SPECIAL GUEST EDITOR SECTION

Determination of α-Solanine and α-Chaconine in Potatoes by High-Performance Thin-Layer Chromatography/Densitometry

PATRICIA BODART, CHARLES KABENGERA, and ALFRED NOIRFALISE
Université de Liège, Toxicologie et Bromatologie, Domaine Universitaire du Sart-Tilman, B-23, 4000 Liège, Belgium
PHILIPPE HUBERT
Université de Liège, Analyse des Médicaments, Domaine Universitaire du Sart-Tilman, B-36, 4000 Liège, Belgium
LUC ANGENOT
Université de Liège, Pharmacognosie, Domaine Universitaire du Sart-Tilman, B-36, 4000 Liège, Belgium

A high-performance thin-layer chromatographic (HPTLC) method was used to determine the glycoalkaloids α-solanine and α-chaconine in potatoes. α-Solanine and α-chaconine are extracted from dehydrated potatoes with boiling methanol–acetic acid (95 + 5, v/v). The analytes are separated on a Silica Gel 60 F_{254} HPTLC plate by a saturated mixture of dichloromethane–methanol–water–concentrated ammonium hydroxide (70 + 30 + 4 + 0.4, v/v), which is used for vertical development of the plate up to a distance of 85 mm. For visualization, the plate is dipped 3 times into a modified Carr-Price reagent, 20% (w/v) antimony(III) chloride in acetic acid–dichloromethane (1 + 3, v/v), and subsequently heated on a hot plate at 105°C for 5 min. The glycoalkaloids all appear as red chromatographic zones on a colorless background. Densitometric quantification is performed at 507 nm by reflectance scanning. After determination of the appropriate response function, the proposed method was validated. Good results with respect to linearity, accuracy, and precision were obtained in the concentration range studied.

The potato, *Solanum tuberosum* L., contains steroidal glycoalkaloids (GAs) generally called solanines. α-Solanine and α-chaconine are the major GAs, accounting for about 95% of the total amount. These compounds contain the same aglycone, solanidine, but differ in the trisaccharide moiety (Figure 1). The ratio of α-chaconine to α-solanine is usually about 60:40 (1).

GAs can be toxic substances: they inhibit cholinesterases and cause gastrointestinal necrosis. Generally, an undamaged tuber contains about 20–150 mg total GAs/kg, depending on the potato cultivar. Several scientists consider 200 mg GAs/kg potato a nontoxic concentration (1, 2). The GA level in a potato tuber is usually higher in the peel than in the flesh. Some varieties of potatoes sometimes contain >200 mg GAs/kg (3, 4), and exposure to light or to mechanical damage can increase GA concentration (1). A routine analytical method for potato GAs is, thus, very useful in a food control laboratory.

GAs (α-solanine and α-chaconine) in potatoes are usually determined by liquid chromatography (LC). The LC method involves time-consuming sample preparation by solid-phase extraction and nonspecific UV detection at 202 nm (5). GAs do not have strong chromophores, and a derivatization step is generally needed to determine them. Therefore, high-performance thin-layer chromatography (HPTLC) can be used.

A few quantitative TLC methods for potato GAs are discussed in the literature, but their validation does not conform to current requirements (6–8). We have, thus, developed and validated a simple, rapid, and accurate quantitative procedure for direct determination of α-solanine and α-chaconine. A solid-phase extraction is not necessary, and GAs are detected by using a modified Carr-Price reagent.

Experimental

Potatoes of 2 cultivars, *Charlotte* and *Désirée*, were purchased at a local farm. A mixture of both varieties was used for method validation.

Apparatus

(**a**) *Densitometer*.—Desaga (Heidelberg, Germany) CD60 TLC plate scanner with user-friendly software installed on a personal computer.

(**b**) *Sample applicator*.—Desaga AS 30 TLC applicator.

(**c**) *TLC chamber*.—Camag (Muttenz, Switzerland) twin-trough chamber for 20 × 10 cm plates.

(**d**) *Chromatogram immersion device*.—Camag.

(**e**) *TLC plate heater*.—Desaga.

(**f**) *Rotary evaporator*.—Büchi (Flawil, Switzerland).

(**g**) *Air-circulation drying oven*.—Memmert (Schwabach, Germany).

(**h**) *Oven*.—Pleuger (Wijnegem, Belgium).

Guest edited as a special report on "Thin-Layer Chromatography/Densitometry" by Joseph Sherma.

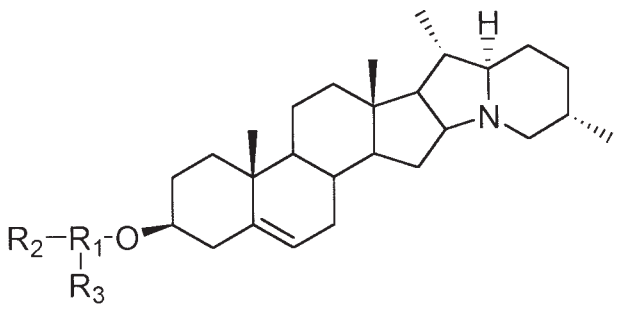

Figure 1. Chemical structures of α-solanine and α-chaconine. α-Solanine: R_1 = D-galactose; R_2 = D-glucose; and R_3 = L-rhamnose. α-Chaconine: R_1 = D-glucose; R_2 = L-rhamnose; and R_3 = L-rhamnose.

Reagents

(**a**) *Deionized water*.

(**b**) *Methanol, dichloromethane, acetic acid, antimony(III) chloride, and concentrated ammonium hydroxide (analytical grade)*.—Acros (Geel, Belgium).

(**c**) *α-Solanine and α-chaconine*.—Sigma (St. Louis, MO).

(**d**) *Chromatographic plates*.—20 × 10 cm Silica Gel 60 F_{254} HPTLC plates (Merck, Darmstadt, Germany).

(**e**) *TLC mobile phase*.—Dichloromethane–methanol–water–concentrated ammonium hydroxide (70 + 30 + 4 + 0.4, v/v).

(**f**) *Modified Carr-Price reagent*.—Solution of 70 g antimony (III) chloride in 280 mL acetic acid–dichloromethane (1 + 3, v/v). The solution is stable for 1 month when stored under nitrogen.

Preparation of Standards

Stock solutions of α-solanine (4 mg/5 mL) and α-chaconine (4 mg/10 mL) were prepared in methanol–acetic acid (99 + 1, v/v). These solutions were diluted with methanol–acetic acid (99 + 1, v/v) to obtain the standards used for method validation (Table 1).

Preparation of Dehydrated Potato

Fresh tubers were peeled, sliced, and then dried at 35°C in an air-circulation drying oven for 48 h. The water loss was assessed at 74%. Dried sliced tubers were then ground.

Preparation of Potato Samples

A sample solution was prepared by extracting 2 g dehydrated potato with three 15 mL portions of boiling methanol–acetic acid (95 + 5, v/v) with mechanical stirring. Each extraction was performed for 10 min. The solution was filtered and evaporated under vacuum at 40°C; the residue was dissolved in 4 mL methanol–acetic acid (99 + 1, v/v).

Chromatography

The plates were prewashed by development with methanol, air-dried, and activated at 110°C overnight. The samples were applied to the plates by using the following program: bandwidth, 4 mm; distance between bands, 8 mm; step volume, 1 μL; rate of application, 8 s/mL; time between applications, 8 s. Applied volumes were always 5 μL. Twenty-one samples can be applied to the same plate.

Plates were heated at 90°C for 30 min in an oven and were placed in the twin-trough chamber immediately afterwards for pre-equilibration with the mobile phase for 1 h. The plates were then developed vertically up to a distance of 85 mm.

Detection

Immediately after development, the chromatograms were air-dried for 15 min and then placed in an oven at 90°C for 60 min. Direct visualization was accomplished by dipping the plates 3 times in the modified Carr-Price reagent and subsequently heating them on a hot plate at 105°C for 5 min. The chromatographed GAs all appeared as red chromatographic zones on a colorless background.

Determination

The densitometric determination was performed at 507 nm by reflectance scanning. Because the GA zones began to turn purple after 30 min, it was necessary to perform quantitation for a maximum of 20 min just after the plates were heated.

Calculations

The amount of GA in fresh tuber was calculated with the following equation:

$$\text{Glycoalkaloid, ppm} = \frac{(100-R)\times(Q_1+Q_2)}{125\times W}$$

where W is the weight (g) of dehydrated potato; Q_1 and Q_2 are the calculated quantities (ng) of α-solanine and α-chaconine, respectively, contained in the applied volume; R is the water loss (%; as determined in the *Preparation of Dehydrated Potato* section).

Q_1 and Q_2 were calculated from calibration curves of α-solanine and α-chaconine according to the following equation:

$$Q = \frac{A-\beta}{\alpha}$$

where A is the sample peak area, β is the intercept of the calibration curve, and α is the slope of the calibration curve.

Results and Discussion

Choice of Development System

Various chromatographic systems previously described for the separation of GAs in potato were tested (8, 9). Silica gel TLC plates as the stationary phase and Carr-Price reagent (10, 11) were used to compare these mobile phases. Four solutions were applied to the plates: standard α-solanine, standard α-chaconine, a mixture of both standards, and the potato extract.

Table 1. Validation of the HPTLC method for the determination of α-solanine and α-chaconine in potatoes

Validation criterion	α-Solanine	α-Chaconine
Linearity	(n = 6, k = 3)	(n = 4, k = 3)
Applied range	100–2000 ng	100–1000 ng
	$y = 0.38x - 23.00$	$y = 0.40x - 24.33$
	$r^2 = 0.9936$	$r^2 = 0.9992$
F-test for slope	$F_1 = 2516$	$F_1 = 12921$
F-test for fit	$F_2 = 1.78$	$F_2 = 2.57$
LOD[a]	50 ng	50 ng
LOQ[b]	100 ng	100 ng
Repeatability, %		
(n = 5, 3 days)		
100 ng	4.6	3.1
500 ng	3.2	4.0
1000 ng		3.3
1500 ng	2.8	
Intermediate precision, %		
(n = 15; 3 days)		
100 ng	4.6	3.5
500 ng	7.6	6.1
1000 ng		4.1
1500 ng	6.9	
Extraction recovery, %		
Added quantity/spot:	50 ng: 108.2 ± 2.9	200 ng: 100.9 ± 1.6
mean ± SD[c] (n = 3)	900 ng: 98.9 ± 0.3	400 ng: 98.0 ± 3.8
	1250 ng: 93.2 ± 4.7	
Accuracy, %		
Added quantity/spot:	50 ng: 118.9 ± 19.8	200 ng: 107.7 ± 6.7
mean ± SD (n = 3)	900 ng: 97.4 ± 0.4	400 ng: 96.7 ± 9.9
	1250 ng: 91.7 ± 5.8	

[a] LOD = limt of detection.
[b] LOQ = limit of quantitation.
[c] SD = standard deviation.

Acidic or neutral mobile phases either did not give narrow bands or did not separate α-solanine and α-chaconine. The best separation of α-solanine and α-chaconine was obtained with an alkaline development system containing chloroform, methanol, and 1, 2, or 4% ammonium hydroxide. Because chloroform is toxic, it was replaced by dichloromethane. The system chosen, dichloromethane–methanol–water–concentrated ammonium hydroxide (70 + 30 + 4 + 0.4, v/v), gave well-separated sharp spots for both GAs. The R_f values of α-solanine and α-chaconine were 0.24 and 0.49, respectively.

Choice of Detection Reagent

Several reagents were compared (10–12). The Carr-Price reagent, 20% (w/v) antimony(III) chloride in chloroform, was chosen because it was the most specific and the most sensitive. Antimony(III) reacts with the double bond of the steroid to yield a red compound (11). The reagent must be anhydrous because water interferes in the reaction with antimony(III) chloride. Thus, several precautions must be taken to decrease the influence of water and to produce a stoichiometric reaction between antimony and the GAs: store the reagent under nitrogen; place the plates in an oven set at 90°C before and after development; and do not expose the plates to air between the different steps of the experiment, or if this is impossible, keep them in a desiccator.

The Carr-Price reagent was modified (20% [w/v] antimony chloride(III) in acetic acid–dichloromethane [1 + 3, v/v]) for 2 reasons: first, when the reagent contains acetic acid, reaction sensitivity increases and the coloration becomes more stable with time; and second, dichloromethane is less toxic than chloroform.

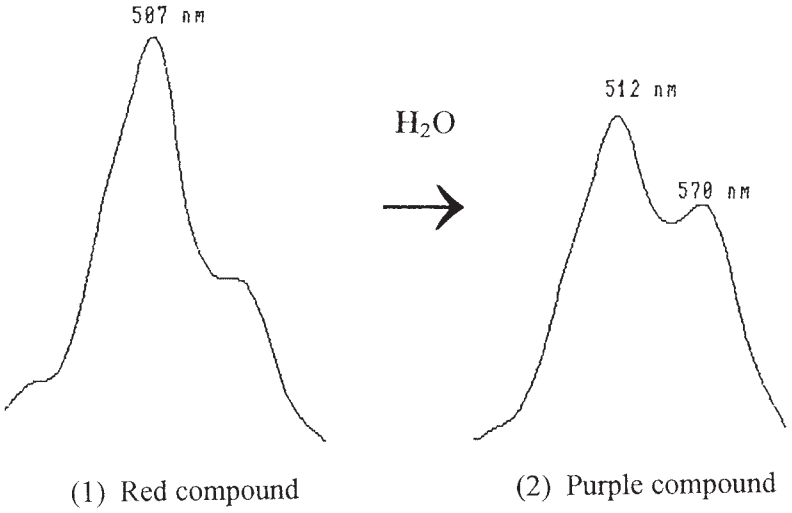

Figure 2. Comparison of the visible spectra of (1) α-solanine just after derivatization and (2) α-solanine 1 h after derivatization.

Immersion Method

To obtain a stoichiometric and sensitive reaction between antimony(III) and the GAs, 3 immersions were necessary. After each dipping in the solution, the plate had to be air-dried for 1 min.

Densitometric Evaluation

When all the precautions to decrease water interference were taken, the in situ background-subtracted spectra of α-solanine and α-chaconine showed a maximum at 507 nm. The GA zones began to turn purple after 30 min because of moisture in the air. The purple compounds had 2 maxima in their spectra, at 512 and 570 nm (Figure 2); thus, it was necessary to perform the quantitation at 507 nm just after dipping (for 20 min maximum).

Validation Data

Selectivity.—Selectivity of an analytical method is defined by its ability to demonstrate the presence of the targeted compound in potentially likely mixtures (13). This criterion was first investigated to assume that the analytical method developed could be used to quantitate α-solanine and α-chaconine in the presence of potential interfering substances from the matrix. Figure 3 shows the densitograms obtained for a potato sample and a mixture of α-solanine and α-chaconine by using the modified Carr-Price reagent. With this specific reagent for the steroid (11), these 2 compounds give clearly separated red spots. With several modifications of the composition of the mobile phase, we checked the absence of interfering endogenous components in potato at the retention factors of both compounds. No endogenous interference sources were observed at the retention factors of the analytes. The separation factor (α) for α-solanine and α-chaconine is 2.

Extraction efficiency.—A dehydrated potato sample containing 120 mg α-solanine/kg and 250 mg α-chaconine/kg was used to assess the extraction efficiency of the procedure. Known quantities of α-solanine and α-chaconine were then added to this matrix (Table 1). Nonspiked and spiked potato samples were extracted, and the extracts were applied to the same HPTLC plate. After subtraction of the responses corresponding to the initial amounts of α-solanine and α-chaconine present in the dehydrated potato sample from those obtained for the spiked potato samples, extraction efficiencies for the whole concentration range were calculated by comparing the resulting responses with those found for standard solutions at the same concentrations. The results are presented in Table 1.

Linearity (response function).—The linearity of an analytical method is its ability within a definite range to obtain results directly proportional to the concentrations of the analyte in the sample (13). To determine the response function, 3 calibration curves ($k = 3$) were constructed with a range of 100–2000 ng for α-solanine and a range of 100–1000 ng for α-chaconine by selecting n concentration levels as shown in Table 1. The lowest concentration was the supposed limit of quantitation (LOQ) foreseen at the end of method development. Once the analyses were performed, the most appropriate calibration curve model function was determined (14, 15).

Because the relationship between response and α-chaconine concentration seemed to be linear in the calibration range considered for this compound, the simplest regression model based on the least-squares method was first selected. The adequacy of the model was then verified by using residual (i.e., the differences between the observed and estimated responses) analysis and lack-of-fit test (F_2). Because no residual curvature was observed, an analysis of variance (ANOVA) was performed on the calibration curve to confirm linearity (F_1) and to test the quality of the fit (F_2). Linearity was assessed with $F_{calc} > F_{(0.95;\,1,\,10)}$ (4.96), as was the fit, with $F_{calc} < F_{(0.95;\,2,\,8)}$ (8.65) (Table 1). Because the adequacy of the selected model was demonstrated, the least-squares model can be used to describe the relationship between peak area and amount of applied α-chaconine. The following equations

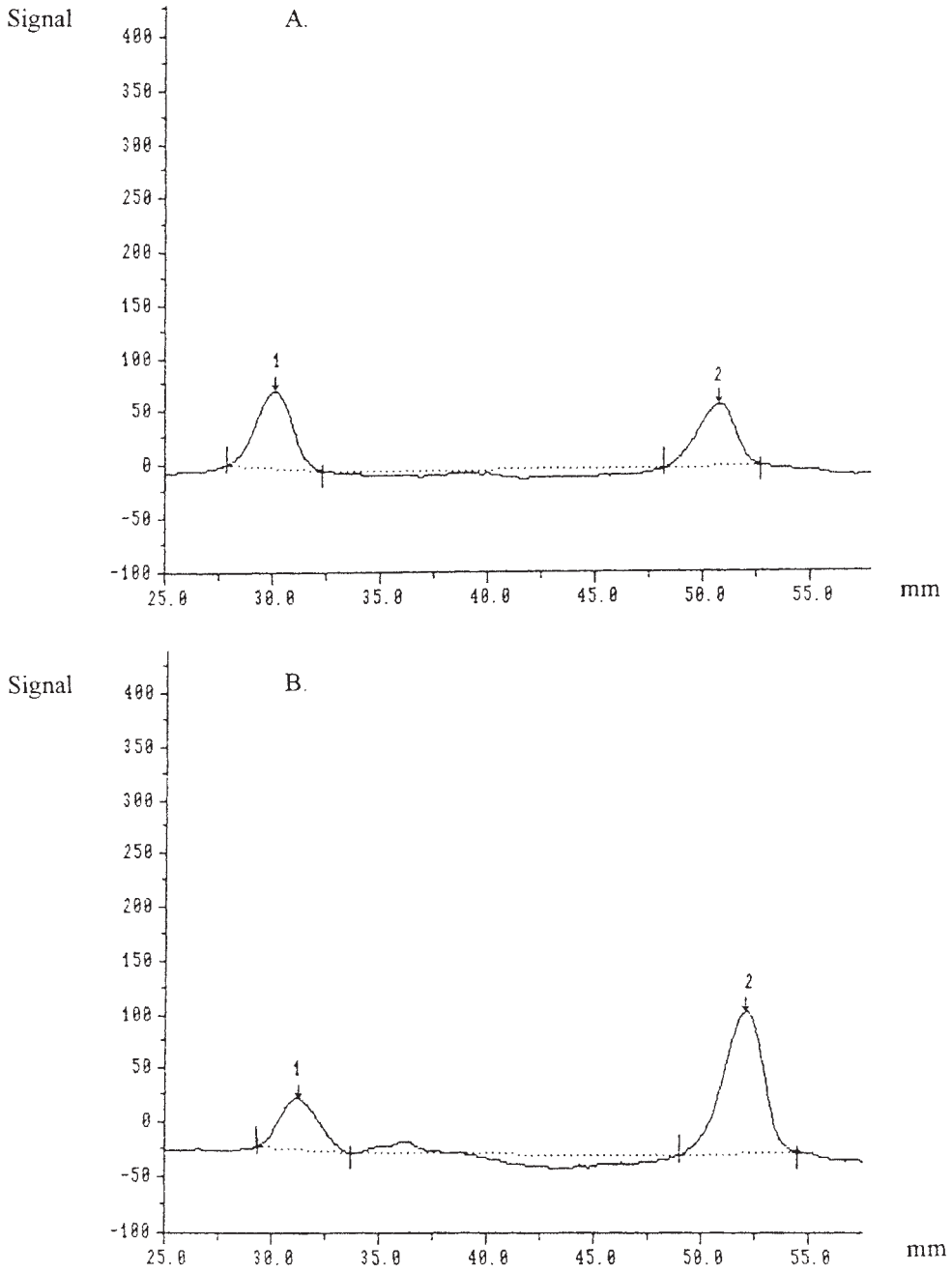

Figure 3. Densitograms obtained for (A) a mixture of α-solanine and α-chaconine and (B) a potato sample. Peak 1: α-solanine; Peak 2: α-chaconine.

were obtained (concentration range: 100–1000 ng; r^2 = coefficient of determination): $y = 0.40x - 24.33$ and $r^2 = 0.9992$.

The linear regression analysis for α-solanine was first made by plotting peak area (y) versus the amount of applied substance in ng (x) and using the regression model based on the least-squares method; however, the plot of residuals seemed to show that variance increased with concentration. This phenomenon was confirmed by the Cochran test (g_{calc} [0.60] < $g_{(0,95;\ 6,\ 2)}$ [0.61]). Indeed, to apply the ordinary least-squares calculation, the pattern of residuals must be homogeneous, i.e., the variance must be constant over the range considered (homoscedasticity). Because the variance homogeneity hypothesis was rejected, application of a weighted regression model was recommended. To select the most appropriate weighting factor, the relationship between variance and concentration was modeled. The weighting factor was then the inverse of the concentration raised to the λth power, where λ was the slope of the line fitted to the data on the logarithmic scale (14, 15). In the present study, it was equal to $1/x^{2.159}$. Under these conditions, the ANOVA was performed for the

range 100–2000 ng and showed that the weighted regression model could be applied. The regression line was assessed with $F_{calc} > F_{(0.95;\,1,\,16)}$ (4.49), as well as the fit, with $F_{calc} < F_{(0.95;\,4,\,12)}$ (3.26) (cf. Table 1). By applying this regression model, the analysis of the response function gave the following equations (concentration range: 100–2000 ng): $y = 0.38x - 23.00$ and $r^2 = 0.9936$.

Note: For a smaller range (100–1500 ng), the simplest regression model based on the least-squares method can be used.

Detectability.—The limit of detection (LOD) is the smallest quantity of the targeted substance that can be detected, but not precisely quantitated, in the sample, and the LOQ is the smallest quantity of the targeted substance in the sample that can be assayed under experimental conditions with well-defined precision and accuracy (13). As shown in Table 1, 100 ng was the minimum amount of α-solanine and α-chaconine that could be reliably scanned. The LOD of the developed method was equal to 50 ng, the minimum amount giving the specific red color that could be reliably scanned for both glycoalkaloids.

Precision.—The precision of the HPTLC method was determined by measuring the repeatability and intermediate precision for α-solanine and α-chaconine at 3 concentration levels (Table 1). Variances of repeatability and time-dependent intermediate precision as well as the corresponding relative standard deviations (RSDs) were computed from the estimated concentrations. The RSD values presented in Table 1 were relatively low, <4.1% and <7.6% for α-chaconine and α-solanine, respectively, and illustrated the excellent precision of the proposed method.

Accuracy.—Accuracy is a measure of the agreement between a conventionally accepted value or a reference value and a mean experimental value (13). The accuracy of the present procedure was assessed by calculating the ratio for the amount of analyte found versus the spiked amount in the same dehydrated potato sample as described above, at different concentration levels (as illustrated in Table 1). To determine accuracy, the peak areas corresponding to the added quantities were calculated by subtracting the peak areas for nonspiked potato samples from those obtained for spiked potato samples. The corresponding quantities of GAs found were then compared with those added. The accuracy, defined as mean % ± interval of confidence ($P > 0.05$), shows that the HPTLC procedure developed for the determination of α-solanine and α-chaconine can be considered accurate within the concentration range investigated (Table 1). Except for the lowest quantity of added α-solanine, mean values were very close to theoretical concentrations, showing method accuracy ranging from 91.7 to 107.3% for the 2 compounds.

Conclusions

The HPTLC method that we propose is rapid and specific for the direct determination of α-solanine and α-chaconine in potatoes. It was validated, and good results with respect to linearity, accuracy, and precision were obtained for the concentration range studied.

We used extraction of dehydrated potatoes in this method instead of the usual disintegration in liquid nitrogen, followed by storage at –18°C (5). Additional experiments will soon be performed to compare both sample preparation methods. Several varieties of potatoes cultivated in Belgium will then be analyzed and compared.

References

(1) Slanina, P. (1990) *Food Chem. Toxicol.* **28**, 759–761

(2) Hopkins, J. (1995) *Food Chem. Toxicol.* **33**, 323–329

(3) Hellenäs, K.E., Branzell, C., Johnsson, H., & Slanina, P. (1995) *J. Sci. Food. Agric.* **67**, 125–128

(4) Hellenäs, K.E., Branzell, C., Johnsson, H., & Slanina, P. (1995) *J. Sci. Food. Agric.* **68**, 249–255

(5) Hellenäs, K.E., & Branzell, C. (1997) *J. AOAC Int.* **80**, 549–554

(6) Cadle, L.S., Stelzig, D.A., Harper, K.L., & Young, R.J. (1978) *J. Agric. Food Chem.* **26**, 1453–1454

(7) Ahmed, S.S., & Mûller, K. (1978) *Lebensm. Wiss. Technol.* **11**, 144–146

(8) Jellema, R., Elema, E.T., & Malingré, T.M. (1981) *J. Chromatogr.* **210**, 121–129

(9) Jadhav, S.J., Sharma, R.P., & Salunke, D.K. (1981) *CRC Crit. Rev. Toxicol.* **9**, 21–104

(10) Wagner, H., & Bladt, S. (1996) *Plant Drug Analysis, A Thin-Layer Chromatography Atlas*, 2nd Ed., Springer-Verlag, New York, NY

(11) Jork, H., Funk, W., Fischer, W., & Wimmer, H. (1990) *Thin-Layer Chromatography, Reagents and Detection Methods*, Vol. 1a, VCH, Weinheim, Germany

(12) E. Merck (1975) *Révélateurs Pour la Chromatographie en Couches Minces et Sur Papier*, E. Merck, Darmstadt, Germany

(13) *Text on Validation of Analytical Procedures: Definitions and Terminology (Q2A)* (1994) Tripartite International Conference on Harmonization (ICH) text, ICH Tech Coordination, London, UK, October 26

(14) Chapuzet, E., Mercier, N., Bervoas-Martin, S., Boulanger, B., Chevalier, P., Chiap, P., Grandjean, D., Hubert, Ph., Lagorce, P., Lallier, M., Laparra, M.C., Laurentie, M., & Nivet, J.C. (1997) *S.T.P. Pharma Pratiques* **7**, 169–194

(15) Hubert, Ph., Chiap, P., Crommen, J., Boulanger, B., Chapuzet, E., Mercier, N., Bervoas-Martin, S., Chevalier, P., Grandjean, D., Lagorce, P., Lallier, M., Laparra, M.C., Laurentie, M., & Nivet, J.C. (1999) *Anal. Chim. Acta* **391**, 135–148