Toxicology International, *31(2) : 219-226; 2024. DOI: 10.18311/ti/2024/v31i2/35679*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi



Quechers Extraction Followed by HPLC Estimation of Steroidal Glycoalkaloids viz, α-Chaconine and α-Solanine in Different Solanaceae Family Vegetable Varieties

Lavanya Nagamalla

Department of Chemistry, Hyderabad Institute of Technology and Management, Hyderabad -502401, Telangana, India; lavanyan.sh@hitam.org

Abstract

Glycoalkaloids are the alkaloid derived chemical constituents naturally produced in various plants species especially Solanaceae family. These chemical constituents protect the plant against predators as well as pathogens. The consumption of high dose of glycoalkaloids was toxic to humans. This study was purposeful to evaluate steroidal glycoalkaloids viz., α-chaconine and α-solanine quantity in different *Solanaceae* family vegetable varieties. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) extraction using acetonitrile as extracting solvent was used for extracting steroidal glycoalkaloids in vegetable samples. In the optimized conditions, the standard chromatogram shows clear resolution of α-chaconine and α-solanine. The analytes were identified at 3.15 min for α-chaconine and 4.88 min for α-solanine. The optimized HPLC (High pressure liquid chromatography) method was validated. The method optimized using acetonitrile and 1.0 mM ammonium acetate buffer at pH 4.2 in 65:45 (v/v) as mobile phase at 0.6 mL/min, µBondapak C18 (10 µm, 4.6x250 mm) column as stationary phase and UV detection is selected at 218 nm. Findings suggest that this method was simple and convenient for the estimation of glycoalkaloids in vegetables. The glycoalkaloids was achieved under the acceptable levels of less than 200 mg/kg as prescribed by USFDA and German Federal Institute for Risk Assessment.

Keywords: Glycoalkaloids, HPLC (High Pressure Liquid Chromatography), *Solanaceae*, α-Chaconine, α-Solanine

1. Introduction

Glycoalkaloids are naturally occurring nitrogen-containing steroidal glycosides found in plants that belong to Solanaceae family such as potatoes, tomatoes etc[1]. These are considered as natural toxins that act as stress metabolites or phytoalexins. These chemical constituents have bitter taste and were protects the plants from insects and fungi[2]. Among the various types of glycoalkaloids present in a plant, α-solanine and α-chaconine are the main compounds and contribute 95 % of total glycoalkaloid content in plants[3].

Glycoalkaloids not only have protective activity but also shows significant benefits to humans at low dose as well as harmful effect at high doses[4]. These compounds show potential anti-cancer activity that shows significant inhibition against various cancer cell lines such as liver (HepG2) cancer cells and human colon (HT29) cells[5,6].

Article Received on: 22.11.2023 Revised on: 15.02.2024 Accepted on: 23.02.2024

At high dose these glycoalkaloids are toxic and cause severe health problems such as diarrhea, vomiting, fever, fast pulse, low blood pressure, gastrointestinal discomfort and gastroenteritis[7]. These are heat stable compounds that were stable and active after cooking of the vegetables[8]. In view of its toxicological effects, the USFDA and German Federal Institute for risk assessment, Germany defined as 200 mg/kg Fresh Weight (FW) or 1000 mg/kg dry weight as consumption dose for total glycoalkaloids in potatoes.

In Andhra Pradesh (India), the consumption of vegetables is very high and consume in both raw and cooked forms. Based on the thermal stability and toxicological effects of the glycoalkaloids, the concentration of glycoalkalods can be regulated and must be under the safety limits before releasing them to the market. In India, very few studies were reported for the evaluation of glycoalkaloids content in various vegetables such as potatoes[9] and Brinjal[10].

In a literature survey, it was observed that analytical methods reported for evaluation of glycoalkaloids in various samples using HPLC[11-15] and LCMS[16-18] UPLC[19] techniques. Based on the literature, it was confirmed that there is no adequate literature reported for the evaluation of glycoalkaloid content in various vegetable marketed in Andhra Pradesh, India. Hence the present work aimed to evaluate the α-solanine and α-chaconine content in various vegetables collected in Andhra Pradesh by a simple, quick, easy, convenient and validated HPLC method. The molecular structure of analytes was shown in Figure 1.

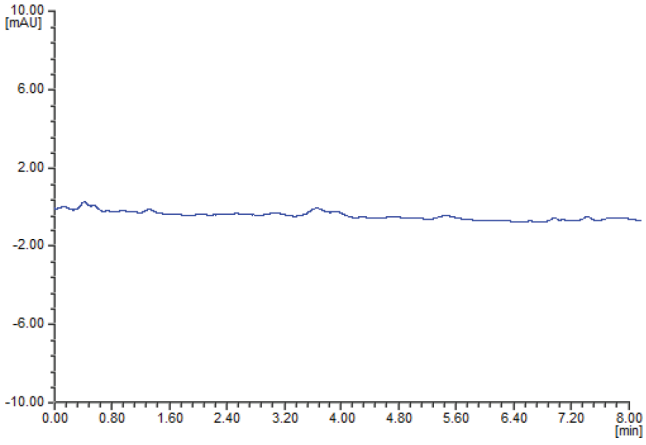


Figure 1. Blank chromatogram observed in the developed method.

2. Materials and Methods

Methodology includes three important steps, QuEChERS extraction, Development of Analytical method and Method Validation

2.1 QUECHERS Extraction

2.1.1 Sample Collection

Twelve numbers of different samples such as potatoes, sprouted potatoes, red sweet potatoes, white sweet potatoes, green tomatoes, red tomatoes, long brinjal, round brinjal, red chillies, green chillies, red and green bell pepper were procured from local retail outlets, Hyderabad. The collected samples were preserved at 5°C and were used when required in the study.

2.1.2 Sample Pre-Treatment and Preparation

The samples collected for the study were grounded thoroughly using an analytical mill and the homogenized samples were preserved in a polyethylene screw container at –20°C. 0.5 g of homogenized crisp samples were weighed separately and was taken in a 50 mL centrifuge tubes having 5 mL water. The content was vortex for 2 min and 25 mL of acetonitrile with 1% formic acid and vortex for 3 min. Then 1 gram of sodium acetate and 2 grams of magnesium sulphate was added and vortexed for 2 min and then kept undisturbed for 5 min to separate the acetonitrile layer. Then the acetonitrile layer was separated, filtered, dried under nitrogen and reconstituted with 1 mL of mobile phase and was used for the study.

2.2 Analytical Method Development

2.2.1 Instrumentation

The HPLC simultaneous analysis of α-chaconine and α-solanine and was performed on Agilent (USA) HPLC system equipped with LC 10ATvp, Agilent 1260 auto-injector with injection capacity of 0.1-100 µL and programmable UV SPD 10 Detector. The chromatographic signals were integrated using Empower 1 software. The standards and samples were weighed through Denver - SI-234 [Bohemia] analytical weighing balance and the solution pH was adjusted using Systronics – Sr. No. S 1326 [India] pH meter. The prepared samples were filtered through 0.2 µMerck Millipore [USA] membrane filter papers.

2.2.2 Chemicals and Reagents

The working standards of α-chaconine (≥98.0%) and α-solanine (phyproof reference substance) were purchased from MilliporeSigma and the samples used in the study were purchased from local market, Vijayawada. The high pure HPLC grade chemicals (Milli-Q® water, methanol and acetonitrile) were procured from Merck chemicals, Mumbai and Millipore make membrane filter papers (0.2 µ) were procured for the study.

2.2.3 Preparation of Ammonium Acetate Buffer at pH 4.2

The precisely weighed 770 grams of ammonium acetate was taken in a 1.0 L flask comprising 500 mL Milli Q water. Shake the flask occasionally till the content dissolves completely in water. Then by utilizing acetic acid (0.1%) solution, the pH was fixed at 4.5. Then by utilizing 0.2 µ membrane filters, the solution filtered and fill the flask mark with MilliQ water. The obtained solution was utilized as buffer for preparing mobile phase and the same was utilized as diluent.

2.2.4 Preparation of Standard Solution

An exactly weighed 25 mg of α-chaconine and α-solanine were taken in a 25 mL flask consisting of 10 mL of methanol separately. It was kept on sonicator 5 min to dissolve the pure compounds completely in the solvent and then by utilizing 0.2 µ filters, the solution was filtered in to a distinct fresh and dry 25 mL flask. Then flask filled till mark with mobile phase as diluent. Then 5 mL of solution was pipetted accurately to 100 mL fresh and dry flask and volume in flask was made till mark with mobile phase as diluent. Then a calibration dilution range of 2.5-250 µg/mL was prepared.

The development of adequate methodology for separation and analysis of α-solanine and α-chaconine was carried using by following systematic steps in a progressive manner. The method parameters like stationary phase nature, liquid phase composition with adequate flow rate as well as wavelength was optimized.

2.3 Method Validation

The developed method for quantification of glycoalkalods was evaluated for its validity in terms of specificity, accuracy, linearity, and detection levels. In the method validation process, guidelines set by ICH[20] along with published literature[21-24] were considered. Further, the developed method was applied for the estimation of α-solanine and α-chaconine in various vegetable samples.

3. Results and Discussion

The study was intended for the establishment of a novel, easy and convenient method for the quantification of α-solanine and α-chaconine in various vegetable samples. The method development was initiated for the identification of suitable wavelengths for simultaneous detection of α-chaconine and α-solanine.

The UV absorption spectrum confirms that α-solanine shows maximum absorbance at 205 nm whereas α-chaconine shows maximum absorbance at 203 nm. The overlay spectrum of these two individual scanning spectra confirms 218 nm as overlay absorption wavelength that shows similar absorbance for both α-chaconine and α-solanine. Hence the detector wavelength at 218 nm was confirmed as suitable wavelength for detection of α-solanine and α-chaconine. Various compositions of mobile phase with various pH ranges, flow rates and various configurations of stationary phases were studied for optimum resolution of α-solanine and α-chaconine. The optimization of mobile phase was performed with the use of various buffers including acetate, phosphate with methanol and acetonitrile as organic modifier. The involvement of phosphate buffers, triethyl amine and tetrahydro furan in mobile phase doesn't produce expected resolution of α-solanine and α-chaconine whereas the use of ammonium acetate buffer produces best resolution of analytes. Finally, acetonitrile and 1.0 mM ammonium acetate buffer at pH 4.2 in 65:35 (v/v) at 0.6 mL/min flow rate, µBondapak C18 (10 µm, 4.6×250 mm) column and UV detection at 218 nm was selected as suitable for resolving α-solanine and α-chaconine.

Standard chromatogram in proposed conditions exhibit clear resolution of α-solanine and α-chaconine was observed with retention time of 4.88 min for α-solanine and 3.15 min for α-chaconine whereas the blank chromatogram doesn't show any detection at the retention of eluents as well as throughout the run time proved that the method specificity. On observing the chromatographic results obtained for the standard solution, it was confirmed that the system suitability results such as number of theoretical plates, tail factor and resolution factor of both the analytes were within the acceptable levels confirms the

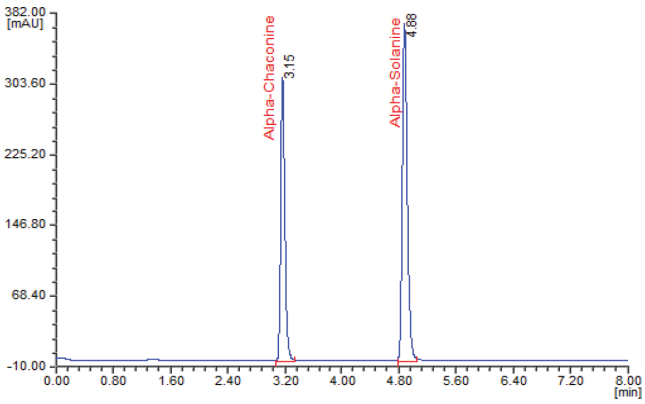


Figure 2. Standard chromatogram observed in the developed method.

method suitability. The blank and standard chromatogram observed in the developed method was shown in Figures 1 and 2 respectively.

The linearity curve was plotted by considering the chromatographic response of individual analyte versus its concentration prepared. The linear calibration curve achieved for studied analyte in the developed method demonstrated that high fit and correlate curve was achieved in the concentration range of 2-200 µg/mL for both and α-chaconine and α-solanine. The regression equation was observed to y = 2822x + 860.12 (R^2 = 0.9995) for α-solanine and y = 2146.8x – 4.5709 (R^2 = 0.9996) for α-chaconine. The beers law curve obtained in the method was observed to be accurately fit with very high correlation coefficient and wide calibration range was observed confirms wide applicability of method. Table 1 represents results and Figure 3 presents calibration curve observed in linearity study.

Repeatability in HPLC refers to the consistency of results obtained by the same analyst using the same

Table 1. Linearity results obtained in the developed method

S. No.	Concentration in µg/mL	Peak area response observed	
		α-chaconine	α-solanine
1	2	6933.7	8142.9
2	10	21268.7	35857.6
3	25	56986.9	69908.1
4	50	100686.9	139584.7
5	100	215189.6	276313.2
6	150	319582.7	421252.3
7	200	432168.9	571251.9

method, equipment, and conditions over a short period. Reproducibility assesses the agreement of results obtained by different analysts when analyzing the same sample under the same conditions. Both are crucial for evaluating the precision and reliability of HPLC methods. The proposed method repeatability and reproducibility was evaluated with standard solution at 50 µg/mL concentration of α-chaconine and α-solanine. The solution was prepared and analyzed in same day (n = 6) for evaluating intraday precision, the same was prepared and analyzed in three consecutive days for inter-day precision and analyzed by three different analysts in ruggedness study. The % RSD was calculated as 0.26, 0.44 and 0.36 for α-chaconine and 0.97, 1.03 and 1.36 for α-solanine in intra-day, inter-day precision and ruggedness study respectively (Table 2). This confirms that the method was precise and reproducible.

Recovery refers to the efficiency with which an analyte is extracted from a sample matrix during sample preparation and subsequently detected by the chromatographic system. It is a measure of the method's ability to accurately quantify the analyte of interest in a complex sample. High recovery

Table 2. Precision and ruggedness results for α-chaconine and α-solanine in proposed method

S. No.	Peak area observed in Intraday Precision		Peak area observed in Interday Precision		Peak area observed in Ruggedness	
	α-chaconine	α-solanine	α-chaconine	α-solanine	α-chaconine	α-solanine
1	100104.4	135523.6	100135.9	135958.7	99815.5	135915.9
2	99900.2	135881.5	99685.9	136598.6	99162.5	136892.3
3	99998.1	136607.0	100415.8	137484.1	99775.1	136912.2
4	99782.0	137393.4	100662.8	138585.2	99797.1	137372.6
5	100069.3	137867.4	99748.2	138748.5	99114.8	139195.1
6	100536.4	139100.2	99582.4	139695.3	99158.2	141033.6
% RSD	**0.26**	**0.97**	**0.44**	**1.03**	**0.36**	**1.36**

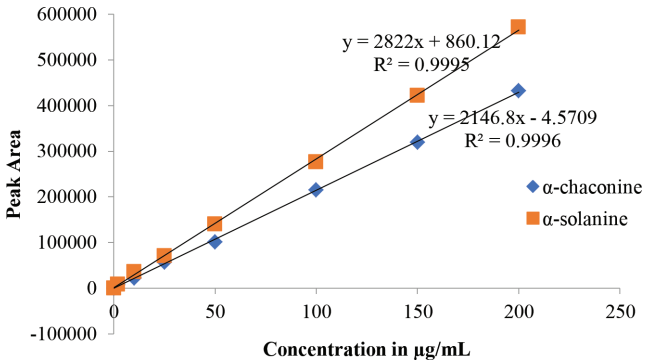


Figure 3. Linearity graph obtained in the developed method.

indicates minimal loss or interference during sample processing, ensuring the accuracy of analytical results. The standard solution at 25 µg/mL, 50 µg/mL and 100 µg/mL was studied for evaluation of method accuracy. The % recovery in each studied concentration was evaluated by correlating results achieved in recovery study with its corresponding standard calibration curve results. The % recovery was within the acceptable level of 98-102 % confirms that the method was accurate.

The impact of small variation in proposed conditions on the chromatographic response of α-chaconine and α-solanine was evaluated in robustness study. In this, small (both positive and negative) deviations in composition, flow and pH of mobile phase were varied and each varied condition, the % change in peak area response was calculated by comparing the results with corresponding standard. Results were observed to be at the acceptable level of less than 2 for both the analytes confirms that small change in the developed conditions doesn't show any significant changes in the results and hence the method was robust. The results achieved in the study were shown in Table 3.

The LOD and LOQ values are crucial parameters for assessing the sensitivity of an analytical method. Sensitivity refers to the ability of the method to detect and quantify analytes at low concentrations. In this method, the LOD of 0.50 µg/mL and LOQ of 1.65 µg/mL indicate that the method is sensitive enough to detect and quantify the analyte even at relatively low concentrations. These values provide valuable information for assessing the performance and suitability of the developed method for trace level quantification of α-chaconine and α-solanine.

Method was applied for evaluation of α-chaconine and α-solanine content in various vegetable samples. The chromatograms observed in the sample analysis confirm that all the samples studied show positive results for the α-chaconine and α-solanine. The peak area response observed in each sample chromatogram was used for calculating the α-chaconine and α-solanine content in samples. Among the samples studied sprouted potatoes showed very high quantity of glycoalkaloids content in which 87.24 ± 0.387 mg/kg of α-chaconine and 33.51 ± 0.149 mg/kg of α-solanine was observed. In red tomatoes and red bell pepper samples the glycoalkaloids content was calculated to be very less. In all the samples studied, the quantity of α-chaconine was observed to be higher than the α-solanine. The samples which are not ripening properly were observed to be high quantity of glycoalkaloids and the mature vegetables were observed to significantly less quantity of glycoalkaloids. Based on the recommendations given by USFDA and German Federal Institute for risk assessment, all the samples contain glycoalkaloids under the permissible levels. The results observed in the sample analysis study were given in Table 4 and chromatograms observed for sprouted potatoes, potatoes and green tomatoes were given in Figures 4, 5 and 6 respectively. Figure 7 shows the comparative quantification results noticed in various vegetable samples studied.

Table 3. Robustness study results in the developed method

S. No.	Changed condition	α-chaconine		α-solanine	
		Peak area	% Change	Peak area	% Change
1	Acetonitrile and buffer in 70:30 (v/v)	99784.02	0.90	137998.1	1.14
2	Acetonitrile and buffer in 60:40 (v/v)	99375.75	1.30	137759.7	1.31
3	0.9 mL/min flow rate	99360.08	1.32	136892.9	1.93
4	0.7 mL/min flow	98923.88	1.75	137407.2	1.56
5	Mobile phase at pH 4.4	99433.85	1.24	138304.5	0.92
6	Mobile phase at pH 4.6	100108.1	0.57	139256.7	0.24

Table 4. Results observed for the analysis of glycoalkaloids in various vegetable samples

S. No.	Sample studied	Amount estimated in mg/kg	
		α-chaconine	α-solanine
1	Potatoes	32.82 ± 0.072	15.56 ± 0.034
2	Sprouted potatoes	87.24 ± 0.387	33.51 ± 0.149
3	Red sweet potatoes	18.79 ± 0.126	14.22 ± 0.096
4	White sweet potatoes	11.88 ± 0.017	9.28 ± 0.013
5	Green tomatoes	53.56 ± 0.294	17.29 ± 0.095
6	Red tomatoes	8.67 ± 0.033	3.04 ± 0.012
7	Long brinjal	15.55 ± 0.052	3.64 ± 0.012
8	Round brinjal	29.65 ± 0.149	7.61 ± 0.038
9	Red chillies	14.74 ± 0.055	7.57 ± 0.028
10	Green chillies	18.53 ± 0.057	15.74 ± 0.048
11	Red bell pepper	11.17 ± 0.022	1.90 ± 0.004
12	Green bell pepper	15.52 ± 0.068	7.36 ± 0.032

Based on results achieved, it's evident that the levels of these glycoalkaloids vary significantly across different plant-based foods. Sprouted potatoes exhibit notably higher concentrations of both α-chaconine and α-solanine compared to regular potatoes, indicating a potential increase in toxicity upon sprouting. Similarly, green tomatoes contain higher levels of these compounds compared to red tomatoes, suggesting a correlation between ripeness and glycoalkaloid content.

Among the different types of peppers, red bell peppers have higher levels of α-chaconine compared to green bell peppers, while α-solanine is more prevalent in green bell peppers. This discrepancy in glycoalkaloid content between pepper varieties may be attributed to differences in genetic factors or ripening stages.

The findings in this study were correlated with literature reported method and it was confirmed that, this method exhibit wide calibration curve, less solvent usage to complete analysis than reported method by Sotelo *et al.*,[12]. The method reported by Ritchie *et al.*,[13] was applicable for quantification of solanine only and not appropriate for evaluation of chaconine. The method reported by Robert *et al.*,[14] involves completed solid phase extraction procedure and hence this proposed method was very easy and convenient than reported method. All the reported authors applied the reported method for quantification

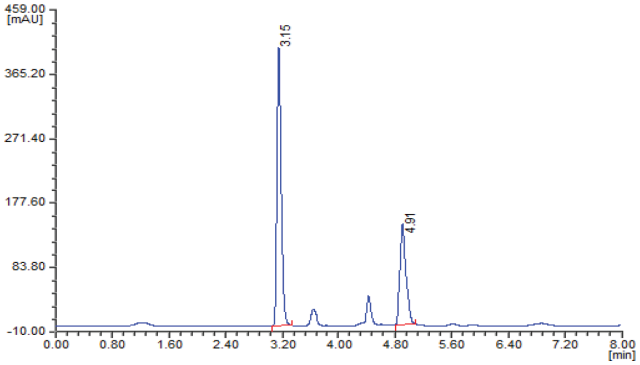


Figure 4. Representative chromatogram observed for the analysis of glycoalkaloids in Sprouted potatoes.

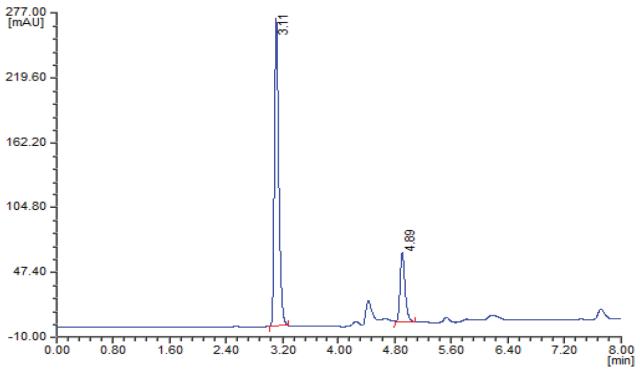


Figure 5. Representative chromatogram observed for the analysis of glycoalkaloids in potatoes.

of glycoalkaloids in one variety of samples such as eggplant or potatoes whereas this method we applied the method for quantification of a variety of samples with different ripening stages. Moreover, no author quantifies the glycoalkaloids in the samples collected in Hyderabad locality. Overall, these findings underscore the importance of considering the type and ripeness of plant-based foods in assessing dietary exposure to glycoalkaloids. Further research and monitoring are necessary to better understand the factors influencing glycoalkaloid levels in food and their potential health implications.

4. Conclusion

This study aimed to establish a novel, facile, and convenient method for quantifying α-solanine and α-chaconine in various vegetable samples. Through meticulous experimentation, it was determined that a wavelength of 218 nm was optimal for simultaneous detection of both

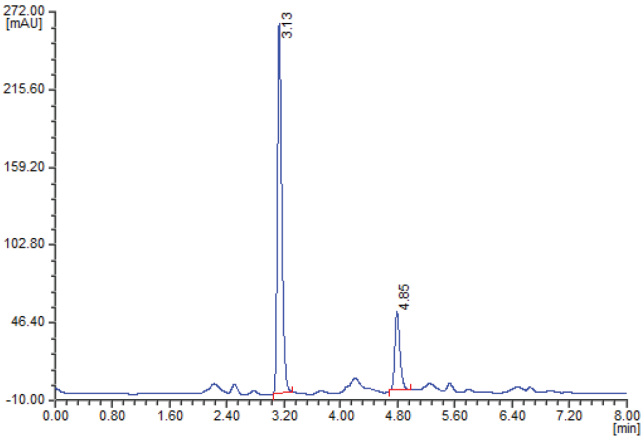


Figure 6. Representative chromatogram observed for the analysis of glycoalkaloids in green tomatoes.

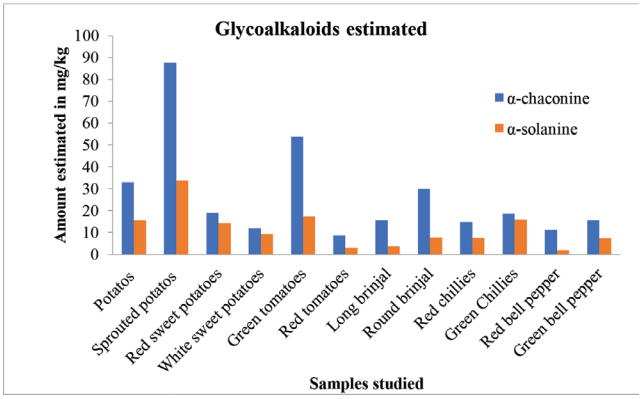


Figure 7. Comparative results observed for the analysis of glycoalkaloids in various vegetable samples.

compounds. Method optimization involved exploring various mobile phase compositions and configurations of stationary phases, leading to the selection of acetonitrile and 1.0 mM ammonium acetate buffer at pH 4.2 in a 65:35 (v/v) ratio at a flow rate of 0.6 mL/min, using a µBondapak C18 column. The developed method exhibited clear resolution of α-solanine and α-chaconine with retention times of 4.88 min and 3.15 min, respectively, while demonstrating specificity and suitability through system suitability parameters. Linearity was established over a concentration range of 2-200 µg/mL for both compounds, with regression equations indicating high correlation coefficients, showcasing the method's accuracy. This method successfully quantified the levels of α-chaconine and α-solanine in various vegetable samples using the developed HPLC method. The data obtained revealed significant variations in the concentrations of these glycoalkaloids across different types of vegetables. These findings highlight the variability in the levels of α-chaconine and α-solanine across different vegetable types, emphasizing the importance of monitoring and regulating these compounds to ensure food safety. Further studies could explore factors influencing the accumulation of glycoalkaloids in vegetables and their potential health implications, aiding in the development of strategies to mitigate their adverse effects on human health.

5. Acknowledgments

Author wishes to acknowledge Hyderabad Institute of Technology and Management, Hyderabad, Telangana, India for their support to complete this research work.

6. References

1. Johns T, Keen SL. Taste evaluation of potato glycoalkaloids by the Aymara: A case study in human chemical ecology. Hum Ecol. 1986; 14:437-52. https://doi.org/10.1007/BF00888308
2. Schrenk D, Bignami M, Bodin L, Chipman JK, del Mazo J, Hogstrand C, Hoogenboom L, Leblanc JC, Nebbia CS, Nielsen E. EFSA Panel on Contaminants in the Food Chain (CONTAM). Risk assessment of glycoalkaloids in feed and food, in particular in potatoes and potatoes-derived products. EFSA Journal. 2020; 18(8):e06222. https://doi.org/10.2903/j.efsa.2020.6222
3. Friedman M, Levin CE. Glycoalkaloids and calystegine alkaloids in potatoes. Advances in Potato Chemistry and Technology. Academic Press. 2016; 167-94. https://doi.org/10.1016/B978-0-12-800002-1.00007-8
4. Friedman M. Potato glycoalkaloids and metabolites: Roles in the plant and in the diet. J Agric Food Chem. 2006; 54(23):8655-81. https://doi.org/10.1021/jf061471t
5. Lee KR, Kozukue N, Han JS, Park JH, Chang EY, Baek EJ, Chang JS, Friedman M. Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells. J Agric Food Chem. 2004; 52(10):2832-9. https://doi.org/10.1021/jf030526d
6. Friedman M, Lee KR, Kim HJ, Lee IS, Kozukue N. Anticarcinogenic effects of glycoalkaloids from potatoes against human cervical, liver, lymphoma, and stomach cancer cells. J Agric Food Chem. 2005; 53(15):6162-9. https://doi.org/10.1021/jf050620p
7. Langkilde S, Mandimika T, Schrøder M, Meyer O, Slob W, Peijnenburg A, Poulsen M. A 28-day repeat dose toxicity study of steroidal glycoalkaloids, α-solanine and α-chaconine in the Syrian Golden hamster. Food Chem Toxicol. 2009; 47(6):1099-108. https://doi.org/10.1016/j.fct.2009.01.045

8. Dinkins CL, Peterson RK. A human dietary risk assessment associated with glycoalkaloid responses of potato to Colorado potato beetle defoliation. Food Chem Toxicol. 2008; 46(8):2837-40. https://doi.org/10.1016/j.fct.2008.05.022
9. Singh B, Raigond P, Barwal S, Mehta A, Chopra S, Joshi A, Dutt S. Glycoalkaloids in peels of Indian potatoes. Potato J. 2016; 43(1).
10. Chadha ML, Sharma CM, Bajaj KL. Inheritance of bitterness in brinjal (*Solanum melongena* L.). Indian J Hortic. 1990; 47(2):244-9.
11. Renata FJT, Mariza AM, Joao PBS *et al*. A validated reverse phase HPLC analytical method for quantitation of glycoalkaloids in *Solanumlycocarpum* and its extracts. Journal of Analytical Methods in Chemistry. 2012; 947836. https://doi.org/10.1155/2012/947836
12. Sotelo A, Serrano B. High-performance liquid chromatographic determination of the glycoalkaloids alpha-solanine and alpha-chaconine in 12 commercial varieties of Mexican potato. J Agric Food Chem. 2000; 48(6):2472-5. https://doi.org/10.1021/jf990755t
13. Ritchie CE, Neslihan T, Oykum K, Anne F, Sami D, Adelia EA. Development of practical HPLC methods for the separation and determination of eggplant steroidal glycoalkaloids and their aglycones. Journal of Liquid Chromatography and Related Technologies. 2008; 31:984–1000. https://doi.org/10.1080/10826070801924741
14. Houben RJ, Brunt K. Determination of glycoalkaloids in potato tubers by reversed-phase high-performance liquid chromatography. Journal of Chromatography A. 1994; 661(1–2):169-74. https://doi.org/10.1016/0021-9673(94)85187-5
15. Everard JE, Andrew HC. Improved high-performance liquid chromatographic method for the analysis of potato (*Solanum tuberosum*) glycoalkaloids. J Agric Food Chem. 1996; 44(9):2705-9. https://doi.org/10.1021/jf9507405
16. Popova I, Sell B, Pillai SS, Kuhl J, Dandurand LM. High-performance liquid chromatography–mass spectrometry analysis of glycoalkaloids from underexploited solanum species and their acetylcholinesterase inhibition activity. Plants. 2022; 11(3):269. https://doi.org/10.3390/plants11030269
17. Nielsen SD, Schmidt JM, Kristiansen GH, Dalsgaard TK, Larsen LB. Liquid chromatography mass spectrometry quantification of α-solanine, α-chaconine, and solanidine in potato protein isolates. Foods. 2020; 9(4):416. https://doi.org/10.3390/foods9040416
18. Distl M, Sibum M, Wink M. Combination of on-line solid-phase extraction with LC-MS for the determination of potentially hazardous glycoalkaloids in potato products. Potato Res. 2009; 52:39-56. https://doi.org/10.1007/s11540-008-9106-1
19. Liu W, Zhang N, Li B, Fan S, Zhao R, Li LP, Wu GH, Zhao Y. Determination of α-chaconine and α-solanine in commercial potato crisps by QuEChERS extraction and UPLC-MS/MS. Chem Pap. 2014; 68(11):1498-504. https://doi.org/10.2478/s11696-014-0617-8
20. ICH Validation of Analytical Procedures: Text and Methodology Q2(R1). 1994; 1-13.
21. Varma RB, Rao BS. Gas chromatography-head space-flame ionization sensor-based assessment of four residuary solvents in rivaroxaban bulk medication. Res J Pharm Technol. 2022; 15:5158-63. https://doi.org/10.52711/0974-360X.2022.00868
22. Varma BHR, Rao BS. Gas chromatography-head space-mass spectrometry sensor-based quality control of dobutamine hydrochloride bulk material for a mutagenic impurity. 2-bromopropane. Res J Chem Environ. 2023; 27:54-61. https://doi.org/10.25303/2702rjce054061
23. Rajesh VB, Battula SR, Kapavarapu MVNR, Mandapati VR. A novel Rivaroxaban degradation impurity detection by RP-HPLC extraction by preparative chromatography, and characterization by LC-MS, NMR and FT-IR: Analysis of novel impurity in batch samples and tablets of Rivaroxaban. Rasayan J Chem. 2022; 15:2373-81. https://doi.org/10.31788/RJC.2022.1547008
24. Rajesh VB, Sreenivasa RB, Maruthi VNRK, Varaprasad RM. Assessment of gas chromatography methodology approach for the trace evaluation of carcinogenic impurity. methyl chloride, in trimetazidine dihydrochloride. Ann Pharm Fr. 2023; 81:64-73. https://doi.org/10.1016/j.pharma.2022.06.012