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Development and validation of methods for quantitative determination of α-solanine, α-chaconine, solanidine in extracts from potato tuber peels BY High-performance liquid chromatography–tandem mass spectrometry

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Promising metabolites of potato tuberosum *(Solanum tuberosum* L., f. *Solanaceae)* are α-solanine, α-chaconine and their aglycone solanidine.

The aim of the work was to develop and validate methods for a quantitative analysis of α-solanine, α-chaconine and solanidine in dry extracts from the potato tuber peels by a high-performance liquid chromatography with a tandem mass-selective detection (HPLC/MS/MS).

Materials and methods. The analysis was performed in a gradient mode on an Ultimate 3000 chromatograph (ThermoFisher, USA) with a TSQ Fortis tandem mass-selective detector and a 4.6 mm×100 mm, 5 µm, 100 Å UCT Selectra C18 column. An electrospray in a positive ionization mode was used in this work. The following mass transitions were used for the quantitative analysis: α-solanine, 868.4→398.3 m/z; α-chaconine, 853.4→706.3 m/z; solanidine, 398.3→98.1 m/z. The following mass transitions were used for the internal standard fexofenadine: 502.3→171 m/z and 502.3→466.2 m/z. The analysis time was 10 min. The developed chromatography conditions were validated for a suitability. The validation was performed according to the following parameters: specificity, analytical range, linearity, correctness, precision and a lower limit of quantification.

Results. The validation procedure showed that the methodology was selective, sufficiently sensitive for α-solanine, α-chaconine and solanidine (lower limits of the quantification were 50, 10 and 2 ng/mL, respectively), the linear in the concentration range of 50–5000, 10–5000 and 2–100 ng/mL, respectively; it was satisfactorily correct (RSD did not exceed 7% for each of the substances) and sufficiently sensitive (RSD for α-solanine did not exceed 5%, for α-chaconine and solanidine – not more than 10%).

Conclusion. A technique for a quantitative determination of *α*-solanine, *α*-chaconine and solanidine in dry extracts obtained from potato tuber peels by HPLC/MS/MS has been developed and validated. This technique can be used in the routine practice of the glycoalkaloids quantitative determination when analyzing their content in food products and combination medicines.

Keywords: validation; glycoalkaloids; *α*-solanine; *α*-chaconine; solanidine; tuberous potato; HPLC-MS/MS

Abbreviations: Gas – glycoalkaloids; HPLC/MS/MS – high-performance liquid chromatography with a mass-selective detection; DMSO – dimethyl sulfoxide; GPM – general pharmacopoeial monograph; SPh RF XV ed. – State Pharmacopoeia of the Russian Federation, XV edition; LLQ – Lower Level of Quantification; ELISA – enzyme-linked immunosorbent assay.

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Разработка и валидация методики количественного определения α-соланина, α-чаконина, соланидина в экстрактах из кожуры клубней картофеля клубненосного методом высокоэффективной жидкостной хроматографии и тандемной масс-спектрометрии

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Перспективными метаболитами картофеля клубненосного (*Solanum tuberosum* L., сем. *Solanaceae*) являются α-соланин, α-чаконин и их агликон соланидин.

Цель. Разработка и валидация методики количественного анализа α-соланина, α-чаконина и соланидина в сухих экстрактах из кожуры клубней картофеля клубненосного методом высокоэффективной жидкостной хроматографии с тандемным масс-селективным детектированием (ВЭЖХ/МС/МС).

Материалы и методы. Анализ выполнялся в градиентном режиме на хроматографе «Ultimate 3000» (ThermoFisher, США) с тандемным масс-селективным детектором TSQ Fortis и колонкой UCT Selectra C18 4,6 мм×100 мм, 3 мкм, 100 Å. В работе использовался электроспрей в положительном режиме ионизации. Для количественного анализа применялись следующие переходы масс: α-соланин – 868,4→398,3 m/z; α-чаконин – 853,4→706,3 m/z; соланидин – 398,3→98,1 m/z. Переходы масс для внутреннего стандарта фексофенадина: 502,3→171 m/z и 502,3→466,2 m/z. Время анализа составило 10 мин. Разработанные условия хроматографирования были проверены на пригодность. Валидацию проводили по следующим параметрам: специфичность, аналитическая область, линейность, правильность, прецизионность и нижний предел количественного определения.

Результаты. Процедура валидации показала, что методика была селективной, достаточно чувствительной в отношении α-соланина, α-чаконина и соланидина (нижний предел количественного определения составил соответственно 50, 10 и 2 нг/мл), линейна в интервале концентраций соответственно 50–5000, 10–5000 и 2–100 нг/мл, обладала удовлетворительной правильностью (RSD не превышали 7% для каждого из веществ), достаточной презиционностью (для α-соланина RSD не превышало 5%, для α-чаконина, соланидина – не более 10%).

Заключение. Разработана и валидирована методика количественного определения α-соланина, α-чаконина и соланидина в сухих экстрактах, полученных из кожуры клубней картофеля клубненосного, методом ВЭЖХ-МС/МС. Данная методика может быть использована в рутинной практике количественного определения гликоалкалоидов при анализе их содержания в пищевых продуктах и комбинированных лекарственных средствах.

Ключевые слова: валидация; гликоалкалоиды; α-соланин; α-чаконин; соланидин; картофель клубненосный; ВЭЖХ/МС/МС

Список сокращений: ГА – гликоалкалоиды; ВЭЖХ/МС/МС – высокоэффективная жидкостная хроматография с масс-селективным детектированием; ДМСО – диметилсульфоксид; ОФС – общая фармакопейная статья; ГФ РФ XV изд. – Государственная фармакопея Российской Федерации XV издания; НПКО – нижний предел количественного определения; ИФА – иммуноферментный анализ.

INTRODUCTION

Plant organisms are unique producers of biologically active substances with a wide range of pharmacological effects, i.e. antiseptic, antimicrobial, antitumor [1]. A secondary metabolism of plants makes it possible to obtain fundamentally new compounds characterized by their own mechanism of action. The complex of plant metabolites – flavonoids, alkaloids and terpenoids in combination with related substances – has an effective and often relatively safe therapeutic effect [2].

More than 90 different glycoalkaloids (GAs) have been described from 300 species of plants in family *Solanaceae)*. Their toxicity is reported in the scientific literature, but a number of useful pharmacological properties, including antitumor, antimicrobial, antifungal, etc., have also been reported. [3]. This fact actualizes the development of GAs extraction techniques, their analysis and a subsequent evaluation of their therapeutic potential with a subsequent determination of their activity.

As a source of raw materials for obtaining a GA pharmaceutical substance, it is promising to use a

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popular food plant *Solanum tuberosum* L., which contains glycosylated alkaloids of the solanidine group: α-solanine and α-chaconine; their waste percentage occupies the third place after cereals and dairy products[1].

To select the optimal GAs extraction techniques, as well as the subsequent standardization of the extracts and evaluation of their pharmacological activity, it is necessary to develop a sensitive, reproducible and selective technique for the quantitative determination of α-solanine, α-chaconine and their aglycone solanidine in an appropriate matrix and to prove its suitability by analyzing its metrological characteristics.

A number of techniques for a GAs quantification have been described in the literature, but some studies focus on individual compounds [4, 5], other techniques are complicated in terms of a sample preparation (e.g., heterogeneous LLQ) [6, 7], some have a low selectivity (HPLC-UV) [8, 9] or an analytical area [10, 11]. The latter criterion is especially important in the selection of extraction techniques, since the GAs level depends both on the native state of the raw materials (a genetically programmed concentration, stress factors, etc.) and on the nature of the extractant used, an isolation mode, and additionally introduced modifications.

The validation method procedure of the quantitative determination of individual GAs in their combined presence in the extract from potato tuber peels according to the requirements of the State Pharmacopoeia of the Russian Federation, XV edition (SPh RF XV ed.) will make it possible to judge unambiguously about the acceptability of the chosen extraction method.

THE AIM of the work was to develop and validate methods for a quantitative analysis of α-solanine, α-chaconine and solanidine in dry extracts from the potato tuber peels (*Solanum tuberosum* L., family *Solanaceae*, by a high-performance liquid chromatography with a tandem mass-selective detection (HPLC/MS/MS).

MATERIALS AND METHODS

The following reagents and chemical substances were used in this work: standard samples of α-solanine, α-chaconine, solanidine (Sigma Aldrich, USA), fexofenadine (United States Pharmacopeia Reference Standard, USA; CAS No. 153439-40-8), methanol for gradient HPLC (Himmed, Russia), 98% formic acid for analytics (Panreac, Spain), HPLC-MS water (VWR, France), dimethyl sulfoxide (DMSO) (OOO SPE PanEco, Russia), a 5% solution of acetic acid (OOO Techplant, Russia), Hanks' Balanced Salt solution without phenol red (HBSS; OOO SPE PanEco, Russia).

The methods development was carried out using the following equipment: Ultimate 3000 chromatograph (ThermoFisher, USA) with a TSQ Fortis tandem mass-selective detector (ThermoFisher, USA) equipped with an autosampler and degasser, Selectra C18 4.6 mm×100 mm, 3 µm, 100 Å column with Selectra C18 SLC-18GDC46-3UM pre-column (UCT, USA). The following kinds of the auxiliary equipment were used: a centrifuge for microcuvettes (Elmi, Latvia); analytical scales LV 210-a (Sartogosm, Russia), a Vortex shaker (Heidolph, Germany). A control of the chromatographic system, as well as mathematical processing of the data were carried out using a Thermo Scientific Xcalibur program (ver. 4.2.47).

The methods development consisted in the selection of the fragmentation method, the choice of the internal standard and the mobile phase composition.

Fragmentation parameters were selected in a semiautomatic mode (fragmentation energies were selected automatically). The two most intense fragments were selected for the registration. Fragmentation conditions were selected under the argon supply at pressures of 1.5, 2.0, 2.5 and 3.0 mTorr.

The chromatographic technique was set up on a water-methanol phase with a C18 column. Initially, an isocratic elution mode with 25, 50 and 80% methanol was used to evaluate the influence of an organic solvent on the separation of the substances, their retention times and chromatographic parameters of the system. Based on the results obtained, the gradient elution profile was selected to optimize the chromatographic parameters. Since satisfactory chromatographic parameters and the sensitivity were already obtained at this stage, testing of the aqueous-acetonitrile phase was deemed unnecessary.

An internal standard was selected from 6 substances: fexofenadine, anastrazole, valsartan, amantadine, amlodipine, metoprolol in concentrations from 1 to 10 ng/mL. The best results in terms of the reproducibility were observed for fexofenadine, which had been selected as an internal standard at a concentration of 1 ng/mL.

A chromatography was carried out in a gradient elution mode: solvent A – 0.1% aqueous formic acid, solvent B – methanol according to Table 1.

The temperature of the test samples was 20°C, the temperature of the chromatography column maintained with a thermostat, was 35°C. The flow rate of the mobile phase was 400 µL/min. The volume sample injection was 20 µL; using an autosampler at 8°C.

The decay products of the molecular ion were recorded using a quadrupole mass detector when exposed to an electrospray in the positive ionization mode.

The flow rate of the Sheath Gas was 50 Arb, of the auxiliary gas (Aux Gas) – 10 Arb, of the Sweep Gas – 1 Arb; the ion transfer tube temperature was 300°C, and evaporator temperature was 350°C. The following mass transitions were used for the detection: α-solanine – 868.4→98.1 m/z, 868.4→398.3 m/z (this transition was used for the quantification) at a collision energy of

[1] Serpova OS, Borzenkov LA. Resource-saving technologies of potato processing: a scientific analytical review. Moscow: Rosinformagrotech; 2009. 84 p. Russian

60 V; α-chaconine – 853.4→398.3 m/z, 853.4→706.3 m/z (this transition was used for the quantification) at a collision energy of 60 V; solanidine – 398.3→98.1 m/z (this transition was used for the quantification) at the collision energy of 43 V, 398.3→382.3 m/z at the collision energy of 48 V; the source fragmentation was 20 V, CID gas 2 mTorr. Mass transitions for fexofenadine were: 502.3→171 m/z and 502.3→466.2 m/z at the collision energy of 27 V. The time per analysis was 10 min.

The structural formulas of the analyzed substances and fragmentation of their molecules are presented in Fig. 1–4.

Validation of analytical methodology

Validation of chromatographic techniques involves proving their suitability for specific purposes and is regulated by a number of domestic regulatory documents[2].

The method was evaluated according to SPh RF, XV ed. by the following parameters: a suitability of the chromatographic system[3], specificity, an analytical range, linearity, correctness, precision (repeatability, in-laboratory precision) and a lower limit of quantification.

Preparation of standard sample solutions

According to the requirements of SPh RF, XV ed., for the evaluation of the validation parameters of the analytical methodology (correctness, precision at the levels of repeatability and in-laboratory precision) it is necessary to use solutions with 100% of the nominal value of the investigated substance, but these concentrations in extracts obtained by different methods can vary significantly. Therefore, to select such concentrations, a preliminary study was carried out to evaluate the content of α-solanine, α-chaconine and solanidine extracted from the insolubilized potato tuber peels using different extractants (pyridine, methanol, ethanol, 5% aqueous acetic acid) according to the physicochemical properties of the substances. The analysis was carried out by HPLC/MS/MS using a partially validated technique (with a satisfactory linearity and reproducibility). The content of α-solanine in the 4 extracts obtained was 252.61±182.85 ng/mL, α-chaconine was 451.33±100.33 ng/mL, and solanidine was 4.60±1.72 ng/mL. The averaged concentrations were further used as theoretical (100%) for the calculation of metrological characteristics [12].

The solutions of α-solanine, α-chaconine and solanidine standard samples were prepared as follows: 1 mg of the substance was dissolved in 1 mL DMSO, incubated at 50°C in an ultrasonic bath for 60 min, diluted 10 times with a DMSO solution, then with Hanks Solution (HBSS) to the concentrations of 50, 250, 300, 500, 1000 and 5000 ng/mL (α-solanine); 10, 50, 50, 400, 450, 450, 500, 1000, 2000, and 5000 ng/mL (α-chaconine); 2, 3, 3.2, 4, 4.8, 5, 10 and 100 ng/mL (solanidine). The sample preparation was performed by a 10-fold dilution with methanol containing the internal standard, fexofenadine (1 ng/mL), followed by a centrifugation at 1500 g for 10 min.

The quantification was performed using a calibration plot, and a normalization of the analytical response was performed using an internal standard:

$$K = \frac{S_{[analyte]}}{S_{[internal\ standard]}},$$

where K – normalized analytical response, %; $S_{[analyte]}$ – peak area of the determined component, %; $S_{[internal\ standard]}$ – peak area of the internal standard, %.

Preparation of test solutions

To validate the developed HPLC methodology, a dry extract of potato tuber peel of the tuberous potato variety Gala (Ryazan region, Klepikovsky district, Tuma settlement, Russia) was analyzed. The tubers pre-treatment included a two-week insolation with a daylight to maximize the GAs accumulation. Then, the dry extract obtained by a 3-fold maceration of the dry peel with a 5% aqueous solution of acetic acid was analyzed.

1 mg of the dry extract was diluted in 1 mL of DMSO, incubated for 60 min at 50°C in an ultrasonic bath, centrifuged for 10 min at 1500 g; the supernatant was diluted 10 times with a DMSO solution, followed by 10 times with HBSS. The sample preparation and calculations were performed according to the methods described above.

Preparation of working solutions for the validation assessment according to the linearity criterion

Solutions of 6 concentrations for α-solanine (50, 250, 300, 500, 1000 and 5000 ng/mL), 8 points for α-chaconine (10, 50, 400, 450, 500, 1000, 2000 and 5000 ng/mL) and 7 for solanidine (2, 3, 3,2, 4, 4,8, 5, 10 and 100 ng/mL), respectively, 3 repetitions for each followed by a sample preparation, were used to determine the linearity criterion.

Preparation of working solutions for validation evaluation by precision, in-laboratory precision, and correctness

The precision validation included the standard solutions preparation of α-solanine (250 ng/mL), α-chaconine (450 ng/mL) and solanidine (4.0 ng/mL) in 6 replicates of each concentration.

[2] 1.1.0012 Validation of analytical techniques. State Pharmacopoeia of the Russian Federation XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-1/validatsiya-analiticheskikh-metodik/. Russian

[3] 1.2.1.2.0001 Chromatography. State Pharmacopoeia of the Russian Federation XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-1/1-2-1-2-khromatograficheskie-metody-analiza/khromatografiya/. Russian

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The solutions for the evaluation of the "in-laboratory precision" index were prepared by another researcher using a similar methodology.

Concentrations of 200, 250 and 300 ng/mL of the test solutions were chosen for an α-solanine to assess correctness. Solutions of α-chaconine were prepared at the concentrations of 400, 450 and 500 ng/mL; solanidine at the concentrations of 3.2, 4.0 and 4.8 ng/mL. All the solutions were prepared in three repetitions. Thus, a total of 9 working solutions of each substance were analyzed.

Statistical processing

The data statistical processing was performed using Microsoft Office 2019 office suite (Microsoft Inc., USA) and Statistica 13.0 program (StatSoft, USA). The nature of the data distribution was determined using the Shapiro-Wilk criterion. Pearson correlation coefficient was used to assess the linearity. MS Excel 2019 program with PKSolver extension was used to construct the weighted regression equation. This parameter was used for a more accurate construction of the calibration plot in the region of low concentrations.

Fisher's criteria were used to prove the absence of variance differences in the analysis of the intra-laboratory precision. The Student's criterion was used to compare the mean values of the analysis results obtained by different researchers. The tables present the following metrological characteristics: arithmetic mean ($\bar{x}$), variance (S^2), standard deviation (SD, S_δ), a relative standard deviation (RSD, $S_{\delta,\%}$), a half-width of the confidence interval of the value (Δx), relative errors of the result of an individual determination (ε), boundary values of the confidence interval of an individual determination ($\bar{x}\pm\Delta x$) result.

Due to the fact that the obtained data had a normal distribution, the results in the text were presented as an arithmetic mean (a mean of 3 parallel measurements) ± standard deviation.

RESULTS

Suitability of chromatographic system

To evaluate the suitability of the chromatographic system, solutions of α-solanine, α-chaconine, and solanidine at concentrations of 250, 450 and 4.8 ng/mL, respectively, were analyzed sequentially in a six-fold replicate.

The main characteristics of the target substances peaks on the chromatograms of the standard sample solutions are presented in Table 2.

Based on the presented data, the RSD values for the peak areas of each of the analyzed substances and the internal standard did not exceed 2%. The number of theoretical plates exceeded 2000. The asymmetry factors corresponded to the acceptable range of 0.7–2.5 [13].

Thus, the developed system meets the requirements of SPh RF, XV ed. 1.2.1.2.2.0001 Chromatography[4].

Validation of the developed methods

Validation of the analytical method for the quantitative determination of α-solanine, α-chaconine, solanidine by HPLC/MS/MS in extracts was performed in accordance with the requirements of SPh RF, XV edition.

Fexofenadine in methanol with a concentration of 1 ng/mL was chosen as an internal standard because of the best reproducibility of the assay results.

The retention times of α-solanine, α-chaconine and solanidine in the dry extract containing the target substances used to test the methodology coincided with those in the chromatograms of the standard solutions (Fig. 5). No accompanying substances eluting at the corresponding time were observed, which confirmed the specificity of the technique.

It should be noted that in the analysis of the obtained plant extracts, the chromatograms showed a substance peak with retention times coinciding with those for α-solanine and α-chaconine (t_R=4.79±0.02 min and t_R=4.78±0.015 min), while the molecular weight corresponded to the aglycone – solanidine (m/z=398.3). Herewith, the retention time for solanidine was t_R=5.38±0.0098 min. Most likely, during the ionization of GAs in the mass detector there was a break of a glycosidic bond with the release of aglycone. The content of solanidine relative to the content of the glycosides sum in the sample was not more than 2% by mass.

The methods linearity was established in the range of the assumed analytical area of the target substances concentrations, including from 80 to 120% of the content of each component in the potato tuber peel extracts, according to Table 3. The calibration plots of the peak area dependence on the concentration of the components, the regression equations and correlation coefficients are presented in Fig. 6–8.

The correlation coefficients obtained were greater than 0.99, meeting the requirements of SPh RF XV ed.

The lower limit of quantification (LLQ) was calculated by comparing the maximum intensity of the detector response when injecting a blank sample (5% DMSO, 95 mL HBSS, 10 mL internal standard methanol) and a sample with minimum analyte concentrations. Concentrations of the target substances that gave a detector response

[4] Ibid.

of at least 10 times the noise level at the time interval t_R of the peak analyte±width at the base obtained after the injection of the sample with the maximum concentration of the working range were taken as LLQ. In addition, the repeatability and correctness of 5 injections of solutions with the concentrations corresponding to the LLQ did not exceed 20%. The LLQ was 50 ng/mL for α-solanine, 10 ng/mL for α-chaconine, and 2.0 ng/mL for solanidine.

The precision (repeatability) of the methodology was evaluated using 6 points of a single concentration equal to 100% of the nominal concentration (Table 4).

For α-solanine at the concentration of 250 ng/mL, RSD did not exceed 5%. For α-chaconine at 450 ng/mL and solanidine at 4 ng/mL, the RSD did not exceed 10%.

An intra-laboratory precision was assessed by 6 points of 100% of nominal concentrations of the test substances prepared by two investigators in the same laboratory, using the same equipment and materials. The data are presented in Table 5.

Fisher's criteria calculated for α-solanine, α-chaconine and solanidine were lower than the table values and were 1.18, 1.19 and 1.15, respectively. The calculated Student's criteria for the substances were 0.46, 0.42 and 1.41, respectively, at a significance level of 95%, the number of degrees of freedom was 10, which was also lower than the tabulated values.

To assess the correctness, the parameters of 9 points were analyzed (Table 6). An openability, a standard deviation, a mean-square deviation were calculated.

As a result, RSDs did not exceed 7% for each of the substances.

DISCUSSION

GAs are promising pharmacological agents due to their wide range of biological effects [14, 15]. The use of extracts from plant raw materials requires a preliminary standardization: first of all, proofs of the percentage content of target substances.

A number of methods for the determination of the GAs content in potato products have been described in the literature: a high-performance thin-layer chromatography [16, 17], a heterogeneous [6, 7] and homogeneous immunoassay (LLQ) combined with capillary electrophoresis [18], a high-performance liquid chromatography with a UV detection [8, 9] or a tandem mass spectrometry [19–21].

The disadvantages of solid-phase LLQ are a low reproducibility, a considerable duration, and a high cost of the analysis. A high-performance capillary electrophoresis in combination with a laser-fluorescence detection is of a limited use in the analysis of the substances poorly soluble in water and water-alcohol solutions. The use of a laser-fluorescence detector also implies a multistage preparation for the analysis (separation, purification, labeling) [22].

GAs have close absorption maxima lying in the range of 200–208 nm, due to which the selectivity and sensitivity of the quantification with the use of an ultraviolet detector are reduced. A high-performance thin-layer chromatography can detect 10 ng of alkaloids, whereas the sensitivity of HPLC/MS/MS is often significantly higher [23, 24]. Also for the described methods (with the exception of the mass detection), the separation of the substances close in physicochemical properties, e.g. α-solanine and α-chaconine, is also a problem, which reduces their selectivity.

Thus, it is urgent to develop a highly sensitive technique that allows a simultaneous analysis of α-solanine, α-chaconine and their aglycone in multicomponent plant extracts with a minimal sample preparation and satisfactory metrological characteristics.

A number of questions about the validation of a methodology that is intended to be used for the quantification of substances in the samples with a potentially wide range of concentrations (using different extractants or extraction techniques) are discouraged.

A determination of the quantification limit is not regulated by SPh RF, XV ed., however, in view of the analysis of unknown concentrations of the target substances during the selection of the extraction technique it was necessary to determine this criterion.

The additive method, classically used in the validation of multicomponent systems [25], was not used in this study, since the tuber peel of tuberous potatoes not subjected to an insolation (assumed matrix) contained the tested substances: 30.74±26.0017 ng/mL α-solanine, 39.19±5.86 ng/mL α-chaconine, 1.71±0.37 ng/mL solanidine. At the same time, the initial level of GAs in the tuber peels of different potatoes varies considerably and can change under the influence of environmental factors (humidity, temperature, etc.) [26]. These peculiarities of the raw materials used served as a basis for the selection of an alternative method using an internal standard.

Study limitations

Changing the conditions of the chromatographic determination (e.g., using a different column or elution mode) can significantly affect the results of the study, and therefore the interlaboratory validation would be more appropriate.

In the present study, the validated methods was tested on the extracts obtained from the peels of potato tubers of the tuber-bearing variety Gala. In case of using another more highly productive potato variety, the GAs concentration values may be outside the working range of the developed methodology.

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Table 1 – Ratio of mobile phase components by volume as a function of elution time

Analysis time, min	0.1% aqueous formic acid	Methanol
1–5	65%	35%
6–8	30%	70%
9	10%	90%
10	1%	99%

Table 2 – Evaluation data of chromatography system suitability

Analyzed substance	Parameter			
	Retention time, min	RSD of peak area, %	Asymmetry factor, A_s	Number of theoretical plates, N
Fexofenadine	5,27±0,037	1,67	1,42	15620,64
α-solanine	4,85±0,0033	1,85	0,96	8827,11
α-chaconine	4,83±0,013	1,73	0,96	8790,29
Solanidine	5,38±0,0098	1,74	0,87	10644,34

Table 3 – Initial concentrations of substances to assess methodology linearity

Concentration of standard sample solution, ng/mL		Peak area of standard sample mAU×min	Peak area of standard sample in terms of internal standard, %	Calculated concentration of standard sample, ng/mL	R, %
α-solanine	50	8003	0.24	54.91	109.82
	250	20994	0.90	237.72	95.088
	300	25957	1.16	308.52	102.84
	500	40719	2.13	573.15	114.63
	1000	72572	3.95	1072.42	107.24
	5000	295813	17.73	4855.96	97.12
α-chaconine	10	124	0.04	8.50	85.00
	50	799	0.04	50.08	100.16
	400	5339	0.29	399.62	99.91
	450	6103	0.32	435.51	96.78
	500	8024	0.40	544.31	108.86
	1000	14759	0.84	1142.53	114.25
	2000	22347	1.44	1949.54	97.48
	5000	52103	3.60	4879.91	97.60
Solanidine	2.0	1355	0.05	2.54	117.00
	3.2	2840	0.11	3.48	111.88
	4.0	2924	0.12	3.65	93.75
	4.8	4093	0.18	4.39	93.54
	5.0	4064	0.19	4.68	95.40
	10.0	13746	0.46	8.62	87.10
	100.0	138737	6.75	101.64	101.37

Table 4 – Results of precision assessment of α-solanine, α-chaconine, solanidine quantitative determination method (repeatability level)

Analyzed component, ng/ml	Peak area of standard sample, mAU×min	Peak area of standard sample in terms of internal standard, %	Calculated concentration of standard sample, ng/mL	Metrological characteristics
α-solanine, 250	21945	0.92	242.33	$\bar{x}$=249.18 ng/mL S^2=136.33 ng/mL SD=11.68 ng/mL RSD=4.69% Δx=±10.12 ng/mL ε=±4.06% $\bar{x}$±Δx=249.18±10.12 ng/mL
	22258	0.90	237.49	
	22453	0.92	243.98	
	20138	0.92	243.41	
	23007	1.00	264.98	
	21626	0.99	262.88	
α-chaconine, 450	7790	0.36	484.60	$\bar{x}$=456.52 ng/mL S^2=1165.93 ng/mL SD=34.15 ng/mL RSD=7.48% Δx=±29.59 ng/mL ε=±6.48% $\bar{x}$±Δx=456.52±29.59 ng/mL
	7997	0.35	476.16	
	7532	0.33	455.45	
	7562	0.36	487.76	
	5339	0.29	399.62	
	6103	0.32	435.51	
Solanidine	3562	0.15	4.08	x=456.52 ng/mL S^2=013 ng/mL SD=036 ng/mL RSD=935% Δx =±032 ng/mL ε=±811% $\bar{x}$±Δx=389±032ng/mL
	2288	0.13	3.89	
	4093	0.18	4.49	
	3483	0.12	3.65	
	3615	0.12	3.75	
	4799	0.19	4.77	
	3562	0.15	4.08	

Table 5 – Results of precision assessment (intra-laboratory precision level) of α-solanine, α-chaconine, solanidine quantitative determination methods

Peak area of standard sample. mAU×min	Peak area of standard sample in terms of internal standard. %	Calculated concentration of standard sample. ng/mL	Peak area of standard sample. mAU×min	Peak area of standard sample in terms of internal standard. %	Calculated concentration of standard sample. ng/mL	Metrological characteristics	
Researcher I			Researcher II				
α-solanine $t_{calculated}$=0.46 <t (95%; 10), $F_{calculated}$=1.18 <F (95%; 5, 5) – differences between results are randomized							
21945	0.92	242.33	22848	0.97	257.67	$\bar{x}$=249.18 ng/mL S^2=136.33 ng/mL SD=11.68 ng/mL RSD=4.69% Δx=±10.12 ng/mL ε=±4.06% $\bar{x}$±Δx=249. 18±10.12 ng/mL	$\bar{x}$=245.91 ng/mL S^2=115.097 ng/mL SD=10.73 ng/mL RSD=4.36% Δx=±9.30 ng/mL ε=±3.78% $\bar{x}$±Δx= 245.91±3.78 ng/mL
22258	0.90	237.49	22635	0.96	253.65		
22453	0.92	243.98	22164	0.89	235.62		
20138	0.92	243.41	22258	0.90	237.72		
23007	1.00	264.98	22935	0.89	235.32		
21626	0.99	262.90	22872	0.97	255.51		
α-chakonine $t_{calculated}$=0.42 <t (95%; 10), $F_{calculated}$=1.19 <F (95%; 5, 5) – differences between results are randomized							
7790	0.36	484.60	8343	0.37	505.77	$\bar{x}$=456.52 ng/mL S^2=1165.93 ng/mL SD=34.15 ng/mL RSD=7.48% Δx=±29.59 ng/mL ε=±6.48% $\bar{x}$±Δx=456.52±6.48 ng/mL	$\bar{x}$=465.14 ng/mL S^2=976.47 ng/mL SD=31.25 ng/mL RSD=6.72% Δx=±27.08 ng/mL ε = ±5.82% $\bar{x}$±Δx=465.14±6.48 ng/mL
7997	0.35	476.16	6103	0.32	503.51		
7532	0.33	455.45	7532	0.33	435.51		
7562	0.36	487.76	2823	0.38	445.61		
5339	0.29	399.62	6318	0.33	444.99		
6103	0.32	435.51	7418	0.37	455.46		
Solanidine $t_{calculated}$=1.41 <t (95%; 10), $F_{calculated}$=1.15 <F (95%; 5, 5) – differences between results are randomized							
3562	0.15	4.08	3615	0.118	3.653	$\bar{x}$=3.89ng/mL S^2=0.13 ng/mL SD=0.36 ng/mL RSD=9.35 % Δx=±0.32 ng/mL ε=±8.11% $\bar{x}$±Δx=3.89±0.32 ng/mL	$\bar{x}$=3.86 ng/mL S^2=0.13 ng/mL SD=0.36 ng/mL RSD=9.16 % Δx=±0.31 ng/mL ε=±7.94% $\bar{x}$±Δx=3.86±0.31 ng/mL
2288	0.13	3.89	3483	0.120	3.681		
4093	0.18	4.49	3155	0.110	3.540		
3483	0.12	3.65	4697	0.147	4.075		
3615	0.12	3.75	4093	0.175	4.486		
4799	0.19	4.77	2921	0.124	3.749		

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Table 6 – Results of correctness assessment of methodology for quantitative determination of α-solanine, α-chaconine, solanidine

Concentration of standard solution, ng/mL	Peak area of standard sample, mAU×min	Peak area of standard sample in terms of internal standard, %	Found, ng/mL	R, %	Metrological characteristics
α-solanine					
200	18565	0.817	214.44	107.22	$\bar{x}$=98.23%
200	19026	0.743	194.24	97.12	SD=5.43
200	19411	0.723	188.69	94.35	RSD=5.53%
250	21945	0.918	242.33	96.93	
250	20138	0.922	243.41	97.36	
250	22453	0.924	243.98	97.59	
300	42062	1.154	307.01	102.34	
300	23007	1.001	264.98	88.33	
300	25957	1.160	308.52	102.84	
α-chakonine					
400	6407	0.290	394.69	98.67	$\bar{x}$=101.25%
400	6545	0.285	388.71	97.18	SD=4.52
400	5339	0.293	399.62	99.91	RSD=4.47%
450	7532	0.334	455.45	101.21	
450	8063	0.370	503.51	111.89	
450	7002	0.320	435.51	96.78	
500	8343	0.371	505.45	101.09	
500	8223	0.371	504.89	100.98	
500	8027	0.381	517.86	103.57	
Solandinine					
3.2	3626	0.092	3.27	97.98	$\bar{x}$=96.81%
3.2	3278	0.082	3.12	102.60	SD=6.28
3.2	3455	0.082	3.13	102.33	RSD=6.49%
4.0	4987	0.147	4.075	98.17	
4.0	3615	0.118	3.65	109.51	
4.0	3483	0.120	3.68	108.67	
4.8	4064	0.194	4.77	100.59	
4.8	6965	0.210	5.01	95.80	
4.8	4697	0.147	4.075	117.81	

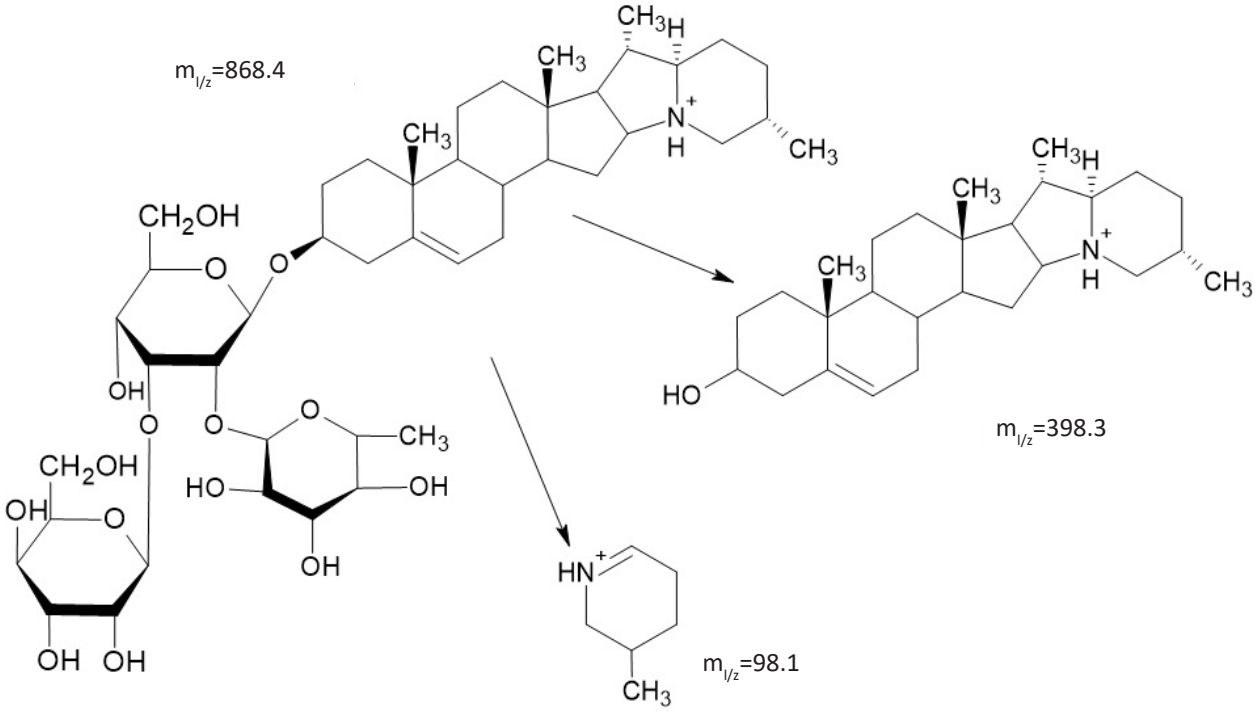

Figure 1 – Structural formula of α-solanine and fragmentation of its molecule

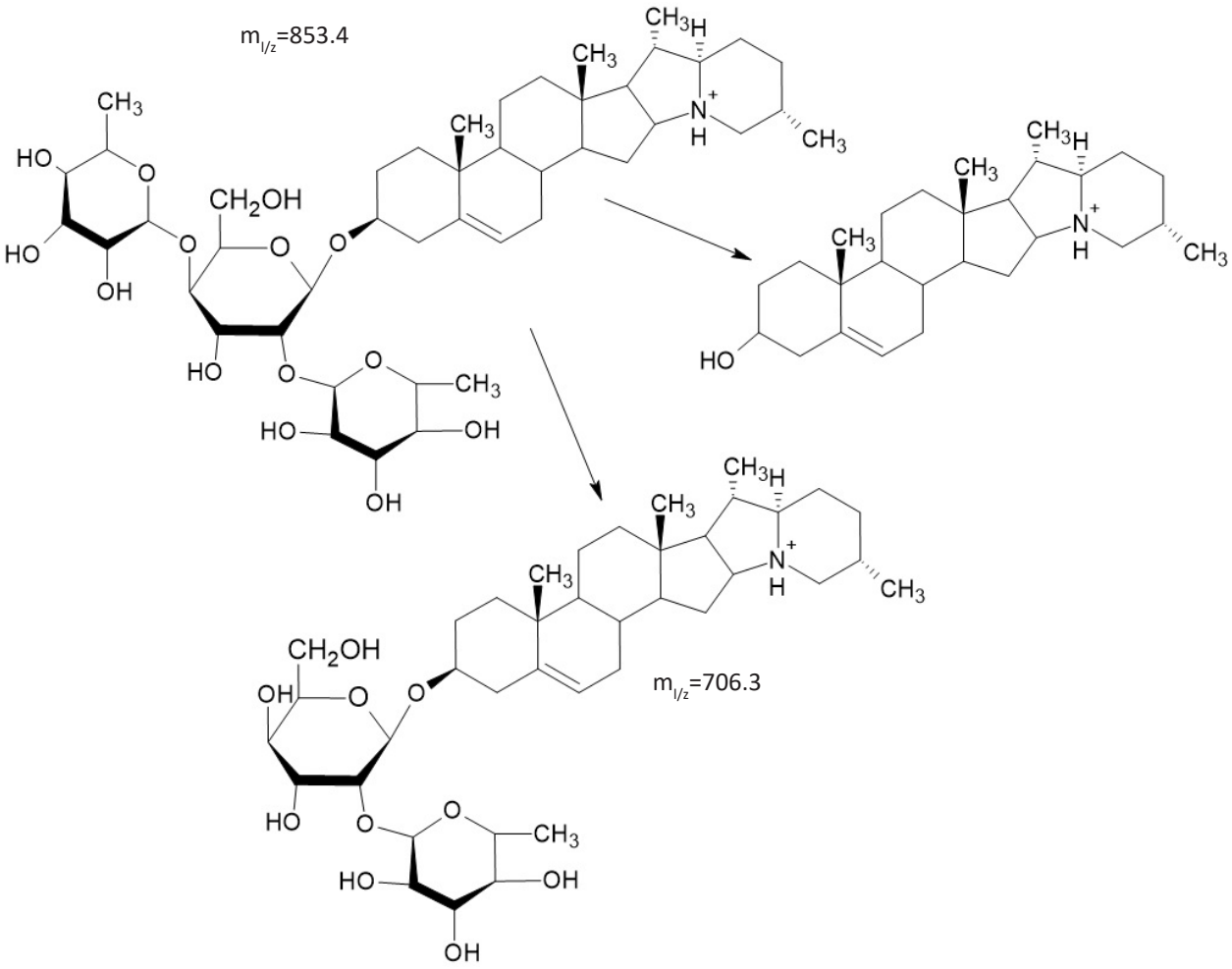

Figure 2 – Structural formula of α-chaconin and fragmentation of its molecule

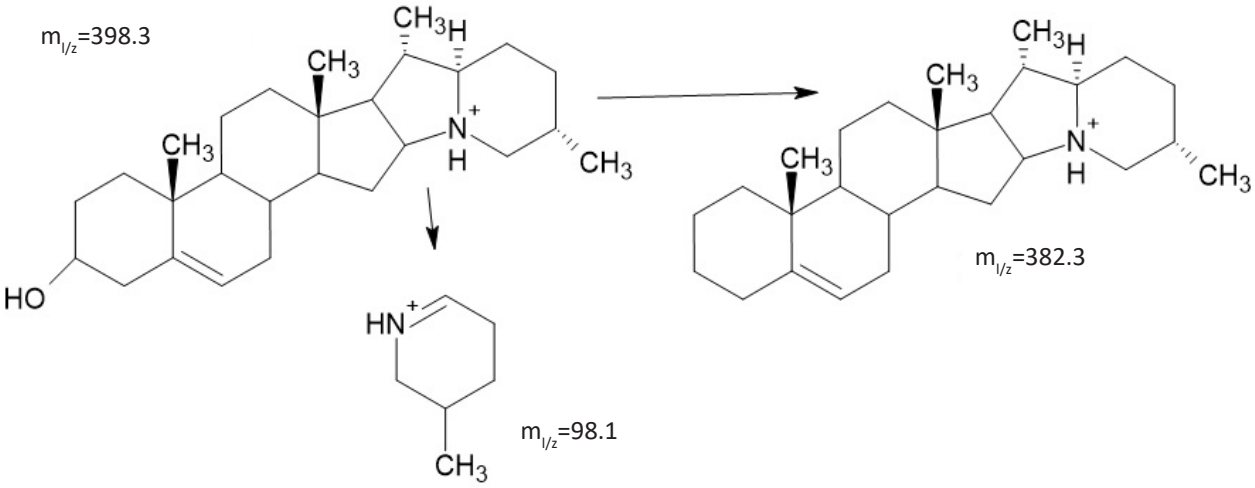

Figure 3 – Structural formula of solanidine and fragmentation of its molecule

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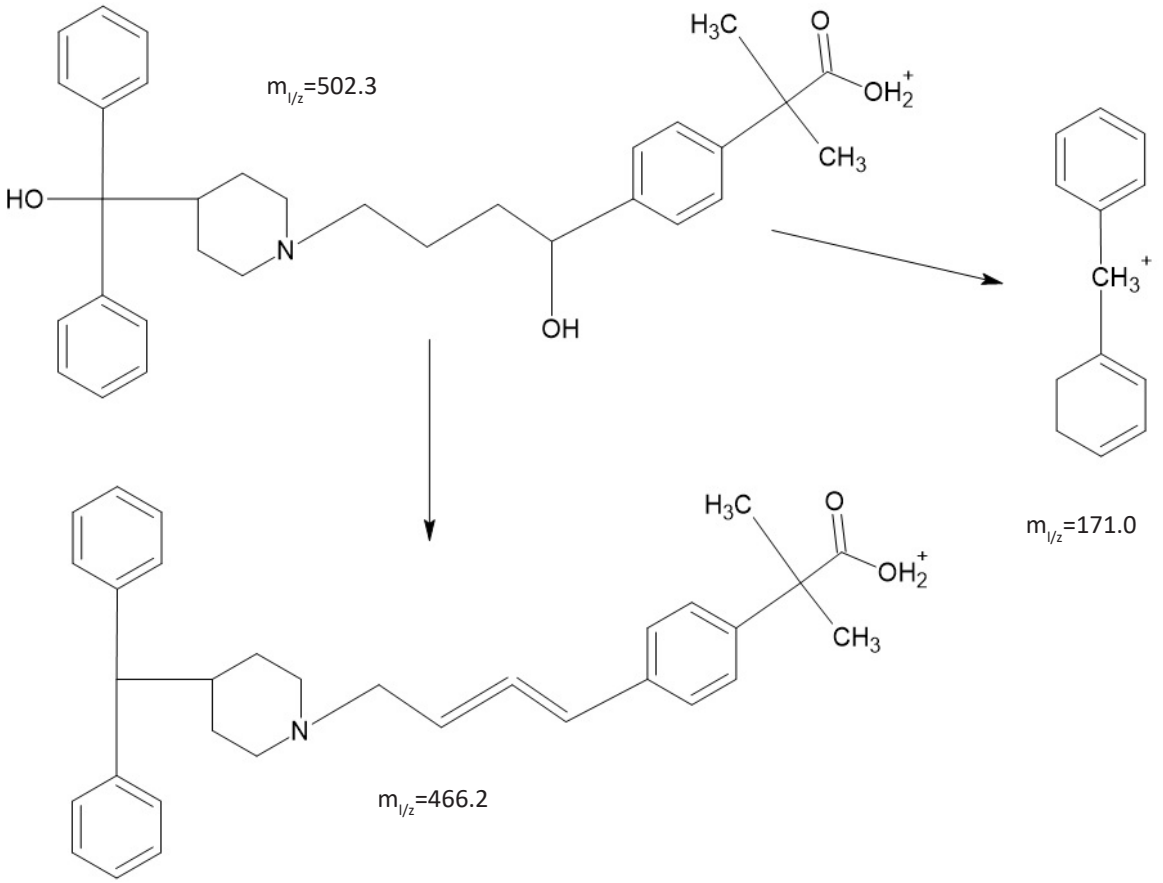

Figure 4 – Structural formula of fexofenadine and fragmentation of its molecule

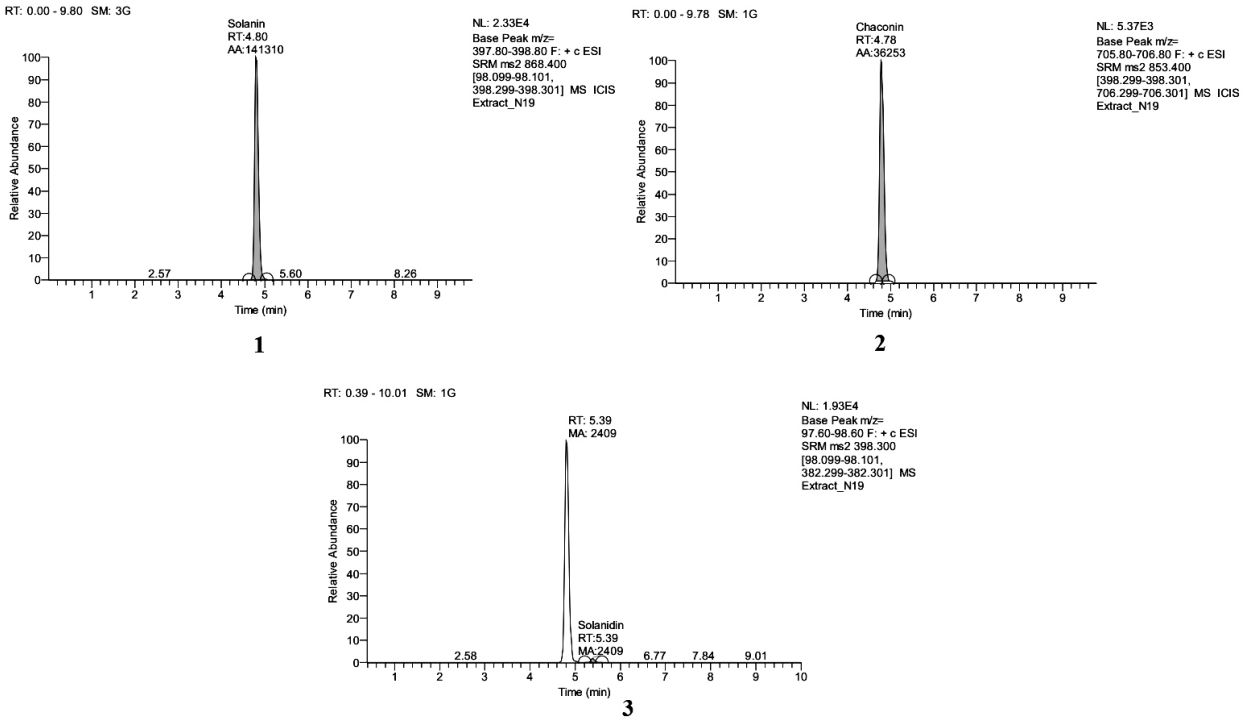

Figure 5 – Sample chromatograms of extract containing α-solanine (1), α-chaconine (2), solanidine (3)

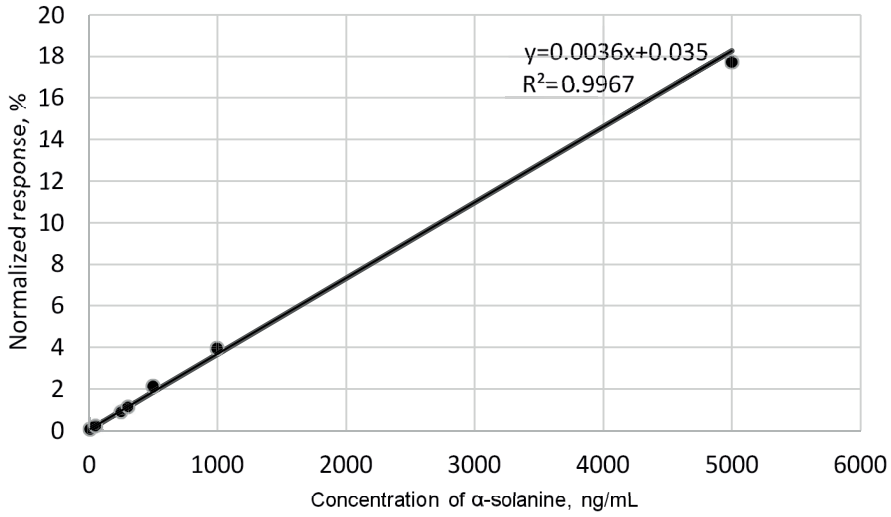

Figure 6 – Dependence graph of α-solanine normalized response on its concentration

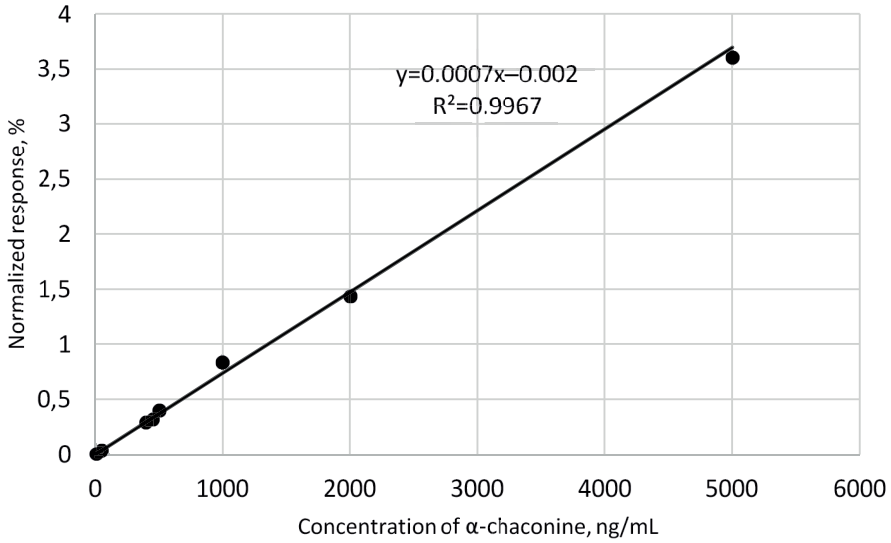

Figure 7 – Dependence graph of α-chaconin normalized response on its concentration

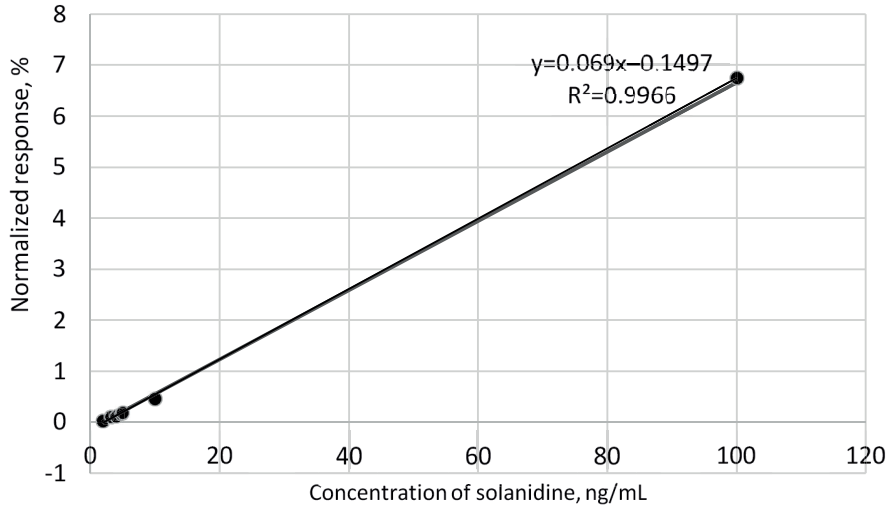

Figure 8 – Dependence graph of solanidine normalized response on its concentration

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CONCLUSION

Thus, an analytical method for the quantitative analysis of α-solanine, α-chaconine and solanidine in the extracts obtained from potato tuber peels *(Solanum tuberosum*, family *Solanaceae)* by HPLC/MS/MS has been developed and validated. The method meets the requirements of the regulatory documentation in terms of the specificity, linearity in the analytical area, correctness, precision (at the levels of a repeatability, an intralaboratory precision).

This technique can be used in the routine practice of the GAs quantification when analyzing their content in food products and compounded pharmaceuticals.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Tatiana O. Ostrikova – conducting the experimental part of the work, statistical processing of the obtained results, preparation of the preliminary version of the manuscript; Nikita G. Bogomolov – conducting the experimental part of the work, review of the literature sources on the topic of the study; Pavel Yu. Mylnikov – conducting the experimental part of the work, statistical processing of the obtained results; Alexey V. Shchulkin – approval of the final version of the manuscript; Ivan V. Chernykh – development of the study concept, approval of the final version of the manuscript. All the authors confirm that their authorship complies with the ICMJE international criteria (all the authors have made a substantial contribution to the concept development, research and preparation of the article, read and approved the final version before publication).

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