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Determination of potato glycoalkaloids and their aglycone in blood serum by high-performance liquid chromatography

Application to pharmacokinetic studies in humans

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ABSTRACT

The development of a high-performance liquid chromatography (HPLC) method for the separation and quantification of potato glycoalkaloids and their aglycone solanidine in blood serum is reported. High selectivity was obtained by using solid-phase extraction followed by off-line dual-column HPLC. Injections were made via a sample enrichment column to achieve maximum sensitivity in the assay. The potato alkaloids in the HPLC effluents were detected by ultraviolet absorption at 200 nm. The detection limits were estimated to be 0.3 ng/ml of serum for each of the alkaloids. The method was used to study the pharmacokinetics of potato glycoalkaloids in humans. α-Solanine and α-chaconine were detected in all blood serum samples collected from seven volunteers 1–25 h after a meal of potatoes. Solanidine was detected in some samples, but there were no traces of the mono- or diglycosides. The average apparent biological half-lives for α-solanine and α-chaconine were 11 and 19 h, respectively.

INTRODUCTION

The glycoalkaloids (GAs) α-solanine and α-chaconine are universally recognized as the compounds causing classical "solanine" poisoning and bitterness in potatoes. They are both triglycosides of the aglycon solanidine, a steroidal hexacyclic alkaloid derived from cholesterol (Fig. 1). The inherent concentrations of α-solanine and α-chaconine may vary substantially between tubers of different cultivars. In addition, the concentrations can increase due to unfavourable

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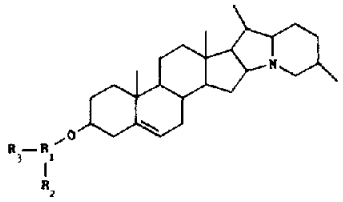

SOLANINES

α-Solanine: R_1 = D-Galactose, R_2 = L-Rhamnose, R_3 = D-Glucose

β_1-Solanine: R_1 and R_2 only

β_2-Solanine: R_1 and R_3 only

γ-Solanine: R_1 only

CHACONINES

α-Chaconine: R_1 = D-Glucose, R_2 and R_3 = L-Rhamnose

β_1-Chaconine: R_1 and R_2 only

β_2-Chaconine: R_1 and R_3 only

γ-Chaconine: R_1 only

AGLYCONE

Solanidine: R_1=H

Fig. 1. Structures of the potato glycoalkaloids and their aglycone.

conditions during growth, harvest and storage [1–3]. A survey of the GA content of Swedish potato samples from the retail market revealed concentrations of between 20 and 300 mg/kg raw unpeeled potatoes [4]. Of a total of 165 samples, nine had GA concentrations in excess of 200 mg/kg, a concentration frequently cited as the upper limit for safe consumption [5]. Peeling the tubers before cooking will normally significantly reduce the concentration of GAs in tubers [4,6].

A review of previously published works gives twelve separate outbreaks of GA poisoning, affecting more than 2000 subjects, some with fatal results [7]. The earliest incident reviewed was in 1865, and the most recent affected 78 children in a British school in 1969. The reported concentrations of GAs in the tubers range from 300 to 800 mg/kg. Using this as a basis, the toxic doses can be estimated to range from about 2 to 5 mg GA per kg body weight. The clinical symptoms observed after a latency period of 2–20 h include gastrointestinal disturbances and, in more severe instances, neurological symptoms.

The toxic dose estimates that can be made from recorded poisonings, however, must be regarded as uncertain, and there is no information available on the correlation between concentrations of GAs in blood and signs of toxicity. There is also a lack of basic pharmacokinetic data, *e.g.* on the absorption of GAs from the gastrointestinal tract. This can be attributed, among other factors, to analytical difficulties. Until a radioimmunoassay (RIA) for the determination of solanidine in human plasma was reported [8], there was no method available for the direct determination of potato alkaloids in body fluids. The RIA was later modified for the determination of solanidine and total potato alkaloids in serum and saliva samples [9,10]. Concentrations in serum samples from healthy subjects from the UK and from Sweden ranged from 3.2 to over 125 nmol/l for total potato alkaloids and from 2.5 to 92.5 nmol/l for solanidine.

The HPLC procedure presented here is the first method that allows the quantification of individual GAs and solanidine in human blood serum samples. From pilot experiments on detection limits, it was concluded that extracts from millilitre amounts of human serum, with an expected GA content in the low nanomolar range, need to be injected to obtain detectable GA peaks. Suitable detection methods are practically limited to measurements of UV absorption at wavelengths of about 200 nm, where absorption is due mainly to the Δ^5 double bond [11]. As most organic compounds absorp at a wavelength of 200 nm, this demands the development of a work-up procedure and a chromatographic system of very high selectivity. In this work, solid-phase extraction (SPE) was used to develop a highly selective procedure for the isolation of GAs and solanidine from serum samples. The extracts were then chromatographed by two-step HPLC: a fractionation step on a cyanopropyl column and an analytical step on a silica column. The sample extracts were introduced into the HPLC systems via miniaturized reversed-phase sample enrichment columns.

This paper also includes a report of the first application of the analytical procedure to pharmacokinetic studies in human volunteers. The study was designed to evaluate the applicability of the method to the determination of GA concentrations following the consumption of normal potatoes and to obtain preliminary information on GA uptake in humans. Such information is of importance for the assessment of dose–toxicity relationships.

EXPERIMENTAL

Materials

α-Solanine, α-chaconine and solanidine were purchased from Sigma (St. Louis, MO, USA). Mixtures of the mono- and diglycosides were prepared by acid hydrolysis of α-solanine and α-chaconine, respectively [12], and the compounds were identified from HPLC retention data [11]. All water used was deiononized and further purified using the Milli-Q purification system from Millipore (Bedford, MA, USA). The acetonitrile used for the preparation of the mobile phases was Fisons' (Fisons Scientific Apparatus, Loughborough, UK) "super gradient", and "far UV grade" for other purposes. The KH_2PO_4 used in mobile phases was Suprapure from E. Merck (Darmstadt, Germany). The ethanol used was 99.5% from KemEtyl (Stockholm, Sweden). All other chemicals and solvents used were of standard analytical-reagent grade.

Blood serum samples, obtained from the blood bank of the University Hospital in Uppsala, were used as blank serum. The SPE cartridges were Bond Elut C_2 containing 500 mg of ethyl phase and the cartridges were used with a Vac Elut processing station (Analytichem International, Harbor City, CA, USA).

The storage bottles and test-tubes used for the standard solutions and sample extracts were Pyrex borosilicate glass with PTFE-lined screw caps.

Apparatus and columns

The HPLC system consisted of a Waters (Milford, MA, USA) Model 6000A pump with built-in pulse dampener, a Rheodyne (Cotati, CA, USA) Model 7125 injector, an LDC/Milton Roy (Riviera Beach, FL, USA) Spectromonitor D variable-wavelength detector and a Shimadzu (Kyoto, Japan) Chromatopac C-R2AX integrator.

A silica column, 150 mm × 2 mm I.D., packed with 3-μm Sperisorb S3W (Phase Separations, Queensferry, UK) and a cyanopropyl column, 75 mm × 4 mm I.D., packed with 5-μm Hypersil CPS (Shandon Southern Products, Astmoor, UK) were used for the HPLC separations. Both were slurry-packed at 550 atm pressure using dichloromethane–hexan-1-ol–Dowfax 9N4 non-ionic surfactant (Dow Chemical, Midland, MI, USA) (70:29:1, v/v/v) as the dispersing medium and ethyl acetate as the packing medium.

LiChroCart 4-4 pre-column cartridges packed with 5-μm RP Select B phase (C_8) from E. Merck were modified as follows before use as sample enrichment columns. The cartridge was emptied and the outlet filters were replaced by a stainless-steel filter of approximately 0.1 mm thickness and 2 μm porosity. A PTFE tube, approximately 5 mm in length, 4 mm O.D. and either 2 or 3 mm I.D. was then inserted into the empty cartridge to reduce its volume and to keep the filter in place. The cartridge was then refilled with RP Select B phase by connecting the outlet end to a vacuum pump and by feeding it with propan-2-ol dispersed phase from the top with a Pasteur pipette. The inlet end was then finally resealed with another stainless-steel filter kept in place by the original PTFE ring.

The modified cartridges were used in Merck LiChroCart cartridge holders connected to the injector by short stainless-steel tubes. The sample enrichment columns were loaded and rinsed by hand pressure via the needle injection port of the injector using Hamilton gas-tight syringes (Hamilton Bonaduz, Bonaduz, Switzerland). The injector connections were arranged so that the flow direction through the enrichment column was the same when it was eluted with mobile phase from the pump, as it was upon loading. The 3 mm I.D. sample enrichment column was used with the cyanopropyl column and the 2 mm I.D. column with the silica column.

Standard solutions

A stock solution containing α-solanine and α-chaconine was prepared by dissolving 10 mg of each substance in 50 ml of methanol. Solanidine was dissolved in ethanol (10 mg per 50 ml). Diluted solutions for HPLC injections were prepared weekly by redissolving evaporated aliquots of the stock solutions in acetonitrile–0.1 *M* potassium phosphate buffer (pH 7.7) (20:80, v/v). The solutions were stored at 4°C.

Solid-phase extraction of potato alkaloids from blood serum samples

Serum (2 ml) was mixed with 3 ml of 0.37 *M* KH_2PO_4 (aqueous), and the mixture was sonicated for approximately 10 min before it was applied to a Bond Elut C_2 extraction cartridge. The cartridge had previously been activated with 2 ml of acetonitrile and washed with 5 ml of acetonitrile–26 m*M* KH_2PO_4 (aqueous) (80:20, v/v), followed by 2 ml of 0.37 *M* KH_2PO_4 (aqueous). After the sample had been passed through, the cartridge was first washed with 5 ml of 0.37 *M* KH_2PO_4 (aqueous) and then with 2 ml of acetonitrile–82 m*M* KH_2PO_4 (aqueous) (10:90, v/v). Following this, the cartridge was carefully rinsed free from any remaining salt by flushing two reservoir volumes (2 × 2.5 ml) of water through it. The water was removed from the cartridge by applying a vacuum for approximately 5 min, after which the cartridge was finally washed with 5 ml of acetonitrile–water (90:10, v/v). The potato alkaloids were then eluted with 1 ml of acetonitrile–26 m*M* KH_2PO_4 (aqeous) (80:20, v/v), and collected in a test-tube. The tube was placed in a water-bath at approximately 70°C and the eluate was evaporated to dryness under a gentle stream of nitrogen.

The solid residue was redissolved in 200 μl of acetonitrile–water (50:50, v/v) by careful agitation and sonication. The solution was then diluted and made slightly alkaline by mixing with 300 μl of 0.11 *M* K_2HPO_4 (aqueous).

HPLC separation and quantification

As a first HPLC step, the sample extracts were fractionated on a cyanopropyl column. For the introduction of extracts into the HPLC system, the injector was first turned into the load position, and the sample enrichment column replacing the loop was washed with 100 μl of water. The sample extract (500 μl) was then passed through the enrichment column, followed by 200 μl of water used for rinsing the test-tube and the syringe. The enrichment column was finally washed with 100 μl of water from a clean syringe. Then, by turning the injector to the inject position, the retained potato alkaloids were eluted from the sample enrichment column by mobile phase from the pump and were chromatographed on the cyanopropyl column.

The mobile phase consisted of acetonitrile–9.8 m*M* KH_2PO_4 (aqueous) (85:15, v/v), and was pumped at a flow-rate of 1 ml/min. The effluent was monitored at 200 nm. Two fractions of the effluent, one containing the various GAs (GA fraction) and the other containing solanidine (solanidine fraction), were manually collected at the detector outlet at intervals after injection. The volume collected for each fraction was 2 ml. The appropriate start and stop times for fractions to be collected were determined from repeated standard runs. After every ten samples, an additional standard run was performed to ensure that the retention was stable. The collected fractions were evaporated and redissolved as described in the previous section for extracts from solid-phase extraction, except that here the K_2HPO_4 solution was 57 m*M*.

In the second HPLC step, GAs and solanidine from the collected fractions were separated on a silica column and quantified. The injection procedure was identical to that described earlier. For chromatographic separation of the GA fraction the mobile phase was acetonitrile–26 m*M* KH_2PO_4 (aqueous) (80:20, v/v) and for the aglycone fraction it was acetonitrile–8.8 m*M* KH_2PO_4 (aqueous) (75:25, v/v). The flow-rate was 0.2 ml/min and the effluent was monitored at 200 nm with a 1 s detector response time. Quantifications were made by comparing the peak areas with those for known amounts of pure standards. The serum concentrations determined in human volunteers were not corrected for losses during the analytical procedure.

The eluates collected from solid-phase extraction, in addition to the effluent fractions collected in the first HPLC step, could be stored in a refrigerator for at least a week. When several samples were to be analysed, the samples were purified by solid-phase extraction on the first day and were fractionated on the cyanopropyl column on the second day. The collected GA and solanidine fractions were then submitted to the final chromatographic separation on the silica column on the third and fourth day, respectively. In this manner, up to twenty samples could be analysed in four days. The same HPLC equipment, with different columns and mobile phases, was used for all separations.

Method validation

The linearity of peak areas *versus* injected amounts was studied for α-solanine, α-chaconine and solanidine over the range 1–200 ng. Various amounts of standards were dissolved in acetonitrile–50 m*M* potassium phosphate buffer (pH 7.7; 20:80, v/v) and 0.5 ml volumes of the solutions were loaded onto the sample enrichment column and chromatographed on the silica column.

For recovery tests, blank serum was spiked with α-solanine, α-chaconine and solanidine at four concentrations (2, 10, 25 and 100 ng/ml) and was stored at −20°C until analysis. Five sets of samples, including one sample (2 ml) from each spike level, were extracted and analysed on different occasions over a two-week period. The recovery was determined by comparing the peak areas of standard solutions to those of spiked samples.

For stability tests, blank serum was spiked with α-solanine, α-chaconine and solanidine to a concentration of 20 ng/ml of each substance. Spiked samples were analysed after 0, 3, 6 and 24 h storage at 37°C and after two, four and eight weeks at −20°C.

Applications

The subjects were seven healthy male doctors and medical students associated with the Department of Medicine at the University Hospital in Uppsala. They were instructed to refrain from eating potatoes and all kinds of potato products for 48 h before beginnning the experiment and for 25 h after they had been given a single potato meal. The meal consisted of mashed potatoes prepared from two parts peeled potatoes and one part low-fat milk. It contained 200 mg/kg GA (82 and 118 mg/kg α-solanine and α-chaconine, respectively) as determined using an HPLC method [13]. The meal was taken in the morning on an empty stomach. The size of the portion served to each subject was adjusted in relation to the subject's body weight to give a total GA intake of 1 mg/kg body weight. Blood samples were collected through venepuncture prior to the potato intake and at intervals during a 25-h period. The serum samples were separated by centrifugation and stored at −20°C. The subjects were kept under medical surveillance at the hospital during the first 8 h of the experiment. The experiment was approved in accordance with Swedish regulations for tests on humans.

RESULTS AND DISCUSSION

Sample pretreatment

This SPE procedure takes advantage of the ion-exchange properties that bonded-phase silicas exhibit due to their residual free silanol groups [14]. The SPE cartridges were first used in a reversed-phase (RP) mode for extracting GAs and solanidine from blood serum samples. Neutral and acidic compounds were then eluted with a strong RP eluent (acetonitrile–water, 90:10, v/v), while the potato alkaloids were still retained on the cartridge because counter-ions had been excluded from the eluent. After this phase reversal the potato alkaloids could be eluted with a slightly weaker RP eluent containing potassium counter-ions.

To obtain the highest possible assay sensitivity, all of the extracted GAs and solanidine were introduced into the HPLC system via a sample enrichment column. From several miniature columns tested, the Merck LiChroCart system was chosen. Its small internal volume and low dead volume connections provided minimal disturbing effects on system equilibrium and low band broadening. During repeated use, however, problems with increasing back-pressure and leakage of packing material from the columns arose. These problems were solved by giving the columns a more robust design with stainless-steel end-filters.

When the evaporated SPE eluates and HPLC fractions were redissolved in water, the recovery was low and variable. This was concluded to be due to adsorption of GA and solanidine onto the surface of the glass tubes during evaporation. The recovery was significantly improved when acetonitrile was included in the solvent. By subsequent dilution with alkaline K_2HPO_4 solution, the potato alkaloids were transformed into their more lipophilic unprotonated form, and thus could still be retained on the RP enrichment column, although 20% acetonitrile was present. At a pH of 7.7 and with an acetonitrile concentration of 20%, the breakthrough volume for GAs

with the 2 mm I.D. sample enrichment column was approximately 800 μl. A higher pH could not be used as this caused precipitation of the GAs. The pK_a of solanine is 6.66 [15].

Separation of the potato alkaloids

Although only α-solanine and α-chaconine are normally present in significant amounts in the human diet, a probable metabolic route would be hydrolysis of their side-chain glycoside. The aim of this work was therefore to develop an HPLC system in which the lower glycosides and solanidine could also be chromatographed. Several types of packing material were tested with combinations of water, acetonitrile and inorganic salts as mobile phase components. This choice of mobile phase components was governed by the use of UV detection at 200 nm.

Good selectivities for the separation of the GAs were obtained with non-polar C_8 and C_{18} silica-bonded phases. Solanidine, however, was strongly retained in these systems and could only be eluted at high solvent strength as a broad and highly asymmetrical peak.

When injections were made via the sample enrichment column, problems with system peaks were experienced with the RP systems.

With bare silica, diol (2OH) and cyanopropyl (CN) phases, solanidine and the glycosides could be eluted within the same chromatographic run. These phases were used with aqueous mobile phases containing potassium counter-ions at concentrations low enough to allow ionic interactions and high concentrations (up to 85%) of acetonitrile as the organic modifier. Elution was controlled both by ionic strength and the concentration of organic modifier. Silica and 2OH gave a typical normal-phase elution order, solanidine being eluted first and α-solanine last, with α-solanine and α-chaconine well resolved from each other and from lower glycosides. Retention and selectivity were increased with increasing acetonitrile concentration. With the CN phase, the elution order was reversed, and the GAs were less resolved from each other.

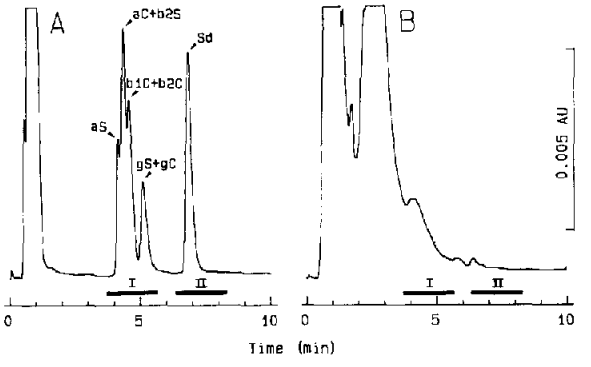

Fig. 2. Chromatograms from CN column used for fractionation of serum extracts. (A) Standard solution (injected amounts: 320 ng total glycoalkaloids and 68 ng of solanidine). (B) Extract from blank serum (2 ml). I = Interval for collection of glycoalkaloid fraction; II = interval for collection of aglycone fraction. Peaks: aS = α-solanine; b2S = β_2-solanine; gS = γ-solanine; aC = α-chaconine; b1C = β_1-chaconine; b2C = β_2-chaconine; gC = γ-chaconine; Sd = solanidine. Column: Hypersil CPS 5 μm, 75 mm × 4 mm I.D. Mobile phase: acetonitrile–9.8 m*M* KH_2PO_4 (aqueous) (85:15, v/v). Flow-rate: 1 ml/min. UV detection: 200 nm.

The highest selectivity between GAs and other serum extract components was achieved with bare silica, but the selectivity was not high enough to allow direct determinations at the desired sensitivity. A two-step HPLC procedure was therefore developed. The CN column was chosen for the first step. Advantage was taken of its relatively low selectivity for separation of the GAs (Fig. 2). The mobile phase composition was optimized so that the GAs could be collected in a single fraction; solanidine was collected in a second fraction. The GAs in the fractions were then separated and quantified in a second HPLC system using the silica column. This yielded chromatograms free from interfering peaks and with a stable horizontal baseline (Fig. 3). The silica column gave baseline resolution for α-solanine, α-chaconine, β_2-solanine and γ-chaconine, whereas β-chaconines and γ-solanine were eluted partly unresolved. The chromatographic behaviour of β_1-solanine was not studied, as it could not be obtained by acid hydrolysis. Different mobile phase compositions were used for chromatographic separation of the GA and aglycon fractions. Substances in the aglycon fraction with retention times close to solanidine prompted the use of a mobile phase with a lower elution strength than was needed for the separation of the GAs.

When not in use, the columns were filled with pure acetonitrile and were stored in a refrigerator. The columns could be used for several

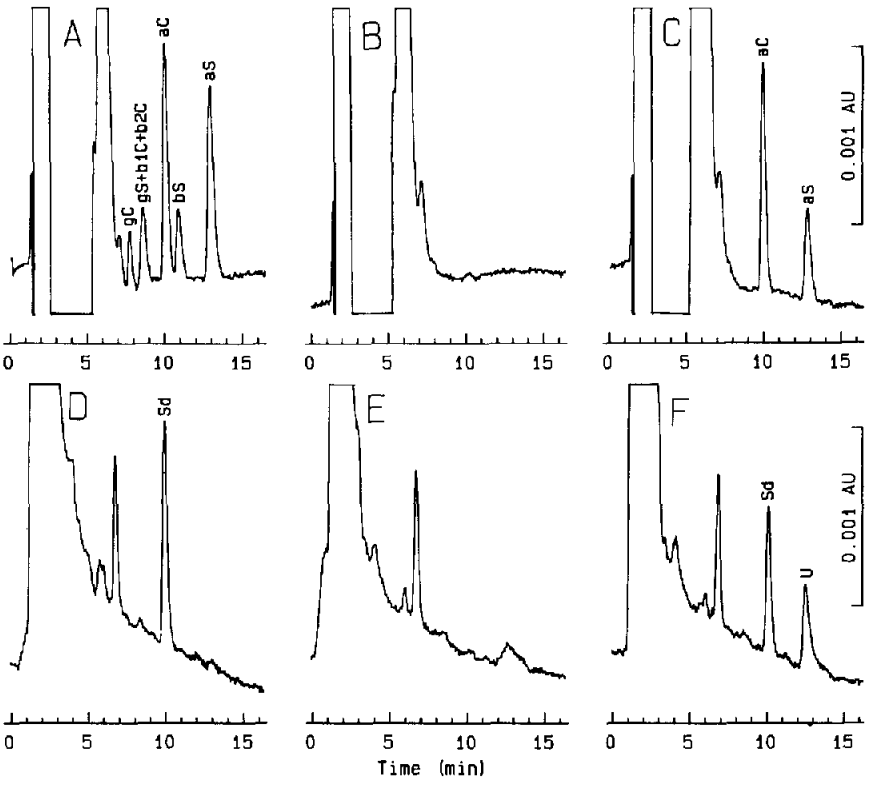

Fig. 3. Chromatograms of HPLC-fractionated serum extracts on a silica column. (A) Glycoalkaloid fraction from blank serum sample spiked with a total of 20 ng GA per ml serum. (B) Glycoalkaloid fraction from volunteer serum following 48 h of potato avoidance. (C) Glycoalkaloid fraction from volunteer serum collected 8 h after a potato meal. (D) Solanidine fraction from blank serum spiked with 4.5 ng solanidine per ml serum. (E) Solanidine fraction from volunteer serum following 48 h of potato avoidance. (F) Solanidine fraction from volunteer serum collected 8 h after a potato meal. Peaks: aS = α-solanine; bS = β_2-solanine; gS = γ-solanine; aC = α-chaconine; b1C = β_1-chaconine; b2C = β_2-chaconine; gC = γ-chaconine Sd = solanidine; U = unknown compound. Column: Hypersil S3W 3 μm, 150 mm × 2 mm I.D. Mobile phases: acetonitrile–26 m*M* KH_2PO_4 (aqueous) (80:20, v/v) for glycoalkaloid fractions; acetonitrile–8.8 m*M* KH_2PO_4 (aqueous) (75:25, v/v), for solanidine fractions. Flow-rate: 0.2 ml/min. UV detection: 200 nm.

months without major changes in the retention characteristics or impairment of the peak shapes.

Method validation

The detector peak area response was linear for injected amounts in the range 1–200 ng. The calibration graphs showed good correlation coefficients ($r^2 \geqslant 0.9992$) and the intercepts were not significantly different from zero ($p > 0.05$).

Results from the recovery experiments with spiked blank serum are summarized in Table I. The average recoveries over the range 2–100 ng/ml serum were 88, 90 and 84% for α-solanine, α-chaconine and solanidine, respectively. The between-day variation of replicates ($n = 5$) was from 1.2 to 7.5%, expressed as coefficients of variation. Linear regression of concentration found on concentration added gave good correlation coefficients ($r^2 \geqslant 0.995$) and the intercepts were not significantly different from zero ($p > 0.05$).

The limit of detection was approximated to 0.3 ng/ml for all three substances when 2 ml of serum were used (three times the baseline noise of blank serum). The concentrations of α-solanine, α-chaconine and solanidine in serum were all stable during the test periods of 24 h at 37°C and two months at −20°C.

Applications

α-Solanine and α-chaconine could be detected in all blood serum samples collected from seven volunteers 1–25 h after an intake of 0.4 and 0.6 mg/kg body weight of α-solanine and α-chaconine, respectively, in a single meal of potatoes.

TABLE I

RESULTS OF THE RECOVERY EXPERIMENT USING HUMAN BLOOD SERUM SPIKED WITH α-SOLANINE, α-CHACONINE AND SOLANIDINE

Concentration added (ng/ml)	Concentration found[a] (ng/ml)	Recovery (%)	Coefficient of variation (%)
α-Solanine			
2	1.83	91.5	4.5
10	8.63	86.3	3.2
25	21.8	87.3	1.2
100	88.3	88.3	3.3
α-Chaconine			
2	1.86	93.0	7.5
10	8.85	88.5	3.9
25	22.3	89.1	4.0
100	88.0	88.0	3.4
Solanidine			
2	1.75	87.6	7.5
10	8.19	81.9	2.4
25	21.1	84.5	4.0
100	81.3	81.3	6.2

[a] Average from five replicates at each spike level analysed on different days.

Detectable amounts of solanidine also appeared after 4–8 h in all subjects, but there were no traces of the β- or γ-glycosides. Average serum profiles are shown in Fig. 4. The occurrence of solanidine might be explained as a result of metabolization of α-solanine or α-chaconine. The presence of an unidentified substance with a retention time between those for γ-chaconine and solanidine on both the CN and silica columns was also noted (Fig. 3). The substance was only detected in samples containing solanidine, and its largest peaks were found in samples collected after 25 h.

The peak serum concentrations for α-solanine and α-chaconine were on average 7.7 and 14.4 ng/ml (8.8 and 16.9 nmol/l), respectively, and were on average reached after 5.1 and 6.0 h, respectively (Table II). The highest solanidine values during the 25-h period were found at 8 or 25 h after the potato meal and varied among the subjects between 1.0 and 4.8 ng/ml (2.5 and 12 nmol/l). The kinetic profiles for α-solanine and α-chaconine were fairly similar among the subjects, as can be seen from the small variations in biological half-lives. It should be pointed out that the calculated biological half-lives are estimates related to the observed time frame. More appropriate determinations would have required serum profiles to be followed for more than 25 h.

The biological half-lives for α-chaconine were longer than those for α-solanine (means 19.1 and 10.7 h, respectively). The slower turnover of α-chaconine was also apparent from the α-solanine/α-chaconine quotient, showing a steady decline during the experiment. In samples collected 1 h after the intake, this quotient was on average 0.81 and after 25 h it was 0.29. The quotient in the potatoes consumed was 0.69.

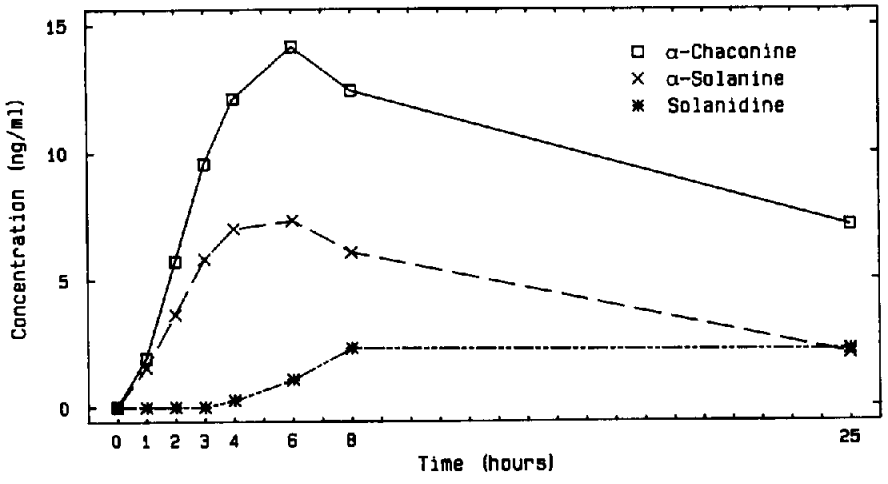

Fig. 4. Average blood serum concentrations of α-solanine, α-chaconine and solanidine for seven volunteers for 25 h after an intake of 0.4 and 0.6 mg/kg body weight of α-solanine and α-chaconine, respectively, from a single potato meal.

TABLE II

PHARMACOKINETIC DATA FOR POTATO GLYCOALKALOIDS IN BLOOD SERUM FROM SEVEN VOLUNTEERS AFTER AN INTAKE OF 0.4 AND 0.6 mg/kg BODY WEIGHT OF α-SOLANINE AND α-CHACONINE, RESPECTIVELY, IN A SINGLE POTATO MEAL

Subject	α-Solanine			α-Chaconine		
	C_{max} [a] (ng/ml)	T_{max} [b] (h)	$T_{\frac{1}{2}}$ [c] (h)	C_{max} [a] (ng/ml)	T_{max} [b] (h)	$T_{\frac{1}{2}}$ [c] (h)
L.L.	5.3	4	13.9	8.8	4	19.7
P.W.	7.1	6	9.6	12.5	6	16.9
B.L.	9.0	6	10.2	17.5	6	21.1
P.G.	9.8	6	11.5	18.9	8	20.1
P.B.	3.9	4	10.0	6.2	6	19.3
M.A.	7.3	6	10.1	15.8	6	16.1
B.K.	11.3	4	9.7	21.4	6	20.4
Mean	7.66	5.1	10.71	14.44	6.0	19.09
S.D.	2.58	1.1	1.54	5.52	1.2	1.88

[a] Maximum blood serum concentration.
[b] Time to reach maximum blood serum concentration.
[c] Biological half-live.

All except one of the volunteers (B.K.) complained about an unpleasant off-taste on ingestion of the potato meal. The nature of the off-taste was consistent with the sensory characteristics described previously for GAs [16,17], *e.g.* bitterness and a burning sensation in the throat. The same subjects also reported signs of toxicity, consisting mainly of light to severe nausea, in one case in combination with diarrhoea (P.G.). In most instances the symptoms started within the first 30 min after the meal and lasted for 3–4 h. No positive correlation between the severity or duration of clinical toxicity symptoms and concentrations of GAs in the serum samples was apparent. Moreover, the rapid onset and short duration of the symptoms in relation to the GA serum profiles suggest that the symptoms might have been mediated by local direct effects on the intestines rather than by systemic interactions. It should be emphasized that the experiment was not particularly designed to study the toxic effects of the GAs (*e.g.* a double-blind study), and therefore the placebo effect cannot be excluded.

The concentration of GAs in the serum samples, and particularly those of solanidine, found in this study were lower than expected from the results of the earlier investigation using RIA [9]. However, the design of the RIA study (a week-long intake at an unspecified dose level) makes comparison difficult. Furthermore, the specificity of the anti-serum used in the RIA appears to be directed against a limited portion of the aglycone molecule, and the possibility of a cross-over reaction with unknown GA metabolites cannot be excluded.

CONCLUSIONS

The HPLC method described here shows a satisfactory specificity, sensitivity and reproducibility to be used in pharmacokinetic and clinical investigations of potato alkaloids. For the first time the concentrations of α-solanine and α-chaconine in blood serum could be monitored in humans after the ingestion of potatoes. Further development of the analytical procedure to make it less tedious, *e.g.* in terms of automating the solid-phase extraction and column-switching procedures, would improve its applicability to routine analytical work.

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