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Short Communication

Rapid determination of solanum glycoalkaloids by thin-layer chromatographic scanning

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ABSTRACT

A rapid quantitative TLC method for the determination of glycoalkaloids in potato leaves and tubers is described. The method includes a microscale extraction, a simple clean-up step and quantification by TLC scanning. The method is valuable for the determination of glycoalkaloids when many samples have to be evaluated as in potato breeding programmes.

INTRODUCTION

The main potato glycoalkaloids (GAs), solanine and chaconine, are trisaccharide glycosides with a common tertiary amine aglycone, solanidine [1]. They represent 95% of the total GAs in cultivated potato varieties and play an important role in the natural defence mechanism against some economically important pests such as fungi, insects and viruses. They are toxic to humans, generally promoting gastrointestinal and neurological disorders [2].

The commercial potato cultivars contain small amounts of GAs in leaves, sprouts and tubers. Concentrations higher than 185 ppm can present risks to human consumers. Concentrations below this limit must be confirmed before delivering new varieties to the market. Wild *Solanum* species, increasingly used in breeding programmes, contain higher GA concentrations, and also GAs not present in the cultivated potato varieties. This determines the need for monitoring the GA content in the breeding processes to avoid hybrids with good resistance

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properties, but unsuitable for human consumption [3]. The determination of GAs in plants, potato tubers and potato products presents considerable difficulties. Almost all the common analytical methods have been tested: spectrophotometric and titrimetric determinations [4,5], quantitative TLC [6,7], GC [8,9] and HPLC [10].

The most satisfactory, from a quantitative point of view, were GC and HPLC. GC requires a hydrolytic step, which implies that only the aglycones can be detected. This represents a loss of information on the individual GAs originally present. The HPLC methods developed require the use of lengthy sample purification procedures and the use of amino columns [10]. The GAs do not have strong chromophores, so the sensitivity with UV detectors is low, and it is necessary to work with short wavelengths, losing selectivity.

In this work, a simple and economic microscale method for the TLC determination of potato GAs, with a purification step using Sep-Pak C_{18} cartridges, was set up.

EXPERIMENTAL

Chemicals and reagents

A glycoalkaloid stock standard solution (1 mg/ml) was prepared by dissolving 10 mg of α-solanine or α-chaconine (Sigma, St. Louis, MO, USA) in 10 ml of methanol–acetic acid (99:1) and working standard solutions (0.1 mg/ml) were prepared by dilution of 1 ml of stock standard solution to 10 ml with the same solvent. Both the stock and working standard solutions were kept at −4°C and were stable for 2 months.

Sep-Pak C_{18} cartridges (Millipore, Milford, MA, USA) were used for the pre-purification step. They were preconditioned by elution with methanol (3 × 3 ml) and water (3 × 3 ml).

Pre-coated silica gel TLC plates (5 × 8 cm, nylon supported, Machery–Nagel, Düren, Germany and 10 × 20 cm, glass supported, Merck, Darmstadt, Germany) were used. Both supports gave similar R_F values. Reversed-phase TLC plates were RP-18 W (Macherey–Nagel). Solvents were of analytical-reagent grade.

Instrumental

TLC scanning determinations were performed on a Shimadzu (Tokyo, Japan) Model 9300 TLC scanner at 560 nm for Carr–Price reagent [11] or 505 nm for Dragendorff reagent [11], using the reflection system in the zig-zag mode with an 8-mm swing width.

Sample preparation

Samples of leaves or tubers (1 g) were minced with 5 ml of 1% acetic acid using a glass rod. The rod was rinsed with 1 ml of the extraction solution and the tube was capped and sonicated for 5 min. The tube was centrifuged (5000 *g*, 5 min), the solution was removed with a Pasteur pipette and the residue was re-extracted as before with 5 ml of 1% acetic acid. The combined extracts were applied to a preconditioned Sep-Pak C_{18} cartridge. The cartridge was washed with 40% aqueous methanol (10 ml) and the glycoalkaloids were eluted with methanol (10 ml). The solvent was evaporated under an air current. The residue was dissolved in 500 μl of methanol and this solution was used for the quantification step.

TLC and quantification procedures

Aliquots (5 μl) of the solutions were applied with an HPLC syringe in duplicate on the TLC plate together with increasing volumes (2, 4, 6 and 8 μl) of the working standard solution (in duplicate). The plate was developed to 10 cm in a 17.5 × 11.0 × 6.2 cm chamber (saturation time 30 min), using chloroform–methanol–2% aqueous ammonia (70:30:5) as the mobile phase, air dried, sprayed with Carr–Price reagent and heated for 2 min at 110°C. The spots were measured within 30 min and GAs concentrations in the samples were calculated using the calibration graph thus constructed. When the plates were developed with Dragendorff reagent, the plate was air dried, sprayed with the reagent, air-dried and measured as before in the same instrument.

RESULTS AND DISCUSSION

Chromatographic system

Both reversed- and normal-phase TLC were tested. In accordance with the results reported

for HPLC [10], the reversed-phase system is unable to give a useful separation of solanine from chaconine. The best results obtained are reported in Table I.

Many solvent systems have been reported [12] for the normal-phase TLC separation of potato GAs. Among the several we tested, the best results were obtained with two mixtures, one containing acetic acid and the other aqueous ammonia. The R_F values are given in Table I.

It was not possible to reproduce the R_F values reported in the literature, and the system was found to be strongly dependent on the saturation of the chamber and the progressive evaporation of the mobile phase, especially with the acetic acid mixture. It is advisable for each laboratory to standardize the system carefully before routine use.

Detection reagents

Several detection reagents have been used with GAs, including Dragendorff, Carr–Price and optical brightener reagents. In early attempts a clinical densitometer and Carr–Price reagent were used, but the detection conditions could not be optimized owing to instrumental limitations [6]. We compared Dragendorff and Carr–Price reagents with respect to their sensitivity and selectivity. The relative response between α-solanine and α-chaconine was similar, 0.97 for Dragendorff and 1.1 for Carr–Price reagent, making correction unnecessary for routine use. Table II summarizes the results obtained.

TABLE II

COMPARISON OF DRAGENDORFF AND CARR–PRICE REAGENTS

Stationary phase, silica gel 60; solvent, chloroform–methanol–2% aqueous ammonia (70:30:5).

Reagent	Detection limit (μg)	Range (μg)	Correlation coefficient
Dragendorff	0.8–1.0	0.8–8.0	0.9991
Carr–Price	0.2–0.3	0.2–2.0	0.9996

Typical solanine, chaconine and extract chromatograms are shown in Figs. 1 and 2. Fig. 1 shows the higher sensitivity obtained with the

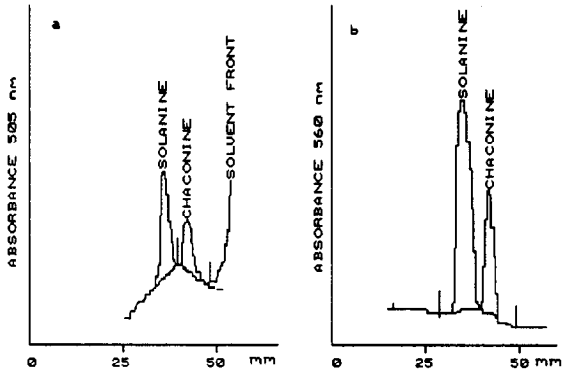

Fig. 1. Comparison of Dragendorff and Carr–Price reagents for standard solutions of α-solanine and α-chaconine. Dragendorff reagent, 5 μg of each; (b) Carr–Price reagent, 0.5 μg. Stationary phase, silica gel 60; solvent system, chloroform–methanol–2% aqueous ammonia (70:30:5).

TABLE I

SOLVENT SYSTEMS FOR THE TLC OF GLYCOALKALOIDS

$hR_F = 100\ R_F$; RP = reversed-phase C_{18}; NP = normal phase. All TLC was carried out on precoated plates.

Stationary phase	Solvent	hR_F	
		Solanine	Chaconine
RP	MeOH–0.2% NH_3 (80:20)	14	17
	MeOH–0.2% NH_3 (75:25)	14	20
NP	$CHCl_3$–MeOH–1% NH_3 (50:50:1)	88	92
	$CHCl_3$–MeOH–1% NH_3 (80:20:1)	22	31
	$CHCl_3$–MeOH–2% NH_3 (70:30:5)	35	45
	$CHCl_3$–MeOH–AcOH (50:45:5)	62	93

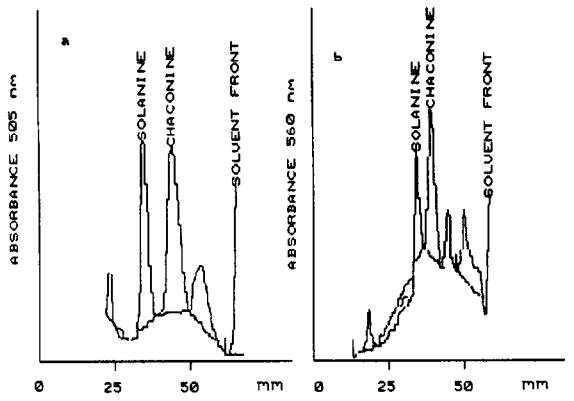

Fig. 2. Comparison of Dragendorff and Carr–Price reagents for extracts of potato leaves. Conditions as in Fig. 1.

Carr–Price reagent compared with Dragendorff reagent. The detection limit with Carr–Price reagent, defined as the concentration at which the signal-to-noise ratio is ≥ 2, was comparable to that reported for optical brightners (OBs) [7], so it is the preferred detection reagent when the sensitivity is the main factor to be considered. The Dragendorff and Carr–Price reagents showed a good linear response ($r_1 = 0.9991$ and $r_2 = 0.9996$, respectively) in the 0.8–8.0 μg and 0.2–2.0 μg concentration ranges, and showed deviations from linearity only at relatively high concentrations.

Dragendorff reagent is more selective than the Carr–Price reagent, as can be seen by comparing Fig. 2a and b, so unless a lower detection limit and sensitivity are required, it is the reagent of choice.

TLC scanning conditions

To test the instrumental error, the same spot was measured 30 times. All the values were within the mean $\pm 1.6s$ range (s = standard deviation) with a relative standard deviation (R.S.D.) of 3.3%.

The precision of the method was determined by applying the same GA concentration five times and measuring each three times. All the values were within the mean $\pm 1.5s$ range with an R.S.D. of 5.6%.

To determine the accuracy of the method, the values obtained with the standard solutions used above were interpolated on the calibration graph and the concentrations obtained were compared with the actual concentrations. The accuracy so determined was 6.2%.

Extraction

GA quaternary ammonium salts are soluble in both water and lower alcohols, but strong acids can hydrolyse the glycosidic linkages, reducing the recovery. Quantitative extraction has proved difficult, and the traditional extraction techniques [10] require the use of large volumes of solvents and several lengthy purification steps.

The proposed method is adequate for quantitative work with many samples, such as in breeding programmes, with good recoveries and simple operation. The overall recovery was measured by adding known concentrations of GAs to control samples, and was 82%. The technique gives "dirtier" extracts than the classical extraction procedures, but the chromatographic system used and the specificity of the detection reagent resolve the resulting mixtures.

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