Combination of On-Line Solid-Phase Extraction with LC-MS for the Determination of Potentially Hazardous Glycoalkaloids in Potato Products

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Abstract A simple and rapid method for the determination of naturally occurring, potentially hazardous glycoalkaloids (GAs) in potatoes and their products has been developed. The procedure is based on the on-line solid-phase extraction of the acetic acid extracts from potato products and combined with liquid chromatography (LC)-mass spectrometry (MS) in a fully automated system (Symbiosis™, Spark Holland Instruments, Emmen, The Netherlands). As sorbent material HySphere™ 18HD was used for alkaloid enrichment. GAs were eluted with the LC gradient and directly analysed by MS. Detection of the analytes was achieved in the sensitive multiple reaction monitoring mode using two characteristic ions (m/z 98 as a qualifier for GAs and m/z 868.3 as a quantifier for α-solanine or m/z 852.4 for α-chaconine). Typical validation data for method precision (v_k α-solanine=5.3–6.5, v_k α-chaconine= 3.4–15.4), accuracy (average recovery of α-solanine=84%, average recovery of α-chaconine=87%) and linearity over the range from 1 to 1,000 ng ml^{-1} (R^2=0.9915 for α-solanine, R^2=0.9939 for α-chaconine) with detection limits of 0.3 ng ml^{-1} for α-chaconine and 0.5 ng ml^{-1} for α-solanine were obtained. GA contents of commercial potato products were determined by the new on-line method and afterwards compared with those obtained with an established high-performance LC routine procedure. Better performance of the on-line procedure was obvious from the standard deviations of both methods. Other advantages included a strong reduction of overall analysis time, human intervention and solvent consumption as well as waste production. The time required for the on-line analysis was 5 min, which would allow processing of almost 100 samples in 8 h.

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Abbreviations

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dw	Dry weight
GA	Glycoalkaloid
HPLC	High-performance liquid chromatography
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MRM	Multiple reaction monitoring
MS	Mass spectrometry
R^2	Coefficient of determination
SPE	Solid-phase extraction
v_k	Coefficient of variation
XLC	Extraction-liquid chromatography

Introduction

As a food plant, the domesticated potato is of global importance. Whereas direct consumption of potato tubers has decreased in developed countries for years (West Germany 1961–1962, 130 kg per capita and year; Germany 2003–2004, 67 kg; ZMP 2005), potato products such as French fries, potato wedges, pancakes, dumplings or mashed potatoes are in great demand. Today about 40% of the potato tubers used in the food industry are processed to several convenience products (ZMP 2005).

Despite their status as food products, potato tubers contain potentially hazardous glycoalkaloids (GAs), which are the result of an evolutionary selection process and serve as defence compounds against herbivores and pathogens (Gemeinholzer and Wink 2002; Wink 2003). Major alkaloids in commercial cultivars are α-solanine and α-chaconine; together they account for 95% of total GAs in tubers. As Fig. 1 illustrates, the two compounds consist of the aglycone solanidine, which has an non-polar lipophilic steroid nucleus fused with two nitrogen-containing heterocyclic rings. The glycosides carry a polar, water-soluble trisaccharide moiety at C-3 (Lachman et al. 2001). This can consist of a glucose, galactose and rhamnose molecule as in α-solanine or two rhamnose molecules and a glucose molecule as in α-chaconine.

The presence of GAs in aerial parts is a desirable trait, because it has been linked to resistance to fungi, bacteria or insects (Uppal 1987), whereas high GA accumulation in tubers can be hazardous. Consumption of GAs at levels >5 mg kg^{-1} body weight leads to poisoning in mammals and other vertebrates (Friedman and McDonald 1997; Maga 1994). Molecular modes of action include the destabilization of cholesterol-containing cell membranes, leading to gastrointestinal disturbances, and inhibition of acetylcholine and butyrylcholine esterase, causing impairment of neuronal functions (Keukens et al. 1995; Friedman and McDonald 1997; Mensinga et al. 2005). At lower doses, mainly gastrointestinal disturbances such as vomiting, diarrhoea and abdominal pain have been reported, whereas at higher doses the toxicity leads to severer

Fig. 1 Structures of the main potato glycoalkaloids α-solanine and α-chaconine with their mass spectrometry (MS) fragmentation patterns (according to Stobiecki et al. 2003)

α-Solanine

$C_{45}H_{73}NO_{15}$

Mw: 868.04 g/mol

α-Chaconine

$C_{45}H_{73}NO_{14}$

Mw: 852.07 g/mol
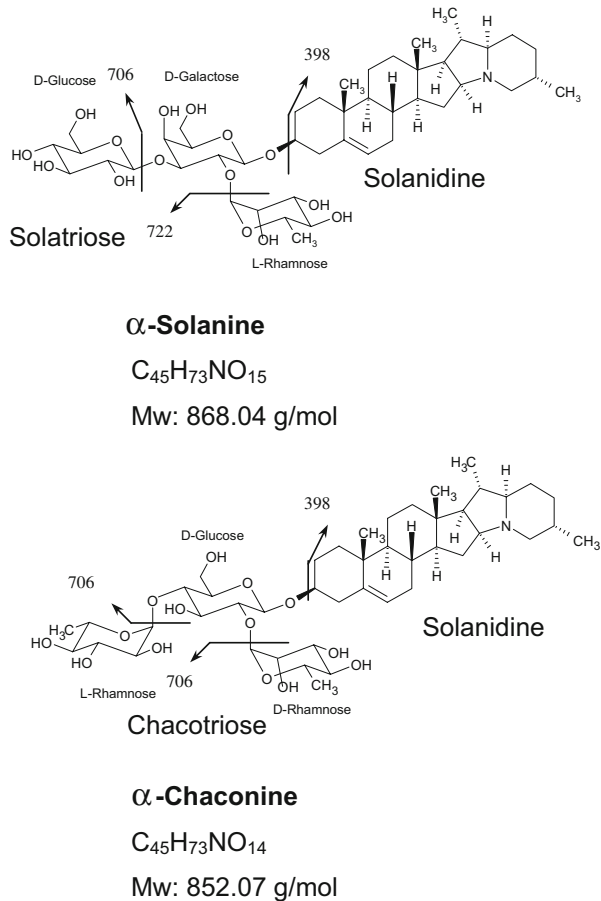
symptoms, including fever, tachycardia, hypotension and neurological disorders (Rayburn et al. 1994). Several cases of lethal poisoning by GAs have been reported (McMillan and Thompson 1979; Morris and Lee 1984). For protection of the consumer, it has been agreed that the GA contents in tubers should be <20 mg GA 100 g^{-1} fresh weight (Lachman et al. 2001).

The highest GA concentrations in potato tubers are found in peels, eyes and sprouts (Uppal 1987). Depending on the potato variety, GA contents can increase after harvest to dangerous levels if tubers are damaged or if they are stored in the light and become green (Papathanasiou et al. 1998; Şengül et al. 2004). In convenience products, the alkaloid content mainly depends on the initial GA level in the raw material, because GAs are largely heat stable (Jadhav and Salunkhe 1975); Porter (1972) mentioned a decomposition temperature of 260–270 °C, which is 70–80 °C above ordinary frying temperatures. Higher levels occur when unpeeled tubers are processed or when potato processing involves removal of water; the alkaloid levels in the dried products can be additionally elevated.

To detect and quantify GAs, several procedures exist, including colorimetric methods (Clarke 1958; Hellenäs 1986; Dao and Friedman 1996), immunoassays

(Hellenäs 1986; Friedman et al. 1998; Driedger et al. 2000), quantitative thin layer chromatography (Cadle et al. 1978; Simonovska and Vovk 2000), gas chromatography (Herb et al. 1975; King 1980), high-performance liquid chromatography (HPLC) (Bushway et al. 1979; Hellenäs 1986; Saito et al. 1990; Edwards and Cobb 1996) and mass spectrometry (MS) (Matsuda et al. 2004; Stobiecki et al. 2003). HPLC and immunoassays are the most commonly used in this context (Edwards and Cobb 1998). For analysis of individual GAs, HPLC is the method of choice. Because a strong chromophore is absent in the molecules, the extracts must be purified prior to the chromatographic analysis. The most frequent method employed is solid-phase extraction (SPE), usually with RP-18 cartridges; however, this purification step is time-consuming and cost-intensive.

In comparison with off-line procedures, automated on-line SPE coupled with HPLC can reduce the analysis time and this is accompanied by higher precision, sensitivity and sample throughput (Rossi and Zhang 2000). On-line SPE is often employed in trace analysis of water samples for organic pollutants (López de Alda and Barreló 2001; Riediker et al. 2002) and the determination of drugs and metabolites in biological samples such as blood or urine (Ding and Neue 1999; Pascual and Sanagustín 1999). Only a few methods have been developed for the analysis of food ingredients so far. Bacaloni et al. (2005), as well as Newkirk et al. (1998), investigated mycotoxins in wine, beer and corn-based feed. In nutraceuticals, ephedrine could be detected with automated SPE-HPLC (Stevens 2003).

The aim of our study was the development of a fast and user-friendly automated method for the purification and sensitive detection of GAs in processed potato products.

Material and Methods

Chemicals and Reagents

HPLC-grade α-solanine, $NaHCO_3$ and phosphoric acid were purchased from Roth (Karlsruhe, Germany). α-Chaconine (purity ≥99%) was from Fluka (Buchs, Switzerland). Methanol (p.a.), 2-propanol and HPLC-grade water were supplied by VWR International (Fontenay sous Bois, France), HPLC-grade acetonitrile, acetic acid and NH_4OH were from Sigma-Aldrich Chemie (Steinheim, Germany). Formic acid (p.a.) was from Merck (Darmstadt, Germany).

Potato Samples

Processed potato products (see Table 3) were purchased from several local stores in Heidelberg, Germany, in 2005.

Sample Preparation

Samples from potato products were prepared for off-line and on-line application in the same way. Dry products such as mashed potato powder were used directly. The remaining samples were lyophilized (Christ, Osterode, Germany). After homogeni-

zation of the dried material with a mortar and pestle, about 3–10 g dry material was sampled, depending on the expected GA content. Extraction was carried out twice, each with 20 ml 1% acetic acid-methanol (70:30, v/v) and 1-min ultrasonication (Sonorex Super RK 102H, Bandelin Electronic, Berlin, Germany). After centrifugation (4,000 rpm, 5 min), 1 ml of the combined supernatants was used for on-line SPE. From the remaining extract an aliquot was purified manually by SPE using Supelclean® ENVI-18 cartridges (Supelco, Bellefonte, PA, USA). In the case of high-lipid-containing samples, such as French fries and crisps, the lipids were removed with *n*-pentane prior to the SPE clean-up.

On-Line SPE-Liquid Chromatography-MS

Extract Clean-up and Concentration

The purification and detection of the GAs were carried out using the Symbiosis™ Pharma extraction system [extraction-liquid chromatography (XLC)—SPE] from Spark Holland Instruments (Emmen, The Netherlands). This was coupled on-line to the mass spectrometer. The SPE system consisted of an automated cartridge exchanger module, which holds two trays, each containing a maximum of 96 cartridges, a solvent delivery unit and a low-pressure six-port valve that is connected directly to the gradient pumps of the HPLC instrument. For enrichment of α-solanine and α-chaconine a HySphere™ 18HD sorbent cartridge (10 mm × 2 mm inner diameter, 20 mg) was used. This was activated and equilibrated consecutively with 1 ml acetonitrile followed by 1 ml 5% acetonitrile in 1% NH_4OH. The flow rate was set to 2 ml min^{-1} for all cleaning steps. After sample loading of 50 µl of the extract, the cartridge was consecutively washed with 2 ml 20% acetonitrile in 1% NH_4OH to remove disturbances.

Liquid Chromatography-MS

The retained GAs were eluted directly onto an analytical Xterra MS C18 column (50 mm × 2 mm inner diameter ×3 µm) from Waters (Manchester, UK) for 2 min with a flow rate of 0.5 ml min^{-1} and a binary liquid chromatography (LC) gradient, consisting of 0.1% formic acid (solvent A) and 0.1% formic acid in methanol (solvent B) and the following profile: 0.0 min, 10% solvent B; 0.1–2.05 min, 10–90% solvent B; 2.05–2.5 min 90% solvent B; 2.5–3.0 min 90–10% solvent B; 3.01–5.0 min 10% solvent B.

MS data were obtained using a API 3000 mass spectrometer from MDS Sciex, (Concord, Canada) equipped with a turbo ion spray interface for ionization. MS detection of positively charged ions was performed in the positive mode using multiple reaction monitoring (MRM). MRM traces were set at *m/z* 868.3 and 98.2 for α-solanine and at *m/z* 852.4 and 98.2 for α-chaconine. The run time was 5 min and the instrument was set to the following tuning parameters: nitrogen was used as the nebulizer gas (15 l h^{-1}) and the curtain gas (10 l h^{-1}), argon was the collision gas with a collision cell potential of 6 V, the ion spray voltage was set to 5 kV and the temperature of the heated transfer capillary was maintained at 400 °C. The complete system was operated and data processing was carried out using Analyst™ 1.4.1 from MDS Sciex (Concord, Canada).

Off-Line SPE

Depending on the GA content, an aliquot of the acetic acid extract was purified using Supelclean™ ENVI™-18 cartridges and the following cleaning protocol. After activation with methanol and conditioning with 1% acetic acid, 5–10 ml of the extract was loaded onto the cartridge, followed by two washing steps each with 2 ml of 5 mM $NaHCO_3$ buffer (pH 8.4) and methanol-5 mM $NaHCO_3$ (60:40, v/v). Elution took place with 2 ml methanol-1% acetic acid (90:10, v/v). The solvent was evaporated to dryness using a vacuum centrifuge (Univapo H 150 concentrator centrifuge, Fröbel, Lindau, Germany) and the residue was suspended in methanol.

High-Performance Liquid Chromatography

Twenty microlitres of the methanol extract was analysed by HPLC using a LiChrospher® 100 RP-18 (250 mm × 4 mm, 5 μm) analytical column (Merck, Darmstadt, Germany). The HPLC instrument consisted of a binary pump (model 125 P solvent module), a photodiode array detector (model 168) set at 202 nm, both from Beckman-Coulter Instruments (Fullerton, USA), and an autosampler from Spark Holland Instruments (Emmen, The Netherlands).

As a binary gradient programme at ambient temperature, the following profile was used with a mobile phase of water (pH 2.5 adjusted with 85% phosphoric acid) (solvent A) and acetonitrile (solvent B): 0 min, 10% solvent B, 1.3 ml min^{-1}; 0.1–10 min, 10–30% solvent B, 1.3 ml min^{-1}; 10–11 min, 30–60% solvent B, 0.9 ml min^{-1}; 11–19 min, 60% solvent B, 0.9 ml min^{-1}; 19–20 min, 60–100% solvent B, 0.9 ml min^{-1}; 20–22 min 100% solvent B, 0.9 ml min^{-1}; 22–24 min, 100–10% solvent B, 1.3 ml min^{-1}.

Peak areas were integrated using Gold Nouveau Chromatography Data System, version 1.72 (Beckman Instruments, Fullerton, USA), with an external standard. A standard calibration curve was plotted by using various concentration ranges of α-solanine (5–480 μg ml^{-1}) and α-chaconine (3.5–450 μg ml^{-1}).

Results and Discussion

Extraction of GAs

Quantitative extraction of GAs from plant material is a challenge. As Driedger et al. (2000) mentioned, the hydrophilic carbohydrate moiety combined with the hydrophobic steroid element leads to an amphiphilic molecule that is at least slightly soluble in most solvents, but not very soluble in any. The nitrogen in solanidine has a pK_a of 8.62 in a water-alcohol solution. By the integration of a proton, the water solubility of the analytes at low pH is enhanced. Friedman and McDonald (1995) evaluated several solvent combinations that have been used for GA extraction. The best results for dry material were obtained with slightly acid aqueous solvents, e.g. 1% acetic acid. Because potato products such as mashed potato powder have a high starch content, the addition of a low amount of an organic

solvent was necessary to reduce starch swelling; therefore, we adopted 1% acetic acid-methanol (70:30, v/v) as the extraction solvent.

Method Development of On-Line XLC-MS

In the course of our investigation a protocol was developed for:

1. The autosampler (injection volume and wash routine)
2. The on-line SPE procedure (sorbent material, clean-up, elution of the analytes)
3. The LC conditions
4. The MS settings

Autosampler

For the autosampler a standard configuration was applied and consisted of an injection volume of 50 µl using the partial loop fill injection routine. For the response of the GA standard solutions in MS, detection was linear up to 1,000 ng ml^{-1}. Washing of the injection system was performed with two solvents: wash solvent 1 was 40% acetonitrile in 0.1% formic acid and wash solvent 2 was 40% acetonitrile-40% methanol-15% water-5% 2-propanol.

SPE Conditions

A number of key parameters were assessed in on-line work to select an appropriate SPE procedure. First, a suitable sorbent material had to be selected. The recovery of interesting compounds and the chromatographic performance (peak shape, peak width and asymmetry factor) are the most important criteria to achieve good selectivity and low limits of detection (LODs) (Patsias and Papadopoulou-Mourkidou 2000). For this purpose, 25 µl of the α-solanine and α-chaconine standard mixture (each 1,000 ng µl^{-1}) was tested for analyte extraction efficiency with eight different sorbent materials (CN, C-2, C-8, C-8ec, C-18ec, C-18HD, GP resin, SH resin), which were located in a method development cartridge tray. The best results were obtained with HySphere™ 18HD material (Table 1). Recovery, as well as the peak shape for the standard solutions, showed the best performance (Fig. 2). For C-8, C-8ec, C-18ec and GP resin, sorbent recovery was tolerable as

Table 1 Recoveries (%) for α-solanine and α-chaconine standard solution (1,000 ng ml^{-1}) using different sorbent materials

Sorbent material	Recovery α-solanine	Recovery α-chaconine
CN	85	93
C-2	59	80
C-8	130	120
C-8ec	144	123
C-18ec	149	106
C-18HD	124	99
GP resin	115	96
SH resin	3	40

a.) C-18HD

■XIC of +MRM (2 pairs): 868.4/98.2 amu from Sample 6 (1000 ng/ml 25 ul C18HD) of sorbent.wiff (Turbo Spray)   Max. 2.5e5 cps.
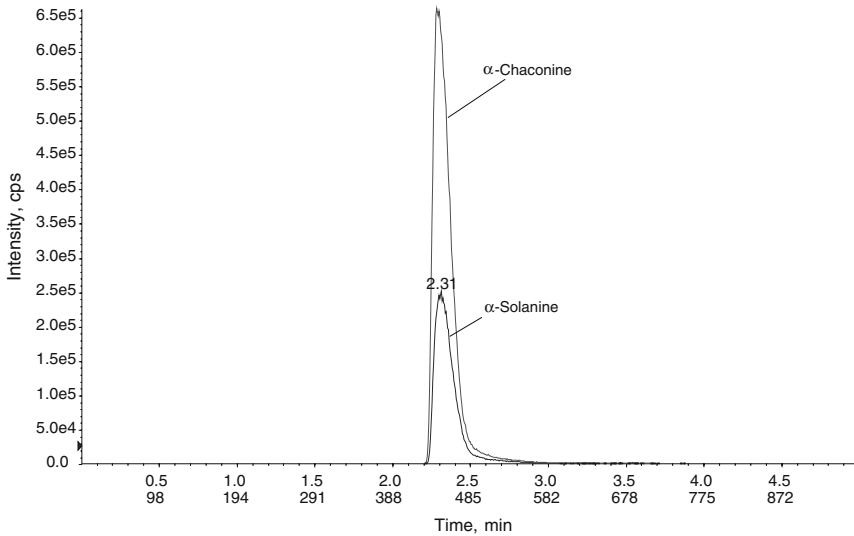
b.) SH-resin

■ XIC of +MRM (2 pairs): 868.4/98.2 amu from Sample 8 (1000 ng/ml 25 ul SH) of sorbent.wiff (Turbo Spray)   Max. 6.8e4 cps.
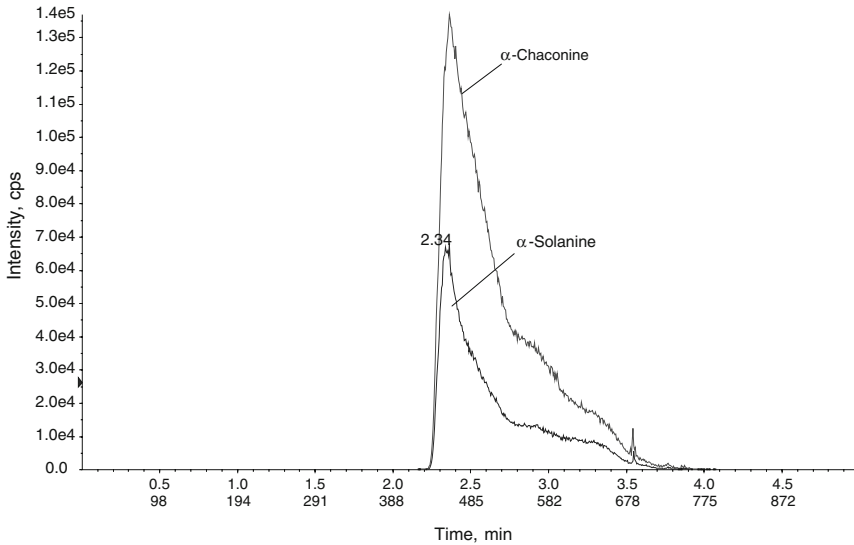

Fig. 2 Method development of on-line extraction-liquid chromatography (XLC)-MS. Comparison of the liquid chromatography (LC)-MS chromatograms after injection of 25 µl α-solanine and α-chaconine standard solution (each 1,000 ng ml^{-1}) when using **a** suitable HySphere 18HD or **b** unsuitable SH resin as the sorbent material for solid phase extraction purification

well. However, the peak shape was unsatisfactory because of peak spreading, which results in inaccurate recoveries over the expected value and poor sensitivity and selectivity. As can be seen in Table 2 and Fig. 2, the SH resin was very unsuitable for GA purification. Because of a high retention of the GAs on this sorbent, recovery for α-solanine was particularly poor and the peak was very broad.

Besides the sorbent material, conditioning of the cartridge, the loading volume of the sample, a washing step to remove disturbances and finally elution directly to the LC-MS system were further important parameters. To moisten the sorbent, 1 ml acetonitrile was utilized for solvation, followed by another 1 ml 5% acetonitrile in 1% NH_4OH for conditioning. The response of 50 µl of the acetic acid extract for all potato products was within the linear range (1–1,000 ng ml^{-1}) with the exception of the potato wedges. The extracts were diluted ten times with 1% acetic acid prior to the injection. After sample loading, the washing step was optimized, for which three variables, namely flow rate, volume and composition of the washing solvent, were important. Optimum conditions were achieved with 20% acetonitrile in 1% NH_4OH, a volume of 2 ml and a flow rate of 2 ml min^{-1}. A higher acetonitrile percentage or a longer washing time resulted in partial loss of the analytes.

Finally, fine-tuning of the elution time was necessary. It is important to elute the analytes without removing disturbing substances, which are still adsorbed to the SPE cartridge. To make the procedure more economical, it is useful to elute the alkaloids with the HPLC gradient; therefore, we raised the methanol content from 10 to 90% within 2 min (compare with gradient described in the next section).

Table 2 Validation parameters for the extraction-liquid chromatography-mass spectrometry (XLC-MS) method developed

	Sample	α-Solanine	α-Chaconine
Measurement precision (%)[a]	Standard solution (50 ng ml^{-1})	3.3	2.5
Method precision (%)[a]	Potato wedges A	5.4	3.4
	Potato crisps A	6.0	5.4
	French fries	5.4	6.0
	Mashed potato powder B	6.5	15.4
	Potato pancake	5.3	5.5
Recovery[b] (%) after spiking with GAs (ng ml^{-1})[c]		83 (104)	88 (94)
		94 (416)	93 (376)
		75 (1,560)	81 (1,410)
Linearity (R^2)		0.9915	0.9939
Limit of determination		0.5	0.3
Limit of quantification		1.5	1.0

GA glycoalkaloid

[a] Precision expressed by the coefficient of variation (v_k)

[b] Recovery calculated as recovered GA amount after subtraction of the initial GA content in the tissue in relation to the spiked amount

[c] Potato starch was spiked with various amounts of GA standard (concentration given in *parentheses*).

Chromatographic Conditions

To achieve a very high throughput, short columns (20–50 mm) with low resolution ability have often been selected. SPE systems coupled with these columns operate very fast and a total analysis time of 5 min or lower can be achieved (Allanson et al. 1996). To shorten and simplify the analytical procedure, we chose a 50-mm Xterra MS C18 column from Waters. Absent or poor resolution of the analytes with this short column cause no problems when MS detection is applied. By use of the MRM mode with two diagnostic ions (qualifier at m/z 98.2 and quantifier at m/z 868.3 for α-solanine and 852.4 for α-chaconine, respectively), a complete separation was not necessary for an exact quantification. Since the main function of the HPLC gradient was the complete elution of the GAs from the SPE cartridge, and not their resolution, the mobile phase was optimized to obtain the highest recovery in the shortest time. The resulting LC gradient was from 10 to 90% 1% formic acid in methanol within 2 min, followed by 1 min with 90% 1% formic acid in methanol and reconditioning of the system. Retention times were 2.3 min for α-solanine and 2.4 min for α-chaconine, with a complete cycle time of 5 min for SPE clean-up and LC-MS analysis.

MS Settings

Tuning of the instrument was optimized for the detection of the GAs, which resulted in the parameters given in "Materials and Methods". The most abundant ions with turbo ion spray were the parent molecular ion $[M + H^+]$ at m/z 868.3 for α-solanine and m/z 852.4 for α-chaconine, which were used in each case for the quantification. For high specificity and confidence in the identification, fragmentation of the GAs was done in a collision cell, which resulted in the daughter ion scan in Fig. 3a for α-solanine and in Fig. 3b for α-chaconine. In each case, the daughter ions were due to the loss of one saccharide unit (glucose in α-solanine, leading to m/z 722, rhamnose in α-chaconine, leading to m/z 706) or all the saccharide units (m/z 398). m/z 98 is probably the result of another decomposition of the steroidal part. As m/z 98.2 emerged in both fragmentation scans with the highest intensity, we chose it as the identification ion. At present, chromatographic separation followed by MRM detection of two ions can be used to precisely identify a molecule (Bacaloni et al. 2005).

Method Validation

Method validation provides assurance of the reliability for a procedure during routine use. The on-line XLC-MS method was formally validated prior to the analysis of the samples. The following parameters were assessed:

Linearity and Quantification Model

For quantification of the GAs in on-line XLC-MS an external method based on peak areas was used. For this purpose five-point calibration curves were generated for both alkaloids between 1 and 1,000 ng ml^{-1} (n=4). In this range the instrumental
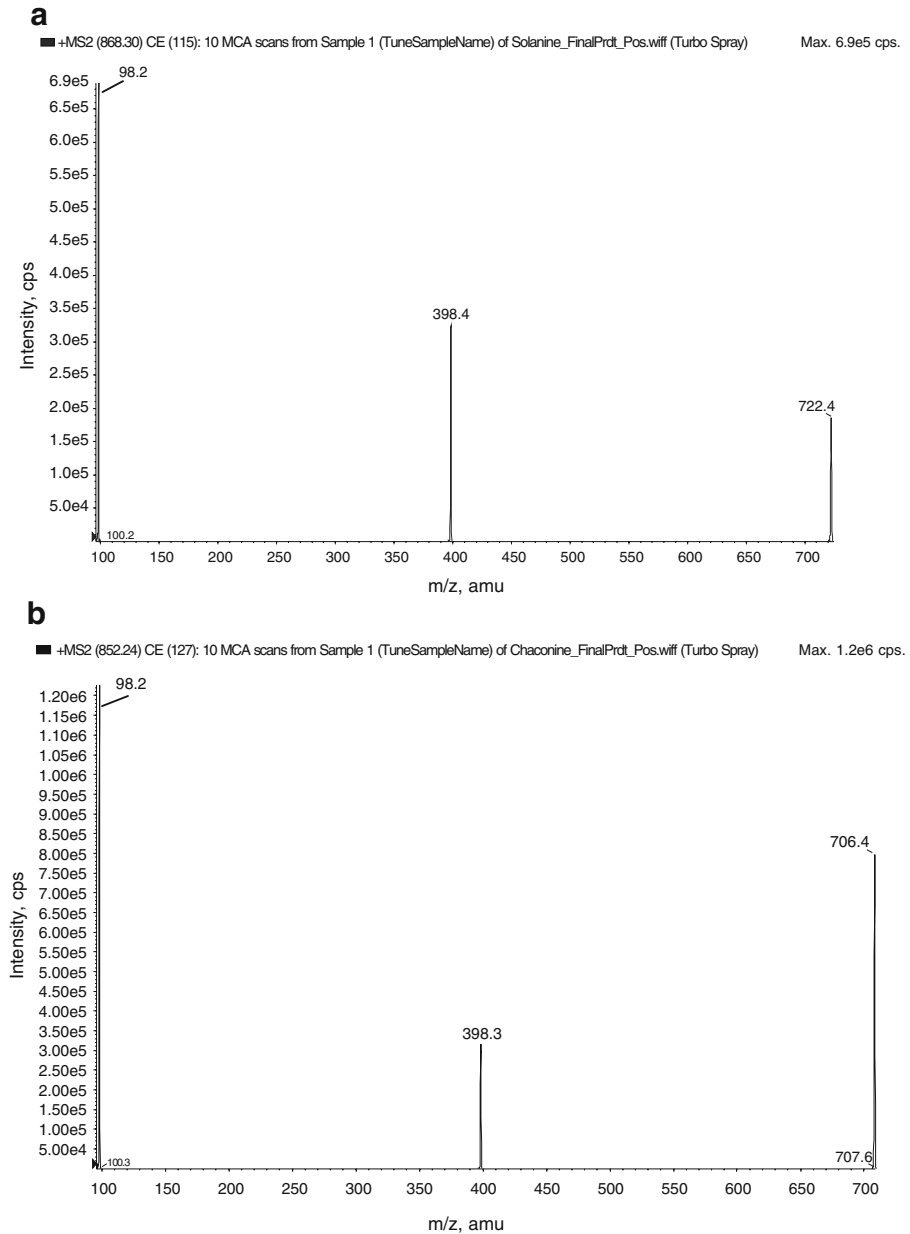
a

b

Fig. 3 MS daughter ion scan of **a** m/z 868 (α-solanine) and **b** m/z 852 (α-chaconine) over the range m/z 95–750 (for the origin of the daughter ions, see "MS settings")

response was found to be linear, showing a coefficient of determination (R^2) of 0.9915 for α-solanine and 0.9939 for α-chaconine. Quantification was performed using the MRM mode with the m/z traces at 868.3 for α-solanine and 852.4 for α-chaconine.

Precision

The instrumental precision is a measure for the deviation of a measured value because of a random error. It is divided into:

a. Measuring precision: variation by the analytical instrument. This is expressed as the coefficient of variation (v_k) after a fourfold analysis of 50 ng ml^{-1} α-solanine or α-chaconine standard solution. With v_k=3.3% for α-solanine and v_k=2.5% for α-chaconine, the measuring precision was acceptable.
b. Method precision: variation within the whole method. The variation within the method was determined by a fourfold analysis of several potato product samples. As can be seen in Table 2, method precision was between 5.3 and 6.5 for α-solanine and between 3.4 and 15.4 for α-chaconine. Because plant material always shows variations, v_k values up to 15% are generally accepted.

Accuracy

The instrumental accuracy is the measure of the deviation of a measured value because of a systematic error. It can be checked by determining the recovery of the analytes after spiking real potato product samples with a standard substance. The difference in the concentrations of the spiked sample and the pure sample in relation to the amount of standard substance added describes the recovery. As a test sample almost-alkaloid-free potato starch was used. Table 2 documents the recovery for several added concentrations of standard solution (75–94%). The comparatively lower recovery for the highest GA amount is attributed to a slight overloading of the SPE cartridge and therefore a partial loss of the analytes.

Specificity

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may also be present in the sample. On-line XLC-MS allows extremely high specificity via retention times and two stages of mass selectivity. With the use of the MRM mode and two characteristic ions for compound identification, specificity is given to a very high degree. Additionally we injected a blank of pure 1% acetic acid-methanol (70:30, v/v) and observed no interfering peaks.

LOD and Limit of Quantification (LOQ)

The LOD is the lowest concentration of an analyte in a sample that can be detected, whereas the LOQ is the lowest concentration that can be determined with acceptable precision and accuracy under the defined operating conditions of the method. In chromatographic analyses the LOD and the LOQ are usually defined as the concentrations for which signal-to-noise ratios of 3 and 10, respectively, are obtained. Because α-chaconine gave a better response in the MS detection, the LOD and the LOQ for α-chaconine (0.3 and 1.0 ng ml^{-1}) were lower than for α-solanine

(0.5 and 1.5 ng ml^{-1}). The LODs and LOQs are generally influenced by matrix effects. With the selective MS detection in MRM mode and the possibility to set mass traces that precisely identify the substance investigated, these matrix effects can be eliminated and the LODs are particularly low, especially for substances with a weak chromophore as in GAs (which are difficult to monitor in HPLC using UV detectors).

Breakthrough

The breakthrough volume is defined as the sample volume above which the analyte starts to be eluted from the cartridge because the retaining capacity is exhausted. It depends on the strength with which the analytes are retained by the sorbent, on the amount of the sorbent and on the packing efficiency of the sorbent bed. To exclude breakthrough of the analytes in the defined range, a standard mixture of α-solanine and α-chaconine (each 1,000 ng ml^{-1}) was injected and the solvent was directly checked by LC-MS. While the recovery was 99.5%, a breakthrough of 0.5% was observed, which is acceptable.

Determination of GAs in Processed Potato Products—Comparison of the On-Line Method with an Established Off-Line HPLC Procedure

The performance of the on-line XLC-MS method developed was evaluated by analysing the GA contents in several commercial potato products (Table 3). The results were compared with those determined by our established off-line SPE-HPLC-UV procedure. On the left hand side of Table 3, we see the α-solanine, α-chaconine and total GA contents which were obtained with off-line SPE-HPLC-UV. Additionally the standard deviation of the total GA contents for each sample is presented. In the centre columns we can see the results for the same samples which were determined by on-line XLC-MS. The GA contents are expressed as the mean value of a duplicate sample preparation, whereby each sample was injected twice. On the right side of Table 3, the proportional correspondence of the on-line XLC-MS method compared with the off-line procedure is shown.

GAs were detected in all products investigated. The contents ranged from 11.05 μg total GA g^{-1} dry weight (dw) in a French fries sample to 156.22 μg total GA g^{-1} dw in potato wedges. Generally, GA levels were in agreement with already published literature data (Saito et al. 1990; Friedman 1992; Friedman and Dao 1992; Easton 1998). Except for potato wedges, most of the products showed similar contents up to 50 μg total GA g^{-1} dw. Since potato wedges are produced and consumed with peel, the higher alkaloid contents are not surprising. As Zitnak and Johnston (1970) reported, the outer layers of the potato contain more than half of the GAs and represent only 14% of the total tuber weight.

We obtained similar GA levels using both procedures. Figure 4 shows the correspondence of the total GA content obtained with the on-line and off-line procedures by representing the line of the best fit and the coefficient of determination (R^2=0.9131). In most of the samples, the difference of the GA content was within the standard deviation for the off-line procedure and was

Table 3 GA contents ($\mu g\ g^{-1}$ dry weight) of several potato products obtained with off-line solid-phase extraction-high-performance liquid chromatography-UV detection (SPE-HPLC-UV) or on-line XLC-MS and comparison of the results

Sample	Off-line SPE-HPLC-UV				On-line XLC-MS[a]				Percentage[b]		
	α-Solanine	α-Chaconine	Total GA	SD	α-Solanine	α-Chaconine	Total GA	SD	α-Solanine	α-Chaconine	Total GA
Potato wedges[c] A	81.25	131.08	212.33	28.55	65.06	91.16	156.22	6.83	80.1	69.5	73.6
Potato wedges[c] B	19.96	37.45	57.41	31.91	22.18	30.42	52.60	8.12	111.1	81.2	91.6
Potato wedges[c] C	53.42	84.11	137.53	43.71	42.28	65.64	107.92	4.80	79.1	78.0	78.5
French fries	5.88	6.47	12.35	8.21	5.77	5.28	11.05	0.57	98.1	81.6	89.5
Potato pancake	11.98	15.22	27.20	10.03	13.87	15.52	29.39	1.32	115.8	102.0	108.1
Mashed potato powder A	14.68	28.82	43.50	8.36	8.04	10.55	18.59	0.94	54.8	36.6	42.7
Mashed potato powder B	12.89	16.77	29.66	9.49	7.99	7.35	15.34	0.76	62.0	43.8	51.7
Mashed potato powder C	32.48	45.65	78.13	16.10	18.59	18.89	37.48	3.11	57.2	41.4	48.0
Mashed potato powder D	9.40	14.25	23.65	5.37	7.07	7.38	14.45	0.78	75.2	51.8	61.1
Shrink-wrapped potatoes[d]	14.08	20.06	34.14	2.95	24.73	25.80	50.53	2.32	175.6	128.6	148.0
Potato crisps A	8.24	14.00	22.24	8.49	9.48	13.49	22.97	1.07	115.0	96.4	103.3
Potato crisps B	13.85	26.69	40.54	4.21	12.72	20.89	33.61	1.52	91.8	78.3	82.9

SD standard deviation of the total GA content (four determinations)

[a] GA contents are expressed as the mean value of a duplicate sample preparation and injection twice

[b] Proportional correspondence of the on-line XLC-MS method compared with the off-line procedure

[c] Potato quarters, consumed with peel, also known as country potatoes

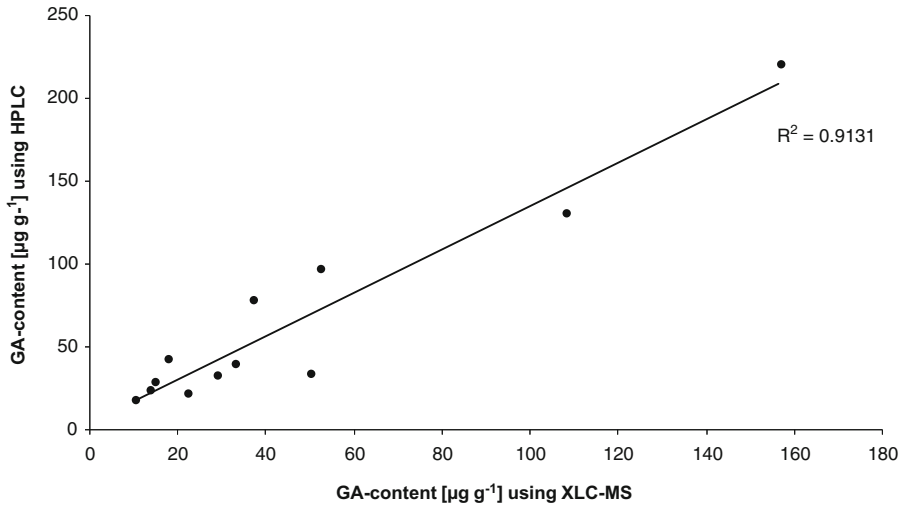[d] Peeled potatoes, wrapped in film for preservation

Fig. 4 Correlation of the total glycoalkaloid contents ($\mu g\ g^{-1}$) using on-line XLC-MS and off-line solid-phase extraction-high performance LC-UV detection

therefore not method-dependent. Already the validation data for the method precision show that a certain variation is normal for natural products. In potato wedges, the alkaloid concentrations seem to be lower for the XLC-MS determination; however, this is attributed to the high GA variation in the off-line procedure, because the alkaloid-rich potato skin was included in the samples. As can be seen in the lower standard deviations of the on-line method, the results are even more accurate than those obtained with the off-line determination. Only the mashed potato powder and shrink-wrapped potato samples were exceptions, despite repeated sample preparation. The reason could not be clarified. The cause was probably matrix effects, for example food additives, which suppress the ionization of the GAs in the MS determination.

The advantages of SPE over conventional methods such as precipitation of GAs are well documented (Väänänen et al. 2000). The drawback of all existing off-line SPE-HPLC procedures is the long and labour-intensive purification of the sample extracts, which leads to expensive analytical methods. With the application of the automated XLC-MS procedure, the cycle times could be reduced to 5 min, so only 8 h is necessary to analyse 100 samples. This offers a fivefold increase in sample preparation time compared with the off-line SPE-HPLC-UV procedure, because preceding work such as weighing and extraction of the GAs can be carried out in parallel. However, in addition to the reduction in time and costs achieved by using an automated purification process, the results are more representative and more accurate because of the reduction in manual sample preparation. This is clearly demonstrated by comparing the standard deviations of both methods: between 0.57 and 8.12 for the automated procedure in contrast to 2.95 to 43.71 for the off-line SPE-HPLC-UV procedure.

Conclusion

On-line procedures are particularly attractive in situations where large numbers of samples or sample series have to be analysed routinely and where the cost pressure is high, such as in food monitoring. As Rossi and Zhang (2000) showed in their report on the perspectives of on-line SPE, several automated procedures are available which concentrate on the examination of biological and environmental samples. The automation of the sample purification using the on-line XLC-MS system Symbiosis™, from Spark Holland Instruments, represents the first example of such a novel application in the food sector, which is forced by strong business competition for efficient and cost-effective analytical procedures for potentially toxic components. It has proven to be an excellent approach for the fast and reliable determination of GAs in processed potato products with an extremely complex matrix. The excellent performance could be verified by checking typical validation parameters as well as by comparing the GA concentrations in processed potato products with an established off-line SPE-HPLC-UV procedure. Consequently, the use of this on-line method in routine analysis could help improve the quality of potato products.

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