

Simple and Sensitive Liquid Chromatography Electrospray Ionization Mass Spectrometry Method for Determination of Glycoalkaloids in Potato (*Solanum tuberosum* L.)

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Abstract A method was developed using enhanced liquid chromatography coupled with electrospray ionization mass spectrometry for the analysis and quantitation of 2 main potato glycoalkaloids, α -chaconine, and α -solanine, without any pre-concentration or derivatisation steps. Calibration curves generated by this technique exhibited a linear dynamic range from 0.025 to 50 $\mu\text{g/mL}$ and from 0.05 to 50 $\mu\text{g/mL}$ for α -chaconine and α -solanine, respectively. Matrix effects were evaluated by comparing calibration curves measured in matrix-matched and solvent-based systems. Ion suppression due to matrix effects was weak and extraction recoveries of 88 to 114% were obtained in different sample matrices spiked with analyte concentrations ranging from 15 to 35 $\mu\text{g/mL}$. Potatoes that had been genetically modified to tolerate glufosinate contained the same glycoalkaloid levels as their non-transgenic counterpart. We suggest complementing compositional comparison assessment strategy by validating quantitative analytical methods for the toxic glycoalkaloids in potato plants.

Keywords: glycoalkaloid, potato, electrospray ionization mass spectrometry, substantial equivalence

Introduction

The concept of 'substantial equivalence' has been adopted as a starting point for the assessment of genetically modified (GM) crops, which requires that a compositional comparison be made between the GM product and its non-transgenic counterpart, with particular emphasis on naturally occurring toxins (1). Toxic steroidal glycoalkaloids are common in plants of the Solanaceae family, including the potato and tomato, and 95% of the total glycoalkaloid content in potato tubers consists of α -chaconine and α -solanine. Moreover, glycoalkaloid poisoning may cause gastrointestinal disorders, confusion, hallucinations, partial paralysis, convulsions, coma, and death (2). Available information suggests that the susceptibility of humans to glycoalkaloid poisoning is high: oral doses in the range of 1 to 5 mg/kg body weight (BW) are marginally to severely toxic to humans (3) whereas the range of 3 to 6 mg/kg BW can be lethal (4). The generally accepted safe upper limit in potato tubers in the United States is 20 mg of total glycoalkaloid/100 g of tuber (5). Glycoalkaloid concentrations in potato tubers depend on several factors, including physical injury, growing conditions, climate, storage conditions, and time of sampling (6,7). Therefore, a strong need exists for a rapid, generally applicable method to monitor the glycoalkaloid content in potato tubers.

Several methods, such as gas chromatography (GC) (8), high performance liquid chromatography (HPLC) (9), high performance thin layer chromatography (10), capillary

isotachopheresis (11), flow injection liposome immunoassay (12), and enzyme biosensing (13), have been used for the determination of glycoalkaloids in potato. HPLC separation coupled with ultraviolet (UV) absorbance detection is rapid, accurate, and reproducible, and has thus become the most widely applied technique. However, HPLC/UV often requires additional sample purification using solid phase extraction techniques or extensive sample preparation such as ammonium hydroxide precipitation. In addition, HPLC/UV detection suffers from poor selectivity, since glycoalkaloid absorbance is typically measured at 200 nm, where many compounds also absorb light. To overcome these drawbacks, glycoalkaloid quantification has recently been demonstrated using hyphenated techniques such as liquid chromatography with electrospray ionization (ESI) and mass spectrometry (LC-ESI-MS) or tandem LC-ESI-MS/MS (14,15). These methods, however, obviate intensive sample cleanup and long analysis times, and MS is often sensitive to interference, especially through ion suppression or enhancement due to matrix effects (16,17). Ion suppression is the process by which the presence of co-eluting ions prevents the ionization of analyte during ESI nebulisation, which occurs most often when the co-eluting ion is present at a much higher concentration than the analyte.

In this study, we established a simple and sensitive LC-ESI-MS/MS method operating in a selected reaction monitoring (SRM) mode for the quantitation of α -solanine and α -chaconine in potato tubers. Matrix effects were performed using several different concentrations of glycoalkaloids. This method represents a major improvement for glycoalkaloid analyses compared to previously published techniques. The glycoalkaloid content of tubers and leaves obtained from both non-transgenic potato and transgenic potato containing the *bar* gene was evaluated for determination

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of the degree of substantial equivalence.

Materials and Methods

Chemicals α -Solanine and α -chaconine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvent-based glycoalkaloid stock solutions (0.33 mg/mL) and serial dilutions thereof were prepared in water:acetonitrile (1:1 by volume) containing 0.01% acetic acid and kept in the dark at -20°C . Samples were prepared with analyte concentrations from 0.025 to 50 μM .

Calibration standards and spike recoveries The % recovery of analyte was determined in matrices composed of tuber peel, tuber pith, and leaf extracts, and each matrix was spiked with a known amount of glycoalkaloid stock solution to generate a matrix-matched solution. The relative enhancement or suppression of analyte signal due to matrix effects was evaluated by calculating the quotient of the slopes of calibration curves obtained in the solvent-based solution with those obtained in each of the aforementioned matrix-matched solutions.

Instrumentation Separation of glycoalkaloids was conducted on a C12 column (250 \times 2.0 mm, 4 μm , Synergi 4 μ MAX-RP 80A; Phenomenex, Torrance, CA, USA) using a HPLC system consisting of a Finnigan Surveyor separation module and diode array detector (Thermo Finnigan, San Jose, CA, USA). The conditions for HPLC elution were as follows: mobile phase A, 0.1% formic acid in 5% acetonitrile; mobile phase B, 0.1% formic acid in 50% acetonitrile. The following elution program was applied: 0 min, 85% A/15% B; 40 min, 35% A/65% B; 45 min, 35% A/65% B; 50 min, 85% A/15% B; 55 min, 85% A/15% B. The flow was set at 200 $\mu\text{L}/\text{min}$.

For tandem MS analysis, the eluent was diverted into a Finnigan LCQ Deca mass spectrometer equipped with a positive electrospray ionization source, operated by Xcalibur software (version 1.4; Thermo Finnigan). The MS was set to the following parameters: capillary voltage, 32 V; capillary temperature, 274°C ; spray voltage, 4.7 kV. MS spectra were recorded with a scanning range of m/z 110 to 1,000. Nitrogen was generated by an NM30LA nitrogen generator (Peak Scientific, Inchinnan, UK) and used as the sheath and auxiliary gas. Mass spectrometric detection was performed in SRM mode, and peak detection and integration were performed using an automated Avalon algorithm.

Plant material Samples of GM potato tuber line, derived from the variety 'Dejima', and its non-GM counterpart (cv. Dejima) were obtained from the National Institute of Highland Agriculture, Korea. Potato was genetically modified by the insertion of the *bar* gene which was isolated from genomic DNA of *Streptomyces hygroscopicus*. Tubers were planted in 24-L pots in a glass house at the National Institute of Agricultural Biotechnology, Korea. The glass house temperature ranged from 27°C during the day to 17°C at night. After 60 days, the leaves produced at the 12th position on each plant were cut, immediately frozen with liquid nitrogen, and kept frozen at -80°C before extraction.

Sample preparation Samples consisted of the leaves

and harvested tubers of the GM and non-GM potato plants described above. Glycoalkaloid extraction was performed according to the method of Zywicki *et al.* (15) with slight modifications. Harvested tubers were rinsed in tap water and wiped with a clean cloth. Tuber samples were divided into skin (approximately 1-mm thick) and pith (heart of tuber). Each sample (100 \pm 2 mg) was disrupted in liquid nitrogen and overlaid with 10 mL of a chloroform:methanol:water (2:5:2, v/v/v) solution. Leaf samples (100 \pm 2 mg) were suspended in 3 mL of the same solution. After vigorously vortexing for 1 min, the leaf extract was centrifuged at 10,000 \times g and 4°C for 10 min; the supernatant was transferred to a new tube. The samples were extracted once more using the same procedure, and the supernatants were combined. The combined supernatant was filtered through a 0.22- μm Teflon PTFE syringe filter for analysis.

Results and Discussion

Optimisation of the ESI-MS/MS detection of α -solanine and α -chaconine SRM settings were chosen according to the observed MS fragmentation pattern. Although Zywicki *et al.* (15) reported that the most abundant fragmentations of α -solanine and α -chaconine were m/z 868.4 \rightarrow 398.4 and m/z 852.4 \rightarrow 706.4, respectively, the most intense product ion observed for both α -solanine and α -chaconine was m/z 706.4. Peak area ratios, shown in Fig. 1, of m/z 868.4 \rightarrow 706.4 (the loss of glucose) to m/z 868.4 \rightarrow 398.4 (the loss of trisaccharide) and m/z 852.4 \rightarrow 706.4 (the loss of rhamnose) to m/z 852.4 \rightarrow 398.4 (the loss of trisaccharide) were 3.7 and 10.4, respectively. A collision energy of 50% was determined optimal for enhanced sensitivity of the glycoalkaloid assay in crude potato extracts (data not shown). SRM data were monitored from 23 to 37 min elution time. All other matrix components were diverted into a waste receptacle by an automated valve switch, thereby minimising ion source contamination.

Calibration curves, detection limits, and comparison with other methods LC-ESI-MS/MS calibration curves, based on the integrated peak areas of α -solanine and α -chaconine, were linear from 0.05 to 50 $\mu\text{g}/\text{mL}$ and from 0.025 to 50 $\mu\text{g}/\text{mL}$, respectively. The line equations and correlation constants (R^2) for each calibration curve were $y = -0.1845 + 1.6528x$ and $R^2 = 0.9994$ for α -solanine and $y = -0.5074 + 4.7323x$ and $R^2 = 0.9995$ for α -chaconine. Detection limits for α -solanine and α -chaconine were 10 and 5 ng/mL, respectively. Standards at this concentration exhibited a signal-to-noise ratio (S/N) greater than 10. The data in Table 1 show that the linear dynamic range for glycoalkaloid quantitation using the present method was larger than that reported for other methods.

In addition, this approach exhibited excellent reproducibility. The amount of glycoalkaloid extracted from a single potato tuber peel, determined in 7 replicate trials, was 185.3 ± 5.6 and 338.2 ± 9.3 $\mu\text{g}/\text{g}$ for α -solanine and α -chaconine, respectively.

The ratio of α -chaconine to α -solanine is a useful number to quickly assess the relative toxicity of a particular sample. A significant positive correlation between α -chaconine and α -solanine was found ($y = 0.48x + 0.1$, $R^2 = 0.93$, $p < 0.0001$) (Fig. 2). For potato samples evaluated herein, the α -

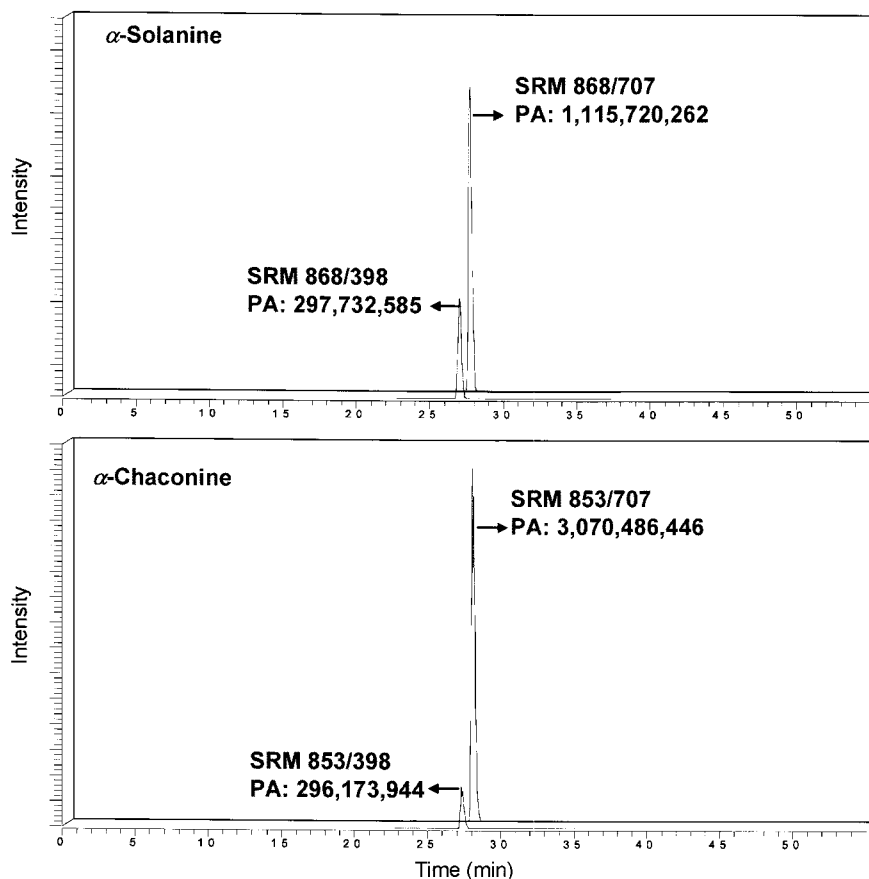


Fig. 1. LC-MS/MS chromatogram of potato extract showing the mass ranges of glycoalkaloids. SRM, selected reaction monitoring (m/z precursor ion)/(m/z product ion); PA, peak area.

Table 1. Comparison of the detection limit and the linear dynamic range for α -solanine using the present methodology with those of previously reported techniques

Method ¹⁾	Detection limit	Linear range	References
GC-FID	3 ng	-	Lawson <i>et al.</i> (8)
HPLC-UV (208 nm)	-	20-1,000 ng	Saito <i>et al.</i> (9)
HP TLC-FL	10 ng	200-2,000 ng	Simonovska and Vovk (10)
Capillary ITP	-	5-25 $\mu\text{g/mL}$	Kvasnicka <i>et al.</i> (11)
Enzyme biosensor	2.0 μM	0.5-100 μM	Korpan <i>et al.</i> (13)
HPLC-CL	24 pg	0.1-200 ng	Kodamatani <i>et al.</i> (5)
Present method	50 pg	0.2-250 ng	

¹⁾FID, flame ionization detector; FL, fluorescence detection; ITP, isotachopheresis; CL, chemiluminescence.

chaconine: α -solanine ratio ranged from 1.70 to 2.28.

Matrix effects Co-eluting matrix components may cause suppression or enhancement of the analyte signal during electrospray ionization. To evaluate matrix effects on the accuracy of the LC-ESI-MS/MS technique, potato extracts were spiked with a known amount of α -solanine and α -chaconine in a concentration range of 10 to 50 $\mu\text{g/mL}$. The line equations for the matrix-matched calibration curves are shown in Table 2, and the R^2 values for the individual calibration curves ranged from 0.9981 to 0.9995. The ratio of the calibration curve slopes obtained in solvent-based and matrix-matched solutions was calculated to determine the influence of matrix components on the signal response (18). A slope ratio of 1 indicates no matrix effects. The %

signal suppression or enhancement was estimated by applying the following equation:

$$[1 - (S_{\text{extr}}/S_{\text{st}})] \times 100 \quad (1)$$

where S_{st} is the slope of the solvent-based standard calibration curve and S_{extr} is the slope of the matrix-matched standard calibration curve. For the samples analysed herein, the observed signal suppression was less than 18%, indicating negligible matrix effects. Extraction recoveries, determined by the ratio between the amount of glycoalkaloid extracted to the amount added into the sample matrix, ranged from 88 to 114%, as shown in Table 3.

Method application The developed method was verified by determining the glycoalkaloid content in non-transgenic

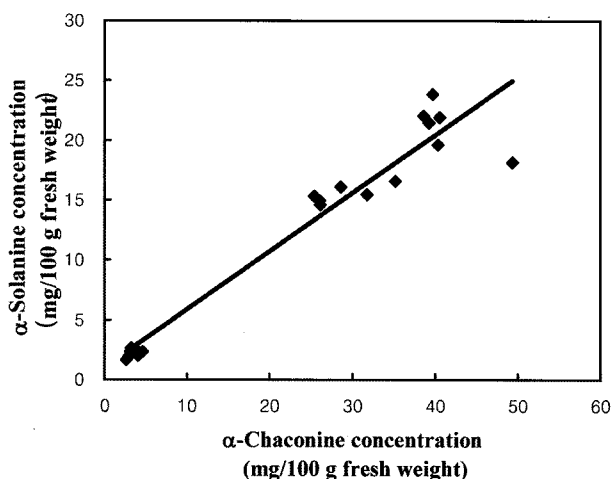


Fig. 2. Correlation between α -chaconine and α -solanine contents in potato tubers and leaves.

and transgenic potato. Sample preparation techniques are of the utmost importance in glycoalkaloid analyses. Although the glycoalkaloid content in potato appears to be largely unaffected by most common food preparation techniques, such as baking, cooking, and frying, it can vary greatly in different potato cultivars and may be enhanced post-harvest by environmental factors such as light, mechanical injury, and storage (19-21). Thus, comparisons should be made at a specific time, sampling the same tuber and leaf from each plant. Tubers and leaves collected for this study were frozen in liquid nitrogen immediately after sampling to quench all biochemical processes.

Substantial equivalence was coined as a means of determining the nutritional quality of newly introduced crop varieties relative to the immediate parent line or consumer-accepted cultivars (22). However, the concept of substantial equivalence applies not only to compositional analysis but also to agronomic characteristics and phenotype. The mean α -chaconine and α -solanine levels in the GM potato were similar to those of the non-GM potato, and none of the potato pith samples exceeded the limit of 20 mg total glycoalkaloid/g potato (Table 4). However, the α -solanine and α -chaconine contents in non-GM potato peels were 20.2 and 38.3 mg/100 g, respectively. Therefore, the peeling of potatoes prior to ingestion usually reduces the

Table 3. Extraction recoveries (%) obtained for spiked glycoalkaloid standards in different potato sample matrices

Glycoalkaloid	Added ¹⁾ ($\mu\text{g/mL}$)	Matrix type		
		Tuber peel	Tuber pith	Leaf
α -Solanine	15	111	95	88
	25	102	93	90
	35	99	92	90
α -Chaconine	15	104	105	114
	25	100	100	102
	35	93	93	96

¹⁾Final concentration of analytes is presented.

Table 4. Glycoalkaloid contents of potato tubers and leaves

Sample	Content ¹⁾ (mg/100 g of fresh weight)	
	α -Solanine	α -Chaconine
Wild type		
Peel	20.0 \pm 1.7	38.3 \pm 1.6
Pith	2.4 \pm 0.2	3.7 \pm 0.2
Leaf	18.4 \pm 2.7	31.3 \pm 4.3
Transgenic		
Peel	18.2 \pm 1.2	40.5 \pm 5.1
Pith	2.0 \pm 0.2	3.4 \pm 0.6
Leaf	17.2 \pm 2.4	30.3 \pm 4.2

¹⁾Values represent mean \pm SD of 3 independent trials.

amount of consumed glycoalkaloids to a safe level. This is especially true for small tubers, which often contain relatively greater quantities of these compounds. When the peels are removed, the glycoalkaloid content can be reduced by 50 to 95% (23).

In conclusion, the LC-ESI-MS/MS method described herein, operated in SRM mode, was shown effective for the quantitation of α -chaconine and α -solanine, the dominant glycoalkaloids naturally present in potato tubers. The technique affords a simplified sample extraction procedure and boasts high sensitivity and selectivity. Moreover, matrix effects on the analytical accuracy were negligible (<18% signal suppression). This study demonstrated that the glycoalkaloid content of the GM potato studied here was substantially equivalent to that of

Table 2. Calibration curve characteristics and percent signal suppression in sample matrices extracted from different potato tissues¹⁾

Analyte	Matrix type	Linear calibration curve equation	R^2	Slope matrix/slope standard ²⁾	Signal suppression (%) ³⁾
α -Solanine	Tuber peel	$y=11.1160+1.4826x$	0.9987	0.90	10.30
	Tuber pith	$y=0.0567+1.4784x$	0.9993	0.89	10.55
	Leaf	$y=2.9015+1.4226x$	0.9987	0.86	13.93
α -Chaconine	Tuber peel	$y=37.0980+4.0597x$	0.9981	0.86	14.21
	Tuber pith	$y=16.5640+4.1285x$	0.9991	0.87	12.76
	Leaf	$y=17.5510+3.8937x$	0.9987	0.82	17.72

¹⁾The line equations of the solvent-based calibration curve for α -solanine and α -chaconine were $y=-0.1845+1.6528x$ and $y=-0.5074+4.7323x$, respectively.

²⁾Matrix effects; the ratio of the matrix-matched calibration curve slope to the solvent-based calibration curve slope.

³⁾Signal enhancement and suppression are indicated by negative and positive signs, respectively.

the parent cultivar.

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