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QUANTITATIVE THIN-LAYER CHROMATOGRAPHY OF *NICOTIANA TABACUM* LEAF SURFACE COMPONENTS

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SUMMARY

A thin-layer chromatographic (TLC) method and scanning densitometry technique have been developed for quantitation of major primary and secondary metabolites of *Nicotiana tabacum* L. leaf surface components. These components were resolved after a single development with isopropanol–chloroform–methylene chloride–hexane (7:8:6:79) using laned silica gel G TLC plates. R_F values were reproducible to $\pm$ 0.01 units. Quantitation of all components of interest was accomplished by charring with 30% fuming sulfuric acid followed by densitometry using white light. Overall, charring results were semiquantitative ($\leqslant$ 10% relative error), but were quantitative ($\leqslant$ 6% relative error) for all major secondary metabolites except the sucrose esters. Quantitation of three secondary metabolites was also accomplished by scanning uncharred plates at a wavelength of 200 nm. In general, UV scanning provided semiquantitative results. For both charring and UV quantitation methods, highly correlated, curvilinear responses between mass and integration area were obtained. Advantages and limitations of these procedures *versus* an existing gas chromatographic procedure and their potential implementation are discussed.

INTRODUCTION

The leaf surface of *Nicotiana tabacum* L. and several closely related species is covered with both a cuticular wax and a gummy exudate, which is synthesized and secreted by glandular leaf trichomes[1–3]. This combined cuticular material consists of both primary and secondary metabolites (Fig. 1). Many of these leaf surface compounds contribute to cured leaf quality[4–7] and to the defense or susceptibility to other organisms[8–14].

Previously, thin-layer chromatography (TLC) of *Nicotiana* green leaf surface components has been limited to qualitative identification of selected constituents. Reid[15] used normal- and reversed-phase TLC to selectively separate major cuticular chemical classes and compounds. Zador and Jones[16] separated *N. stocktonii* alkaloids

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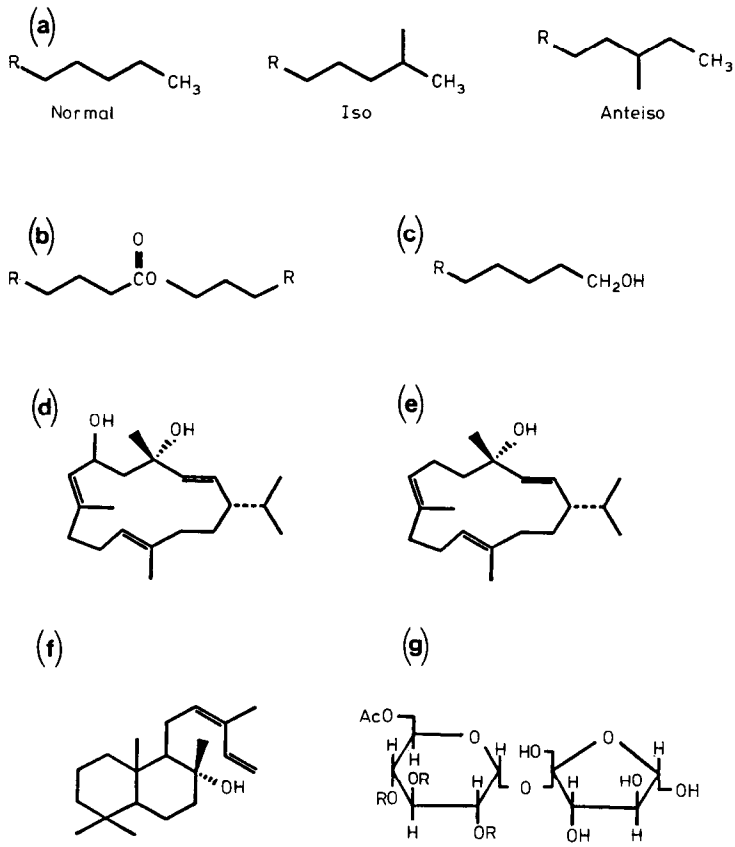

Fig. 1. Major primary and secondary metabolites of *Nicotiana tabacum* leaf surface constituents. (a) Hydrocarbons.; (b) wax esters; (c) fatty alcohols; (d) α- and β-4,8,13-duvatriene-1,3-diol; (e) α- and β-4,8,13-duvatriene-1-ol; (f) *Z*-abienol; (g) sucrose esters (R = C_3–C_8 fatty acids). AC = acetyl.

on Kieselguhr TLC plates using chloroform–methanol (100:40) and chloroform–methanol–ammonium hydroxide (90:10:1). General screening of plants for labdane and duvane diterpenoid production has been performed using Kieselgel TLC plates developed in chloroform–methanol (100:2)[17–19].

To date, no single TLC procedure exists by which the major metabolites of the *Nicotiana* leaf surface (Fig. 1) can be resolved and quantitated. Severson *et al.*[20] has developed an analytical gas chromatographic procedure for quantitation of major cuticular wax components; but this procedure requires sample derivatization before analysis and an analysis time of approximately 1 h. Due to the influence these plant products have on crop quality and the plant's ecology, development of a rapid, high-throughput screening procedure would benefit entomologists, plant pathologists, and plant breeders who are interested in these chemical traits and who require rapid results on large numbers of phenotypes. The purpose of this work was to develop a quantitative TLC (QTLC) procedure to screen leaf surface extracts of *N. tabacum* and related species.

EXPERIMENTAL

Preparation of standards and leaf surface extracts

Isolation of α-4,8,13-duvatriene-1,3-diol (ADVT), β-4,8,13-duvatriene-1,3-diol (BDVT), α- and β-4,8,13-duvatriene-1-ols (DVT-monols), *Z*-abienol (*Z*-AB), and mixtures of 6-O-acetyl-2,3,4-tri-O-acyl-α-D-gluco-pyranosyl-β-D-fructofuranoside, in which the 2, 3, and 4 positions are esterified with C_3–C_8 fatty acids (sucrose esters, SE) was as described elsewhere[21]. *n*-Tetracosane (C_{24} hydrocarbon, HC), octadecyl stearate (wax ester, WE), and *n*-docosanol (fatty alcohol, DOC) were purchased from Analabs/Foxboro (North Haven, CT, U.S.A.). Mixed standard solutions of 1 and 0.1 mg/ml/component were prepared in chloroform immediately prior to analysis.

Preparation and extraction of green bud leaves of field-grown Samsun, greenhouse-grown Burley 21, and greenhouse-grown flue cured [experimental (Coker 209 × NC602) × *N. africana* maternal derived haploids] varieties of *N. tabacum* were as described previously[22]. The resulting volumes (20 ml) from sample workups were quantitatively transferred to calibrated test tubes and reduced to 1 ml under a stream of nitrogen at 40°C on a Pierce Reactitherm® heating block. Samples were stored 1–2 days under nitrogen atmosphere at −80°C until needed.

Plate preparation

Analtech (Newark, DE, U.S.A.) Uniplate silica gel G precoated TLC plates (10 × 20 cm) were predeveloped at least three times in methylene chloride–methanol (1:1). A tight band of contamination concentrated at the top of the plate and was removed. Plates were divided into ten 1-cm wide lanes. The plates were then activated and equilibrated to 52% humidity over a saturated aqueous solution of sodium dichromate[23].

Spotting

Equilibrated plates were removed from the humidity chamber and quickly covered with a clean glass plate to maintain activation[23]. Appropriate volumes of mixed standard solutions and 4 μl of crude leaf surface extract were applied in 1-μl doses 1.5 cm from the bottom of the plate using a Nanomat spotting device (CAMAG Scientific). Complete drying was allowed between applications when multiple doses were required. For establishing calibration curves, sample dosages were randomly assigned to the lanes of individual plates. Each plate represented a replicate, and three replicates were run.

Development

After mixed standard solution or sample applications had completely dried, plates were developed in a rectangular chamber designed to accommodate a 20 × 20 cm thin-layer plate. The mobile phase was isopropanol–chloroform–methylene chloride–hexane (7:8:6:79). The developing tank was lined with a saturation pad and was pre-saturated with the vapor phase of the developing solvent. Development was carried out for 15 cm and allowed to overrun[23] 5 min. Plates were then removed from the developing chamber and dried 5 min using a hand-held hot air gun. Fresh mobile phase was used for each development.

Charring

The charring procedure employed was a slight modification of that developed by Martin and Allen[25]. The charring apparatus consisted of a Corning PC-101 hot plate upon which was placed a 30.5 × 30.5 × 1.3 cm aluminum block. A 25.4 × 25.4 cm Corning pyroceram® plate was used to cover the aluminum block to protect it from liquid acid. A 10 × 20 × 0.16 cm glass plate rested on the pyroceram plate and served as a platform on which the TLC plate was placed in order to prevent liquid acid from contacting the adsorbent layer during charring. To complete the charring chamber, a pyrex® casserole dish lid was used as a cover to secure an atmosphere of sulfur trioxide fumes.

To char the separated components, a glass wool plug was soaked with 30% fuming sulfuric acid (Fisher Scientific, St. Louis, MO, U.S.A.) and spread over the undersurface of the casserole lid using acid resistant tongs. Two glass wool plugs saturated with 30% fuming sulfuric acid were placed on either side of the TLC plate to ensure a continuous supply of sulfur trioxide fumes. Charring began by covering the TLC plate and glass wool plugs with the reagent-coated casserole lid. The hot plate was steadily heated by adjusting a transformer to a setting predetermined to heat the plate from room temperature to 180°C in 30 min. After the 30-min period, charring was terminated by removing the casserole lid.

Quantitation

Plates were scanned and quantitated using a CAMAG TLC Scanner II equipped with a Spectra-Physics SP4290 integrator and accompanying TLC scanning software. A slit width of 0.6 mm, a slit length of 8 mm, and a monochromator bandwidth of 30 nm was employed for scanning. Plates were scanned in reflectance mode with white light at a rate of 1 mm/s. Plates not subjected to charring treatments were scanned at a wavelength of 200 nm in the UV.

RESULTS AND DISCUSSION

Mobile phase selection

Mobile phase composition was optimized using Snyder's solvent classification[26] and its application to TLC as proposed by Heilweil[27]. Because of the chemical complexity and diversity of most tobacco leaf surface extracts, three modifiers were selected (isopropanol, chloroform and methylene chloride), each from a different classification group. Therefore, each modifier contributed different solvent characteristics to the mobile phase.

These three modifiers and hexane were combined in seven different mobile phase mixtures of equal chromatographic strength[27]. All seven were screened for optimum resolution of Galpao Commun (a Brazilian domestic variety of *N. tabacum* L.) leaf surface extracts, as its leaf surface chemistry is one of the most chemically complex. Thus, optimizing resolution of Galpao leaf surface extracts virtually ensured satisfactory resolution of other *N. tabacum* leaf surface extracts (Figs. 2 and 3). Under the described chromatographic conditions, a quaternary mixture of isopropanol–chloroform–methylene chloride–hexane (7:8:6:79) provided resolution of major tobacco leaf surface constituents in both standard mixtures and green leaf surface extracts after just one development. Resolution was sufficient for quantitation. The HC and WE classes were not separated in this system, but codeveloped as a single spot.

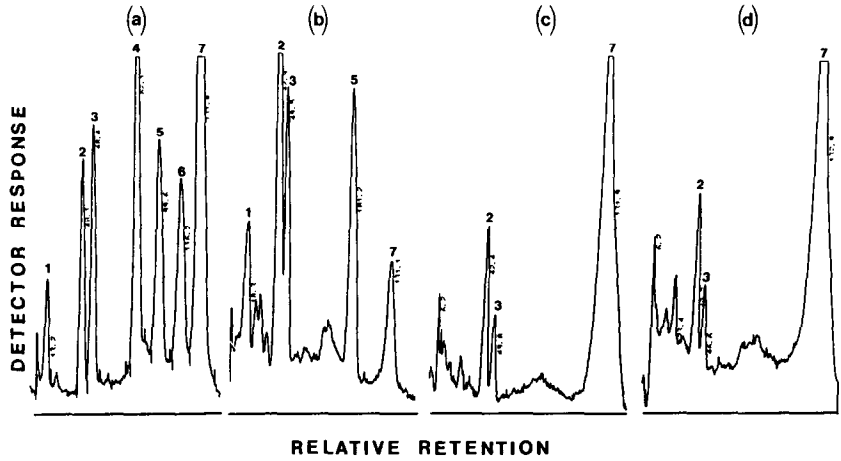

Fig. 2. Scanning densitometry traces of charring treatments. (a) Standard mixture; (b) field-grown Samsun leaf surface extract; (c) greenhouse-grown Burley 21 leaf surface extract; (d) greenhouse-grown experimental flue-cured [(Coker 209 × NC602) × *N. africana* maternally derived haploid]. Peaks: 1 = sucrose esters, 2 = ADVT, 3 = BDVT, 4 = DOC, 5 = *Z*-AB, 6 = DVT-monols, 7 = HC/WE. TLC plates: silica gel G, 0.25 μm equilibrated to 52% humidity. Mobile phase: isopropanol–chloroform–methylene chloride–hexane (7:8:6:79). Developing distance: 15 cm. Detection: charring with 30% fuming sulfuric acid followed by scanning with white light in reflectance mode.

Reproducibility

R_F values of each compound were reproducible to $\pm$ 0.01 units in comparison between lanes of individual plates and between separate plates (Table I). When R_F values are consistent, one need not run standards alongside samples on the same plate, but only compare sample R_F values to those of a single set of standard runs. Reproducibility also ensures that resolution between components will be constant, and therefore will not affect quantitation of closely developing solutes.

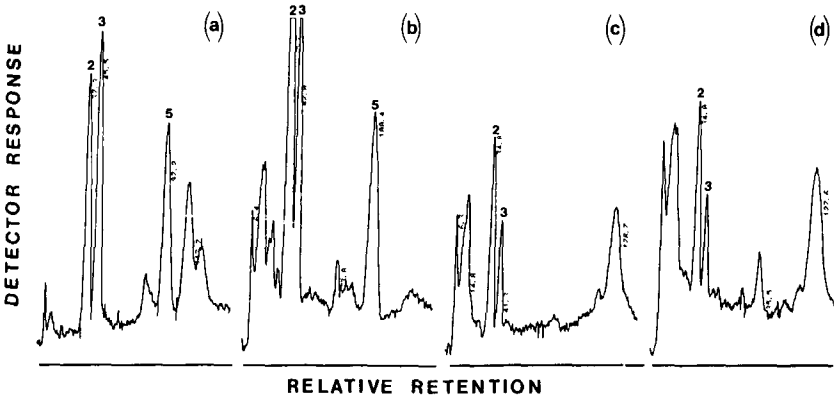

Fig. 3. Scanning densitometry traces of UV scanned plates. Details as for Fig. 2 except detection: scanned without charring at 200 nm in the reflectance mode.

TABLE I

MEAN R_F VALUES ± STANDARD DEVIATION OF *NICOTIANA TABACUM* LEAF SURFACE COMPONENTS

Conditions are as in Fig. 2.

Replication No.	*Compound*						
	SE	*ADVT*	*BDVT*	*DOC*	*Z-AB*	*DVT-monols*	*HC/WE*
1	0.05 ± 0.01	0.21 ± 0.01	0.27 ± 0.01	0.50 ± 0.01	0.62 ± 0.01	0.73 ± 0.01	0.83 ± 0.01
2	0.05 ± 0.01	0.22 ± 0.01	0.28 ± 0.01	0.50 ± 0.01	0.63 ± 0.01	0.74 ± 0.01	0.83 ± 0.01
3	0.05 ± 0.01	0.21 ± 0.00	0.25 ± 0.00	0.48 ± 0.01	0.62 ± 0.01	0.73 ± 0.00	0.83 ± 0.01
Average	0.05 ± 0.01	0.21 ± 0.01	0.27 ± 0.01	0.49 ± 0.01	0.62 ± 0.01	0.73 ± 0.01	0.83 ± 0.01

Reproducibility was accomplished through careful consideration of major parameters which affect TLC. Humidity and plate (adsorbent) activity are two parameters which are closely related and which can be adjusted to optimize resolution[28]. Since laboratory conditions can change daily, humidity must be rigidly controlled in order to maintain reproducible TLC results.

Effects of humidity on plate activation were controlled by equilibrating fully activated plates in a chamber whose atmosphere had been adjusted to 52% relative humidity using a saturated aqueous solution of sodium dichromate[23]. To maintain plate activation, equilibrated plates were covered with clean glass plates during spot application.

Reproducibility was also kept by allowing developments to "overrun"[23]. Dallas[23] noted that if development was stopped immediately after the mobile phase reached the developing distance, upper portions of the plate were less saturated with mobile phase than lower portions. Overrunning until the entire plate was fully saturated with developing solvent produced equal mobile phase to stationary phase ratios across the entire plate. R_F values increased slightly, but were more reproducible.

Equal rates of development between lanes of individual plates were accomplished by using a fully saturated developing chamber. The developing chambers were saturated with solvent vapor of the mobile phase, which was changed routinely after each development. Presumably, differences in rates of development between lanes would suggest different rates of solvent evaporation at the solvent front, as is the case with edge effects[24].

Quantitation: charring

Curvilinear relationships between mass and integration area were observed for each compound (Figs. 4 and 5 and Table III). Curvilinear responses were anticipated based on QTLC theory and on previous reports of quantitative densitometry of lipids. According to QTLC theory, light scattering caused by the thin-layer medium results in a non-linear relationship between substance mass and absorbance (ref. 29, reviewed in ref. 30). Previous work in quantitative densitometry of lipids has reported curvilinear responses at masses $\leqslant$ 10 μg[31]. Linear relationships have been reported at masses above 10 μg[32,33].

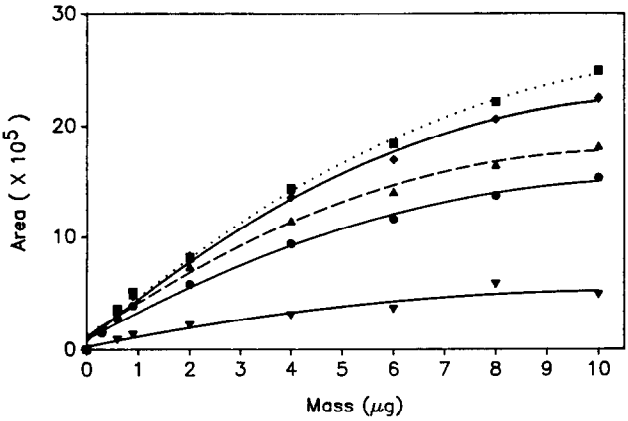

Fig. 4. Relationship between mass and integration area of *N. tabacum* secondary metabolites subjected to charring treatments. Conditions as in Fig. 2. (●) ADVT; (▲) BDVT; (■) DVT-monols; (▼)sucrose esters; (◆) *Z*-AB.

Overall, this technique provided at least semiquantitative results ($\leqslant$ 10% relative error) (Table II). Quantitation was most consistent between 0.6 μg and 2.0 μg. Results were quantitative ($\leqslant$ 6% relative error) in nearly all instances for all major secondary metabolites except the SE.

Quantitation at 0.3 μg was generally not reliable. At this mass, spots of most compounds produced detector responses, but these responses were not sufficient for reliable integration under the described scanning conditions. This is evidenced by the absence of reliable data for the sucrose esters at this mass (Table II).

Nonetheless, quantitation was reliably extended to 0.3 μg for ADVT and BDVT (Table II). These isomers represent two of the more important secondary products on the tobacco leaf surface because of their relative abundance compared to other leaf

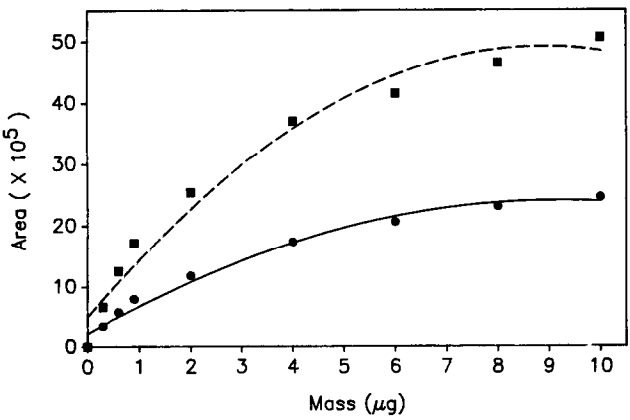

Fig. 5. Relationship between mass and integration area of *N. tabacum* primary metabolites subjected to charring treatments. Conditions as in Fig. 2. (●) DOC; (■) HC/WE.

TABLE II

RELATIVE PERCENT ERRORS OF QUANTITATION OF *NICOTIANA TABACUM* LEAF SURFACE COMPOUNDS

Chemical	*Mass applied (μg)*								
	0.0	*0.3*	*0.6*	*0.9*	*2.0*	*4.0*	*6.0*	*8.0*	*10.0*
Charring technique									
ADVT	0	11	4	11	2	6	5	5	3
BDVT	0	5	3	6	3	6	5	5	4
Z-AB	0	6	3	2	2	9	3	1	2
DVT-monols	0	18	2	6	5	12	3	6	5
SE	0	–	4	3	28	11	0	1	3
DOC	0	8	6	3	5	7	4	8	10
HC/WE	0	11	6	2	6	10	7	8	7
UV absorbance technique									
ADVT	0	4	8	9	3	2	2	4	4
BDVT	0	2	7	5	3	6	4	7	9
Z-AB	0	4	9	17	11	12	7	4	8

surface components[20], their known contribution to the ecology of the plant[8–14], and their eventual contribution to tobacco leaf quality[4–7].

Quantitation of the sucrose esters was not as consistent as other secondary metabolites (Table II). One possible explanation for this is the chemical nature of these these compounds (Fig. 1). The carbohydrate backbone is susceptible to oxidation to carbon dioxide during charring, thus resulting in carbon losses. Even with standardization of charring methods, variations in oxidation may have occurred among replicates.

With the primary metabolites, DOC and HC/WE, semiquantitative results were obtained (Table II). Semiquantitative data would generally be sufficient for HCs and

TABLE III

REGRESSION EQUATIONS AND R^2 VALUES FOR RELATIONSHIP BETWEEN MASS AND INTEGRATION AREA OF LEAF SURFACE CONSTITUENTS DETECTED BY CHARRING TREATMENT OR UV (200 nm) AND DENSITOMETRY

Chemical	*Regression equation*	R^2
Charring		
ADVT	$\text{Area} = -0.11x^2 + 2.52x + 0.91$	0.99
BDVT	$\text{Area} = -0.15x^2 + 3.12x + 1.18$	0.99
Z-AB	$\text{Area} = -0.17x^2 + 3.81x + 0.81$	0.99
DVT-monols	$\text{Area} = -0.16x^2 + 3.94x + 0.85$	0.99
SE	$\text{Area} = -0.04x^2 + 0.93x + 0.28$	0.97
DOC	$\text{Area} = -0.26x^2 + 4.79x + 2.25$	0.99
HC/WE	$\text{Area} = -0.56x^2 + 9.95x + 5.01$	0.99
UV 200 nm		
ADVT	$\text{Area} = -0.12x^2 + 2.59x + 2.07$	0.99
BDVT	$\text{Area} = -0.15x^2 + 3.10x + 2.83$	0.99
Z-AB	$\text{Area} = -0.56x^2 + 3.00x + 0.87$	0.99

WEs in actual screening applications, as interest in their contribution to ecological interactions and quality are small relative to tobacco secondary plant products. Typical of most tobaccos, DOC was below detection limits in tested leaf surface extracts (Fig. 2).

These charring procedures enabled quantitation of all major primary and secondary metabolites of the tobacco leaf surface. While potentially less accurate and less sensitive than the GC procedure developed by Severson *et al.*[20], a more rapid analysis of large numbers of leaf surface extracts can be performed with less sample preparation. Because TLC is a parallel technique rather than a serial technique, such as liquid chromatography (LC) and gas chromatography (GC), many samples can be chromatographed simultaneously. No special sample preparation is required before chromatography as would be necessary for GC analysis of these mixtures[20]. Also, the lack of a UV chromophore in all but one of these compounds would prevent universal detection on LC systems using UV detectors.

Total analysis time for eight samples, including sample application, development, charring, scanning, and integration, was approximately 2 h; using the GC procedure of Severson *et al.*[20], analysis time would be approximately 8 h for eight samples. For twenty samples, charring QTLC analysis time would increase only to approximately 3 h, *versus* 20 h using GC. For the QTLC procedure the most time-consuming steps of the analysis (development and charring) remain constant, whether eight or twenty samples are analyzed. Sample application time for twenty samples would increase approximately 12–15 min over that of eight samples, and scanning and integration time would increase approximately 35–40 min.

The decreased sensitivity of this procedure relative to GC is not necessarily a drawback. The leaf surface products of primary interest are almost exclusively those existing in greatest abundance (*i.e.*, ADVT, BDVT, *Z*-AB and SE)[12,17–20] for which quantitative results were obtained in all but one case.

Quantitation: UV

Detection and quantitation was much more limited using this procedure. Only ADVT, BDVT, and *Z*-AB were reliably detected. Using this procedure, culvilinear responses were obtained between mass and integrator response (Fig. 6 and Table III). High correlations were observed in all three cases. Despite the lack of universality, this procedure represents a rapid means for quantitating these three constituents, as plates were scanned immediately following development without further treatment. Since no modification for detection and quantitation were required, this procedure would be extremely attractive to those researchers who require simple procedures and rapid results.

Overall, semiquantitative results were obtained (Table II). ADVT and BDVT results were most consistent and quantitative in many instances, even in the absence of a strong UV absorbing chromophore. Results of *Z*-AB were less consistent. Scanning at 200 nm in order to quantitate all three components in a single scan decreased sensitivity to *Z*-AB, whose *in situ* absorbance maximum is at a wavelength of 229 nm. More quantitative and consistent results would probably be obtained for *Z*-AB if it were scanned at this wavelength. However scanning at two wavelengths to optimize quantitation of all three metabolites would require two separate scans and would decrease efficiency.

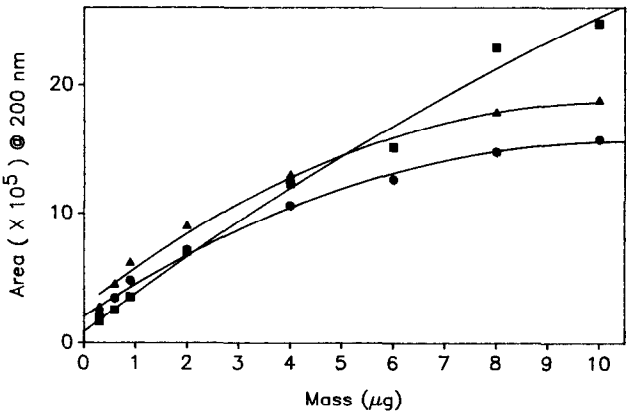

Fig. 6. Relationship between mass and integration area of *N. tabacum* secondary metabolites scanned at a wavelength of 200 nm. Conditions as in Fig. 3. (●) ADVT; (▲) BDVT; (■) *Z*-AB.

For both the charring and the UV procedures, statistical analysis revealed that different TLC plates significantly contributed to variation in quantitation. The contribution of plate effects to variation was small relative to mass, but nonetheless significant. Based on these results, it is recommended that standard curves be produced periodically and routinely to account for variation that may exist between plate lots. Likewise, for most accurate results, replicates of each sample should be run. Replicates of each sample would not be necessary if one requires only "rough" quantitative comparisons, which may be appropriate, for example, in plant breeding selections.

Plate variation may be reduced through use of higher quality high-performance thin-layer chromatography (HPTLC) plates; however organic binders commonly used in their preparation make them incompatible with sulfur trioxide charring procedures. Only one manufacturer was found which produced HPTLC plates without organic binder. In addition, HPTLC plates are more expensive, and thus may be economically prohibitive to some screening programs.

These procedures were tested against crude leaf surface extracts of fresh, green, bud leaf material of field-grown Samsun, greenhouse-grown Burley 21, and a greenhouse-grown experimental flue-curred plant [(Coker 209 × NC602) × *N. africana* maternally derived haploids]. Samsun was selected because of its complex leaf surface chemistry, which includes all the components comprising the mixed standard solutions. Burley 21 was selected because it represented a common tobacco variety, and the experimental flue-cured plant was selected to illustrate the procedure's effectiveness in screening experimental phenotypes. For both charring and UV scanning procedures, peak-to-noise ratios were similar to that of mixed standard scans. Thus, resolution was satisfactory for quantitation of major metabolites of all three crude leaf surface extracts.

Figs. 2 and 3 illustrate the effectiveness of the charring and UV scanning procedures, respectively, to identify differences that exist in the leaf surface chemical compositions of Samsun, Burley 21, and the experimental cross. The charring trace of Samsun (Fig. 2b) confirms the presence of the major tobacco secondary metabolites, SE, ADVT, BDVT, and *Z*-AB, while charring traces of Burley 21 (Fig. 2c) and the

experimental cross (Fig. 2d) properly identify these tobaccos as duvane (*i.e.*, ADVT, BDVT) producers and show no *Z*-AB. Similarly, the UV scanning traces in Fig. 3 confirm the presence of ADVT and BDVT in all three tobaccos (Fig. 3a–d), but the presence of *Z*-AB in Samsun only (Fig. 3b).

CONCLUSION

QTLC procedures have been developed for rapid quantitation of major *N. tabacum* leaf surface metabolites. These procedures should be useful tools in plant breeding and other research programs interested in rapid screening of these chemical traits in large numbers of samples. Utility may also be found in general screening of *Nicotiana* species for taxonomic or other purposes.

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REFERENCES

1 M. J. Michie and W. W. Reid, *Nature (London)*, 218 (1968) 578.
2 C. K. Keene and G. J. Wagner, *Plant Physiol.*, 79 (1985) 1026.
3 A. W. Johnson, R. F. Severson, J. Hudson, G. R. Carner and R. F. Arrendale, *Tob. Sci.*, 29 (1985) 67.
4 A. Colledge, W. W. Reid and R. A. Russell, *Chem. Ind. (London)*, 1975 (1975) 570.
5 C. R. Enzell, *Rec. Adv. Tob. Sci.*, 2 (1976) 32.
6 W. W. Reid, *Ann. Tabac. Sect. 2*, 12 (1975) 33.
7 I. Wahlberg, K. Karlson, D. J. Austin, N. Junker, J. Roeraade, C. R. Enzel and W. H. Johnson, *Phytochemistry*, 16 (1977) 1217.
8 H. G. Cutler, *Science (Washington, D.C.)*, 170 (1970) 856.
9 H. G. Cutler, W. W. Reid and J. Deletang, *Plant Cell Physiol.*, 18 (1977) 711.
10 H. G. Cutler, R. F. Severson, P. D. Cole and D. M. Jackson, in M. B. Green and P. A. Hedin (Editors), *Natural Resistance of Plants to Pests*, Am. Chem. Soc., Washington, DC, 1986, p. 178.
11 A. W. Johnson and R. F. Severson, *Tob. Sci.*, 26 (1982) 98.
12 A. W. Johnson and R. F. Severson, *J. Agric. Entomol.*, 1 (1984) 23.
13 D. M. Jackson, R. F. Severson, A. W. Johnson and G. A. Herzog, *J. Chem. Ecol.*, 12 (1986) 1349.
14 I. A. M. Cruickshank and D. R. Perrin, *Phytopath. Z.*, 90 (1977) 243.
15 W. W. Reid, *Ann. Tabac. Sect. 2*, 11 (1974) 151.
16 E. Zador and D. Jones, *Plant Physiol.*, 82 (1986) 479.
17 H. Tomita, M. Sato and N. Kawashima, *Agric. Biol. Chem.*, 44 (1980) 2517.
18 T. Komari, T. Kuko and M. Sato, *Tob. Sci.*, 30 (1986) 159.
19 T. Kubo, M. Sato, H. Tomita and N. Kawashima, *Tob. Sci.*, 26 (1982) 126.
20 R. F. Severson, R. F. Arrendale, O. T. Chortyk, A. W. Johnson, D. M. Jackson, G. R. Gwynn, J. F. Chaplin and M. G. Stephenson, *J. Agric. Food Chem.*, 32 (1984) 566.
21 D. R. Lawson and D. A. Danehower, in preparation.
22 D. A. Danehower, *Tob. Sci.*, 31 (1987) 32.
23 M. S. J. Dallas, *J. Chromatogr.*, 17 (1965) 267.
24 E. Stahl, in E. Stahl (Editor), *Thin-Layer Chromatography*, Springer Verlag, New York, 2nd ed., 1969, Section C, p. 52.
25 T. T. Martin and M. C. Allen, *J. Am. Oil. Chem. Soc.*, 48 (1971) 752.
26 L. R. Snyder, *J. Chromatogr. Sci.*, 16 (1978) 223.

27 E. Heilweil, in J. C. Touchstone and J. Sherma (Editors), *Techniques and Applications of Thin Layer Chromatography*, Wiley Interscience, New York, 1985, Ch. 3, p. 37.
28 F. Geiss, H. Schlitt, F. J. Ritter and W. M. Weimar, *J. Chromatogr.*, 12 (1963) 469.
29 U. B. Hezel, in A. Zlatkis and R. E. Kaiser (Editors), *HPTLC: High Performance Thin-Layer Chromatography* (*Journal of Chromatography Library*, Vol. 9), Elsevier, Amsterdam, 1987, Ch. 8, p. 149.
30 F. A. Huf, in L. R. Treiber (Editor), *Quantitative Thin-Layer Chromatography and Its Industrial Applications*, Marcel Dekker, New York, 1987, Ch. 2, p. 17.
31 J. Bitman and D. L. Wood, *J. Liq. Chromatogr.*, 5 (1982) 1155.
32 M. L. Blank, J. A. Schmit and O. S. Privett, *J. Am. Oil. Chem. Soc.*, 41 (1964) 371.
33 L. J. Nutter and O. S. Privett, *J. Chromatogr.*, 35 (1968) 519.