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Liquid chromatographic determination of β-naphthoxyacetic acid in tomatoes

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Abstract

An alternative high-performance liquid chromatographic method for the determination of β-naphthoxyacetic acid (BNOA) in tomatoes is described. BNOA was extracted from tomatoes with acetone–dichloromethane (2:1). The extract was cleaned up by Bio-Beads S-X3 gel-permeation chromatography and by partitioning. A reversed-phase C_{18} column was used for HPLC analysis. The mobile phase was acetonitrile–2% acetic acid in water (50:50, v/v) pumped at a flow-rate of 1.0 ml/min. Retention time of BNOA was ca. 7 min with a percentage coefficient of variation of 0.71. Resolution of BNOA was good on the column. Percentage recoveries of BNOA were 79.5±6.82, 94.8±2.70 and 86.4±16.43 for the corresponding spiking levels of 0.5, 1.0 and 2.0 μg per g tomato, respectively. Analysis of 10 greenhouse tomato samples from local markets in Ankara showed no BNOA residue. © 1998 Elsevier Science B.V.

Keywords: Tomatoes; Fruits; Food analysis; Naphthoxyacetic acid; Auxins

1. Introduction

The use of synthetic auxins is an important growing practice in regions with winters that are mild and greenhouses that are not heated. It is an effective method for obtaining marketable fruits under conditions where pollination is deficient. Short cloudy days, which lack sunlight, and cool nights under 15°C, are conditions that are particularly unfavorable for flower pollination. Tomatoes, which might otherwise be lost due to poor pollination, can be set by the application of chemicals such as β-naptoxyacetic acid (BNOA) or 4-chlorophenoxycetic acid (4-CPA) [1,2].

In Turkey, BNOA is registered as a plant growth regulator. The effective concentration used on tomato is 50 to 500 mg/l as an aqueous solution [3]. BNOA is regarded as a slightly hazardous chemical by the World Health Organization (WHO) based on its acute toxicity in rats [4]. A maximum permitted concentration of BNOA has not been established. At present, there is no information concerning the amounts of BNOA residues in greenhouse tomatoes. Such tomatoes are widely consumed during winter and spring in Turkey.

A simple method for monitoring BNOA is needed. A limited number of methods, based on liquid and gas chromatography, have been reported for measuring BNOA in plant materials [5–8]. In this article, an alternative liquid chromatographic method is described for the analysis of BNOA in tomatoes.

2. Experimental

2.1. Tomato

Tomato samples were supplied from the local

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markets in Ankara and kept in a deep-freezer (−18°C) until analysis.

2.2. Chemicals

Acetone, dichloromethane, ethyl acetate, cyclohexane were extra pure while methanol and acetonitrile were HPLC grade (Merck, Darmstadt, Germany). NaOH (1 *M*), $NaHCO_3$ (4%, w/w), H_2SO_4 (20%, w/w), Na_2SO_4 (anhydrous) were all reagent grade (Merck). Bidistilled water was used throughout the experiments.

2.3. BNOA working standards

One hundred mg of BNOA (Sigma, UK) was dissolved in 100 ml of methanol to obtain a stock solution with a concentration of 1000 μg/ml. Working standards were prepared by diluting this stock solution with methanol to yield concentrations of 1, 2, 3, 4, 5 and 10 μg/ml.

2.4. UV–Vis spectrophotometer

A Shimadzu model 2101 spectrophotometer was used to record the UV spectra of BNOA.

2.5. Gel chromatograph

The column used to clean up the tomato extract was packed with Bio-Beads S-X3 (Bio-Rad, UK) to form a 32×2-cm bed. Beads were swelled in the eluent for 24 h before the column was packed. The eluent was ethyl acetate–cyclohexane (1:1, v/v) pumped at a flow-rate of 2.5 ml/min with a Bio-Rad Econo model pump.

2.6. Gas chromatograph

A Hewlett–Packard 5890 II gas chromatograph equipped with a capillary injection system, ^{63}Ni electron-capture detector and capillary column (30 m×0.32 mm I.D.) coated with SE-54 was used. The detector and injector temperatures were 300°C and 250°C, respectively. The oven temperature was programmed from 80 to 250°C at a rate of 10°C/min with a final hold time of 3 min. The carrier gas was nitrogen at a column flow-rate of 2.5 ml/min (10 p.s.i. of column head pressure; 1 p.s.i.=6894.76 Pa). Splitless injection was used with an injection volume of 1 μl.

2.7. High-performance liquid chromatograph

A Varian Star model liquid chromatograph with a Varian 9010 gradient solvent delivery system, Varian 9050 variable-wavelength UV–Vis detector set at 270 nm, Rheodyne 7163 six-way injection valve with 10-μl sample loop, and Varian 4400 integrator were used.

2.8. HPLC column

A Shimpak C_{18} column, 300×4 mm (I.D.), stainless steel, operated at ambient temperature was used. It was protected by a C_{18}, 40×4 mm (I.D.) guard column.

2.9. Mobile phase

A mixture of acetonitrile–2% acetic acid in water (50:50, v/v) was used with a flow-rate of 1.0 ml/min. It was filtered through a 0.45-μm filter and degassed ultrasonically before use.

2.10. Sample preparation

Tomatoes were blended in a Waring blender at medium speed for 5 min. Fifteen ml of 1 *M* NaOH and 60 ml of water were added to 25 g of blended tomato and the mixture hydrolyzed in a water bath at 95°C for 2 h to convert the bound and conjugated BNOA to the free acid form. After cooling, water lost due to evaporation during the hydrolysis was replaced and the mixture acidified by adding 5 ml of 20% H_2SO_4. The hydrolyzed material was homogenized with 200 ml of acetone for 2 min at medium speed in a Virtis homogenizer and filtered through S&S (Schleicher and Schuell) no. 589^3 filter paper into a graduated cylinder. The appropriate volume of the filtrate that is equivalent to 20 g tomato was transferred into a 1000-ml separation funnel. Twenty-five g of NaCl was added and the filtrate was shaken vigorously for 3 min. One hundred ml of dichloromethane was added and the filtrate was extracted by shaking vigorously for 2 min. After

phase separation, the aqueous phase was discarded and the organic phase quantitatively transferred into a flask containing 25 g of anhydrous Na_2SO_4 and dried for 20 min. The organic phase was then filtered through a S&S no. 589[3] filter paper into a 1000 ml separatory funnel. The filter paper was washed with 20 ml of ethyl acetate. The washing was combined with the organic phase and the combined material concentrated to an approximate volume of 1 ml using a rotary evaporator at 40°C. The remaining residue was completely dried under a gentle stream of nitrogen. Dried residue was dissolved immediately in a mixture of ethyl acetate–cyclohexane (1:1, v/v) and made up to a volume of 10 ml. Half of this solution containing 10 g of tomato extract was injected onto a Bio-Beads S-X3. The sample was eluted through the column with ethyl acetate–cyclohexane (1:1, v/v) at a flow-rate of 2.5 ml/min. The first 35 ml of the eluate was discarded. Eluate from 35 ml to 60 ml was collected and transferred into a 100-ml separatory funnel with 15 ml of ethyl acetate–cyclohexane (1:1, v/v). It was then extracted with 2×30 ml of 4% $NaHCO_3$. Combined bicarbonate phases were acidified with 3–4 ml of 20% H_2SO_4 and shaken gently to remove CO_2. The bicarbonate phase was extracted with 2×30 ml of dichloromethane. Combined organic phases were filtered through a S&S no. 589[3] filter paper covered with anhydrous Na_2SO_4 and evaporated to dryness using the rotary evaporator at 40°C. Residue was further dried under the gentle stream of N_2. Remaining residue was dissolved in a minimum amount of methanol and injected into the HPLC column. A portion of the sample was analyzed on a gas chromatograph after conversion of the acidic residue to the methyl ester form as described elsewhere [7].

2.11. Expression of the results

Results are expressed as μg residue in g of tomato sample using the following formula:

$$\text{2,4-D in tomato } (\mu g/g) = C_f \times \frac{V}{m}$$

where C_f is the concentration of the final solution, μg/ml; V is the volume of the final solution, ml; and m is the amount of sample in the extract, g.

3. Results and discussion

UV spectra for methanolic solutions of BNOA, at concentrations of 5 and 10 μg/ml, exhibited a maximum absorbance at ca. 270 nm (Fig. 1). Therefore, the HPLC detector was set as 270 nm to obtain maximum sensitivity.

Correlation between concentration and detector response as peak height was high ($r^2 = 0.997$, $n = 7$). As low as 1 ng BNOA injected into the column could be detected. Retention time of BNOA was ca. 7 min with a percentage coefficient of variation of 0.71 for seven repetitive injections indicating high precision.

Five ml of a standard solution of BNOA, 10 μg/ml, was injected onto the Bio-Beads S-X3 column in order to determine the elution volume of BNOA. Eluate fractions collected as 5 ml portions up to a total eluate volume of 100 ml were analyzed for BNOA content. One hundred percent of the BNOA injected was recovered within the eluate volume of 45–60 ml (Fig. 2). Injecting tomato extract containing BNOA caused faster elution through the column and the elution limit changed to 35–50 ml. Elution volume of BNOA during gel chromatography on Bio–Beads S-X3 has been reported as 110–140 ml using similar conditions by Specht and Tillkes [9].

In order to verify the accuracy of the method, tomato samples were spiked with 0.5, 1.0 and 2.0 μg/g of BNOA and extracted. Percentage recoveries

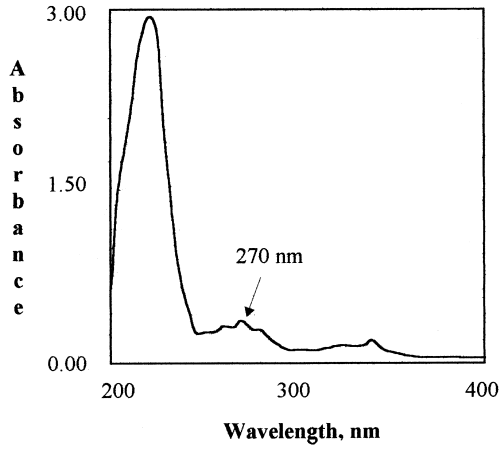

Fig. 1. UV spectrum of BNOA (10 μg/ml BNOA in methanol).

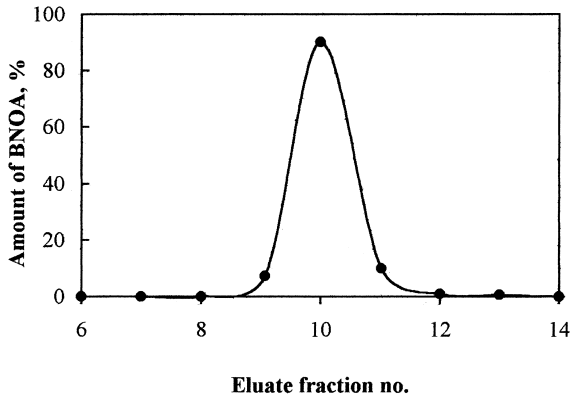

Fig. 2. Gel chromatographic elution limit of BNOA.

Table 1
Percentage recovery of BNOA in tomato

Spiking level (μg/g)	Recovery (%)	S.D. ($n=4$)
0.5	79.5	6.82
1.0	94.8	2.70
2.0	86.4	16.43

obtained show that the method described can be used for the analysis of BNOA in tomatoes (Table 1). Standard deviation of the percentage recovery was high when large amounts (2.0 μg/g) of BNOA were present in tomato.

Archer and Stokes [6] reported recoveries of 76.0% to 92.0% from tomatoes spiked with BNOA.

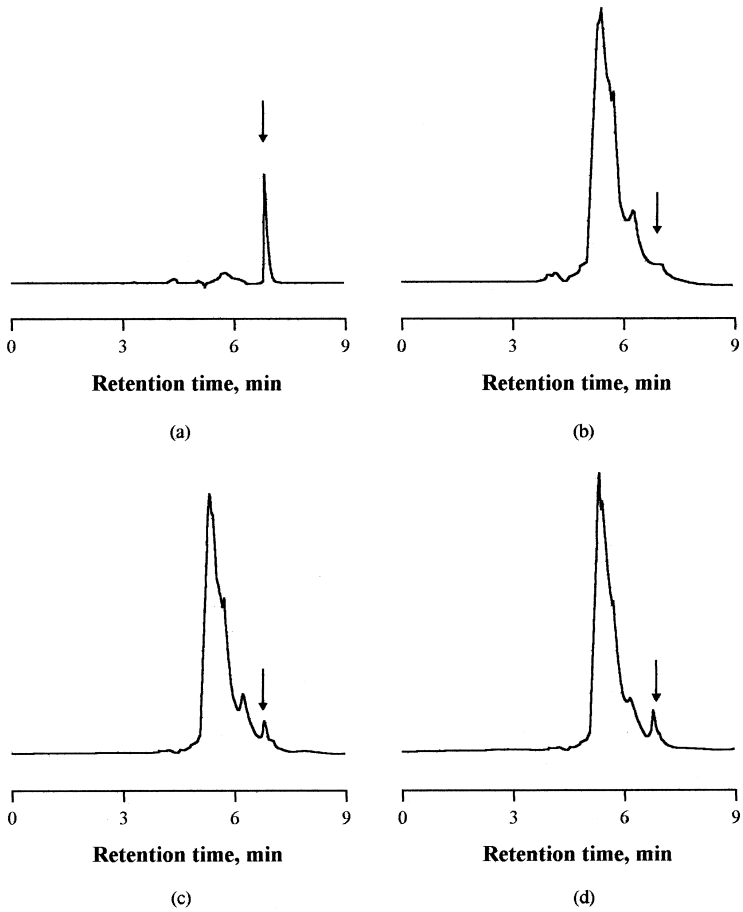

Fig. 3. Chromatograms of BNOA residues in tomato, (a) 5 μg/ml BNOA standard (b) no spiking (control, 0.0 mg/kg), (c) spiking level of 0.5 mg/kg BNOA, (d) spiking level of 1.0 mg/kg BNOA (arrows show BNOA peaks).

However, in their studies the retention time of BNOA was ca. 25 min while BNOA was analyzed effectively in an elution time of 10 min using the chromatographic conditions described in this study.

BNOA peaks were separated from the coextractives of tomato as shown in Fig. 3. The Bio-Beads S-X3 gel chromatographic cleanup played an important role in the sample preparation by removing much of the interfering substances that coextracted with the BNOA.

Using this method, no BNOA was detected using HPLC in greenhouse tomato samples ($n=10$) obtained from the local markets in Ankara. These results were confirmed by gas chromatographic analysis of the samples as a methyl ester form.

Separation of BNOA by GC is better than on HPLC. However, phenoxyacetic acids such as BNOA cannot be analyzed directly by GC because of their high polarity [10]. Derivatization that is applied to render them volatile has important disadvantages regarding time, economics and reproducibility.

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