Investigation of Steroidal glycoalkaloid concentrations in different potato cultivars through Liquid chromatography-Tandem Mass Spectrometry

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ABSTRACT

A robust analytical method employing solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) was optimized and validated for the determination of α-solanine and α-chaconine in different potato cultivars. Calibration and validation were conducted using a blank matrix derived from sweet potato powder. The method demonstrated excellent linearity, low detection limits, and high precision and accuracy.

Keywords:Liquid chromatography-Tandem Mass Spectrometry, Glycoalkaloids, α-Solanine, α-Chaconine, Potato analysis

١. INTRODUCTION

Steroidal glycoalkaloids (SGAs) are secondary metabolites naturally occurring in plants of the Solanaceae family[١]. These compounds serve as chemical defenses against pests, pathogens, and environmental stresses. Among SGAs, α-solanine and α-chaconine are the most abundant toxic compounds in potatoes, constituting up to ٩٥٪ of the total glycoalkaloid content[٢], [٣]. As shown in Figure ١, the core structures of α-solanine and α-chaconine are similar, with the only difference being in the structure of the trisaccharide chain attached to the solanidane triterpenoid molecule. Studies have indicated that α-chaconine is more toxic than α-solanine[٢]. While SGAs can have antimicrobial and anticancer properties at low concentrations, their excessive accumulation poses significant risks to human health, including gastrointestinal and neurological effects[٤]. To ensure consumer safety, regulatory guidelines limit the total glycoalkaloid (TGA) content to ٢٠٠ µg g-١ of potato fresh weight or ١ µg g-١ of potato dry weight[٥]. Exceeding these thresholds makes potatoes unsuitable for consumption. The concentration of SGAs in potatoes is influenced by various factors, including cultivar, agricultural practices, and storage conditions. These compounds are typically concentrated in the peel, with lower levels found in the tuber's inner tissues. Therefore, accurate quantification of α-solanine and α-chaconine is essential for monitoring compliance with safety standards. Advances in analytical techniques, particularly liquid chromatography-tandem mass spectrometry (LC-MS/MS), offer

sensitive and selective methods for SGA analysis. Integrating LC-MS/MS with solid-phase extraction (SPE) enhances method accuracy by mitigating matrix effects[٦], [١].

This study develops and validates an LC-MS/MS method for the quantification of α-solanine and α-chaconine in four popular commercial potato cultivars. The optimized approach ensures compliance with global safety standards while providing a reliable tool for monitoring glycoalkaloid levels in agricultural products.

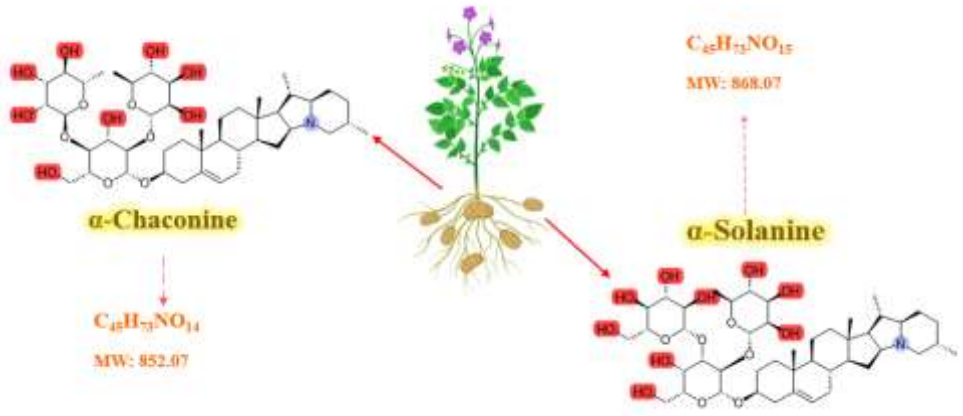


Fig. ١. *Chemical structures and molecular weights of α-chaconine and α-solanine*

٢. Materials and Methods

١.٢. Standards and Reagents:

- Glycoalkaloids: α-Solanine, α-Chaconine, and α-Tomatine (≥٩٥٪ purity, Sigma-Aldrich).
- Solvents: LC-MS grade methanol and acetonitrile (Merck).
- Buffer: Ammonium formate (١٠ mM, pH adjusted with ٠.١٪ formic acid).

٢.٢. Optimization of Mass Spectrometry

To optimize the mass spectrometry parameters, direct infusion analysis was performed using a standard solution at a concentration of ٠.٥ µg/mL. The following settings were determined empirically: capillary voltage −٥١٠٠ V, skimmer voltage ٣٠ V, Oct ١ DC voltage ١٤ V, Oct ٢ DC voltage ١.٦٠ V, trap drive voltage ٥٨.٢ V, Oct RF voltage ١٨٣.٧ V, capillary exit voltage ١٢١.٨ V, Lens ١ voltage −٦ V, and Lens ٢ voltage −٧٠ V. Each analyte was monitored under single multiple reaction monitoring (MRM) mode in the positive ESI mode using the optimized "SPS mode" of the mass spectrometer's tuning program. Verification was achieved through flow injection analysis of a ٠.٥ µg/mL standard solution under the optimized chromatographic conditions. Table ١ presents the specific MRM transitions for α-chaconine, α-solanine, and α-tomatine standards. Protonated ions [M+H]+ were observed at m/z ٨٥٢.٦, ٨٦٨.٦, and ١٠٣٤.٧ for α-chaconine, α-solanine, and α-tomatine, respectively.

Table ١. *Optimized MRM transitions for α-chaconine, α-solanine, and α-tomatine.*

Compounds	Parent ion (m/z)	Production (m/z)
α-Chaconine	٨٥٢.٦	٧٠٦.٥, ٥٦٠.٤, ٣٩٨.١
α-Solanine	٨٦٨.٦	٧٢٢.٤, ٥٦٠.٤, ٣٩٨.١

α-Tomatine	۱۰۳٤.٦	۱۰۱٦.٦, ۹۰۲.٥, ٤۱٦.۱

۳. Instrumentation:

Chromatographic separation was achieved using a HILIC column (٤.٦ × ۱٥۰ mm, ٥ µm) with a mobile phase of ۱۰ mM ammonium formate:acetonitrile (۲۰:۸۰, v/v) at a flow rate of ۰.۳ mL/min. The HPLC system was coupled to an Agilent ٦۳۳۰ ESI-ion trap mass spectrometer equipped with an electrospray ionization interface. Hardware operation and data analysis were performed using Agilent ChemStation software. Direct injection of standard solutions into the mass spectrometer was carried out using a syringe pump (model KDS-CE ۱۰۰, KDScientific, U.S.A.).

٤. Sample Preparation

Potato samples were collected from local markets across different provinces of Iran. The potatoes were washed, chopped, and ground using an industrial grinder. The ground material, including both peel and tuber, was freeze-dried and further pulverized into a fine powder. A ۰.٥ g portion of the dried sample was extracted with ۱٥ mL of ۱۰٪ acetic acid and sonicated for ۳۰ minutes. The extracts were centrifuged, filtered, and purified using solid-phase extraction (SPE) under a vacuum manifold.

٥. Result and Discussion

Under the optimized chromatographic conditions, the retention times for α-chaconine, α-solanine, and the internal standard (α-tomatine) were determined to be ٤۰.٥, ٦۱.۲ and ۷۰.٥ minutes, respectively (Figure ۲). The method demonstrated excellent quantification capability for both α-solanine and α-chaconine, with calibration curves exhibiting high linearity (R^2 = ۰.۹۹۹۳ and R^2 = ۰.۹۹٥٥, respectively). The sensitivity of the method was further highlighted by its low detection (LOD) and quantification (LOQ) limits, as detailed in Table ۲.

The accuracy and precision of the method were evaluated at three concentration levels (low, medium, and high) for each analyte. Precision was assessed through intra-day (n=٥) and inter-day (n=۳) measurements, expressed as the relative standard deviation (RSD%). The intra-day RSD values ranged from ۱.۲۲٪ to ۷.٦۷٪, while inter-day RSD values ranged from ۲.۰٥٪ to ۷.۸۲٪, all of which are below the acceptable threshold of ۱٥٪, as recommended by the USFDA for bioanalytical methods. The highest RSD observed was ۷.٦۷٪, indicating high reproducibility across different analyses (Table ۳). Accuracy was determined by calculating the relative recovery percentage (RR%) using Equation ۱. The recovery values for α-solanine and α-chaconine were close to the theoretical value of ۱۰۰٪, ranging from ۹٥.۳۱٪ to ۱۰۷.٥۳٪. These results demonstrate that the method is both accurate and precise for the quantification of α-solanine and α-chaconine in potato samples.

To evaluate the practical application of the method, the total glycoalkaloid (TGA) content was analyzed in four popular commercial potato cultivars in Iran: Agria, Sante, Jelly, and Spirit. The measured TGA levels are presented in Table ٤ and Figure ۳. Among the cultivars, Agria had the highest TGA concentration (۱۳۹.۹ mg/۱۰۰ g dry weight), followed by Sante, Jelly, and Spirit, which had concentrations of ۱۲۰.٥, ۱۱۰.۳, and ۹٥.۷ mg/۱۰۰ g, respectively. As illustrated in Figure ۳, the bar chart clearly demonstrates these differences, with Agria showing a significantly higher TGA level compared to the other cultivars, while Spirit exhibited the lowest concentration. These findings highlight significant variations in glycoalkaloid content among different cultivars, likely due to genetic differences and environmental factors.

Importantly, the results indicate that the TGA levels in most cultivars exceeded the safety threshold of ۱۰۰ mg/۱۰۰ g dry weight, which is considered the upper limit for safe consumption. This underscores the need for intervention measures to reduce glycoalkaloid concentrations. One effective strategy involves peeling the potatoes, as glycoalkaloids are predominantly localized in the peel. Studies have shown that removing the peel can significantly lower the overall glycoalkaloid content, making the potatoes suitable for

consumption[٨]. The robustness of the developed method and its applicability for assessing glycoalkaloid levels were demonstrated through its successful application to these cultivars. These findings not only validate the method but also emphasize the importance of routine monitoring and processing interventions in ensuring the safety of potato-based foods, particularly for cultivars with high glycoalkaloid content.

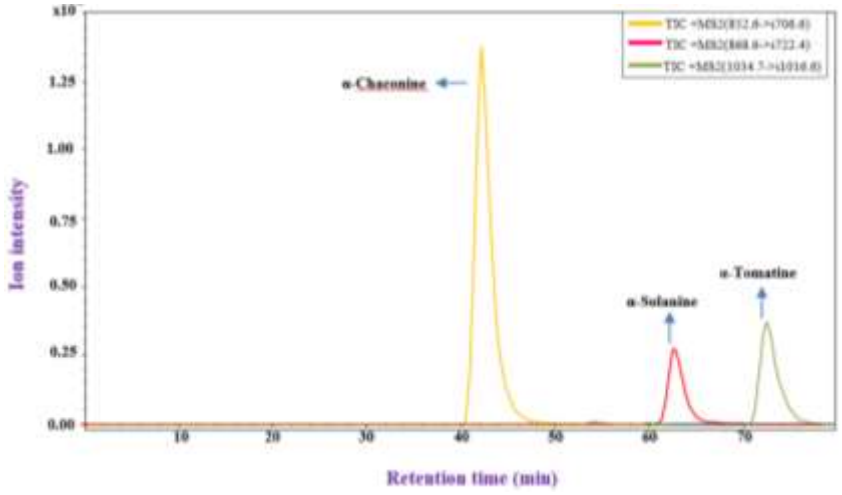


Fig. ٢. *Total ion current chromatogram of α-Chaconine (٨٥٢.٦ m/z]M+H[+ ion), α-Solanine (٨٦٨.٦ m/z]M+H[+ ion) and α-Tomatine (١٠٣٤.٧ m/z]M+H[+ ion) in standard solutions.*

Table ٢. *Correlation coefficients, LODs and LOQs of α-solanine and α-chaconine*

Compounds	Correlation coefficient	LOD (mtas)	LOQ (mg/١٠٠ g DW)
α -Solanine	٠.٩٩٩٣	٢.٠٢×١٠$^{-٣}$	٥.٨×١٠$^{-٣}$
α -Chaconine	٠.٩٩٥٥	٠.٨٨×١٠$^{-٣}$	٣.٤٠×١٠$^{-٣}$

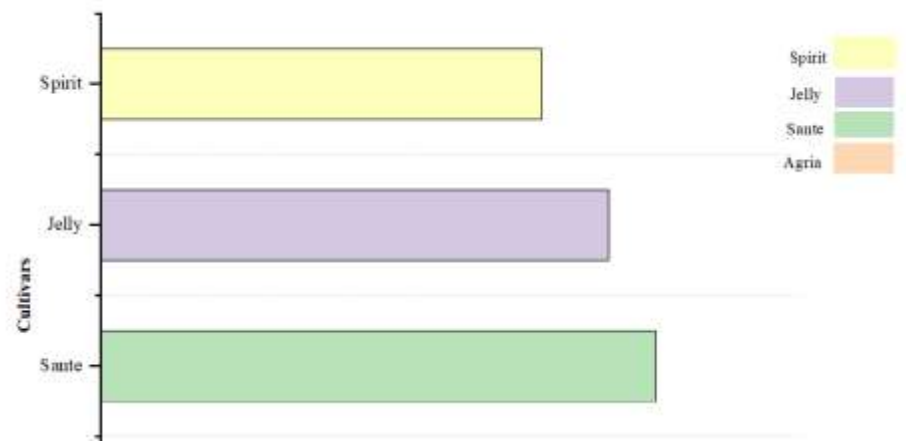
Table ٤. *TGA content in potato cultivars*

Cultivar	TGA (mg/١٠٠g dry weight)
Agria	١٣٩.٩
Sante	١٢٠.٥
Jelly	١١٠.٣
Spirit	٩٥.٧

Table ٣. *Evaluation of the precision (RSD%) and accuracy (Relative recovery%) of the LC-MS/MS method for quantification of α-Solanine and α-Chaconine at different concentration levels.*

Compounds	Spiked concentration (mg/١٠٠ g DW)	RSD % Intra-day (n=٥)	RSD % Inter-day (n=٣)	Relative recovery (RR%) (n=٣)
α-Solanine	٠.٠٠٨٥	٣.٠٦	٧.٨٢	١٠٧.٥٣±١.٠٣
	٠.٥٣٢	١.٥٠	٢.٤٠	٩٥.٣١±٢.١٥
	٥.٧٠٠	٧.٦٧	٦.٢٠	١٠٣.٥٥±٢.٣٧
α-Chaconine	٠.٠٠٥٥	٢.٥٣	٣.١٦	٩٨.٩٣±١.٤٦
	٠.٥٣٢	١.٢٢	٢.٠٥	١٠٥.٧٤±١.٤٥
	٨.٤٣٥	٥	٦.٢١	١٠٠.٢١±١.٢٢

$$RR\% = \frac{Observed\ Amount - Actual\ concentration\ in\ real\ sample}{Spiked\ amount} \times ١٠٠ \quad (١)$$



٦. Conclusion

This study successfully developed and validated a sensitive and reliable method for the quantification of α-solanine and α-chaconine in potato samples using SPE coupled with LC-MS/MS in MRM mode. The method demonstrated excellent linearity, low detection limits, and high precision, making it suitable for glycoalkaloid analysis in diverse potato cultivars. Application of the method to four commercial potato varieties in Iran (Agria, Sante, Jelly, and Spirit) revealed significant variations in total glycoalkaloid (TGA) content among cultivars. Most of the tested varieties exceeded the safety threshold of ١٠٠ mg/١٠٠ g dry weight, highlighting the necessity of monitoring glycoalkaloid levels to ensure consumer safety. Peeling was shown to be an effective intervention, significantly reducing TGA content and improving the safety of the final product. This validated method provides a robust tool for the accurate assessment of glycoalkaloids in potatoes, supporting food safety efforts and compliance with international health standards. Future studies should focus on understanding the effects of agricultural and environmental factors on glycoalkaloid content and exploring additional strategies to further improve potato safety.

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