Journal of Chromatography, 219 (1981) 403–410
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,192

ANALYSIS OF POTATO GLYCOALKALOIDS WITH RADIALLY COMPRESSED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC CARTRIDGES AND ETHANOLAMINE IN THE MOBILE PHASE

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(First received May 22nd, 1981; revised manuscript received July 13th, 1981)

SUMMARY

All recorded potato glycoalkaloids of the solanidine series are separated by the use of two reversed-phase (C_8 and C_{18}) and a silica column. The use of radially compressed cartridges and ethanolamine in the mobile phase enables rapid analysis in 3–6 min. The interaction of ethanolamine and the acetonitrile concentration in the mobile phase with the cartridge packing is examined, and, from this information, the optimal mobile phase for each cartridge is determined for maximum separation and minimum retention time. The optimal wavelength for detecting glycoalkaloids is found to be 200 nm.

INTRODUCTION

Screening of the glycoalkaloid content of potato breeding lines is now widely practised[1,2] following the disclosure by Zitnak and Johnston[3] of dangerously high levels of glycoalkaloids in a newly released potato cultivar. Further, a considerable amount of work is in progress to devise techniques for the control of glycoalkaloid synthesis in potatoes, which may occur due to exposure to light, low temperature or injury. Both lines of research are severly hampered by the slow and laborious methods available for glycoalkaloid analysis[4,5].

Current methods of glycoalkaloid analysis virtually all depend on reactions with the stereoid part of the glycoalkaloid molecule and give a result only for total glycoalkaloids[6–9]. Moreover, lengthy extraction and purification processes are necessary in order to obtain accurate analysis, and these processes can introduce considerable errors[4,10]. Recently, gas–liquid chromatographic techniques have been described that are capable of analysis of the individual glycoalkaloids[11–13]. However, these methods require chromatography at high temperatures, which shortens column life, and lengthy hydrolysis and/or derivatization of the glycoalkaloids. Individual

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steroidal alkaloids[14] and glycoalkaloids[15] may also be separated by thin-layer chromatography (TLC). Recent work has considerably shortened the time of analysis, and the application of densitometers enables quantitative analysis[16,17], but a large number of manipulative and preparative steps is still necessary, and the errors involved still make TLC only semiquantitative[5].

The method with the greatest potential for quantitative glycoalkaloid analysis is high-performance liquid chromatography (HPLC); it has the merit of minimal sample preparation, separation into individual glycoalkaloids and rapid, accurate analysis. Several recent papers have explored the HPLC of potato glycoalkaloids; as yet, none has described a method encompassing all the merits potentially achievable. Hunter *et al.* described a preparative[18] and an analytical[19] HPLC method for the individual steroidal alkaloids. Crabbe and Fryer[5] found that, to separate into individual glycoalkaloids, a bonded-phase (Carbohydrate) column was required, with analysis times of 15–20 min. These columns are easily contaminated and have a much shorter life than C_{18} and C_8 reversed-phase columns[5]. Bushway *et al.*[20] reported the separation of glycoalkaloids on bonded-phase (NH_2 and Carbohydrate) columns. The Carbohydrate column was superior, with analysis times of 15–20 min. Neither group of workers[5,20] could separate the major glycoalkaloids on reversed-phase columns.

This paper reports the separation of glycoalkaloids on more robust reversed-phase columns with greatly increased speed and sensitivity of analysis. This was achieved with radially compressed cartridges and the addition of ethanolamine to the mobile phase.

MATERIALS AND METHODS

The HPLC system consisted of a Waters Assoc. (Milford, MA, U.S.A.) M45 solvent-delivery system, an Altex 210 injector, a Waters RCM-100 compression module and a Varian LC flow cell (Model 02-00173-00), connected to a Varian 635 spectrophotometer. The columns used were Waters Silica, C_8 and C_{18} Radial-Paks, with 10 cm × 8 mm I.D. cartridges of each packing used; a 10 cm × 5 mm I.D. C_{18} cartridge was also used. The volume between injection and the solvent front for the 8 mm cartridges was *ca.* 3 ml; for the 5 mm column, this volume was 1.5 ml. The flow-rate for all analyses was 3 ml/min.

Acetonitrile of HPLC grade was obtained from Waters and ethanolamine (LR grade) and orthophosphoric acid (AR grade) from Ajax Chemicals (Sydney, Australia). Samples of α-solanine and α-chaconine were obtained from Sigma (St. Louis, MO, U.S.A.). Solanidine, solanadiene and β_2-chaconine were prepared from potato sprouts according to Coxon *et al.*[21]. The remaining glycoalkaloids, β- and γ-solanine, β_1- and β_2-chaconine and γ-chaconine were prepared by hydrolysis of α-solanine and α-chaconine, followed by TLC separation according to Lavintman *et al.*[22].

Separations were calculated from the resolution (R_s) equation $R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'}{k' - 1}\right)$ (see ref. 23), where α is the separation factor, k' is the capacity factor and N is the number of theoretical plates calculated as $16\left(\frac{V}{W}\right)^2$, for V = retention time and W = duration of peak.

RESULTS AND DISCUSSION

Glycoalkaloid detection

The column effluent was initially monitored with a refractive-index detector (Waters, Model 401), but sensitivity was low, the limit of detection (2 × noise) being 5 μg.

The limit of detection was improved greatly when the column effluent was monitored with a UV detector. The UV absorbance of glycoalkaloids is significant only at wavelengths shorter than 220 nm, (previous workers have used 208–215 nm[2,4] and 205 nm[10]). The UV absorbance of glycoalkaloids is due to the Δ^5-double bond in the alkaloid portion of the molecule[5]; therefore, the absorbance should be identical on a molar basis for glycoalkaloids with a common alkaloid group. The UV spectra of a 1 μg/25 μl injection of α-solanine and of α-chaconine were determined and were virtually identical; this was to be expected, since their molecular weights are extremely close. The maximum UV absorbance would appear to occur in the region 190–195 nm, although high solvent absorbance did not permit operation of the detector in this range. At 195 nm and below, sugars begin to absorb significantly[2,4], and, since the UV absorbances of individual sugars vary, the equal absorbance of glycoalkaloids on a molar basis no longer applies. The optimal wavelength for detection and quantitation of glycoalkaloids without interference from sugars was therefore 200 nm. The absorbance at 200 nm was only 7% lower than that at 195 nm, and, further, the high background absorbance of the solvent was reduced. Detection of glycoalkaloids at 200 nm with acetonitrile–water was 55% more sensitive than that with methanol–water at 205 nm (ref. 5) and 960% more sensitive than that with tetrahydrofuran–water–acetonitrile at 215 nm (ref. 20). The limit of detection at 200 nm was 0.1 μg, a considerable improvement over that with a refractive index detector.

The present work confirmed the difficulty of separating α-solanine and α-chaconine with a μBondapak C_{18} column[5,20], although addition of ethanolamine to the mobile phase resulted in a slightly forked peak. As separation was still inadequate, C_{18} Radial-Pak cartridges were assessed with encouraging results, but retention times were lengthy and the effect of ethanolamine was unpredictable. In order to understand the effect of ethanolamine more clearly and to reduce retention times, mobile phases of varying acetonitrile and ethanolamine concentrations were examined for the three packings available in Radial-Pak cartridges.

Initially, a negative peak occurred after the solvent front with some mobile phases containing ethanolamine. This was caused by too rapid addition of the orthophosphoric acid when adjusting the pH of the mobile phase from 11 to 3–5, which was the optimal range for peak shape and background absorbance. A reaction in the solvent mixture of ethanolamine with high concentrations of orthophosphoric acid is the most likely cause of this problem, as slow addition of orthophosphoric acid to the mobile phase (with thorough mixing) prevented the negative peak. This solvent mixture is most likely to be unstable at higher ethanolamine and acetonitrile concentrations due to the insolubility of ethanolamine under these conditions.

Addition of ethanolamine to the mobile phase greatly improved the speed of analysis (Fig. 1a–c). The retention time of α-chaconine on the C_8 cartridge was reduced from 18.4 min with a mobile phase of acetonitrile–water (60:40) and 0.01% of ethanolamine to 1.5 min with the same mobile phase containing 0.2% of ethanol-

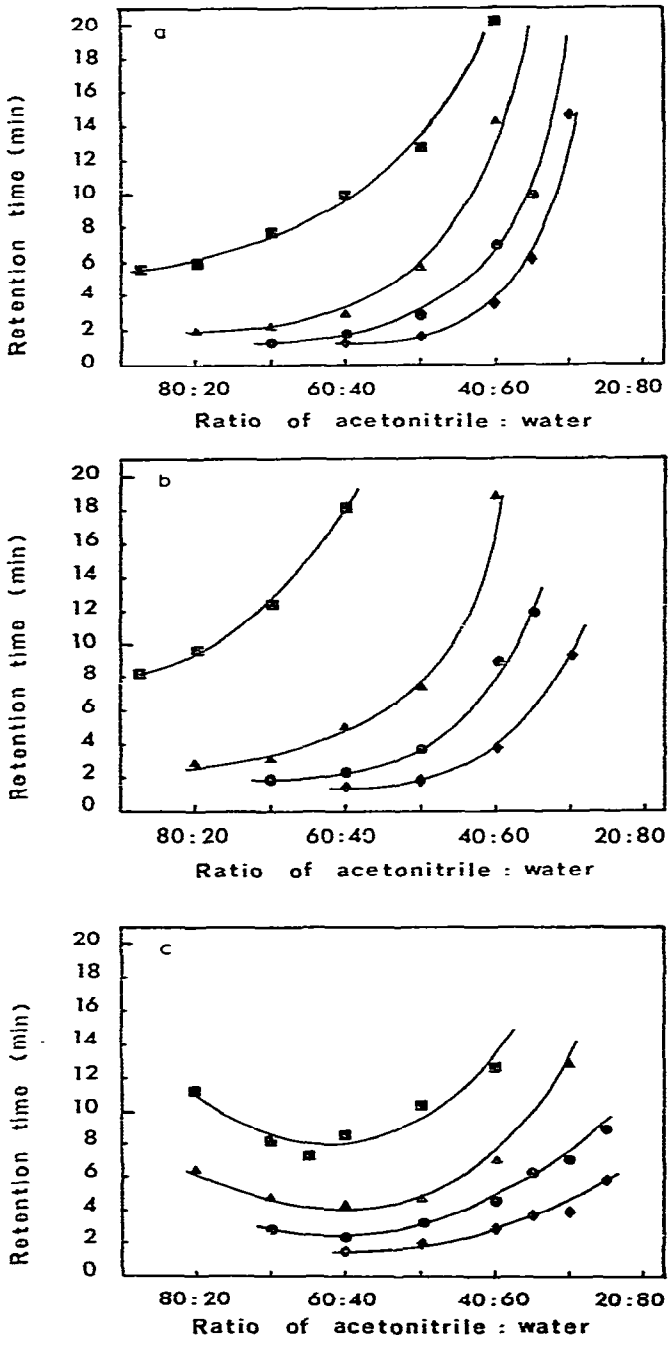

Fig. 1. Effect of concentration of ethanolamine and acetonitrile proportion on retention time. (a) C_{18} 8-mm cartridge; (b) C_8 8-mm cartridge, retention time for α-chaconine (which elutes last from C_{18} and C_8 cartridges); (c) Silica 8-mm cartridge, with retention time for α-solanine, as this elutes last from this cartridge. Ethanolamine concentrations: ■, 0.01%; ▲, 0.05%; ●, 0.1%; ◆, 0.2%.

amine. Moreover, the ratio of resolution (R_s) to retention time (t_R) or R_s/t_R, is greatly improved from 0 without ethanolamine (no separation) to 0.75 with 0.2% of ethanolamine.

The levels of acetonitrile and ethanolamine in the mobile phase also affected retention time and resolution. For example, with the C_8 cartridge and the mobile phase containing 0.05% of ethanolamine, R_s/t_R was 0.3 for acetonitrile–water (80:20) 0.43 for (70:30) and 0.05 for (40:60). With the C_{18} column, the maximum of R_s/t_R was at acetonitrile–water (60:40), whereas R_s/t_R for the Silica column was increased with concentrations of acetonitrile and ethanolamine up to the limits of solubility of ethanolamine in acetonitrile–water. These limits are 90:10 for 0.01% of ethanolamine, 80:20 for 0.05%, 75:25 for 0.1% and 65:35 for 0.2%.

Optimal mobile-phase composition for separation of α-solanine and α-chaconine

An R_s of 1.25 for α-solanine and α-chaconine was considered adequate for analytical work, and the optimal mobile-phase composition that minimised retention time but still achieved this separation was determined for each column. The optimum mobile phase for the C_{18} cartridge was acetonitrile–water–ethanolamine 35:65:0.2; for the C_8 column, the proportions were 50:50:0.2. However, at 200 nm, these mobile phases had high UV absorbance due to ethanolamine. At 0.1% of ethanolamine, this problem was eliminated, the optimal mobile-phase composition then becoming 55:45:0.1 for C_8 and 45:55:0.1 for C_{18}. A 5 mm I.D. C_{18} cartridge gave even more

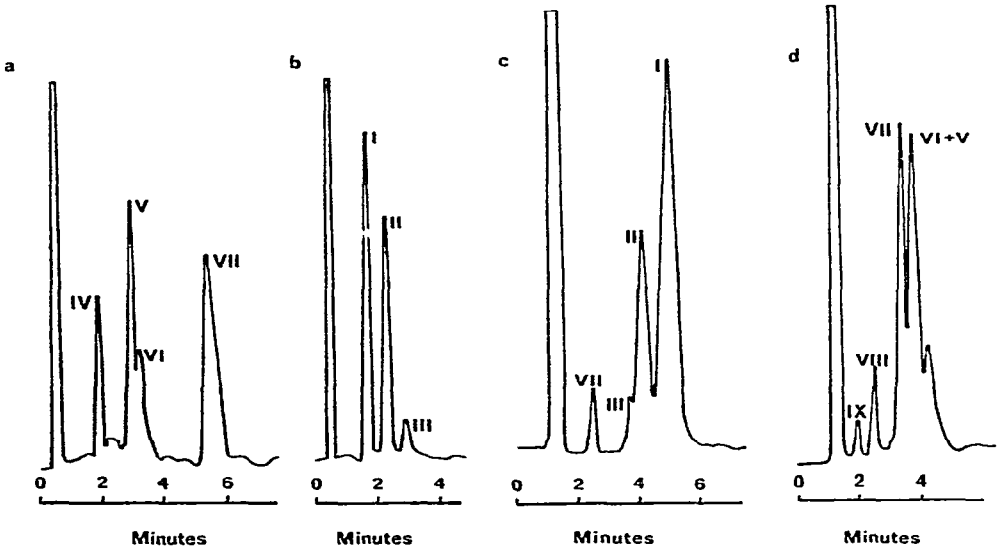

Fig. 2. HPLC separation of glycoalkaloids of the α-solanine and α-chaconine series. Conditions of chromatography: (a) α-Solanine hydrolysate, C_{18} 5-mm cartridge, mobile phase acetonitrile–water–ethanolamine (45:55:0.1), a.u.f.s. 0.025. (b) α-Chaconine hydrolysate, other conditions as in (a). (c) α-Solanine hydrolysate, Silica 8-mm cartridge, mobile phase proportions 77.5:22.5:0.05, a.u.f.s. 0.025. (d) α-Chaconine hydrolysate, other conditions as in (c). Peaks: I = α-solanine; II = β-solanine; III = γ-solanine; IV = α-chaconine; V = β_1-chaconine; VI = β_2-chaconine; VII = γ-chaconine; VIII = solanidine; IX = solanadiene.

rapid analysis with the latter mobile phase, without any significant loss of resolution compared to that from the 8 mm I.D. C_{18} cartridge.

The Silica cartridge showed a behaviour different from that of the C_{18} and C_8 reversed-phase cartridges. At high acetonitrile concentration (>60%), the order of elution from this cartridge was the reverse of that from the C_{18} and C_8 cartridges, α-chaconine being eluted first and being separated from α-solanine. At a lower acetonitrile concentration (<50%), the column operated in a reverse manner, α-solanine and α-chaconine being eluted as one peak, followed by the less polar solanidine. The optimal mobile phase composition for the separation of α-solanine and α-chaconine was established as 77.5:22.5:0.5. For maximum sensitivity, although without separation of α-solanine and α-chaconine, the optimal composition was 45:55:0.1.

Separation of other glycoalkaloids in the solanidine series

Besides α-solanine and α-chaconine, significant levels of β_2-chaconine and solanidine can occur in potato extracts[15,25]. Therefore, the optimal mobile phase–cartridge combinations established for the separation of α-solanine and α-chaconine were tested with other glycoalkaloids; the results (for the C_{18} column only are presented in Figs. 2a and 2b. However, the C_8 column is also capable of separating the hydrolytic products of both α-solanine and α-chaconine. The separation of the combined products of α-solanine and α-chaconine hydrolysis is possible, except for γ-

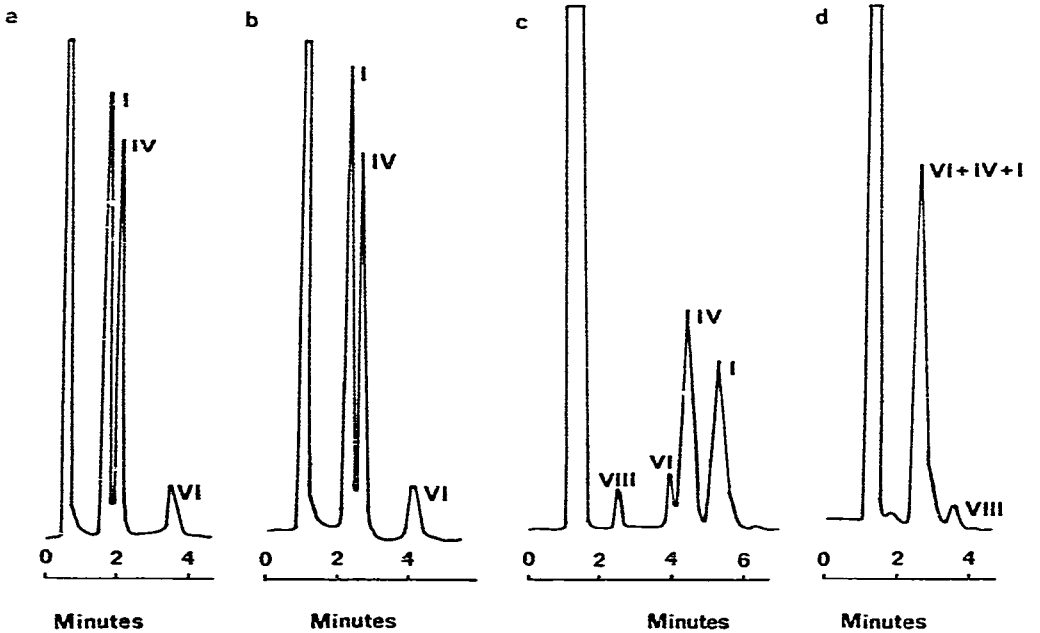

Fig. 3. Separation of glycoalkaloids of potato tubers (cv. Pentland Crown). Glycoalkaloids were extracted with 2% acetic acid, precipitated with ammonia, redissolved in 1 ml of 2% acetic acid and drawn through a filter tip of porosity 2 μm (Supelco, Bellefonte, PA, U.S.A.). Conditions of chromatography: (a) C_{18} 5-mm cartridge, mobile phase acetonitrile–water–ethanolamine (45:55:0.1), a.u.f.s. 0.025; (b) C_8 8-mm cartridge, mobile phase 55:45:0.1, a.u.f.s. 0.025; (c) Silica 8-mm cartridge, mobile phase 77.5:22.5:0.05, a.u.f.s. 0.025; (d) Silica 8-mm cartridge, mobile phase 45:55:0.01, a.u.f.s. 0.05.

solanine and β_1-chaconine, which have similar retention times. Solanidine and solanadiene were retained on the reversed-phase cartridges with all mobile phases tested.

On Silica cartridges, separation and peak shape of the hydrolytic products are not as good as with C_{18} and C_8 cartridges (Fig. 2c and d). However, the hydrolytic products of both α-solanine and α-chaconine were separated, except for β_1- and β_2-chaconine, which were eluted as one peak. The separation of all combined hydrolytic products was not possible, as β- and α-solanine have retention times similar to α-, and to β_1- and β_2-chaconine, respectively. Unlike the reversed-phase cartridges, both solanidine and solanadiene can be eluted and separated with the Silica cartridge at the same time as higher-order glycoalkaloids (see Fig. 2d).

Analysis of tuber extracts

The methods developed were evaluated with acetic acid extracts from a cultivar that contained higher than normal levels of β_2-chaconine and solanidine (levels per g FW in this sample were α-solanine 54.4 μg, β_1-chaconine 79.6 μg, β_2-chaconine 9.7 μg and solanidine 10.8 μg). On the reversed-phase C_{18} and C_8 packings, the peaks were clearly separated from the solvent front with adequate resolution of α-solanine, α-chaconine and β_2-chaconine; however the solanidine peak was not eluted (Figs. 3a and b). On the Silica column, with the 77.5:22.5:0.05 mobile phase, all four compounds were eluted with acceptable resolution (Fig. 3c). With the 45:55:0.1 solvent mixture, α-solanine, α-chaconine and β_2-chaconine were eluted as one peak, solanidine following shortly afterwards (Fig. 3d). Where only total glycoalkaloids are required, this column–mobile phase combination is desirable, both for its speed and increased sensitivity of analysis.

This work reports for the first time a rapid separation of all known glycoalkaloids in the solanidine series, including the separation of β_1- from β_2-chaconine. Similar separations have been made by TLC, but analysis times of up to several hours are required[15,22,26]. For routine analysis of potato glycoalkaloids, the reversed-phase cartridges are recommended, owing to their robust characteristics and long life. These should permit a routine 3-min analysis time, although, when significant levels of β_2-chaconine are present, the time of analysis may be 5 min. When solanidine is expected, the Silica cartridge should be used, either with 45% of acetonitrile, which elutes α-solanine and α-chaconine as one peak followed by solanidine (analysis time 4 min), or with 77.5% of acetonitrile, which elutes solanidine first, followed by α-chaconine and α-solanine peaks (analysis time 6 min).

ACKNOWLEDGEMENTS

The advice and assistance of W. R. Day (Waters Associates, Sydney, Australia) is gratefully acknowledged. This work was carried out with the assistance of a Rural Credits Development Fund grant from the Reserve Bank of Australia.

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