glycosidic steroidal alkaloids in potatoes are an important concern to potato producers given their known toxicity. It is recommended that newly developed varieties should be routinely screened for these naturally occurring antipathogenic secondary metabolites as part of the prerelease safety assessment [1]. This is not without foundation as at least two cases of adverse symptomatic development have been recorded as a result of the consumption of potatoes of classically bred varieties subsequently found to have accumulated high levels of glycoalkaloids: Lenape [2] and Magnum Bonum [3]. Such cases demonstrate the susceptibility of even classically bred cultivars to the inheritance of undesirable traits and have subsequently meant that potatoes also produced through nonconventional means are justifiably required to meet the same criteria. The concept of substantial equivalence [4] was introduced to provide a framework within which the evaluation of the degree of similarity between a transgenic crop line and a suitable accepted comparator was addressed. As part of this process known toxicants represent a vital component, particularly as glycoalkaloids, which are resistant to typical food processing methods [5]. The total glycoalkaloid content of newly introduced potato varieties is limited by informal guidelines to 200 mg/kg tuber fresh weight [6].

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A range of steroidal alkaloids have been reported in potato tubers [7], although consistently and overwhelmingly the most dominant are α-chaconine and α-solanine, which together are held accountable for up to 95% of the total glycoalkaloid content in tubers [8]. Structurally, they are esters of an aglycone base (solanidine) and a trisaccharide, differing in only one of the three attached monosaccharides (Fig. 1). Except in instances of abnormally high content, glycoalkaloids are not uniformly distributed throughout tubers but rather are localized to areas of increased metabolic activity such as sprouts and flowers and tend to accumulate in the surface 2 mm [9].

Fructans are reserve carbohydrates found in about 15% of all flowering plants and consist of a β2-1-linked fructosyl chain with a terminal glucose residue. They are potentially beneficial to human health, as they have been shown to preferentially promote the growth of bifidobacteria in the colon, which in turn are thought to confer digestive tract pathogen resistance [10]. They also lead to increased fecal short-chain fatty acid content and therefore a decrease in tumor-promoting metabolites such as ammonia [11].The insertion of fructan biosynthetic machinery derived from crops such as the Jeruselem artichoke (*Helianthus tuberosus*), which have high natural fructan content, is therefore of significant interest [12]. According to the model suggested by Edelman and Jefford [13], two enzymes are required for fructan biosynthesis in the presence of sucrose. First, 1-SST (sucrose:sucrose 1-fructosyltransferase) transfers the fructosyl moiety from one sucrose molecule to the other, thereby synthesizing 1-kestose and liberating a free glucose ($G-F_2+G$). Second, 1-FFT (fructan:fructan 1-fructosyltransferase) elongates the 1-kestose fructan chain further ($G-F_n$) by transferring fructosyl moieties between fructans [14], thus generating longer-chain fructans.

GC-MS techniques have been most often used as a tool for metabolic profiling [15], although glycoalkaloids are too large and consequently nonvolatile to permit their detection using this method. Therefore, in the assessment of substantial equivalence a rapid complementary technique is necessary. Previous work on this subject using ultraviolet (UV) detection has necessitated additional sample purification using solid phase extraction [16,17] or extensive sample preparation, including ammonium hydroxide precipitation [18,19]. The work presented here demonstrates the accurate, rapid, high-throughput quantification of the two major glycoalkaloids routinely observed in potato tubers in a large number of samples from conventional cultivars and those genetically modified for inulin production.

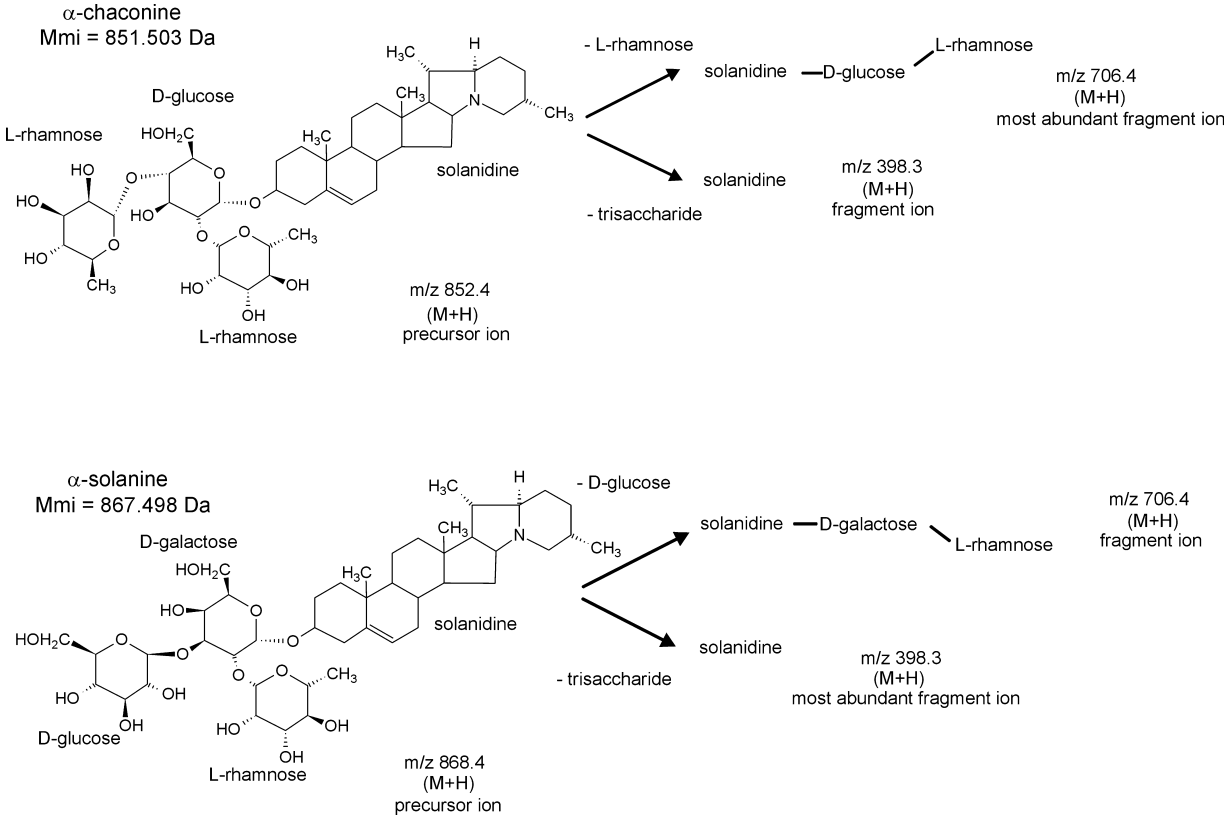

Fig. 1. Structural composition of the glycoalkaloids α-chaconine and α-solanine and their mass spectrometric detection (positively charged precursor ions) including observed fragment ions.

Materials and methods

Chemicals and reagents

HPLC-grade α-solanine and α-chaconine, methanol (LiChrosolv), chloroform (LiChrosolv), acetonitrile (biotech grade), acetic acid (HPLC grade), and formic acid (puriss p.a.) were all purchased from SAF (Taufkirchen, Germany). A Purelab Plus water system (USF Seral, Ransbach-Baumbach, Germany) was used for the further purification of deionized water.

Stock solutions and calibration standards

Glycoalkaloid stock solutions (0.5 mg/ml) were prepared in water:ACN[1] (1/1 v/v) containing 0.01% acetic acid and stored at 4 °C. Standard solutions containing both glycoalkaloids were freshly prepared every second day from stock solutions, despite observed stability over 1 week. Serial dilution was carried out with a mixture of pure water and acetonitrile (1/1 v/v) to the following concentrations: 0.5, 1, 3, and 5 μg/ml.

Plant material

The potato tubers used here were grown as a part of a large-scale field trial undertaken by the German BBA (Biologische Bundesanstalt für Land- und Forstwirtschaft) in 2001 and 2003 in Dahnsdorf, Germany, in which six GM lines altered in carbohydrate metabolism were cultivated alongside six accepted non-GM cultivars in a randomized block design. Three GM groups were single transgenic lines, transformed with a 1-SST gene and the remaining three GM groups were 1-SST transgenic lines supertransformed with a 1-FFT gene. Transgenic plants were generated and supplied by Dr. Arnd Heyer, Max-Planck Institute of Molecular Plant Physiology, Potsdam, Germany. A comprehensive description of the transformation process is given by Hellwege et al. [20,21] in which the polysaccharide complement of the tubers is also described. Following harvest, tubers were stored in the dark at 4 °C for 2 days and then at 10 °C for up to 5 days as samples were prepared.

Sample preparation

Potato tuber samples were prepared using an established high-throughput GC-MS metabolite profiling protocol [22]. A transverse tuber core was removed with an 8-mm-ID cork borer; a 2-mm-thick disk from one end of such a core was immediately frozen in liquid nitrogen for potato peel samples and subsequent 2-mm-thick disks were frozen for potato flesh samples. The weight of these samples varied considerably, so only those within 123.2 ± 34.8 mg (mean ± 1 SD) were included in the analysis. Each sample was simultaneously homogenized and extracted in 2 ml chloroform:methanol:water (2:5:2, v/v/v) at −15 °C using an Ultra-Turrax device. Extraction was completed by shaking for 5 min at 4 °C before centrifugation at 14,000 rpm (Eppendorf centrifuge 5417C) to remove solids. Potato peel samples were diluted 1:10 with a mixture of water and acetonitrile (1:1 v/v) prior to analysis; potato flesh samples were used without dilution.

Instrumentation

The LC-MS system consisted of a Finnigan Surveyor autosampler, a Surveyor MS pump with integrated degasser, and a TSQ Quantum mass spectrometer (ThermoFinnigan, San Jose, CA). This was operated under Xcalibur software (version 1.3, ThermoFinnigan). Nitrogen, generated by an Ecoinert ESP nitrogen generator (DWT, Gelsenkirchen, Germany), was used as sheath and auxiliary gas. Argon 5.0 was used as collision gas (Spectron, Messer, Berlin, Germany). An electrospray interface with a 34-gauge metal needle was used for ionization. HPLC analyses were performed on a 100 × 2-mm Hyperclone ODS (C18) reversed phase (RP) column with a 3-μm particle size (Phenomenex, Aschaffenburg, Germany). Separation at ambient temperature was achieved using a binary gradient system consisting of water (A) and acetonitrile (B), both containing 0.1% formic acid. A 100 × 2.1-mm polyhydroxyethyl A hydrophilic interaction (HILIC) column with 3-μm particle size (PolyLC, Columbia, MD) was used for comparison for which a binary gradient system which consisted of 5 mM ammonium acetate (adjusted to pH 5.5 with acetic acid) (A) and acetonitrile (B) at ambient temperature was also used for separation. A post-column flow splitting of 1:5 was employed. The elution profiles for the investigated columns are given in Table 1. Sample volumes of 3 μl were injected on each column.

Mass spectrometric detection of positively charged ions was performed using the very selective MRM mode, for which the mass spectrometer was set to the following tune parameters: sheath gas pressure of 10 and auxiliary gas pressure of 20 arbitrary units. Spray voltage was set to 3.5 kV and the temperature of the heated transfer capillary was maintained at 270 °C. The collision gas pressure was 1.5 mTorr and the ion gauge pressure was 8e-06 Torr. The resolution for Q1 and Q3 was 0.7 mass units and the scan width for all MRMs was 0.3 mass units. Collision energy of 60 eV was used for all recorded transitions (displayed in Fig. 1). Chromatograms were processed using LCquan (Xcalibur Software, version 1.3; ThermoFinnigan) and statistical analyses were undertaken using Matlab version 6.5 (The MathWorks, MA, USA).

[1] *Abbreviations used:* ACN , acetonitrile; GM, genetically modified; RP, reversed phase; HILIC, hydrophilic interaction; MRM, multiple reaction monitoring; TIC, total ion chromatogram.

Table 1
Gradient used for glycoalkaloid separation on HILIC (A) and RP (B) columns. HILIC: (A) 5.5 mM NH_4Ac (pH 5.5) and (B) ACN. RP: (A) H_2O and (B) ACN, both containing 0.1% formic acid

t in min	% A	% B	Flow in µl/min
(A)			
0	20	80	450
3	35	65	400
8	35	65	300
9	100	0	180
11	100	0	180
12	20	80	200
13	20	80	300
15	20	80	450
(B)			
0	60	40	200
7	45	55	200
7.5	0	100	200
9	0	100	300
12	0	100	300
13	60	40	200
20	60	40	200

Results and discussion

Separation and selective quantification of glycoalkaloids

The elution profiles from both RP and HILIC columns were optimized for run time and chromatographic resolution of the two glycoalkaloids. Typical TIC chromatograms of all recorded transitions of a standard mixture (abs. inj. = 3 ng each) from RP and HILIC columns are shown in Figs. 2A and B. The RP method was applied on samples garnered from potato peels, which are known to have higher concentrations of glycoalkaloids compared to potato tuber flesh. Extracts from tuber flesh samples were analyzed by the HILIC method. Retention times of α-chaconine and α-solanine were 7.3 and 6.8 min using the RP column and 4.4 and 5.4 min on HILIC. However, the HILIC column lifespan was severely reduced as a result of operating conditions, which consequently led to a loss of peak shape after 100 analyses. For this reason, only 288 representative tuber flesh samples were analyzed, compared to 864 potato peel extracts analyzed by the RP method. Despite this difference in total number of samples analyzed, all genotypes and field plots were included in both sets, due to the randomized run sequence detailed below. The glycoalkaloid peaks remained well separated and symmetrical using the RP column for over 1000 analyses.

Selective and sensitive detection of these target analytes was achieved using multiple reaction monitoring with tandem mass spectrometry on a triple quadrupole instrument. Peak detection and integration were performed using the automated Avalon algorithm. MRM settings were chosen according to the observed

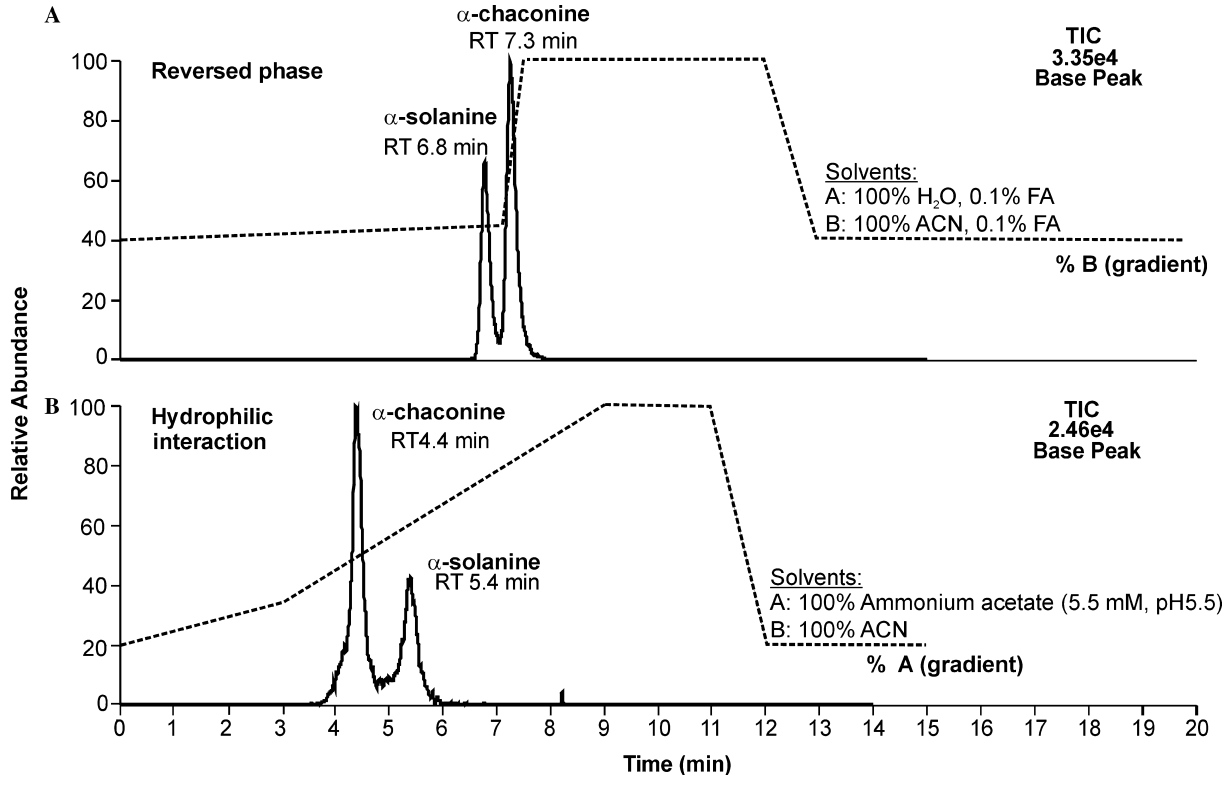

Fig. 2. Reversed phase (RP) vs hydrophilic interaction (HILIC) chromatography of the glycoalkaloids, α-chaconine and α-solanine. Typical TIC chromatograms of all recorded transitions; α-chaconine (*m/z* 852.4 → 706.4 and 852.4 → 398.4) and α-solanine (*m/z* 868.4 → 398.4 and 868.4 → 706.4) for a standard mixture (abs. inj. = 3 ng each) for RP column (A) and HILIC column (B).

fragmentation pattern (Fig. 1). For quantification of α-chaconine and α-solanine, the most abundant transitions were used with *m/z* 852.4 → 706.4 and *m/z* 868.4 → 398.4, respectively, although the transitions *m/z* 852.4 → 398.4 and *m/z* 868.4 → 706.4 were additionally recorded for both compounds to have a further control of compound identity in potato extracts. The ratio of the peak areas of the recorded transitions proved stable for standards and potato samples with 1.8 for α-chaconine (ratio *m/z* 852.4 → 706.4 to 852.4 → 398.4) and 2.5 for α-solanine (ratio *m/z* 868.4 → 398.4 to 868.4 → 706.4). This procedure ensured that LC-MS-MS analyses in MRM mode resulted in a highly selective and sensitive detection of glycoalkaloids in crude potato extracts. A comparison with full-scan detection (*m/z* 300–1000) is illustrated in Fig. 3. The α-chaconine and α-solanine peaks were clearly chromatographically separated from all other major compounds present in the mixture, which limited the risk of unwanted ionization cosuppression by matrix interference. Contamination of the ion source was minimized by diverting all matrix components into the waste by automated valve switching; therefore MRM data were monitored only from 2.5 to 8.5 min of each analytical run.

Method validation

To validate this analytical method for high-throughput analysis of potato samples, the sequence of analytical runs was defined with respect to the order of calibration curve runs, method blank runs, and potato sample runs to eliminate bias based on injection order. This sequence also formed the basis of a quantitative comparison of both LC-MS/MS methods.

All sequences were run in partly randomized blocks. The potato samples were run by blocking the classes of genotypes of a total sample size of N in subsets of n samples with $n = N^{0.5}$. This blocking scheme is a compromise between ultimate statistical rigidity (which would demand to have each sample from each class in each sequence) and practicability in laboratory routines: for each year of field trial, over 2200 potato samples from 12 potato plant genotypes and four field plots had been harvested and stored in 48 different boxes in −80 °C freezers, from which representative samples were taken in randomized fashion. The size of each sequence was 30 potato samples (RP method) and 24 samples (HILIC method). Between each block of potato samples, a series of calibration standards was analyzed, starting with a method blank control sample to check for carry-over effects or cross-contaminations and then increasing concentrations of glycoalkaloids from 0.5 up to 5 μg/ml for the RP method and 0.2 up to 1 μg/ml for the HILIC-MS/MS method. Concentrations of calibration standards were lower in the HILIC-MS/MS method due to the expected lower levels of glycoalkaloids in potato flesh extracts. Daily calibration curves were constructed from merging three of such calibration sequences, i.e., applying the resulting regression equation to 60 samples using weighted least squares regression analysis. The weighting factor 1/x was used due to data heteroscedascity and because of the consistently better results produced. For the example of the RP method, overall 165 calibration standards were analyzed resulting in daily coefficients of

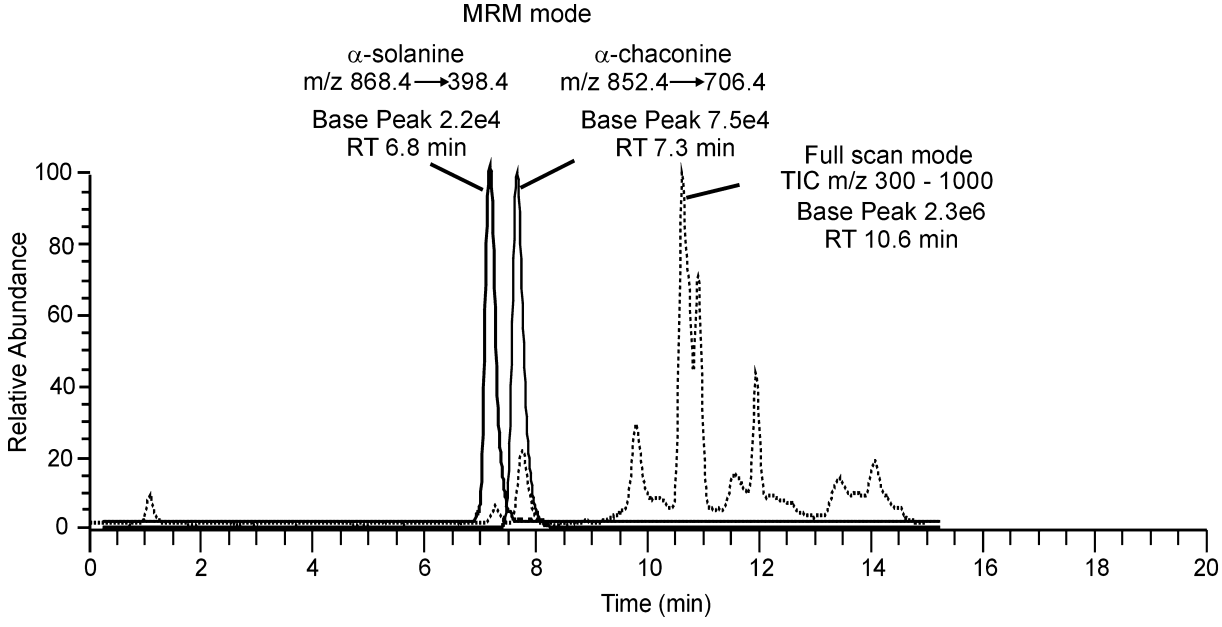

Fig. 3. Mass spectrometric detection of the α-chaconine and α-solanine content of a potato peel extract diluted 1:10 with ACN:water (1:1 v/v), comparing the total ion chromatogram (TIC) in full-scan mode (in the mass range *m/z* 300–1000) and MRM detection.

regression of $r^2 = 0.9985 \pm 0.0004$ for α-chaconine and $r^2 = 0.9986 \pm 0.0003$ for α-solanine. The overall r^2 values obtained from the regression over the entire duration of analysis were $r^2 = 0.989$ for α-chaconine and $r^2 = 0.990$ for α-solanine. This demonstrated that an overall calibration curve would have been sufficient for quantification, which can also be seen in the comparable precision and accuracy results (Table 2).

Randomization of run sequences was done in a slightly different way for the HILIC-MS/MS method. Due to the lower glycoalkaloid levels in potato flesh, it was expected to have less accuracy and precision using the HILIC-MS/MS method. To counteract this, potato samples were blocked in run sequences sizes of only 24 samples per sequence, with subsequent calibration series runs. For each of these 24-sample potato blocks, a calibration curve was generated from the preceding and the subsequent calibration series and applied for quantifying the 24 samples in between. It turned out that this batch-wise quantification was necessary because there was a strong loss of peak shape over time. The peak shape could be recovered by flushing with 100% solvent A (Table 1) or longer equilibration steps. This loss of peak shape may be explained by a compression of the column bed. In total, 288 potato samples and 96 calibration standards were analyzed in HILIC-MS/MS mode.

The glycoalkaloid content determined in the potato extracts was well represented by the chosen range of the calibration curves, which was extended in exemplary calibration series to a broader range of 0.1–10 μg/ml which still resulted in high linear correlation. The lower limit of quantification was below 0.02 μg/ml, far lower than the minimum content measured in tuber samples. Standards at this concentration still showed a signal-to-noise ratio $s/n > 10$. The reproducibility of the analyses was obtained by multiple injection ($n = 5$) of single potato extracts. This experiment was carried out in triplicate on potato tuber peels that had roughly average glycoalkaloid contents. Reproducibility was found to be 91.5, 91.6, and 88.8% for α-chaconine, and 93.7, 93.8, and 90.8% for α-solanine. Replica potato extracts were spiked with α-chaconine and α-solanine to evaluate matrix effects of the established RP-LC-ESI-MS-MS method. Triplicate injection of a potato extract with average glycoalkaloid content (0.998 μg/ml α-solanine and 2.439 μg/ml α-chaconine) was spiked with 1 μg/ml α-chaconine and α-solanine. The detected amounts of glycoalkaloids in the spiked potato sample were found to be 1.936 μg/ml α-solanine and 3.465 μg/ml α-chaconine. Therefore observed recoveries of 93.8% for α-solanine and 101.0% for α-chaconine were calculated. In addition, accuracy and precision need to be determined for comparison and validation of methods. The precision of an established analytical method is indicated by the percentage relative standard deviation (% RSD) at a specific concentration level, and accuracy is expressed as the average percentage error of the calculated concentration to the nominal concentration of each calibration standard. Detailed accuracy and precision data are summarized in Table 2. For the RP method, daily precision and accuracy were calculated from all replicates ($n = 3$) of the four concentration levels run each day. Overall precision and accuracy were calculated from all replicates ($n = 33$) at a specific concentration over the 15 consecutive days of measurement. The daily precision and accuracy were greater than 95%, with an RSD of <8.3% for α-chaconine and <6.8% for α-solanine. The overall precision and accuracy for the determination of both glycoalkaloids had an error of <8.7% with an RSD of <11.5%. In comparison to the RP method, the HILIC-MS/MS method proved to be less precise and accurate. For the level of 1 μg/ml, the data for imprecision and inaccuracy were

Table 2
Accuracy (% average error) and precision (% standard deviation) of calibration standards for both day-by-day calibration curves ($n = 3$) and overall calibration curve ($n = 33$)

External calibrants	α-Solanine		α-Chaconine	
Concentration level (μg/ml)	Accuracy % Average error	Precision % STDEV	Accuracy % Average error	Precision % STDEV
RP method				
Day-by-day ($n = 3$)				
0.5	4.9	8.3	4.3	6.8
1	3.3	4.0	1.3	4.1
3	3.0	3.5	5.6	3.4
5	2.2	2.6	0.5	2.4
Overall ($n = 33$)				
0.5	8.7	11.5	8.6	10.8
1	6.6	8.2	6.1	8.0
3	5.8	7.3	5.7	7.9
5	6.6	5.2	5.6	6.9
HILIC method				
Day-by-day ($n = 3$)				
0.2	8.5	7.9	6.9	7.4
0.5	9.3	12.5	4.7	5.3
1	7.1	10.1	4.8	6.7

roughly doubled for both glycoalkaloids in the HILIC method compared to the RP data. However, given the high biological variability found for field-grown potato tubers (see Fig. 4), this level of imprecision and inaccuracy was clearly acceptable for both the HILIC and the RP method and both methods can therefore be regarded as validated for this purpose. With respect to long time robustness, however, the HILIC method was found to be less suitable than to the RP method due to the hampered peak shapes after prolonged run sequences. Hence, the reversed phase LC-MS/MS method is recommended as suitable for glycoalkaloid determination in potato tubers.

Glycoalkaloid concentrations are substantially equivalent in GM and non-GM potatoes

The α-chaconine and α-solanine content of potato peels from the 12 potato groups is displayed in Fig. 4. This shows the dominance of α-chaconine over α-solanine in the tubers under study and may reflect an evolutionary advantage conferred by the former, which is considered the more toxic [23,24] and therefore confers superior pathogenic resistance. The acceptable limit of total glycoalkaloids is considered to be 200 mg/kg total tuber fresh weight (FW) and the average total level recorded here ranged from 460 to 868 mg/kg peel FW depending upon group (cultivar or line). It should be emphasized that these levels do not represent those found in the whole tuber but rather represent those in the glycoalkaloid-rich peel. For example, it has been shown that when peel contained 300–600 mg/kg FW, whole tuber contained 75 mg/kg FW [6]. The apparent high levels recorded in the present study undoubtedly arise as a result of the region of the tubers sampled, that being from the surface 2 mm, where reportedly between 80 and 95% of total glycoalkaloids are located in commercial cultivars [20]. However, this demonstrates levels comparable to those in previous work where up to 1068 mg/kg peel FW has been recorded [9]. A significant positive correlation between α-chaconine and α-solanine was found ($y = 0.42x + 15.21$, $r^2 = 0.8$, $p = 0$), revealing that an overall ratio of 2.3:1 (α-chaconine: α-solanine) was recorded. A variety of α-chaconine: α-solanine ratios have been reported, a point of importance given the greater toxicity of the former. That found in the present study approximates those reported in eight commercial potato varieties, where 1.4:1–2.2:1 was observed in peel samples [22]. In fact in the current study, ratios of 1.8:1–2.3:1 were recorded in all groups under investigation, except for the cultivar Solara in which a ratio of 1.2:1 was observed (r^2 values ranged from 0.80 to 0.93, $p < 0.001$ in every case).

The six GM lines were assessed with regards to their glycoalkaloid contents solely in comparison to their immediate parent line, cv. Désirée. Analysis of variance (for which data were log transformed) indicated that four GM lines contained increased levels of α-chaconine ($F = 5.9$, $p < 0.01$), the three double transgenics and SST 18, while the α-solanine level was increased in only SST 18 ($F = 4.3$, $p < 0.01$).

However, substantial equivalence is founded upon the basic idea of comparison of a newly introduced crop variety with consumer-accepted cultivars. To this end, the mean α-chaconine and α-solanine contents of the six GM lines were additionally compared to all six non-GM

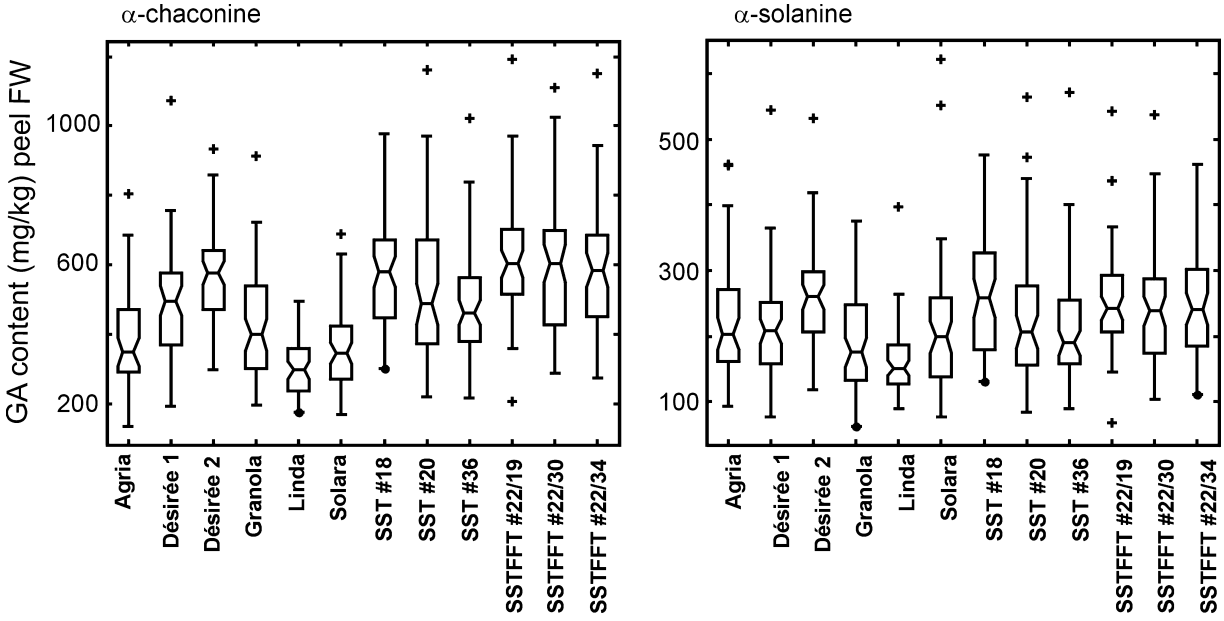

Fig. 4. Box plots illustrating the α-chaconine and α-solanine content of potato tuber peel samples from six classically bred cultivars and six transgenic lines derived from Désirée tubers as determined by RP-LC-MS/MS analysis. Each box demonstrates the group median, the 25th and 75th percentiles and outliers (defined as being greater than 1.5× the interquartile range from the 25th or 75th percentile).

lines through determining the range of each seen in non-GM tubers. This was defined from the mean content of each non-GM line ± 1 SD and then using the maximum and minimum determined values to represent acceptable limits. If the mean content of each glycoalkaloid recorded in GM lines was found to be within this range, the samples were considered equivalent. This comparison indicated the α-chaconine and α-solanine mean levels of the GM lines were within the range found in non-GM tubers that are accepted for consumption and therefore should not be rejected upon these grounds alone. A previous investigation has shown transgenic potato tubers to contain elevated glycoalkaloid levels compared to the wild type control [25], although this was not wholly surprising given that the transgenic plants had been designed to confer improved protection against a viral pathogen, the main role of glycoalkaloids in potato tubers. Although this increase did not elevate the levels above the 200-mg/kg-FW limit using the estimation of peel/total tuber glycoalkaloid ratios [6], the case illustrates that newly introduced crop varieties should be evaluated on a meritocratic basis, independent of the technique used to generate the new variety, be it classical breeding or targeted genetic modification. This also demonstrates that choice of comparator is of vital importance [26], and while this should undoubtedly include the direct parent line from which transgenics were generated, there is a case for assessing substantial equivalence with respect to a range of accepted cultivars.

Conclusions

The work presented here demonstrates the validity [27] of rapid LC-ESI-MS/MS analyses of α-chaconine and α-solanine, the dominant glycoalkaloids naturally present in potato tubers. The technique presented was shown to be applicable to crude tuber extracts prepared using standard extractions used for metabolomic purposes, without further cleanup or sample preparation. In this respect, both the HILIC and the RP LC-MS/MS methods proved to be powerful additions to standard metabolomic approaches in gas chromatography/mass spectrometry. It is noteworthy that the HILIC method disclosed its lack of ruggedness with respect to chromatographic peak shapes only when hundreds of samples were analyzed. Despite this difficulty, quantitative data obtained by either of the two methods are validated to be trustworthy, if randomized run sequences are performed and enough quality control runs between the biological samples are carried out. The reversed phase LC-MS/MS method fulfills all criteria for use of monitoring the levels of naturally occurring toxic glycoalkaloids in potato tubers, for example in the framework of testing substantial equivalence of GM crops to classical cultivars.

Acknowledgments

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