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CAPILLARY GAS CHROMATOGRAPHY OF STEROIDAL ALKALOIDS FROM SOLANACEAE

RETENTION INDICES AND SIMULTANEOUS FLAME IONIZATION/NITROGEN-SPECIFIC DETECTION

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SUMMARY

A capillary gas chromatography method for identification and quantification of *Solanum* and *Lycopersicon* C_{27}-steroidal alkaloids (SAs) was developed. Hydrogen was used as the carrier gas to achieve a relatively short analysis duration. Simultaneous flame ionization and nitrogen-specific detection (FID and NPD) enabled unambiguous differentiation between SAs and closely related non-nitrogen-containing compounds, such as sterols and steroidal sapogenins. Calibration plots for FID and NPD were linear for all individual SAs tested. The NPD/FID response ratios appeared to be an aid in identification and characterization of the compounds. Tuber samples of *Solanum* species varied in contents of solanidine glycosides from 600 to 6220 mg kg^{-1} fresh weight. Because of these large differences, identification by retention times was not possible, but retention indices were sufficiently reproducible even for identification of the closely eluting $\Delta5$- and 5α-aglycone pair solanidine and demissidine. Although, SAs are not usually easily quantified by capillary gas chromatography, quantitative analyses can be done routinely provided sufficient attention is paid to the accuracy and precision of the sample injection.

INTRODUCTION

C_{27}-steroidal alkaloids (SAs) are natural toxins of the Solanaceae. They occur mainly as glycoalkaloids, which are conjugates of a steroidal alkaloid (aglycone) and a sugar moiety, often a tri- or tetrasaccharide[1]. The edible parts of potato and tomato cultivars usually contain small amounts of SAs. In The Netherlands, most household potato varieties have contents of solanidine glycosides of 20–85 mg kg^{-1} unpeeled tuber[2] (in all cases contents are expressed as mg kg^{-1} fresh weight). Contents up to 150

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mg kg^{-1} have been reported to be normal for potatoes[3,4]. Levels above 200 mg kg^{-1}, which may arise from various environmental factors[4,5], are regarded as a potential health risk to the consumer. In Sweden in 1986, large amounts of the variety Magnum Bonum had to be withdrawn from commerce because of incidences of illness due to high levels of solanidine glycosides. Levels of tomatidine glycosides up to 5 mg kg^{-1} red-ripe fruits have been reported for tomato cultivars[5–7].

Wild species of the genera *Solanum* and *Lycopersicon* may contain more than 1000 mg glycoalkaloids kg^{-1} in tubers and fruits, respectively[7–9]. Such species are increasingly being used in plant breeding programmes to introduce resistance to diseases, pests or climate stress. However, it must be emphasized that little is known about the complete profile of the SAs of Solanaceae food plants. This is especially true for the wild species, in which even novel SAs may be found. Therefore, plant breeders should be careful not to introduce either new types of SAs or high levels of solanidine or tomatidine glycosides into cultivars. In the United States, a commercial potato variety with disease resistance, inherited from wild species, has been withdrawn because of its high levels of glycoalkaloids in the tubers (160–350 mg kg^{-1})[10]. Consequently, wild species and sometimes the hybrid progeny have to be analyzed qualitatively and quantitatively for their SAs.

A method for assessing the SA composition of potato breeding material by capillary gas chromatography has recently been published[11]. This method proved to be applicable to tomato breeding material as well[7]. By this method, several unidentified components were detected in Solanaceae species, in addition to the available reference alkaloids[7,9,12]. In the screening of breeding material for SAs, the characterization of each unknown peak in a chromatogram by costly spectrometric techniques is hardly feasible. This paper describes a method that enables differentiation between peaks of unknown SAs and peaks of non-alkaloidal constituents. The method involves a dual detector system consisting of a flame ionization detector and a nitrogen–phosphorus detector, which is connected to a two-channel system for interactive processing of the two sets of data. As a further aid for identification of the SAs, a retention index system was introduced. The chromatographic variables were optimized, and hydrogen was used as the carrier gas in order to decrease the duration of the analyses. Spectrometric techniques had to be employed for characterization only when new SAs were present. The advantages and the problems of this method are discussed.

EXPERIMENTAL

Apparatus

A Packard 439 microprocessor-controlled gas chromatograph equipped with flame ionization detection (FID) and nitrogen–phosphorus detection (NPD) and with two split/splitless-type injectors was used. One injector was in the split sampling mode, splitting ratio 1:50; the vaporization tube was a modified Jennings tube[13] provided with a fritted glass filter and partly filled with deactivated quartz-wool. The other injector was used for controlled supply of 20 ml nitrogen min^{-1} as a make-up gas for a four-way effluent-splitting device, which consisted of a quartz tube (9 mm × 1.3 mm I.D.) and two two-hole vespel ferrules in a stainless-steel housing. The effluent splitter connected the column with both detectors via two deactivated fused-silica restriction

tubes (20 cm × 0.22 mm I.D.). The effluent-splitting ratio for FID and NPD, which depends on the length ratio of the restriction tubes, was kept at 1:1 in order to obtain identical retention times for the FID and the NPD traces. The column was fused silica (50 m × 0.22 mm I.D.) coated with CP-Sil 5 CB, film thickness 0.12 μm (Chrompack Nederland, Middelburg, The Netherlands). The oven temperature was 270 or 280°C. The temperature of the detector was 325°C and of the injector initially 300°C but after the optimization experiment it was 350°C. Hydrogen was used as the carrier gas, linear velocity 49 cm s^{-1}, unless stated otherwise. The flow-rates of hydrogen and air were 25 and 250 ml min^{-1} respectively for FID, and 5.0 and 50 ml min^{-1} for NPD. The detectors were each supplied with 30 ml nitrogen min^{-1} as a make-up gas (unless stated otherwise), including 10 ml min^{-1} each of the make-up gas from the effluent splitter. For the injector, Chromsep Red, high-temperature (up to 450°C) septa (Chrompack) were employed. Two Shimadzu C-R3A data processors were used for recording the FID and NPD signals and for interactive processing of the data. The processors were interconnected by a PC14N current loop interface, an RS232C and a MIC-loop.

Software

For communication between the data processors, a program was written partly in BASIC and partly in machine language. After the introduction of the identification system using NPD/FID response ratios and retention indices, a BASIC program was written for automated calculation of these values. Integrated into the BASIC program, concentrations were measured using the ROM-software of the data processors.

Alkaloid standards and other chemicals

Solanidine, solanthrene, demissidine, solasodiene, solasodine, tomatidine, 5α-cholestane, the solvents and other chemicals were prepared or obtained as described[11,14]. For comparison of SAs and test compounds with respect to the relationship between injection volumes and peak areas, a commercially available test mixture (Chrompack, Cat. No. 6606) was used. This contained *n*-octanal, *n*-octanol-1, *n*-undecane, 2,6-dimethylphenol, methyl *n*-octanoate, 2,6-dimethylaniline, naphthalene, *n*-dodecane, *n*-tridecane and methyl *n*-decanoate, each in a concentration of 1% in cyclohexane. Cholesterol and stigmasterol were obtained from Roth GmbH & Co (Karlsruhe, F.R.G.), diosgenin, *n*-octacosane and *n*-tetratriacontane from Sigma (St. Louis, MO, U.S.A.) and jervine and tigogenin from ICN Pharmaceuticals (Plainview, NY, U.S.A.).

Plant material

Tuber samples of *Solanum* species were obtained from the Potato Breeding Department of the Foundation for Agricultural Plant Breeding (SVP). They included accessions of *S. vernei*, *S. sucrense*, *S. brevicaule*, *S. oplocense*, *S. spegazzinii* and *S. leptophyes*.

Sample preparation and quantification of solanidine glycosides

Sample preparation was done using described methods for extraction[11] and two-phase hydrolysis[14]. The following modifications were made. A sample of 6.25 g of water-homogenized tuber, corresponding to 5 g fresh tuber, was extracted twice, with 100 and 75 ml of methanol–chloroform (2:1, v/v), respectively. The hydrolysis

conditions were: 50 ml of 2 mol l^{-1} hydrochloric acid; 100 ml chloroform; reaction period 4 h. Solanidine glycosides were quantified using the peak areas of solanidine and of solanthrene.

Calculation of retention indices

Retention indices[15], *I*, on CP-Sil 5 CB at 270°C were calculated using the formula

$$I_{270}^{\text{CP-Sil 5 CB}} = 100\ C_1 + 100\ (C_2 - C_1) \cdot \frac{\log\ (t_{R,i} - t_{R,0}) - \log\ (t_{R,1} - t_{R,0})}{\log\ (t_{R,2} - t_{R,0}) - \log\ (t_{R,1} - t_{R,0})}$$

where C_1 = the carbon number of the first reference compound (*n*-octacosane, $C_{28}H_{58}$), C_2 = the carbon number of the second reference compound (*n*-tetratriacontane, $C_{34}H_{70}$), $t_{R,0}$ = the retention time of an unretarded component (methane), $t_{R,i}$, $t_{R,1}$ and $t_{R,2}$ = the retention times of the component of which *I* has to be determined, of the first reference compound and of the second reference compound, respectively.

RESULTS AND DISCUSSION

Separation of C_{27}-steroidal alkaloids

The effect of the injector temperature on the retention time, relative response, chromatographic stability and resolution was investigated by increasing this temperature from 300 to 450°C by increments of 25°C. The temperature increase gradually

TABLE I

RETENTION TIMES t_R AND RESOLUTION, *R*, OF C_{27}-STEROIDAL ALKALOIDS (SAs) USING NITROGEN, HELIUM AND HYDROGEN AS CARRIER GASES

Column: fused silica, 50 m × 0.22 mm I.D., CP-SIL 5 CB, film thickness 0.12 μm. Optimized average linear velocities of carrier gas ($\bar{\mu}$); injector temperature 350°C.

SA	*Oven temperature (O.T.) 280°C*						*O.T. 270°C Hydrogen $\bar{\mu}$ 49.0 cm s^{-1}*	
	Nitrogen $\bar{\mu}$ 13.5 cm s^{-1}		*Helium $\bar{\mu}$ 24.3 cm s^{-1}*		*Hydrogen $\bar{\mu}$ 48.8 cm s^{-1}*			
	t_R (min)	*R*	*t_R (min)*	*R*	*t_R (min)*	*R*	*t_R (min)*	*R*
Solanidine	30.74		16.98		8.60		10.96	
		2.00		1.84		1.76		1.92
Demissidine	31.28		17.27		8.74		11.15	
		3.64		3.04		3.01		3.19
Solasodiene	32.28		17.83		9.02		11.57	
		35.8		30.3		33.2		33.2
Solasodine	46.56		25.30		13.03		17.16	
		6.71		6.13		6.29		6.23
Tomatidine	49.85		27.10		13.94		18.43	

resulted in shorter retention times of the SAs but the effect was negligible (<2%) even at 450°C. The compound ratios, *e.g.*, that of solanidine and tomatidine, did not change at the various injector temperatures, which meant that all the SAs were vaporized to the same extent at the various injector temperatures. Thus, the injector temperature did not influence the possible discrimination of the SAs. However, increasing the injector temperature deteriorated the baseline stability and peak symmetry, and caused ghost peaks, especially at temperatures >375°C. These phenomena probably resulted from septum bleeding and/or sample decomposition. The resolution of the early eluting compounds solanidine and demissidine was optimum at an injector temperature of 350°C, whereas for the late eluting compounds such as solasodine and tomatidine it was optimum at 375–400°C. Because the chromatographic stability deteriorated at the higher temperatures and an optimum resolution of the Δ5- and 5α-aglycone pair solanidine and demissidine was important, 350°C was chosen as the injector temperature.

Table I shows the retention times and the resolution of the SAs chromatographed on the CP-Sil 5 CB column at an oven temperature of 280°C using helium, nitrogen and hydrogen as the carrier gases at optimized average linear velocities. The highest resolution was obtained with nitrogen, but this was attended with long retention times. The separation efficiencies obtained with helium and hydrogen were similar, but using hydrogen the analysis duration was half as long. Comparison of the separation efficiencies with hydrogen at oven temperatures of 260, 270, 280 and 290°C showed that the optimum temperature for the separation of solanidine, demissidine and solasodiene was 270°C. Compared with helium as the carrier gas and the oven temperature of 280°C used before, 270°C was favourable with regard to the column durability (maximum operating temperature 300°C), and a 30% shorter analysis duration was obtained (Table I). Under the experimental conditions described and with careful maintenance of the injection system and column, the column performance was still satisfactory after 550 SA analyses of leaf and tuber samples of *Solanum* species and inter-specific hybrids, the resolution of solanidine and demissidine being 1.73.

Split injection of C_{27}-steroidal alkaloids

In isothermal capillary gas chromatography (GC) the split injection technique is preferable, especially when automated injection is applied. However, when quantitative results are required many problems may arise[16]. Therefore, the splitting process was investigated in order to study whether quantitative results could be obtained for the individual SAs.

For quantitative analyses it is necessary that the splitting is linear both for various concentrations and sample sizes. This was checked by constructing FID calibration curves for the internal standard 5α-cholestane, using two injection volumes, 0.9 and 1.9 μl. For both curves, the peak areas were found to be directly proportional to the concentrations. So, splitting was independent of concentration. However, the normalized FID responses of 5α-cholestane found for the 0.9-μl injection volumes were only 80% of those found for 1.9 μl. This suggested that the splitting ratio varied depending on the sample size. For quantitative analyses, the normalized peak areas must be similar for the various sample sizes and for each steroidal alkaloid. Therefore the concentrations of the compounds were kept constant, whilst the sample sizes were varied. Table II gives the average of the normalized peak areas of solanidine,

demissidine, solasodiene, solasodine and tomatidine for each sample size. The normalized peak areas were expressed as percentages of the peak areas, averaged for the SAs, at the injection volume of 2.4 μl. For each sample size the variation (range and % S.D.) in normalized peak areas between the individual SAs is also given. The experiments showed that the normalized peak areas decreased with decreasing sample sizes. The linearity of the calibration plots (see hereafter) showed that this was not due to irreversible adsorption of the components onto the column. Therefore, it is most probable that the pressure pulse caused by flash vaporization of the sample decreases at smaller sample sizes, and as a consequence, the splitting ratio is lowered, which in turn results in a reduction of the peak areas. Concomitantly with this reduction in normalized peak areas, the variation in these areas between the individual SAs increased, as is shown by the range and % S.D. From replicate analyses it was clear that this variation was not due to a lack in precision of operation or detection ($n = 5$; % S.D. for the peak areas of solanidine 2.22, demissidine 2.06, solasodiene 2.15, solasodine 4.31 and tomatidine 3.08). Comparison of the peak areas of the individual SAs showed that at injection volumes ≤ 1 μl the relative peak areas of the compounds decreased with their order of elution. This can be ascribed to discrimination of the compounds according to their boiling points. Discrimination is due to variation of splitting ratio for the different compounds present in the splitter[16]. This non-linearity of the splitting process apparently increased at smaller sample sizes. So, discrimination occurred in spite of the precautions taken with respect to the configuration of the vaporization tube, to ensure that the sample is heated sufficiently rapidly for a complete vaporization during injection (see Experimental section).

A similar experiment was done using a commercially available mixture of test compounds of well defined chromatographic behaviour. Also in this experiment, although to a lesser extent, the normalized peak areas decreased (relative to 1.9 μl) with decreasing sample sizes (Table II). This shows the characteristics of the splitter configuration used. However, the decrease in the peak areas was similar for all compounds of the test mixture.

TABLE II

VARIATION IN NORMALIZED PEAK AREAS FOR C_{27}-STEROIDAL ALKALOIDS (SAs) AND FOR TEST COMPOUNDS DEPENDENT ON THE INJECTION VOLUME

Normalized peak areas in percentages of the area at 2.4 μl for the SAs and at 1.9 μl for the test compounds, respectively. SA: solanidine, demissidine, solasodiene, solasodine and tomatidine; for the composition of the test mixture see Experimental section.

Injection volume (μl)	*SA*			*Test compounds*		
	Average	*Range*	*% S.D.*	*Average*	*Range*	*% S.D.*
2.4	100	–	–			
1.9	98	95–103	4.3	100	–	–
1.4	94	91–98	3.1	100	99–107	2.4
1.0	67	59–80	9.9	85	78–86	2.7
0.8	53	46–57	10.7	80	77–81	1.5
0.4	30	17–39	31.6	76	72–79	3.1
0.3				69	64–72	4.1

The results obtained for the SAs and for test compounds show that the SAs are difficult compounds for capillary GC. For quantitative results, extra care is needed. A constant injection volume is essential, which disallows correction of peak areas by changing the injection volume when the column is overloaded, or when the peaks are too small to be detected. Also an assessment of the smallest volume that gives uniform results for the different SAs is required. In our study 1.4 μl was found to be appropriate, even more so because at this volume the results for 5α-cholestane agreed well with those for the SAs, allowing this compound to be used as an internal standard.

Nitrogen-specific detection of C_{27}-steroidal alkaloids

The selectivity and sensitivity of a nitrogen–phosphorus-specific detector depend on the position of its alkali metal source, on the supply of thermal energy for emission of the alkali metal ions and on the composition and flow of the gas surrounding the source[17–19]. Each of these factors is the resultant both of detector characteristics determined by the instrumental design and of adjustments of chromatographic conditions and detector variables. The selectivity and sensitivity of the detector used in this study were mainly determined by the distance between the rubidium bead and the detector jet, by the flow of hydrogen used for combustion, by the flow of the nitrogen make-up gas and by the current of the electrically heated bead for adjustment of the background ionization current (BIC). As an instrumental prerequisite, the position of the rubidium bead was carefully tuned and the hydrogen flow adjusted to 5.0 ml min^{-1}. Then, the influence of the flow-rate of the make-up gas and of the BIC on the selectivity and sensitivity were studied. In studying the SA composition of Solanaceae, the former is more important than the latter, because the selectivity allows differentiation between SAs and non-nitrogen-containing compounds (non-N compounds), whilst for quantification of SAs the sensitivity of FID is usually sufficient. A number of experiments were carried out, using SAs and several more or less related non-N compounds, to compare different background ionization

TABLE III

INFLUENCE OF MAKE-UP GAS FLOW AND BACKGROUND IONIZATION CURRENT (BIC) ON THE SELECTIVITY AND SENSITIVITY OF NPD FOR SOLASODINE AND DIOSGENIN IN COMPARISON TO THE FID RESPONSE

Background ionization current (pA)	*Nitrogen make-up gas flow (ml min^{-1})*	*Relative sensitivity NPD/FID*		*Selectivity* solasodine/diosgenin*	
		Solasodine	*Diosgenin*	*NPD*	*FID*
80	20	3.2	0.119	25	0.91
80	25	3.1	0.019	139	0.87
80	30	2.9	0.006	418	0.88
80	35	2.4	0.005	462	0.86
40	20	1.5	0.082	16	0.90
40	25	1.5	0.011	117	0.88
40	30	1.1	0.003	389	0.90
40	35	0.9	0.002	409	0.91

* Normalized for concentration diosgenin = 7.11 · concentration solasodine. Injection volume: 3 μl.

currents and make-up gas flows. An example is given in Table III. The relative sensitivity of NPD (NPD/FID) was defined as the ratio of the NPD and FID responses to the same amount of a compound. The selectivity was defined as the ratio of the response to a compound under study, *i.e.*, solasodine and the normalized response to a reference compound, *i.e.*, diosgenin. At flow-rates of 20, 25 and 30 ml nitrogen min^{-1} and a BIC of 80 pA, the relative sensitivity of NPD to solasodine (22α*N*-spirosol-5-en-3-ol) remained similar, whilst the relative sensitivity to diosgenin, the non-N (22αO) analogue, strongly decreased when the flow-rate was increased. As a result the selectivity of NPD for the nitrogen-containing compound greatly improved. At 35 ml min^{-1} and 80 pA the selectivity was further improved, but the sensitivity to N became rather low for subtraction of FID from NPD traces (see hereafter). Lowering the BIC to 40 pA reduced the sensitivity of NPD to solasodine more than to diosgenin, and the selectivity slightly decreased. Increasing the BIC above 80 pA negatively affected the baseline stability and the noise level.

In summary, the make-up gas flow especially influenced the NPD sensitivity to non-N compounds and so it had a strong effect on the selectivity for SAs whereas the BIC mainly influenced the sensitivity of NPD to the SAs. After these experiments a make-up gas flow of 30 ml nitrogen min^{-1} and a BIC of 80 pA were chosen as optimal for the present study.

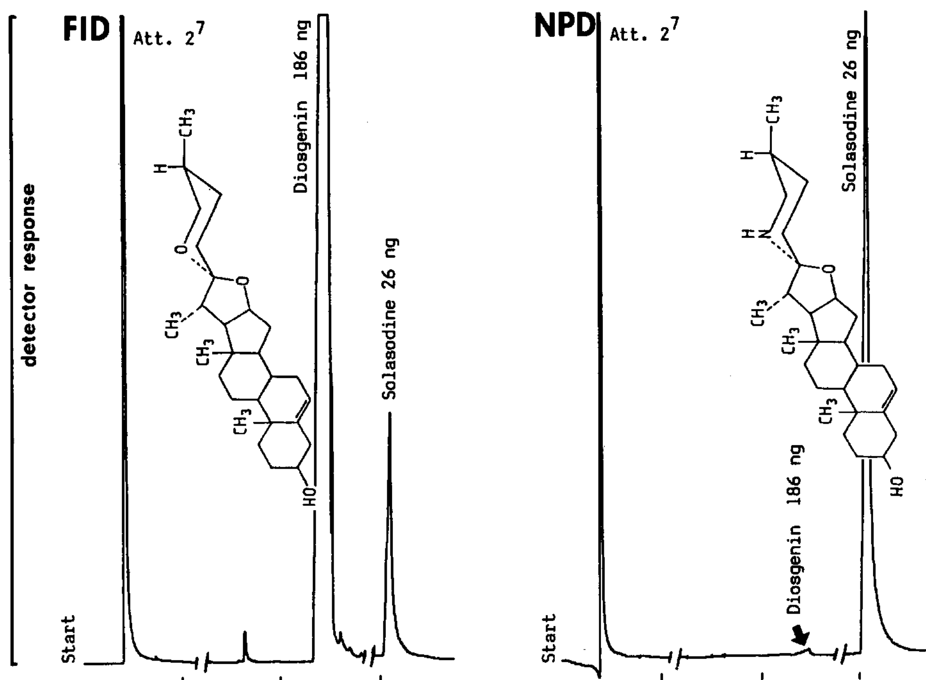

Fig. 1. Gas chromatograms obtained simultaneously by FID and NPD, showing the selectivity of NPD for solasodine (22αN), compared to the 22αO analogue diosgenin. Column: fused silica, 50 m × 0.22 mm I.D., CP-Sil 5 CB, film thickness 0.12 μm. Carrier gas: hydrogen, linear velocity 53 cm s^{-1}. Oven temperature: 270°C. Injection volume: 3 μl. Splitting ratio: 1:50. Effluent splitting ratio 1:1 (FID:NPD). Detector sensitivity: 1 pA mV^{-1}. Attenuation: 2^7. For further details see Experimental section.

The relative sensitivity and the selectivity of NPD for the SAs were rather low compared with the corresponding values for pyridine: NPD/FID response ratio 22; selectivity pyridine/cyclohexane 2900. This might be explained by the low N/C ratio of the SA, *i.e.*, 1:27.

Fig. 1 shows the gas chromatograms of solasodine and diosgenin, recorded simultaneously by NPD and FID. Only high levels of the non-N compound, far above the theoretical sample capacity of the column, were able to interfere with the nitrogen-specific detection. This means that simultaneous detection by NPD and FID unambiguously differentiates between SA and non-N compounds.

Quantification of C_{27}-steroidal alkaloids

Calibration lines were constructed for FID and NPD. As samples, solutions of solanidine, of demissidine plus tomatidine and of solasodiene plus solasodine were used. Each compound was used in eleven concentrations, varying from *ca.* 0.05 to 10 mg ml^{-1}. Although concentrations between 4 and 10 mg ml^{-1} may cause overloading of the column, they were used because preliminary experiments showed that tubers of wild *Solanum* species may contain levels of solanidine glycosides > 6000 mg kg^{-1} fresh weight.

The calibration lines for FID and for NPD were linear over the entire concentration range, for all SAs tested (Table IV). Under the conditions described for sample preparation and chromatography, 50- and 150-fold concentrations for the individual SAs could be quantified by FID and NPD respectively, without exceeding the column capacity. Under these conditions and using the NPD calibrations of Table IV, 2.7 mg kg^{-1} fresh weight was the minimum content for accurate quantification. The actual detection limit that can be achieved using modified procedures for sample preparation is lower, but because of irrelevancy for the application described in this paper, this limit was not determined. The concentrations between 4 and 10 mg ml^{-1}, which exceeded the column capacity, had no effect on the peak symmetry. Thus, when no closely eluting SAs are present, extremely high levels of SAs can be quantified without dilution of the samples.

Identification of C_{27}-steroidal alkaloids

The reproducibility of the retention times expressed as % S.D. ($n = 6$) was 0.045

TABLE IV

SLOPE, m, INTERCEPT, c, AND CORRELATION COEFFICIENT, r, OF THE CALIBRATIONS $y = mx - c$ FOR C_{27}-STEROIDAL ALKALOIDS (SAs) WITH FID AND NPD

y = Response (counts · 10^3); x = concentration (mg SA ml^{-1}).

SA	*FID*			*NPD*		
	m	*c*	$r \cdot 10^4$	*m*	*c*	$r \cdot 10^4$
Solanidine	70	4.4	9997	190	15.5	9997
Demissidine	70	5.3	9996	186	1.4	9984
Solasodiene	70	6.7	9996	183	8.0	9983
Solasodine	58	4.0	9999	149	11.7	9994
Tomatidine	62	13.6	9980	153	9.5	9994

for solanthrene and 0.037 for solanidine; for demissidine, solasodiene, solasodine and tomatidine it varied from 0.040 to 0.068. These values agreed with those which have been reported for accurate analyses[20]. However, such determinations are usually made under ideal conditions. This means that injections are made successively within one day and using one concentration. In studying the SA composition of Solanaceae, samples with widely different concentrations of SAs must be analyzed in the course of weeks or months. Progressive phase stripping of the column due to the high oven temperature, and especially a large variation in peak heights due to variation in the concentrations of the SA, might result in less reproducible retention times. Therefore, we also determined the reproducibility of the retention times of the SAs routinely. Nine different tuber samples of six *Solanum* species with various contents of solanidine glycosides (600–6220 mg kg^{-1}) were analyzed for their SA composition. The analyses were done between routine analyses in the course of a 5-day period. Most of the samples contained only solanthrene and solanidine, so calculations were made for these compounds. The reproducibility of the retention times deteriorated strongly; the % S.D. was now 0.864 and 0.690, respectively. Due to the large variations in retention time, it was not possible to set a time window for solanidine along with one for demissidine, in order to identify these peaks by retention times.

For certain *Solanum* species, complex chromatograms showing large numbers of SAs may be obtained. As potato breeding may require very many SA analyses, the characterization and identification of compounds by costly spectrometric techniques is hardly feasible. Therefore a cheap and relatively simple system for characterization and identification using retention indices[15] and NPD/FID response ratios was developed.

TABLE V

RETENTION TIMES, t_R, RETENTION INDICES, *I*, AND NPD/FID RESPONSE RATIOS FOR C_{27}-STEROIDAL ALKALOIDS, 5α-CHOLESTANE, STEROLS AND STEROIDAL SAPOGENINS

Column: fused silica, 50 m × 0.22 mm I.D., CP-SIL 5 CB, film thickness 0.12 μm. Oven temperature: 270°C. Carrier gas: hydrogen, linear velocity 49 cm s^{-1}.

Peak number	*Compound*	t_R	*I*	*NPD/FID response ratio*
1	Octacosane ($C_{28}H_{58}$)*	5.87	2800.0	–
2	5α-Cholestane	6.61	2866.5	–
3	Solanthrene	7.61	2941.8	3.04
4	Cholesterol	10.13	3068.3	–
5	Solanidine	11.07	3129.3	2.96
6	Demissidine	11.25	3136.8	3.01
7	Solasodiene	11.59	3151.1	3.06
8	Stigmasterol	13.22	3212.9	–
9	Diosgenin	13.43	3220.4	–
10	Tigogenin	13.78	3232.4	–
11	Solasodine	17.36	3337.6	3.02
12	Tomatidine	18.65	3369.8	2.88
13	Tetratriacontane ($C_{34}H_{70}$)*	19.96	3400.0	–
14	Jervine	28.73	3559.4	3.43

* Reference compound.

The retention indices shown in Table V were sufficiently reproducible. Under ideal conditions, injections being made successively within one day and using one concentration, the % S.D. of the indices of the SAs varied from 0.006 to 0.010. In the above mentioned routine experiment the % S.D. of the retention index was 0.046 for solanthrene and 0.028 for solanidine. Tests with widely different concentrations of solanidine and demissidine showed that typical identification windows for the retention indices of these closely eluting compounds would be 3129.3 $\pm$ 1.7 and 3136.8 $\pm$ 1.7 units, respectively. This meant that the retention index system could be used as an aid in the identification of the SA. For automated identification in our laboratory, the indices are compared, using the BASIC program, with the values of stored retention index windows. These values have been determined for individual SAs, using samples of plant material or standard solutions, with a wide concentration range. The accuracy and precision of the retention indices may be further improved by using for each SA two reference compounds differing by only one in their number of carbon atoms[21]. For practical reasons this was not done. Future studies on the SA composition of Solanaceae will reveal whether improvement of the retention index system is required.

The NPD/FID ratios for the SAs were quite similar (Table V) and are useful in the characterization of unknown compounds. There are a number of nitrogen-containing plant compounds which potentially could be coextracted with the SAs and hence could interfere with their identification. However, no compounds of this nature have been detected so far. This is due to the current procedure for sample preparation, which is rather specific for the SAs because the bisolvent extraction and two-phase hydrolysis exploit the difference in polarity between the glycoalkaloids and their aglycones. Moreover, because of their high boiling points, the SAs would be separated during capillary GC from most of the coextracted compounds. As the NPD/FID ratios of the SAs differed markedly from that of pyridine, 22, it should be worthwhile to investigate whether NPD/FID response ratios are sufficiently specific for different types of nitrogen-containing compounds to discriminate between SAs and other nitrogen-containing compounds which may be present in plant extracts. If so, this could allow a considerable simplification of the complex procedure for sample preparation currently in use.

Fig. 2 shows gas chromatograms of a potato extract spiked with SAs and related non-N compounds (5α-cholestane, sterols and steroidal sapogenins) obtained by FID (A) and NPD (B). The peak numbers correspond to those of the compounds listed in Table V. The FID chromatogram shows that, under the conditions applied, all compounds were well separated. The *Solanum* and *Lycopersicon* SAs tested were eluted within 19 min. The *Veratrum* SA jervine ($C_{27}H_{39}NO_3$) had a retention time of almost 29 min. This shows the necessity of a temperature rise after elution of tomatidine (or the reference compound tetratriacontane) to facilitate the detection of other, late eluting, SAs as stated earlier[11]. Comparison of the FID chromatogram with the NPD chromatogram reveals the peak vacancies in the latter, which correspond with the non-N compounds of Table V. Fig. 2C shows the result of post-analysis reprocessing of the chromatograms, which was realized by the software mentioned in the Experimental section. By subtraction of the FID chromatogram from the NPD chromatogram, the peak vacancies in the NPD trace are converted into negative peaks. So, the differentiation between SAs and non-N compounds is unambiguously achieved.

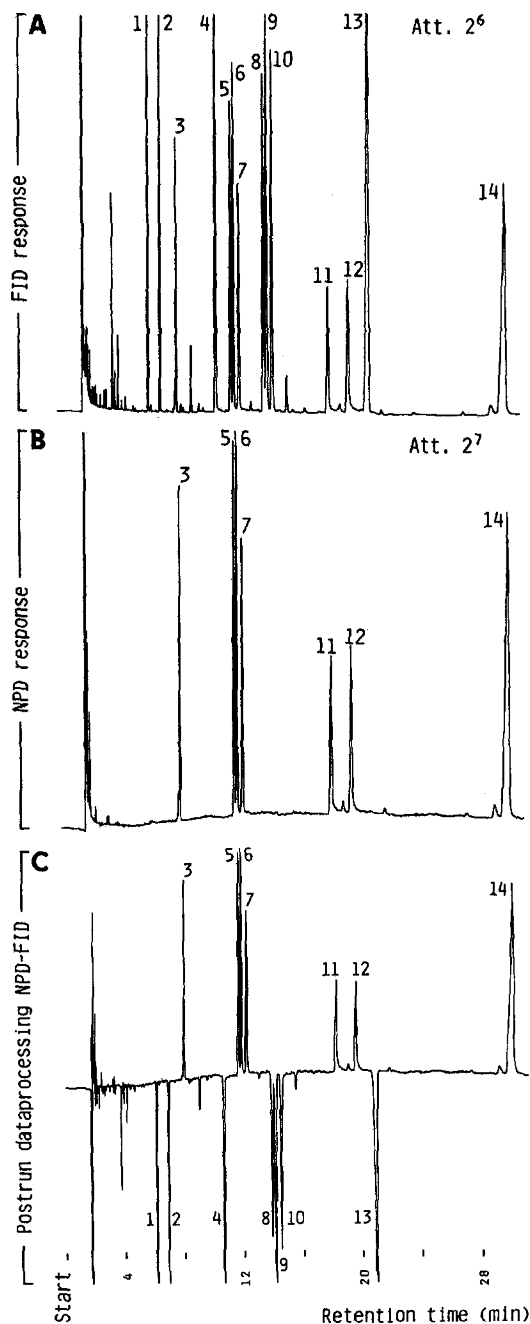

Fig. 2. Gas chromatograms obtained simultaneously by FID (A) and NPD (B) for a potato extract spiked with C_{27}-steroidal alkaloids, 5α-cholestane, sterols and steroidal sapogenins. Post-analysis reprocessing of the raw data from A and B (subtraction of the FID trace from the NPD trace) is shown in C. Conditions as in Fig. 1, except: linear velocity of carrier gas, 49 cm s^{-1}; injection volume, 1.4 μl; attenuation, FID 2^6, NPD 2^7. Amounts (ng) of compounds in the column: 1 = octacosane, 19.0; 2 = 5α-cholestane, 13.7; 3 = solanthrene, 12.9; 4 = cholesterol, 14.4; 5 = solanidine, 15.2; 6 = demissidine, 16.0; 7 = solasodiene, 13.6; 8 = stigmasterol, 14.1; 9 = diosgenin, 20.9; 10 = tigogenin, 14.2; 11 = solasodine, 15.7; 12 = tomatidine, 17.4; 13 = tetratriacontane, 17.9; 14 = jervine, 31.3.

CONCLUSION

Quantification of individual SAs using capillary GC can be done routinely either by FID or by NPD, provided extra care is taken over the injection of the samples. Usually FID is more stable than NPD, but the latter will be more sensitive. The NPD/FID response ratios can be used together with retention indices in the identification and characterization of SAs. Gas chromatographic–mass spectrometric analysis can probably be restricted to compounds with NPD/FID response ratios which correspond to those of the SAs and with retention indices which do not agree with those of the known SAs.

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REFERENCES

1 K. Schreiber, in R. H. F. Manske (Editor), *The Alkaloids*, Vol. X, Academic Press, New York, London, 1968, p. 1.
2 W. M. J. van Gelder, *Bedrijfsontwikkeling*, 16 (1985) 474.
3 W. Lepper, *Z. Lebensm.-Unters.-Forsch.*, 88 (1949) 264.
4 S. L. Sinden and R. E. Webb, *U.S. Dep. Agric. Tech. Bull.*, (1974) 1472.
5 S. J. Jadhav, R. P. Sharma and D. K. Salunkhe, *CRC Crit. Rev. Toxicol.*, 9 (1981) 21.
6 H. Sander, *Planta*, 47 (1956) 374.
7 W. M. J. van Gelder and O. M. B. de Ponti, *Euphytica*, 36 (1987) 555–561.
8 S. F. Osman, S. F. Herb, T. J. Fitzpatrick and P. Schmiediche, *J. Agric. Food Chem.*, 26 (1978) 1246.
9 W. M. J. van Gelder, in A. Beekman (Editor), *Potato Research for Tomorrow*, Centre for Agricultural Publishing and Documentation, Wageningen, 1986, p. 166.
10 A. Zitnak and G. R. Johnston, *Am. Potato J.*, 47 (1970) 256.
11 W. M. J. van Gelder, *J. Chromatogr.*, 331 (1985) 285.
12 W. M. J. van Gelder, in J. Bojanowski (Editor), *Proc. 11th Congress Eucarpia, Warsaw, 1986*, in press.
13 R. R. Freeman, in R. R. Freeman (Editor), *High Resolution Gas Chromatography*, Hewlett-Packard Company, Palo Alto, CA, 2nd ed., 1981, Ch. 3.
14 W. M. J. van Gelder, *J. Sci. Food Agric.*, 35 (1984) 487.
15 E. Kovats, *Helv. Chim. Acta*, 41 (1958) 1915.
16 K. Grob, Jr. and H. P. Neukom, *J. Chromatogr.*, 236 (1982) 297.
17 J. Ševčik, *Detectors in Gas Chromatography*, Elsevier, Amsterdam, New York, 1976, Ch. 6.
18 G. R. Verga, *J. Chromatogr.*, 279 (1983) 657.
19 E. B. Shmidel, L. I. Kalabina and L. N. Kolomiets, *J. Chromatogr.*, 365 (1986) 353.
20 T. A. Rooney and R. R. Freeman, in R. R. Freeman (Editor), *High Resolution Gas Chromatography*, Hewlett-Packard Company, Palo Alto, CA, 2nd ed., 1981, Ch. 7.
21 G. Schomburg and G. Dielman, *J. Chromatogr. Sci.*, 11 (1973) 151.