Improved Method for HPLC Analysis of Polyamines, Agmatine and Aromatic Monoamines in Plant Tissue

Robert D. Slocum*, Hector E. Flores, Arthur W. Galston, and Leonard H. Weinstein

Department of Biology, Williams College, Williamstown, Massachusetts 01267 (R.D.S.), Biotechnology Institute, Pennsylvania State University, State College, Pennsylvania (H.E.F.), Department of Biology, Yale University, New Haven, Connecticut 06520 (A.W.G.), Environmental Biology Program, Boyce Thompson Institute, Ithaca, New York 14853 (L.H.W.)

ABSTRACT

The high performance liquid chromatographic (HPLC) method of Flores and Galston [1982 Plant Physiol 69: 701] for the separation and quantitation of benzoylated polyamines in plant tissues has been widely adopted by other workers. However, due to previously unrecognized problems associated with the derivatization of agmatine, this important intermediate in plant polyamine metabolism cannot be quantitated using this method. Also, two polyamines, putrescine and diaminopropane, also are not well resolved using this method. A simple modification of the original HPLC procedure greatly improves the separation and quantitation of these amines, and further allows the simultaneous analysis of phenethylamine and tyramine, which are major monoamine constituents of tobacco and other plant tissues. We have used this modified HPLC method to characterize amine titers in suspension cultured carrot (Daucus carota L.) cells and tobacco (Nicotiana tabacum L.) leaf tissues.

Polyamines are thought to play essential roles in plant growth and development [19]. The PA1 contents of various plant tissues have been analyzed using several ion exchange, thin-layer and high-performance liquid chromatographic procedures, each of which has its advantages and limitations.

Perhaps the most widely used routine analytical method for PAs involves their separation by TLC, following prechromatographic derivatization with dansyl chloride [14]. The highly fluorescent products permit excellent detection sensitivity, but quantitation of Dns-PAs on the TLC plate is cumbersome and suffers from the fact that fluorescence yield is modified by temperature, pH, and solvent polarity and the Dns derivatives are photolabile [9]. Furthermore, DnsCl reacts not only with amines, but with phenolics and some alcohols, including sugars [16], and these derivatives may mask or interfere with the separation of Dns-amines on the chromatogram. Finally, the quantitation of Agm, or similar polar Dns-amine derivatives in PA mixtures, has not proved feasible using this method. DnsCl shows poor reactivity toward the quanidino function [5] and the polar Dns-Agm derivative exhibits little mobility on silica gel plates developed in the nonpolar solvent systems commonly used to separate Dns-PAs [14]. Because agmatine (Agm), whose formation is catalyzed by arginine decarboxylase (ADC; EC 4.1.1.19) in plants and bacteria [19, 22], is an important intermediate in PA biosynthesis [Fig. 1], it is desirable to have a reliable method to quantitate this metabolite. Two separation procedures, involving postchromatographic derivatization, have proved useful in the analysis of both Agm and PAs in the same mixtures: ion-exchange chromatography followed by o-phthalaldehyde fluorescence detection, using an amino acid analyzer [23], and ninhydrin visualization following separation on cellulose TLC plates [21]. However, with the latter TLC method, Put and Cad are poorly separated and Spm comigrates with N-carbamylputrescine.

Recent advances in the separation and quantitation of PAs have been facilitated by the development of HPLC procedures for analysis of these amines and their derivatives in tissue extracts [3, 6, 11, 15, 17, 20, 24, 25]. Of these, the method of Flores and Galston [3], involving the separation of benzoylated PAs followed by UV detection, has been widely adopted for plant studies. This method utilizes a simple isocratic elution protocol to separate both the naturally occurring diamines and polyamines, as well as Agm, by reverse-phase HPLC. We have since discovered that the peak previously identified as Agm is, in fact, a benzoyl-urea breakdown product of Agm (benzoyl-urea) formed during derivatization under certain sample preparation conditions. Undegraded-Agm is not eluted from the column at all under the isocratic elution conditions originally described [3]. However, a simple modification of this method, involving a gradient elution of these benzoylated amines, allows simultaneous analysis of Agm and PAs, with improved resolution of diaminopropane (Dap) and

---

1 Abbreviations: PAs, polyamines; ADC, arginine decarboxylase; Agm, agmatine; Cad, cadaverine; Dap, diaminopropane; DFMA, ω-difluoromethylarginine; DnsCl, dansyl chloride (5-[dimethylamino]-1-naphthalenesulfonyl chloride); ODC, ornithine decarboxylase; ODS, octadecylsilane; PCA, perchloric acid; Phen, phenethylamine (2-phenylethylamine); Put, putrescine; Spd, spermidine; Spm, spermine; Tyr, tyramine (4-(2-aminoethyl)phenol).

---

Figure 1. Polyamine biosynthesis in plants via the arginine decarboxylase and ornithine decarboxylase pathways. A, Arginine decarboxylase; B, agmatine iminohydrolase; C, N-carbamoylputrescine amidohydrolase; D, arginase; E, ornithine decarboxylase; F, spermidine/ spermine N-acetyltransferase.
putrescine (Put), from plant extracts. Additionally, the monoamines phenethylamine (Phen) and tyramine (Tyr), which occur as major hydroxybenzoic acid amide constituents of tobacco (23), are well-resolved from the other amine peaks, permitting their quantitation in the same sample.

**MATERIALS AND METHODS**

**Chemicals**

Benzoyl chloride was purchased from Baker Chemical Co. DFMA was generously supplied by Dr. Peter P. McCann of the Merrell Dow Research Institute, Cincinnati, OH. All other reagents and chemicals were obtained from Sigma Chemical Co.

**Plant Materials**

Tobacco (*Nicotiana tabacum* L. cv Wisconsin 38) plants were grown under standard greenhouse conditions in vermiculite. Mature leaf tissues from 2 month old plants were homogenized at 100 mg fresh weight tissue/mL of 5% PCA on ice. After 30 min, the extracts were centrifuged for 10 min at 27,000g. The supernatant was then acid-hydrolyzed in 6 N HCl for 18 h at 110°C. The hydrolysate was dried under a stream of air at 80°C, then resuspended in PCA prior to derivatization.

Carrot (*Daucus carota* L.) suspension cultures were grown and prepared for PA analysis as previously described (7).

**TLC Analysis of PAs**

Amine samples were dansylated, separated on Whatman LK6D high-performance silica TLC plates, and quantitated according to Flores and Galston (3).

**HPLC Analysis of PAs**

Polyamine standards and unknowns were benzoylated according to the procedure of Redmond and Tseng (12). HPLC analysis of benzoyl-PAs was performed using a programmable Altex-Beckman model 322 liquid chromatograph employing 254 nm detection. Derivatized PAs were injected into a fixed-volume 20 μL loop and chromatographed at ambient temperature (25°C) through a 4.6 × 250 mm, 5 μm particle size C18 reverse-phase column (ODS; Rainin "Microsorb") using a Hewlett-Packard 3390A integrator for quantitation. Benzoyl PAs were eluted at a flow rate of 1.0 mL/min using one of two water (solvent A)/MeOH (solvent B) stepped gradient programs followed by a column cleaning/regeneration cycle. Program I: 50 to 65% B in 7 min/65 to 80% B in 6 min/80% B for 5 min/80 to 100% B in 6 min/100% B for 5 min/100 to 50% B in 4 min/50% B equilibration for 7 min. Program II: 60% B for 7 min/60 to 80% B in 4 min/80 to 100% B in 10 min.

**Figure 2.** Separation of benzoylated diamines, polyamines, and agmatine standards by reverse-phase HPLC. Samples were chromatographed using an aqueous MeOH gradient system (Program I; see "Materials and Methods" section). A, Complete standards mix (5 nmol each of Put, Dap, Cad, Spd, Spm; 10 nmol Agm); B to G, Identification of peaks by spiking a 1:5 diluted standards mixture with 5 nmol...
Table I. Retention Times (Rt) for Amine Standards Separated by Reverse-Phase HPLC Using Gradient Elution Program II

<table>
<thead>
<tr>
<th>Amine</th>
<th>Rt (min)</th>
<th>± (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Put</td>
<td>6.46</td>
<td>±0.11</td>
</tr>
<tr>
<td>Dap</td>
<td>6.93</td>
<td>±0.11</td>
</tr>
<tr>
<td>Cad</td>
<td>7.35</td>
<td>±0.12</td>
</tr>
<tr>
<td>Phen</td>
<td>10.67</td>
<td>±0.09</td>
</tr>
<tr>
<td>Spd</td>
<td>11.26</td>
<td>±0.12</td>
</tr>
<tr>
<td>Spm</td>
<td>14.43</td>
<td>±0.04</td>
</tr>
<tr>
<td>Tyr</td>
<td>16.12</td>
<td>±0.07</td>
</tr>
<tr>
<td>Agm</td>
<td>17.64</td>
<td>±0.01</td>
</tr>
</tbody>
</table>

Values represent means ± se of four individual separations with 0.5, 1.0, 2.0, or 5.0 nmol of each amine in the standards mixture.

Figure 3. HPLC elution profile for agmatine standard (5 nmol) benzoylated at pH 9 for 30 min, 25°C and chromatographed as in Figure 1. Note approximate stoichiometric amounts of the Put and urea degradation products (~0.46 nmol each).

4 min/100% B for 2 min/100 to 60% B in 2 min/60% B equilibration for 4 min.

Urea Assay

The urea content in the Agm standard was assayed using the procedure of Archibald (1), based on the colorimetric detection of urea after reaction with 1-phenyl-1,2-propanedione-2-oxime.

RESULTS AND DISCUSSION

Good separation of the benzamide derivatives of the common PAs (Put, Spd, Spm) by reverse-phase HPLC employing isocratic (64% MeOH) elution from a standard octadecylsilane (ODS) column was reported by Flores and Galston (3). However, Put and Dap, a PA oxidation product, were not fully resolved using this method. In order to achieve better separation of these amines, we have adopted a gradient elution protocol which improves their separation characteristics. Typical elution profiles for the common diamines (Dap, Put and Cad), polyamines (Spd and Spm), and Agm are shown in Figure 2. Each amine peak was identified by 'spiking' the sample with an authentic standard. Mean retention times for each amine standard at 25°C are listed in Table I. Retention time values vary little over a 10-fold concentration range (0.5–5.0 nmol each).

The elution order for Put and Dap in our present studies (Fig. 1, B and C) was the reverse of that reported previously (3), but it is difficult to directly compare these elution data for Dap and Put since reverse-phase ODS columns (5 μm, 4.6 x 250 mm) supplied by different manufacturers (Altex 'ODC Ultrasphere,' Ref. 3; Rainin 'Microsorb,' present study) were used in the two studies. These columns, ideally, should exhibit similar chromatographic characteristics, although differences in silanol end-capping efficiencies, or other parameters, may have contributed to differences in column selectivity and elution order for Dap and Put. We did not address this latter possibility, but it would be useful to determine whether trimethylhydrochlorosilane blocking of free silanols, or the use of amine modifiers to mask the silanol groups (2, 10), would standardize the retention behavior of these amines on different columns.

As seen in Figure 2 G, the authentic benzoyl-Agm standard elutes only at high MeOH concentrations, much later than the other amines, probably due to ionic interactions between its guanidino function and the column matrix. This is in contrast to our previous study (3), in which Agm was reported to elute between Cad and Spd. In fact, the benzoyl-Agm standard did not elute from the column in 64% MeOH, even after considerable run times (60–80 min). The reason for this...
anomalous behavior became apparent when the true identity of the ‘Agm’ peak in the early study was discovered to be benzoyl-urea. We accidentally discovered this when an Agm sample was prepared in 10% PCA, instead of the usual 5% PCA, then benzoylated under standard conditions. When this standard was chromatographed (Fig. 3), we noted the presence of Agm, as well as two non-Agm components; one cochromatographed with Put and the second peak was later identified as urea by cochromatography with a benzoyl-urea standard. This latter urea peak corresponded to the Agm peak in 64% MeOH, therefore we concluded that it represented either an artefact of benzoylation or, perhaps, a urea contaminant in the Agm standard. We were unable to detect any urea in this sample, using a specific colorimetric assay (1), although the Spm standard contained ~5% urea (Fig. 2F). The only other obvious difference between the Agm standards was the concentration of PCA, which would be expected to modify the final reaction pH.

It is well-known that unprotonated amines (-NH₂) react with carboxic or sulfonic acid chlorides (e.g. benzoyl and dansyl chlorides) at moderately basic pH to form amide derivatives. In the normal reaction mixture (amine in 5% PCA), the pH was ~11; in contrast, the pH of the reaction mixture for the 10% PCA Agm standard was ~9. Dansylation of the Agm standard at pH 9 also produced Dns-urea + Dns-

Put and the second peak was later identified as urea by cochromatography with a benzoyl-urea standard. This latter urea peak corresponded to the Agm peak in 64% MeOH, therefore we concluded that it represented either an artefact of benzoylation or, perhaps, a urea contaminant in the Agm standard. We were unable to detect any urea in this sample, using a specific colorimetric assay (1), although the Spm standard contained ~5% urea (Fig. 2F). The only other obvious difference between the Agm standards was the concentration of PCA, which would be expected to modify the final reaction pH.

It is well-known that unprotonated amines (-NH₂) react with carboxic or sulfonic acid chlorides (e.g. benzoyl and dansyl chlorides) at moderately basic pH to form amide derivatives. In the normal reaction mixture (amine in 5% PCA), the pH was ~11; in contrast, the pH of the reaction mixture for the 10% PCA Agm standard was ~9. Dansylation of the Agm standard at pH 9 also produced Dns-urea + Dns-

Put and the second peak was later identified as urea by cochromatography with a benzoyl-urea standard. This latter urea peak corresponded to the Agm peak in 64% MeOH, therefore we concluded that it represented either an artefact of benzoylation or, perhaps, a urea contaminant in the Agm standard. We were unable to detect any urea in this sample, using a specific colorimetric assay (1), although the Spm standard contained ~5% urea (Fig. 2F). The only other obvious difference between the Agm standards was the concentration of PCA, which would be expected to modify the final reaction pH.

It is well-known that unprotonated amines (-NH₂) react with carboxic or sulfonic acid chlorides (e.g. benzoyl and dansyl chlorides) at moderately basic pH to form amide derivatives. In the normal reaction mixture (amine in 5% PCA), the pH was ~11; in contrast, the pH of the reaction mixture for the 10% PCA Agm standard was ~9. Dansylation of the Agm standard at pH 9 also produced Dns-urea + Dns-

Put and the second peak was later identified as urea by cochromatography with a benzoyl-urea standard. This latter urea peak corresponded to the Agm peak in 64% MeOH, therefore we concluded that it represented either an artefact of benzoylation or, perhaps, a urea contaminant in the Agm standard. We were unable to detect any urea in this sample, using a specific colorimetric assay (1), although the Spm standard contained ~5% urea (Fig. 2F). The only other obvious difference between the Agm standards was the concentration of PCA, which would be expected to modify the final reaction pH.

It is well-known that unprotonated amines (-NH₂) react with carboxic or sulfonic acid chlorides (e.g. benzoyl and dansyl chlorides) at moderately basic pH to form amide derivatives. In the normal reaction mixture (amine in 5% PCA), the pH was ~11; in contrast, the pH of the reaction mixture for the 10% PCA Agm standard was ~9. Dansylation of the Agm standard at pH 9 also produced Dns-urea + Dns-

Put and the second peak was later identified as urea by cochromatography with a benzoyl-urea standard. This latter urea peak corresponded to the Agm peak in 64% MeOH, therefore we concluded that it represented either an artefact of benzoylation or, perhaps, a urea contaminant in the Agm standard. We were unable to detect any urea in this sample, using a specific colorimetric assay (1), although the Spm standard contained ~5% urea (Fig. 2F). The only other obvious difference between the Agm standards was the concentration of PCA, which would be expected to modify the final reaction pH.

It is well-known that unprotonated amines (-NH₂) react with carboxic or sulfonic acid chlorides (e.g. benzoyl and dansyl chlorides) at moderately basic pH to form amide derivatives. In the normal reaction mixture (amine in 5% PCA), the pH was ~11; in contrast, the pH of the reaction mixture for the 10% PCA Agm standard was ~9. Dansylation of the Agm standard at pH 9 also produced Dns-urea + Dns-

Put and the second peak was later identified as urea by cochromatography with a benzoyl-urea standard. This latter urea peak corresponded to the Agm peak in 64% MeOH, therefore we concluded that it represented either an artefact of benzoylation or, perhaps, a urea contaminant in the Agm standard. We were unable to detect any urea in this sample, using a specific colorimetric assay (1), although the Spm standard contained ~5% urea (Fig. 2F). The only other obvious difference between the Agm standards was the concentration of PCA, which would be expected to modify the final reaction pH.

It is well-known that unprotonated amines (-NH₂) react with carboxic or sulfonic acid chlorides (e.g. benzoyl and dansyl chlorides) at moderately basic pH to form amide derivatives. In the normal reaction mixture (amine in 5% PCA), the pH was ~11; in contrast, the pH of the reaction mixture for the 10% PCA Agm standard was ~9. Dansylation of the Agm standard at pH 9 also produced Dns-urea + Dns-

Put and the second peak was later identified as urea by cochromatography with a benzoyl-urea standard. This latter urea peak corresponded to the Agm peak in 64% MeOH, therefore we concluded that it represented either an artefact of benzoylation or, perhaps, a urea contaminant in the Agm standard. We were unable to detect any urea in this sample, using a specific colorimetric assay (1), although the Spm standard contained ~5% urea (Fig. 2F). The only other obvious difference between the Agm standards was the concentration of PCA, which would be expected to modify the final reaction pH.

It is well-known that unprotonated amines (-NH₂) react with carboxic or sulfonic acid chlorides (e.g. benzoyl and dansyl chlorides) at moderately basic pH to form amide derivatives. In the normal reaction mixture (amine in 5% PCA), the pH was ~11; in contrast, the pH of the reaction mixture for the 10% PCA Agm standard was ~9. Dansylation of the Agm standard at pH 9 also produced Dns-urea + Dns-

Put and the second peak was later identified as urea by cochromatography with a benzoyl-urea standard. This latter urea peak corresponded to the Agm peak in 64% MeOH, therefore we concluded that it represented either an artefact of benzoylation or, perhaps, a urea contaminant in the Agm standard. We were unable to detect any urea in this sample, using a specific colorimetric assay (1), although the Spm standard contained ~5% urea (Fig. 2F). The only other obvious difference between the Agm standards was the concentration of PCA, which would be expected to modify the final reaction pH.

It is well-known that unprotonated amines (-NH₂) react with carboxic or sulfonic acid chlorides (e.g. benzoyl and dansyl chlorides) at moderately basic pH to form amide derivatives. In the normal reaction mixture (amine in 5% PCA), the pH was ~11; in contrast, the pH of the reaction mixture for the 10% PCA Agm standard was ~9. Dansylation of the Agm standard at pH 9 also produced Dns-urea + Dns-

Put and the second peak was later identified as urea by cochromatography with a benzoyl-urea standard. This latter urea peak corresponded to the Agm peak in 64% MeOH, therefore we concluded that it represented either an artefact of benzoylation or, perhaps, a urea contaminant in the Agm standard. We were unable to detect any urea in this sample, using a specific colorimetric assay (1), although the Spm standard contained ~5% urea (Fig. 2F). The only other obvious difference between the Agm standards was the concentration of PCA, which would be expected to modify the final reaction pH.

It is well-known that unprotonated amines (-NH₂) react with carboxic or sulfonic acid chlorides (e.g. benzoyl and dansyl chlorides) at moderately basic pH to form amide derivatives. In the normal reaction mixture (amine in 5% PCA), the pH was ~11; in contrast, the pH of the reaction mixture for the 10% PCA Agm standard was ~9. Dansylation of the Agm standard at pH 9 also produced Dns-urea + Dns-

Put and the second peak was later identified as urea by cochromatography with a
reaction occurs is unknown. This result was somewhat surprising, since the quanidino group is strongly basic (pK_a ~ 12.5) and, therefore, would be positively charged and weakly nucleophilic toward the acid chlorides at pH 9. The degree of Agm hydrolysis in these samples (approximately 10% total) was also unexpected since these reactions were carried out for only 30 min at 37°C. Obviously, the quantitation of Put and Agm would be seriously compromised in tissue extracts prepared under these conditions.

We investigated the effect of pH on the dansylation of Agm by varying the ratio of PCA/KOH in the reaction mixture, Agm concentration remaining constant. The results are seen in Figure 5. At pH < 10, significant quantities (8–10%) of Agm were hydrolyzed to Put + urea; at pH 10, very little (<1%) degradation of Agm was observed; at pH > 10%, Agm hydrolysis was not detected. These data suggest that care should be taken to standardize sample preparation such that amine derivatization is carried out in the pH 10 to 11 range (e.g. 250–500 μL amine sample in 5% PCA + 1.0 mL of 2 N NaOH prior to benzylation). Under such conditions, little or no degradation of Agm would be expected; in fact, this result has been confirmed by mass spectral characterization of benzamine derivatives of PAs separated by HPLC (13).

One final point to consider, with regard to Agm quantitation, is that the benzoyl-Agm derivative may be inherently unstable even after the benzylation reaction has been terminated, particularly if it is stored in aqueous MeOH solutions. We reported (3) that benzoyl-PAs, with the exception of Agm and Spd, were stable for several months in MeOH at -20°C. The A_{254} of the benzoyl-Agm (i.e. benzoyl-urea) peak tended to increase when the sample was stored longer than 2 weeks at -20°C. This suggests that degradation of the Agm derivative may have contributed to this benzoyl-urea peak absorbance. The appearance of several unknown peaks was also noted in chromatograms of benzoylated samples which had been stored at -20°C after only a few days. For this reason, we recommend performing HPLC analyses on freshly benzoylated samples.

Misidentification of the Agm peak in the original report (3) may require reevaluation of some data presented in later studies employing this HPLC method (4, 8). For example, Kaur-Sawhney et al. (8) reported a nearly fivefold increase in Agm titers of apical bud tissues of potato tubers during sprouting, although ADC activity in these tissues did not increase. However, a fivefold increase in ODC activity was observed to accompany markedly elevated PA levels in these tissues. This apparent inconsistency may now be explained in light of our finding that the Agm peak in these chromatograms corresponded to urea. Elevated ODC activities are frequently linked to low ADC and high arginase activities in several plant tissues (18, our unpublished observations). Consequently, one would expect increased levels of urea, rather than Agm, in the sprouting bud tissues.

Table II. Polyamine Titer in Carrot Suspension Cultured Cells Grown for 8 h in the Absence (Control) or Presence of 1 mM DFMA

<table>
<thead>
<tr>
<th>Polyamine Titer</th>
<th>Agm</th>
<th>Put</th>
<th>Spd</th>
<th>Spm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1198</td>
<td>213</td>
<td>520</td>
<td>37</td>
</tr>
<tr>
<td>+1 mM DFMA</td>
<td>194 (16)</td>
<td>412 (79)</td>
<td>Trace^b</td>
<td></td>
</tr>
</tbody>
</table>

^a Not detected. Percent control values are shown in parentheses. ^b Peak area not integrated.

Figure 7. HPLC separation of Phen (A) and Tyr (B) alone (5 nmol each), and in complete standards mix (C: 1 nmol each) using gradient elution Program II. D, Analysis of total PCA-soluble PAs and Tyr in acid hydrolysate of tobacco leaf tissue. E, Same sample diluted 1:1 and spiked with 1 nmol each of Phen and Tyr.
Table III. Titers of Polyamines and Aromatic Monoamines (Free and Conjugated) in Acid-Hydrolyzed PCA-Soluble Fraction from Tobacco Leaf Tissue

<table>
<thead>
<tr>
<th>Polyamine Titer</th>
<th>nmol/g fr wt tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Put</td>
<td>252</td>
</tr>
<tr>
<td>Spd</td>
<td>550</td>
</tr>
<tr>
<td>Spm</td>
<td>51</td>
</tr>
<tr>
<td>Phen</td>
<td>trace*</td>
</tr>
<tr>
<td>Tyr</td>
<td>42</td>
</tr>
</tbody>
</table>

* Peak area not integrated.

The modified HPLC method described in the present report has successfully been used to quantitate PAs and Agm in carrot suspension cell cultures (7). We adopted gradient elution Program I for use in these studies, since Agm was better resolved from a number of peaks which eluted from the column rather late in the run. One such analysis is shown in Figure 6, where the effect of an irreversible ADC inhibitor, DFMA, on PA and Agm titers in carrot cells is clearly seen. As compared with the control suspension culture, cells grown in the presence of 1 mM DFMA exhibit a marked decrease in Agm, the product of ADC activity and precursor of Put and Spd, whose titers are also lowered (Table II).

A second gradient elution Program II was later developed to facilitate the simultaneous separation of PAs and two aromatic monoamines, Phen and Tyr, in tobacco tissues. As can be seen in Figure 7, A to C, these amines are well resolved from each other and also from Agm in a standards mix. A typical elution profile for total PCA-soluble amines from tobacco leaf tissues is seen in Figure 7D and amine titers are given in Table III. In this analysis, Tyr was the major monoamine constituent, while trace amounts of Phen were also detected. Confirmation of peak identities for these monoamines was carried out by spiking the sample with authentic standards (Fig. 7E). Trace amounts of Agm were also detected.

In summary, we have identified several problems related to the analysis of PAs, and especially Agm, in plant tissues using the HPLC method of Flores and Galston (3), as well as problems associated with the derivatization of Agm itself. Only slight modification of the original procedure is required to fully exploit the usefulness of the method, while permitting the simultaneous analysis of the aromatic monoamines Phen and Tyr. Consequently, this method should continue to find broad application in future studies of plant polyamine metabolism.

ACKNOWLEDGMENT

We thank Dr. Richard A. Blatchly, Department of Chemistry, Williams College, for his helpful comments during the preparation of this manuscript.

LITERATURE CITED