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Comparison of commercial solid-phase extraction sorbents for the sample preparation of potato glycoalkaloids

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Abstract

In this study five different commercial sorbents C_{18}, SCX, CN, Certify and Oasis HLB were compared for the solid-phase extraction of potato glycoalkaloids. The recoveries were determined using α-solanine, α-chaconine and α-tomatine, which contained dehydrotomatine as an impurity, as standard compounds. The samples were analysed by reversed-phase liquid chromatography under gradient elution conditions using a Zorbax Rx-C_{18} column and acetonitrile–25 m*M* triethylammonium phosphate buffer (pH 3.0) as the mobile phase. The highest recovery (~100%) was achieved with Oasis HLB (60 mg) cartridges. An acetic acid extract of wild *Solanum brevidens* leaf material was used for the testing of a clean-up procedure. The SCX proved to be the most selective and efficient for removing the undesired components from the leaf extract.

Keywords: *Solanum brevidens*; Extraction methods; Solid-phase extraction; Glycoalkaloids; Alkaloids

1. Introduction

Steroidal glycoalkaloids (SGAs) are present in all *Solanum* species, e.g., in the cultivated potato *Solanum tuberosum*. An SGA aglycone consists of a non-polar steroid unit and either indolizidine or oxa-azaspirodecane basic portion. Natural SGAs have a tri- or tetrasaccharide moiety attached to the 3-position of the aglycone. It is assumed that the role of the SGAs is to protect the plant from abiotic and biotic stress. On the other hand, the SGAs are toxic to mammals [1]. The recommended safety limit for the total SGA content in potato cultivars is 200 mg/kg fresh mass of tubers. The commercial varieties do not generally exceed this limit. The genome and growing conditions have the primary effect on the types and concentration of SGAs.

Since wild potato species showing divergent SGA profiles are used in potato breeding, new types of SGAs are introduced into *S. tuberosum*. The commonly known SGAs in *S. tuberosum* are α-solanine and α-chaconine. For example, the wild species *Solanum brevidens* has been found to contain α-tomatine, dehydrotomatine and other minor components [2,3]. The dried leaves of the interspecific hybrid *S. brevidens*(+)*S. tuberosum* contain these SGAs and some minor compounds at 700 mg/kg of dry matter [4] or SGA aglycones even at 7700 mg/kg of dry matter [5].

The extraction of SGAs from different kinds of tuber materials with several solvent systems has been evaluated by Friedman and McDonald [6]. Precipitation with ammonia is often used for clean-up of SGAs prior to liquid chromatographic analysis. This

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method, however, is not a quantitative method since some of the SGAs, e.g., α-chaconine, are still found in liquid phase at pH 10 [7]. Furthermore, a successful precipitation procedure is largely dependent on the concentration of SGAs in the extract. For liquid–liquid extraction (LLE) of SGAs from aqueous media butanol has been used by Dao and Friedman [8]. The most commonly used clean-up method for SGA determination by liquid chromatography (LC) is solid-phase extraction (SPE). In most cases, silica-based octadecyl (C_{18}) [9,10] or amino (NH_2) [11] sorbents have been used for sample preparation from potato tuber extracts. Jonker et al. have reported the clean-up of tuber extracts also with cyano (CN), phenyl (Ph) and octyl (C_8) cartridges [12]. The efficient clean-up method is especially needed for green plant extracts because of pigments and other undesired components. SPE is a powerful method to concentrate and purify the analytes of the complicated matrixes if the procedure is optimised.

The aim of this study was to develop an SPE clean-up procedure for determination of SGAs by LC. The recoveries for α-solanine, α-chaconine, tomatine and dehydrotomatine, using five different SPE sorbents with different retention mechanisms, were determined by our LC method [13].

The selected sorbents were a non-polar C_{18}, a benzenesulphonate cation exchanger (SCX), a polar cyanopropyl (CN), a combined C_8 and SCX (Certify) and a macro porous copolymer (Oasis HLB).

The acetic acid extract of *S. brevidens* leaves was cleaned up with all the sorbents mentioned above. An external standard method was used for the semi-quantitative analysis of *S. brevidens* SGAs. The selectivity of the sorbents and their efficiency to remove undesired compounds are also discussed.

2. Experimental

2.1. Chemicals and reagents

α-Solanine (purity approx. 95%), α-chaconine (approx. 95%) and tomatine (no label purity), obtained from Sigma (St. Louis, MO, USA) were used as standard compounds.

For HPLC experiments distilled water was further purified with Gelman's Water I apparatus (Gelman Sciences, Ann Arbor, MI, USA). HPLC-grade acetonitrile (Labscan, Dublin, Ireland) was filtered through a 0.45-μm membrane filter (Millipore, Milford, MA, USA) and thoroughly degassed with helium bubbling. 1.0 *M* Triethylammonium phosphate (TEAP) buffer in water was obtained from Fluka (Buchs, Switzerland). A 25 mmol TEAP eluent of pH 3.0 was prepared by diluting 25 ml of 1.0 *M* TEAP to 1000 ml with water. The pH of the buffer was measured before addition of the organic modifier.

For the extraction 5% acetic acid (AcOH) was diluted from 99.5% acetic acid (Riedel-de Haen, Seelze, Germany). For the SPE experiments, 2.5% ammonia (NH_3) in methanol was prepared from 25% ammonia (FF-Chemicals, Yli-Ii, Finland). HPLC-grade methanol (MeOH) was obtained from J.T. Baker (Deventer, The Netherlands).

2.2. Sample preparation and the SPE procedure

A mixture of four compounds for the recovery determinations was prepared by dissolving 25 mg of α-solanine, 25 mg of α-chaconine and 50 mg of tomatine, which contains dehydrotomatine [14], in 200 ml of 5% acetic acid. Aliquots (2 ml) of this solution were applied to SPE cartridges. In addition, 2-ml aliquots were taken from the standard mixture and evaporated to dryness under an argon stream at 50–60°C. The residue was dissolved in 1 ml of methanol and this was used as a reference mixture for the LC analyses.

The dried leaves of *S. brevidens* ($2n=2x=24$) CPC 2451 were ground to fine powder. A 500-mg amount of the powder was extracted with 100 ml of 5% acetic acid by application of ultrasonication for 10 min at room temperature and finally the suspension was filtered. From the filtrate, 2-ml aliquots were evaporated to dryness under an argon stream at 50–60°C. The residue was dissolved in 250 μl of methanol and this was used as a reference sample in the SPE recovery determinations. The rest of the filtrate was applied to the pre-conditioned SPE cartridges. In separate experiments quantitation of the acetic acid extraction of dried leaves of *S. brevidens* was studied by adding 5 mg of pure α-chaconine as an internal standard to the acetic acid suspension and treating it in the same manner as the

reference sample. The result was controlled with a standard solution containing 1 mg of α-chaconine in 1 ml of methanol.

The SPE cartridges used in this study were: Bond Elut C_{18} (100 mg, Varian, Harbor City, CA, USA), SCX (100 and 500 mg, Varian), Certify (300 mg, Varian), Oasis HLB (60 mg, Waters, Milford, MA, USA) and CN (500 mg, J.T. Baker). The recovery determinations were performed using three cartridges of each sorbent type and the results were calculated as the mean value of three injections per cartridge.

The preconditioning, loading, washing and eluting conditions for the SPE sorbents are described in Table 1. All the solvent volumes are chosen to be at least the given bed volume. The total amount for each cartridge was 1 mg of pure SGAs/100 mg of sorbent or 2 ml of extract/100 mg of sorbent, except for the Oasis HLB cartridges (1 mg/60 mg and 2 ml/60 mg) and for the 500 mg SCX cartridges (1 mg/500 mg and 2 ml/500 mg), respectively. The eluates were evaporated to dryness under the argon stream. The residue of standard mixture was dissolved in 1 ml (2 ml loading), 3 ml (6 ml loading) or 5 ml (10 ml loading) of methanol and the extraction samples in 250 μl (2 ml loading), 750 μl (6 ml loading) or 1250 μl (10 ml loading) of methanol. All the SPE experiments were performed at room temperature.

2.3. HPLC instrumentation and separation conditions

A Hewlett-Packard HP 1090A liquid chromatographic system (Waldbronn, Germany) with an autoinjector, a built-in diode-array detection (DAD) system HP 1040A, a HP 3392A integrator, a HP 85B computer control and a HP 9121D disk memory was used. The effluent from the column was monitored at 205 nm. The on-line UV absorbance spectral data were collected from 190 to 400 nm with DAD. The analytical chromatography column was a Zorbax Rx-C_{18} (250×4.6 mm I.D.) of 5 μm particle size (Hewlett-Packard, Rockland Technologies, Newport, DE, USA). The gradient elution with acetonitrile–buffer followed the stepwise gradient of 20, 25, 35, 45 and 65% acetonitrile at times 0, 12, 15, 17 and 25 min. The equilibration time between the runs was at least 8 min. The columns were operated at a constant temperature of 40°C. The flow-rate of eluent was 1.0 ml/min. The injection volume for the recovery determinations was 10 μl. All the results are calculated from the peak areas.

3. Results

Table 2 shows the recoveries of SGAs with

Table 1
The SPE conditions on different sorbents[a]

Sorbent	Preconditioning	Sample (ml)	Washing	Elution	Final volume of extract (μl)
C_{18} (100 mg)	1 ml MeOH 1 ml 5% AcOH	2	1 ml 5% MeOH	1 ml MeOH	250
SCX (100 mg)	1 ml MeOH 1 ml Water	2	1 ml 5% MeOH	1 ml 2.5% NH_3 in MeOH	250
SCX (500 mg)	2 ml MeOH 2 ml Water	2	1 ml 5% MeOH	1.5 ml 2.5% NH_3 in MeOH	250
Certify (300 mg)	2 ml MeOH 2 ml Water	6	3 ml 5% MeOH	3 ml 2.5% NH_3 in MeOH	750
Oasis (60 mg)	1 ml MeOH 1 ml Water	2	1 ml 5% MeOH	1 ml MeOH	250
CN (500 mg)	2 ml MeOH 2 ml 5% AcOH	10	3 ml 5% MeOH	5 ml 25% AcOH in MeOH	1250

[a] The loaded sample in 5% AcOH was a standard mixture of α-solanine, α-chaconine, α-tomatine and dehydrotomatine (100 mg/200 ml) or a sample of *S. brevidens* leaf extract (500 mg of dried leaves/100 ml).

Table 2
The recoveries (%) of standard SGAs on different SPE sorbents determined by LC[a]

Sorbent	Recovery (%)			
	Dehydrotomatine	α-Tomatine	α-Solanine	α-Chaconine
C_{18} (100 mg)	98±0.6	97±0.7	67±5.4	84±4.0
SCX (100 mg)	89±12.0	99±1.0	95±1.5	96±1.5
SCX (500 mg)	63±6.6	69±1.4	59±0.7	69±2.8
Certify (300 mg)	92±28	83±23	67±38	76±32
Oasis (60 mg)	106±12	104±6.0	104±2.9	102±1.3
CN (500 mg)	59±2.1	70±3.4	67±5.1	63±2.9

[a] The results are the mean percentage recoveries of standards±standard deviations, where $n=3$.

different SPE cartridges. The results of Table 2 indicate that the highest recoveries of SGA standards are achieved for all the compounds using Oasis HLB (60 mg), with virtually 100% recovery. C_{18} (100 mg) and SCX (100 mg) also gave very high recoveries for all the compounds apart from the recovery of α-solanine with C_{18} (100 mg) cartridge. Certify (300 mg) showed high recoveries 83% and 92% for tomatine and dehydrotomatine and slightly lower for α-solanine and α-chaconine. This cartridge showed exceptionally low reproducibility. SCX (500 mg) and CN (500 mg) gave the lowest recoveries for all the compounds.

The repeatability of three successive injections from each cartridge eluate as relative standard deviation (RSD) was 2.3% for α-solanine, 2.5% for α-chaconine, 4.8% for tomatine and 9.4% for dehydrotomatine.

The recovery for α-chaconine added to the extraction suspension was approximately 90% calculated from the α-chaconine standard. The extract of *S. brevidens* contained two main compounds, dehydrotomatine and tomatine on the basis of the report by Laurila et al. [5] and our preliminary results. The SPE recoveries of *S. brevidens* compounds on different SPE sorbents are listed in Table 3.

The total SGA content of *S. brevidens* leaves was 1.4±0.2% (w/w) of dry mass, which is in accordance with value reported by Laurila et al. [5]. Pure dehydrotomatine and α-tomatine standards are needed for the accurate quantitative analysis.

Fig. 1 shows representative chromatograms of the *S. brevidens* extract sample before the clean-up and after SPE procedure with C_{18}, Certify and SCX. The chromatograms of the extract sample after Oasis or CN SPEs were similar to that of C_{18}.

4. Discussion

The 5% acetic acid was chosen for the extraction of SGAs from the plant material, because it is an inexpensive and non-toxic solvent. The acetic acidic sample can be applied into the most SPE sorbents. The SGAs are in ionized form in this solvent, which is prerequisite for their retention in SCX.

The possible reasons for the big differences in the recoveries between the 100 mg and 500 mg SCX cartridges are too small elution volume for the latter and varying elution rates when using manual operation.

The SGAs are recommended to be eluted with 100% methanol or ammonia–methanol to avoid acidic environment if the eluates are stored before the analyses. The SGAs did not elute from CN at all when methanol was used as elution solvent, so 25% acetic acid in methanol had to be used as reported previously by Jonker et al. [12].

The interactions between the SGAs and the sorbent can not be explained only with ionic interac-

Table 3
The recoveries (%) of dehydrotomatine and α-tomatine of *S. brevidens* extract on different SPE sorbents determined by LC[a]

Sorbent	Recovery (%)	
	Dehydrotomatine	α-Tomatine
C_{18} (100 mg)	69±2.1	61±15.6
SCX (100 mg)	99±3.5	86±14
SCX (500 mg)	49±2.2	47±5.6
Certify (300 mg)	109±4.2	114±2.8
Oasis (60 mg)	103±2.8	110±13
CN (500 mg)	48±4.9	44±5.7

[a] The results are the mean percentage recoveries of standards±standard deviations, where $n=3$.

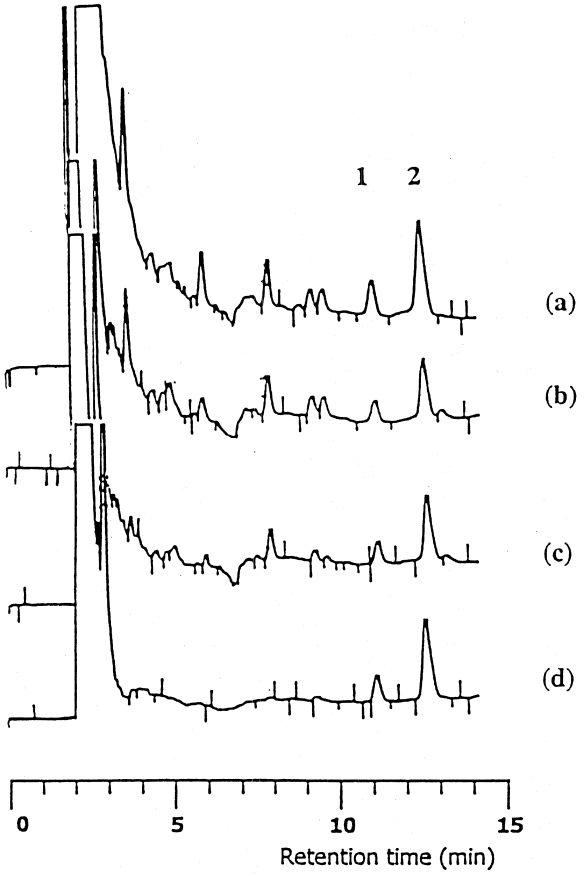

Fig. 1. The RPLC chromatograms of *S. brevidens* extract (a) before the clean-up, and after the SPE with (b) C_{18}, (c) Certify and (d) SCX sorbents. Chromatographic conditions: column, Zorbax Rx-C_{18} (250×4.6 mm I.D.), 5 μm; column temperature 40°C; flow-rate 1 ml/min; stepwise acetonitrile–25 m*M* TEAP buffer (pH 3.0) gradient 20, 25, 35, 45 and 65% acetonitrile at times 0, 12, 15, 17, 25 min, respectively; UV absorbance detection at 205 nm. Peaks: 1=dehydrotomatine and 2=α-tomatine.

tions in SCX, Van der Waals forces between the steroidal and C_{18} groups or possible hydrogen bonding between hydroxyl groups and CN. Interactions between free silanol groups in silica-based sorbents and basic SGAs may cause lower recoveries. The amount of residual surface silanols are dependent on the sorbent manufacturer.

The approximate content of SGAs in plant sample has to be estimated before SPE application because the interferences in the sample matrix reduce the capacity of the sorbent. The overloading causes the loss in the recovery.

The high recovery, selectivity and reproducibility are the most important factors when selecting the SPE cartridge. In our experiments, SCX sorbent was the most selective with a high recovery for SGAs. For C_{18}, CN, Certify and Oasis HLB sorbents the washing or eluting conditions should be changed to eliminate undesired compounds from potato leaf extract.

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