Plant Physiol. (1977) 59, 821-826

Rapid Separation and Quantification of Abscisic Acid from Plant Tissues Using High Performance Liquid Chromatography[1, 2]

Received for publication June 15, 1976 and in revised form November 29, 1976

ALLAN J. CIHA,[3, 4] MARK L. BRENNER,[5] AND WILLIAM A. BRUN[3]
University of Minnesota, St. Paul, Minnesota 55108

ABSTRACT

Abscisic acid (ABA) was purified from soybean (*Glycine max* [L.]) seed extract using a preparative high performance liquid chromatography (HPLC) procedure. The preparative procedure was rapid (70 minutes per sample), required no prior partitioning for purification and was quantitative as demonstrated with an internal standard of [2-^{14}C]ABA, of which 98.9% was recovered.

Following purification by the preparative HPLC procedure, the ABA in a soybean seed extract was quantified using either GLC with an electron capture detector (GLC-EC) or by analytical HPLC with a UV detector. For soybean seed extracts, two analytical HPLC column packing materials were found adequate: μPorasil and μBondapak-NH_2 (Waters Associates). However, with complex tissue extracts, such as soybean leaf and nodule tissues, only GLC-EC had the necessary selectivity and sensitivity.

The determination of ABA from plant tissues has been time-consuming in the past. It usually consists of a preparative and an analytical procedure. The preparative procedure normally involves the partitioning of a plant extract against a number of organic solvents (6, 11) and is followed by either TLC and/or paper chromatography (1, 10, 13, 20). The analytical procedure has been achieved either by bioassay (12, 17, 18) or by GLC (4, 8, 15). More recently, HPLC[6] has been used for the analytical quantification of ABA (5, 16).

Carnes *et al.* (3) have recently described a preparative HPLC procedure for the purification and fragmentation of the cytokinin complex in tomato root pressure exudate. This procedure, using Bondapak C_{18}/Porasil B packing material, achieved in $^1/_{30}$ the time the same resolution of tomato root cytokinins as did conventional column chromatography on Sephadex LH-20.

The objective of this work was to determine the usefulness of HPLC procedure for both the preparative and the analytical aspects of ABA determinations on soybean tissues.

[1] This work was supported in part by grants from the National Soybean Crop Improvement Council, The Green Giant Foundation, and University of Minnesota Graduate School.

[2] Agricultural Experiment Station, University of Minnesota, Scientific Journal Series No. 9568.

[3] Dept. of Agronomy and Plant Genetics.

[4] Present address: USDA ARS, Dept. of Agronomy, Washington State University, Pullman, Wash. 99164.

[5] Dept of Horticultural Science and Landscape Architecture.

[6] Abbreviations: HPLC: high performance liquid chromatography; GLC-EC: GLC with electron capture detector; K_d: partition coefficients.

MATERIALS AND METHODS

EXTRACTION PROCEDURE

The soybean (*Glycine max* [L.] Merr. cv. Clay) plants used in the following experiments were grown under greenhouse or field conditions. Harvested plant material was immediately placed on dry ice and then brought to the laboratory. The tissue was weighed, homogenized in 80% (v/v) methanol (20 ml methanol/g fresh weight of tissue) at 4 C, transferred to a 250-ml Erlenmeyer flask, and shaken for 24 hr at 4 C. The homogenate was centrifuged at 1,545*g* for 10 min and the supernatant filtered through methanol-washed Miracloth (Chicopee Mills, Inc.), and taken to dryness *in vacuo* at 35 C. The sample was then frozen and stored at −20 C.

PARTITION COEFFICIENTS FOR ABA

Although several organic solvents have been used for partitioning ABA, to our knowledge the literature does not contain a direct comparison of the effectiveness of such solvents. To achieve such a comparison, the partition coefficients were determined for the partitioning of ABA between water at four pH values and seven organic solvent systems commonly used for this purpose.

ABA standards (1 μg/ml) in charcoal-filtered, deionized, distilled H_2O were adjusted to either pH 2.5, 5, 7, or 9 with 0.1 N HCl or 0.1 N KOH. Ten ml of the ABA standards were placed in 60-ml culture tubes and partitioned once against an equal volume of glass-distilled organic solvent. The organic fractions were removed and dried *in vacuo* at 40 C. The ABA in the control (no partitioning) was determined by taking to dryness a 10-ml ABA standard for each pH treatment. All samples were reconstituted in 1 ml of 5% (v/v) methanol in 0.2 N acetic acid. A 200-μl aliquot was injected into a 316 stainless steel column (30 cm × 4 mm i.d.) packed with μBondapak C_{18} (Waters Associates). The sample was eluted with 0.2 N acetic acid in 45% (v/v) methanol at a flow rate of 4.5 ml/min, and a pressure of 262 to 290 bars. The ABA was detected with a fixed wavelength UV detector at 254 nm. The instrumentation used in this HPLC procedure is further described below.

The ABA eluted with a very narrow peak width which permitted quantification based on peak height.

Partition coefficients were determined as:

$$K_d = \frac{\text{Concentration in aqueous phase}}{\text{Concentration in organic phase}} = \frac{a - b}{b}$$

where a = initial concentration of ABA in the aqueous phase, and b = concentration of ABA in the organic phase after partitioning.

PREPARATIVE HPLC

The dried samples from the extraction or from the partitioning procedure were reconstituted in a known volume of 5% (v/v) methanol in 0.2 N acetic acid and centrifuged at 1,239*g* for 10 min using a horizontal rotor. The supernatant was microfiltered through a 5-μm pore size PTFE filter (Millipore Corp.) on top of a 0.2-μm pore size Fluoropore filter (Millipore Corp.).

A known volume of filtered sample (an aliquot of the supernatant) was subjected to preparative HPLC as described by Carnes *et al.* (3) by injecting it through a 5-ml external loop valve (Valco 7000 series) connected to a stainless steel precolumn (3 cm × 2 mm i.d.) and then to two stainless steel columns (1 m × 6.5 mm i.d.) in series, all packed with Bondapak C_{18}/Porasil B (Waters Associates). The sample was then eluted with a linear gradient of methanol (0–100% in 60 min) in 0.2 N acetic acid, delivered by two constant flow, nonpulsed, high pressure piston pumps (Waters Associates, model 6000) controlled by a solvent programmer (Waters Associates, model 660). The eluate was delivered to the column at 9.9 ml/min at a pressure of 172 to 276 bars. The detection system used was a UV monitor (Waters Associates, model 440).

The eluate fraction containing ABA was collected based upon the retention time of an ABA standard (Burdick and Jackson Laboratories, Inc.). The fraction was taken to dryness *in vacuo* at 35 C and stored at −20 C for subsequent analysis.

To test the efficiency of the preparative HPLC procedure with and without partitioning, 2 μCi of DL-[2-^{14}C]ABA (6.1 μCi/mol) (Amersham/Searle) were added to the 80% (v/v) methanol extract of 54.6 g of soybean seed (Fig. 1, sample point A). The extract was then divided into subsamples each representing 3.2 g of seed. These subsamples were then processed through progressively larger portions of the partitioning scheme. Partition phases normally discarded were taken to dryness *in vacuo* at 35 C, reconstituted in 2 ml of deionized, distilled H_2O, and ^{14}C activity measured to determine any loss of ABA during the partitioning. Partition phases containing ABA were taken to dryness *in vacuo* at 35 C, reconstituted in 10 ml of 5% (v/v) methanol in 0.2 N acetic acid, and prepared for preparative HPLC. Aliquots were removed for scintillation counting after centrifugation, after filtering, and after preparative HPLC. The scintillation solution was 30% Triton X-100 in xylene containing 5.3 g/l butyl-PBD(2-[4′-*tert*-butylphenyl]-5-[4″-biphenyl]-1,3,4-oxadiazole) and 0.55 g/l POPOP. The ^{14}C activity was determined using a liquid scintillation spectrometer (Packard Tri-Carb, model 3375). Efficiency was 79% for ^{14}C.

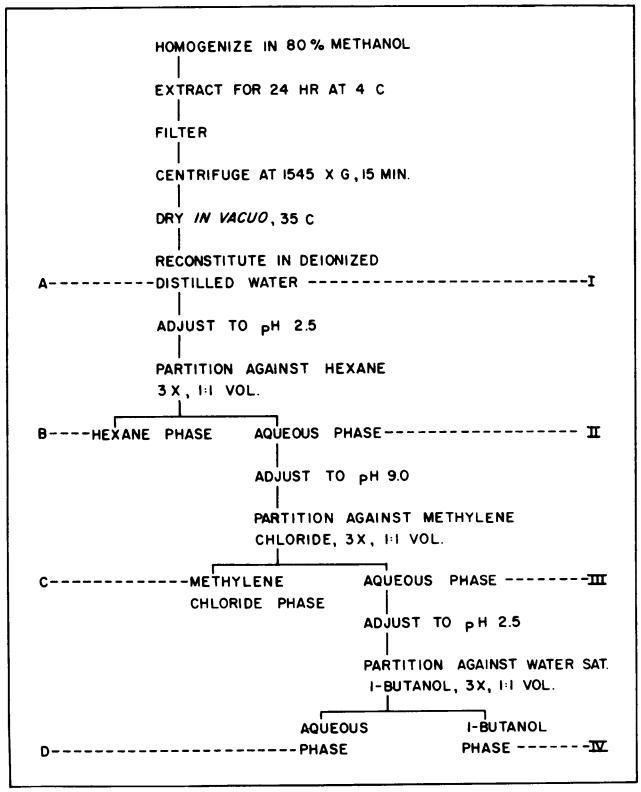

FIG. 1. Flow chart for the purification of ABA from soybean tissue extract. At points I through IV, samples were processed through the preparative HPLC procedure. [^{14}C]ABA was added at point A and its recovery determined at points B through D, as well as in the ABA fractions from the preparative HPLC procedures at points I through IV.

ANALYTICAL PROCEDURE

Gas-Liquid Chromatography. All ABA samples for GLC-electron capture (GLC-EC) analysis (15) were methylated using diazomethane (14), dried, and reconstituted (details under "Results and Discussion"). One-μl samples were injected into a gas chromatograph (Beckman GC-45) fitted with one of three columns. These were a glass column (1.05 m × 4 mm i.d.) packed with 4% SE-30 on 100/120 Gas-chrom Q; a glass column (1.84 m × 2 mm i.d.) packed with 3% SP-2100 on 100/120 Supelcon; or a glass column (1.05 m × 4 mm i.d.) packed with 3% Dexsil 300 on 100/120 Gas-chrom Q. The glass columns were silylated with Sylon (Supelco, Inc.) prior to packing, and the supports were silylated with Silyl 8 (Pierce Chemical Co.) after packing. Column and injection port temperatures were 215 and 235 C, respectively. A mixture of 5% methane in argon served as the carrier and make-up gases at flow rates of 30 and 40 ml/min, respectively. A wide range electron capture detector (Analog Technology Corp., model 140A) with a scandium-tritide source was used at 280 C. The electron capture detector was linear from 10 pg to 100 ng ABA.

Gas-Liquid Chromatography-Mass Spectrometry. Multiple ion detection was performed using a LKB 9000 combined gas-liquid chromatograph-mass spectrometer (GC-MS). A glass column (1.4 m × 2 mm i.d.) packed with 3% OV-1 on Supelcoport 100/120 mesh was used. Helium was used as a carrier gas at a flow rate of 25 ml/min. The column and source temperatures were 220 and 290 C, respectively. The ionizing source was 70 ev. Data were reduced using a data acquisition system (digital mini computer, model PDP 8/e, Digital Equipment Corp.).

Analytical HPLC. Three analytical HPLC procedures were compared to the standard GLC-EC technique used for quantification of ABA. The HPLC columns were commercially packed with μBondapak C_{18}, μPorasil, and μBondapak-NH_2 (Waters Associates). Each column was 30 cm × 4 mm i.d. with a particle size of 10 μm. The HPLC system was the same as the preparative system, except that a 200-μl injection loop valve (Chromatronix HPSV-20) was connected to the respective analytical columns.

An extract of 15.05 g of soybean seeds was taken through the preparative HPLC procedure and then divided into subsamples for separate triplicate analyses through the chromatographic systems of μBondapak-NH_2, μPorasil, and GLC-EC. Recoveries were established with reference to an internal standard of 44 μg of [^{14}C]ABA added to the fraction from the preparative HPLC procedure (containing approximately 160 μg of endogenous ABA) before subdivision. To determine if further purification was necessary before GLC-EC for accurate quantification, the solvent elution zone containing the ABA peak was collected from the μBondapak-NH_2 and μPorasil columns. These fractions were then taken to dryness *in vacuo* at 35 C and prepared for GLC-EC analysis as previously described. The ABA in the respective subsamples was quantified on the basis of peak height compared to ABA standards. The ^{14}C quantification was done as previously described.

RESULTS AND DISCUSSION

Preparative Procedure. The partition coefficients (K_d) of ABA when partitioned between water at four pH values and seven organic solvent systems are shown in Table I. Also shown are the per cent retention values of ABA in the aqueous phase. From the data of Table I, the following three-step partitioning sequence was calculated to provide theoretically maximum recovery of ABA:

1. Partitioning an aqueous phase at pH 2.5 against hexane and discarding the hexane which retains 1.1% of the ABA.
2. Partitioning the aqueous phase at pH 9 against methylene chloride and discarding the methylene chloride which retains 0.8% of the ABA.
3. Partitioning the aqueous phase at pH 2.5 against water-saturated 1-butanol which retains 98.1% of the ABA, and is retained.

The theoretical recovery of ABA from this partitioning sequence, with each step performed three times, is 94% calculated as: $([0.989]^3 \times [0.992]^3 \times 0.981\ [1 + 0.019 + (0.019)^2])$.

To test the usefulness of this partitioning sequence, the amount of ABA in a soybean seed extract was determined before and after each of the three partitioning steps involved.

Table I. Partition coefficients (K_d) for ABA when partitioned between equal volumes of organic solvents and water at four pH values.

Organic solvent	Dielectric constant (20C)	pH of aqueous phase 2.5	5.0	7.0	9.0
Hexane	1.9	93.0 (98.9)[1]	55.9 (98.2)	82.0 (98.8)	72.0 (98.6)
Diethyl ether	4.3	0.302 (23.2)	2.13 (68.1)	6.61 (84.9)	62.9 (98.4)
Chloroform	4.81	0.330 (24.8)	16.3 (94.4)	165 (99.4)	382 (99.7)
Ethyl acetate	6.0	0.100 (9.1)	0.736 (42.4)	4.32 (81.2)	138 (99.3)
Methylene chloride	9.09	1.05 (51.2)	6.88 (87.3)	35.1 (97.2)	123 (99.2)
1-Butanol	17.8	0.198 (16.0)	0.186 15.7)	0.625 (38.5)	126 (68.4)
1-Butanol-water saturated	---	0.019 (1.90)	0.295 (22.8)	0.419 (29.5)	2.14 (68.2)

[1] Values in parenthesis show the percentage of ABA remaining in the aqueous phase $(\frac{k_d}{k_d+1} \times 100)$.

Table II. Recovery of ABA from soybean seed extract after various steps of purification.[1]

Sample Point	Experiment I	Experiment II
	ng ABA/g fresh wt	% Recovery of ^{14}C-ABA
A		100
I	830	98.9 ± 0.3
B		2.4 ± 1.5
II	800	94.7 ± 3.3
C		9.3 ± 6.5
III	801	86.9 ± 12.0
D		0.3 ± 0.0
IV	758	84.0 ± 3.7

[1] See Figure 1 for purification flow chart.

The results (Table II, experiment I) suggested that the partitioning procedure might not be necessary.

To test this possibility further, another soybean extract was labeled with an internal standard of [^{14}C]ABA and submitted to the partitioning sequence (Table II, experiment II). Eighty-four per cent of the radioactivity was recovered when the sample was passed through the entire sequence (Fig. 1, sample point IV). Most of the loss of radioactivity occurred while partitioning against methylene chloride at pH 9 (Fig. 1, sample point C). It was difficult to obtain a clean phase separation at this step, presumably due to lipid-like, surface-active material in the seed extract.

In contrast, 98.9% recovery of radioactivity was obtained when the seed extract was simply centrifuged, microfiltered, and passed through the preparative HPLC column (Fig. 1, sample point I). The centrifugation and microfiltration were necessary to prevent the column from plugging, and yet caused no detectable loss of radioactivity. The [^{14}C]ABA was collected in 63 ml of eluent recovered from the column between 34.6 and 41 min (343-406 ml). The selection of this particular fraction was based upon the retention time of the ABA standard. The 1.1% loss of radioactivity could be caused by taking slightly too narrow a fraction of the eluate, or it could be due to impurities in the [^{14}C]ABA. The 98.9% recovery of ABA from the preparative HPLC column without any initial partitioning represents a considerable improvement over recoveries of 60 to 94% reported using other purification procedures (2, 8, 18).

The concentration of [^{14}C]ABA used in the previous experiment was representative of ABA concentrations found in seed extracts. This insured that if absorption did occur during the handling procedure such losses would not be masked by excessive quantities of [^{14}C]ABA.

A typical trace of the UV absorbance (254 nm) of a seed

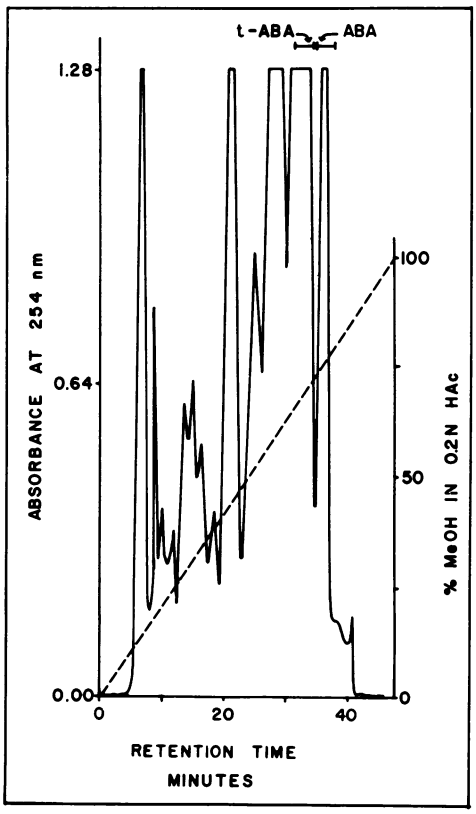

FIG. 2. Typical trace of the UV absorbance (254 nm) of a seed extract eluted from the preparative HPLC column. Dotted line indicates the concentration gradient of methanol in acetic acid used as a solvent.

extract eluted from the preparative HPLC column is shown in Figure 2. After 10 min of the linear program, the ABA was completely eluted from the column. The column was then flushed with 100% methanol in 0.2 N acetic acid for 15 min at 9.9 ml/min to remove the remaining nonpolar compounds. When the tissue is extracted with a less polar solvent (lower dielectric constant), such as 1-butanol, a solvent of similar polarity is required to clean the column. Initial conditions of the column were reestablished after flushing the column for an additional 15 min with 0.2 N acetic acid at 9.9 ml/min. The minimum time between sample injections of the preparative HPLC column using this column design was thus 70 min.

The preparative HPLC procedure described may make it possible to recover and purify other plant hormones from a single extraction, since 80% methanol is a common extracting solvent for a number of plant hormones. Preliminary work in our laboratory demonstrated that *t*-zeatin, IAA, phaseic and dihydrophaseic acids were each readily resolved from the ABA fraction (Andersen, Brenner, Mondal, Hein and Brun, unpublished).

Analytical Quantification by GLC-EC. ABA quantification can be achieved by GLC-EC of plant extracts after a preparative HPLC procedure described above. Due to the selectivity of the electron capture detector for molecules with high electron affinity, very few compounds other than ABA were observed. With the detector used in this study, the lower limit of sensitivity was 10 pg ABA/injection.

A few problems were possible as a result of the minimum amount of sample purification used. A large quantity of organic compounds other than ABA was present in the ABA fraction from the preparative HPLC column. Some of these did not dissolve well in the 10% (v/v) methanol in ether solution used for methylation. To determine whether ABA was entrapped in the poorly dissolved components preventing complete methylation of all of the ABA, methanol was added initially to dissolve the sample, and then ether was added to the desired concentration. Concentrations of methanol from 10% to 50% in ether were found to have little effect on the final yield of methylated ABA.

After methylation, the sample was taken to dryness with N_2 stream at 30 C and then reconstituted in a suitable solvent for GLC. Hexane, ethyl acetate, pyridine, and acetonitrile are commonly used solvents for this purpose. In choosing a solvent, the following considerations must be made: (*a*) ABA must be completely soluble; (*b*) solvent peak tailing must be minimal; and (*c*) the time required for the detector signal to return to base line between sample injections must be minimal. Hexane, ethyl acetate, and acetonitrile gave very little solvent peak tailing before the time of ABA elution, but did not completely dissolve the ABA sample. Pyridine completely dissolved the sample, but had a large solvent peak tailing.

A 10% (v/v) solution of pyridine in hexane was the final choice. The methylated sample was dissolved in 50 μl of pyridine to which 450 μl of hexane were added. Solubility in the pyridine was excellent and solvent peak tailing was acceptable. However, the choice of solvent can vary with the plant material examined, the GLC column packing material used, and with the electron capture detector employed. Therefore, one should examine carefully various solvents to achieve efficient, reproducible quantification of ABA by GLC-EC.

Multiple ion detection on GC-MS for the major fragments of ABA (91, 125, 134, 162, 190, m/e) (7) substantiated the presence of ABA in the seed sample. A fragmentation pattern characteristic of ABA was observed only at the retention time matching that of ABA standard.

Analytical Quantification by HPLC. Of the three HPLC columns examined, μPorasil and μBondapak-NH_2 were found suitable for ABA quantification of a soybean seed extract following preparative HPLC as described above.

ABA can be quantified from μPorasil using 15% (v/v) aceto-

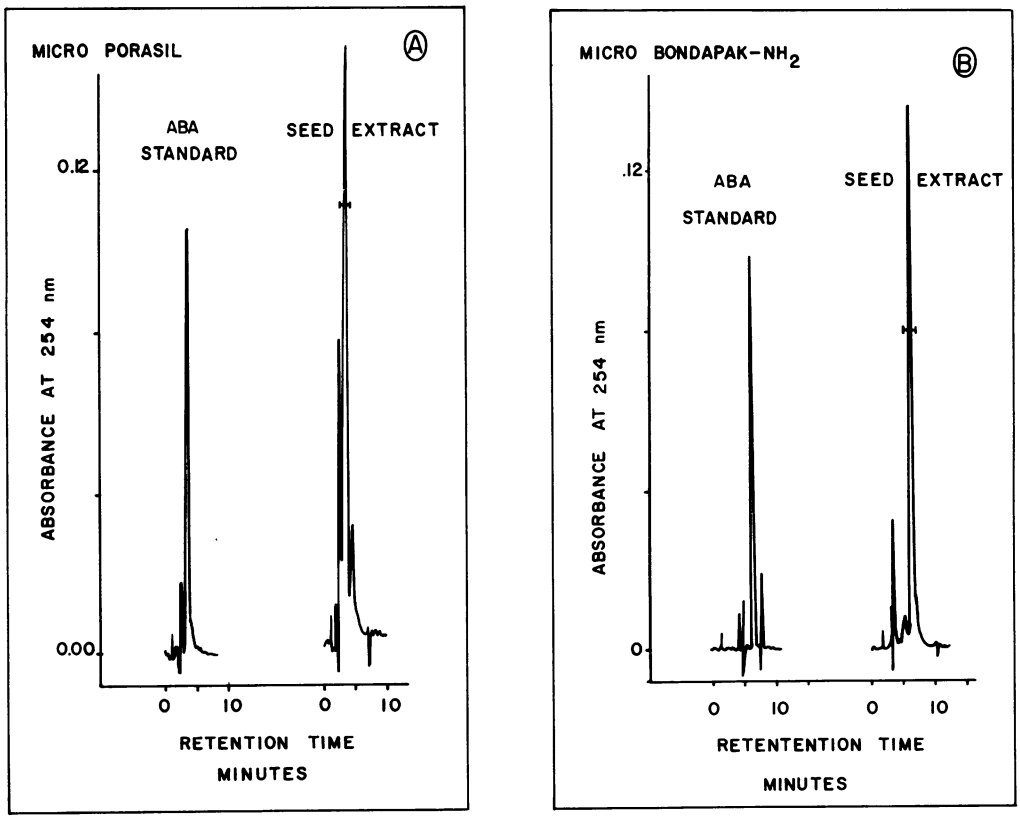

FIG. 3. Absorbance (254 nm) of standard ABA (500 ng) and a preparatively purified soybean seed extract eluted from μPorasil (A) and μBondapak-NH_2 (B) analytical HPLC columns.

nitrile in chloroform acidified with 0.17 N acetic acid at a flow rate of 2 ml/min (Fig. 3A). The lower limit of detection was 500 pg ABA/injection, and quantification was linear to 20 μg using a UV detector (254 nm). Sample injections of 20 μl or 200 μl gave similar ABA quantification.

Quantification of ABA from μBondapak-NH_2 was achieved with 45% (v/v) acetonitrile in chloroform acidified with 0.17 N acetic acid at a flow rate of 2 ml/min (Fig. 3B). The lower limit of detection and the concentration linearity were similar with μBondapak-NH_2 and μPorasil.

The μBondapak C_{18} column separated the ABA, *t*-ABA standards with a solution of 45% (v/v) methanol in 0.2 N acetic acid at a flow rate of 4.5 ml/min, but quantification of ABA from a plant extract following preparative HPLC was not achieved due to interfering UV absorbing compounds. This was presumably due to the similarities in packing material and elution solvents used in the two systems. The desired separation may be possible by changing the organic solvent and/or pH used to elute the column or by derivatizing the ABA.

Table III compares the ABA quantifications by the two analytical HPLC columns alone and followed by GLC-EC. The ABA quantifications by the various procedures were similar showing that the peaks from the two analytical HPLC columns were in fact only ABA. The standard deviations associated with GLC-EC were much larger and possibly related to the following problems characteristic of GLC-EC: (*a*) difficulty in achieving reproducible sample injections; (*b*) fluctuation in the EC detector sensitivity due to chemical build-up in the detector; and (*c*) very small injection volumn relative to the sample volume.

Filtering caused no reduction of ABA concentration (Table IV). These samples were handled on an aliquot basis to correct for volume loss. Ninety-five per cent of the radioactivity was recovered in the ABA-containing fraction from each column.

Table V shows the minimum amount of ABA that can be quantified using either analytical HPLC or GLC-EC. HPLC using μPorasil and μBondapak-NH_2 columns have a lower limit of detection of 500 pg ABA/injection while the lower limit of detection using GLC-EC is 10 pg/injection. An advantage of the analytical HPLC system is the ability to use large injection volumes, thus making it possible to quantify a larger portion of the total sample per injection. Therefore, the minimum quantity of ABA needed in a sample is relatively similar for the two techniques. Quantification by analytical HPLC requires 40 times less sample dilution than by GLC-EC. Thus the precision achieved should be greater with the analytical HPLC than with GLC-EC.

The ABA elution times and turn-around times are very similar for the analytical HPLC and the GLC-EC. The analytical HPLC procedure does not require the sample to be derivatized and offers easier sample collection after quantification. The major limitation of analytical HPLC compared to GLC-EC for quantification of ABA is that a UV detector is not as selective as GLC-EC. This is a problem when ABA is present at levels similar to that of interfering compounds. Additional purification is necessary for such samples before analytical HPLC. Very likely a different type of HPLC separation would be adequate. The advent of more selective detectors will overcome this problem thereby permitting less purification of very complex samples.

Additionally, any laboratory planning extensive work with ABA (and perhaps other plant hormones) will find that HPLC offers the opportunity to do both the preparative clean up and the analytical quantification using the same instrumentation. However, one important note of caution is that the use of HPLC for identification and quantification of plant hormones may be quite tenuous unless the peaks being identified are first carefully characterized as being pure by either bioassay or preferably GC-MS.

Table III. ABA content of a preparatively purified extract determined using two analytical HPLC packing materials and GLC-electron capture.

ABA quantification[2]	μPorasil	μBondapak-NH_2
	ng ABA[1]	
by analytical HPLC[3]	5076 ± 67	4901 ± 155
by GLC-EC after analytical HPLC	6217 ± 869	7018 ± 598

[1] The reported values are calculated to reflect the % recovery of an internal ^{14}C-ABA standard.

[2] Direct analysis by GLC-EC showed a value of 5884 ± 673 ng ABA.

[3] K' values for μPorasil and μBondapak-NH_2 were 1.2 and 4.8, respectively.

Table IV. Percent recovery of ^{14}C-ABA[1] concentration using two analytical HPLC packing materials.

	Analytical HPLC Column	
	μPorasil	μBondapak-NH_2
	%	
after filtration	100.0 ± 1.9	100.0 ± 11.4
after chromatography		
Fraction I[2]	3.6 ± 1.8	2.9 ± 0.4
Fraction II (ABA)	94.5 ± 5.9	95.5 ± 4.6
Fraction III	1.4 ± 0.1	1.3 ± 0.5

[1] Added to the fraction from the preparative HPLC column presumed to contain the ABA.

[2] Fractions I, II, and III represent material eluted from the repsective columns, before, during, and after the ABA containing fraction.

Table V. Hypothetical quantification of ABA using GLC-EC vs. analytical HPLC.

	Analytical HPLC	GLC
Sample size	1 ml	0.5 ml
Injection volume	200 μl	2.5 μl
Detection limit[1]	500 pg[2]	10 pg[3]
Minimum sample[4]	2.5 ng	2 ng

[1] The signal to noise ratio was always greater than 5

[2] UV, 254 nm.

[3] EC.

[4] Calcualted as: $\frac{\text{sample size x detection limit}}{\text{injection volume}}$

LITERATURE CITED

1. Beardsell MF, D Cohen 1975 Relationships between leaf water status, abscisic acid levels, and stomatal resistance in maize and sorghum. Plant Physiol 56: 207-212
2. Browning G, GV Hoad, P Gaskin 1970 Identification of abscisic acid in flower buds of *Coffea arabica* (L.). Planta 94: 213-219
3. Carnes MG, ML Brenner, CR Andersen 1975 Comparison of reversed-phase high-pressure liquid chromatography with Sephadex LH-20 for cytokinin analysis of tomato root pressure exudate. J Chromatogr 108: 95-106
4. Davis LA, DE Heinz, FT Addicott 1968 Gas-liquid chromatography of trimethylsilyl derivatives of abscisic acid and other plant hormones. Plant Physiol 43: 1389-1394
5. Düring H, O Bachmann 1975 Abscisic acid analysis in *Vitis vinifera* in the period of endogenous bud dormancy by high pressure liquid chromatography. Physiol Plant 34: 201-203
6. Goldschmidt EE, R Goren, Z Even-Chen, S Bittner 1973 Increase in free and bound abscisic acid during natural and ethylene-induced senescence of citrus fruit peel. Plant Physiol 51: 879-882
7. Gray RT, R Mallaby, G Ryback, VP Williams 1974 Mass spectra of methyl abscisate and isotopically labelled analogues. J Chem Soc Perkin Trans II: 919-924
8. Lenton JR, VM Perry, PF Saunders 1971 The identification and quantitative analysis of abscisic acid in plant extracts by gas-liquid chromatography. Planta 96: 271-280
9. Loveys BR, PE Kriedemann 1973 Rapid changes in a abscisic acid-like inhibitors following alterations in vine leaf water potential. Physiol Plant 28: 476-479
10. Mayak S, AH Halevy 1972 Interrelationships of ethylene and abscisic acid in the control of rose petal senescence. Plant Physiol 50: 341-346

11. Mielke EA, FG Dennis Jr 1975 Hormonal control of flower bud dormancy in sour cherry (*Prunus cerasus* L.). I. Identification of abscisic acid. J Am Soc Hort Sci 100: 285-287
12. Nitsch JP, C Nitsch 1956 Studies on the growth of coleoptile and first internode sections. A new, sensitive, straight-growth test for auxins. Plant Physiol 31: 94-111
13. Ramsay J, GC Martin 1970 Isolation and identification of a growth inhibitor in spur buds of apricots. J Am Soc Hort Sci 95: 574-577
14. Schlenk H, JL Gellerman 1960 Esterification of fatty acids with diazomethane on a small scale. Anal Chem 32: 1412-1414
15. Seeley SD, LE Powell 1970 Electron capture-gas chromatography for sensitive assay of abscisic acid. Anal Biochem 35: 530-533
16. Sweetser PB, A Vatvars 1976 High-performance liquid chromatographic analysis of abscisic acid in plant extracts. Anal Biochem 71: 68-78
17. Tillberg E 1975 An abscisic acid-like substance in dry and soaked *Phaseolus vulgaris* seeds determined by the *Lemna* growth bioassay. Physiol Plant 34: 192-195
18. Tucker DJ, TA Mansfield 1971 A simple bioassay for detecting "antitranspirant" activity of naturally occurring compounds such as abscisic acid. Planta 98: 157-163
19. Zabadal TJ 1974 A water potential threshold for the increase of abscisic acid in leaves. Plant Physiol 53: 125-127
20. Zeevaart JAD 1974 Levels of (+)-abscisic acid and xanthoxin in spinach under different environmental conditions. Plant Physiol 53: 644-648