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Short Communication

High-performance liquid chromatographic separation and determination of small amounts of 2-naphthaleneacetic acid in 1-naphthaleneacetic acid[a]

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ABSTRACT

A simple and rapid high-performance liquid chromatographic method was developed for the separation and determination of small amounts of 2-naphthaleneacetic acid in 1-naphthaleneacetic acid. The separation was achieved on a reversed-phased μBondapak CN column using 0.15 *M* ammonium sulphate–2-propanol (97.5:2.5, v/v) as the eluent. Reaction mixtures collected during the course of condensation of naphthalene with monochloroacetic acid were analysed by the proposed method and the yields of 1-naphthaleneacetic acid were monitored. The limit of detection of 2-naphthaleneacetic acid in a large excess of 1-naphthaleneacetic acid was $3 \cdot 10^{-9}$ g.

INTRODUCTION

1-Naphthaleneacetic acid (1-NAA) is an important plant growth regulator used not only to prevent the preharvest drop of apples but also as a fruit thinning agent [1,2]. It is manufactured generally by the alkylation [3,4] of naphthalene with acetic anhydride or by the condensation of naphthalene with monochloroacetic acid (MCA). Depending on the conditions, a mixture of 1-NAA, 2-NAA and di-, tri- and tetraacetic acid is obtained. High-purity 1-NAA is often required in vegetable cultivation. It is applied along with a recommended concentration of 2-NAA in controlling grape tendril atrophy [5]. Therefore a simple method is needed for the determination of 1-NAA and 2-NAA in mixtures.

No single method for the determination of 2-NAA in 1-NAA appears to have been reported. However, several methods [6,7] for the determination of 1-NAA in

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apples, grapes and oranges are available. Recently, Bertrand *et al.* [8] separated naphthaleneacetic acids by derivatizing them with cyanoethyldimethylsilane followed by gas chromatography–mass spectrometry. Ion-pair reversed-phased thin-layer chromatography and liquid chromatography (RP-LC) are used extensively [9,10] for the separation of naphthaleneacetic and sulphonic acids. Ion-pairing reagents such as tetrabutylammonium dihydrogenphosphate and cetyltrimethylammonium bromide are added to the mobile phase containing acetonitrile or methanol during RP-LC. However, using these reagents it is observed [11] that the peaks are often not only split but also show irreproducible shapes. Jandera and co-workers [12–14] have successfully overcome this problem by using solutions of inorganic salts as mobile phases for the separation of naphthalenesulphonic acids. They also compared the retention behaviours of various naphthalenesulphonic acids and concluded that the separation using inorganic salts is superior to that in systems containing ion-pair reagents [15]. Several workers [16–18] have also recommended the use of eluents containing inorganic salts for the separation of aromatic sulphonic and carboxylic acids.

In this paper, we describe a simple and rapid high-performance liquid chromatographic (HPLC) method for the separation and determination of 1-NAA and 2-NAA in standard and reaction mixtures using a μBondapak CN column and an eluent containing 0.15 *M* ammonium sulphate at ambient temperature.

EXPERIMENTAL

Materials and reagents

All reagents were of analytical-reagent grade unless stated otherwise. Glass-distilled water, 2-propanol (Spectrochem, Bombay, India), 1-NAA and 2-NAA (Fluka, Buchs, Switzerland), ammonium sulphate (BDH, Poole, UK) and sodium 3-nitrobenzenesulphonate (3-NBSS) (Aldrich, Milwaukee, WI, USA) were used.

Apparatus

A high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) with a 20-μl loop injector having a high-pressure six-way valve was used with a Model SPD-6AV variable-wavelength UV–VIS spectrophotometric detector (Shimadzu). A μBondapak CN (10 μm) column (300 mm × 3.9 mm I.D.) (Waters Assoc., Milford, MA, USA) was used for separation. The chromatograms and the integrated data were recorded by a Chromatopac C-R3A processing system.

Chromatographic conditions

The mobile phase was 0.15 *M* ammonium sulphate–2-propanol (97.5:2.5, v/v). Samples were dissolved in the mobile phase. The analysis was carried out under isocratic conditions at a flow-rate of 1 ml/min and a chart speed of 2.5 mm/min at room temperature (27°C). Chromatograms were recorded 223 nm.

Analytical procedure

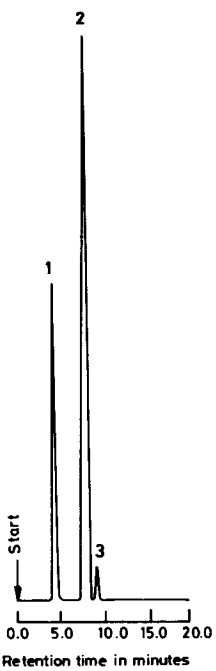
Standard mixtures containing 5 mg of internal standard, 27–30 mg of 1-NAA and 0.3–3.0 mg of 2-NAA were prepared by dissolving known amounts of the compounds in 25 ml of the mobile phase. A 5-μl volume of each standard mixture was injected and chromatographed under the above conditions. From the peak areas, the

response factors of 1-NAA and 2-NAA with respect to the internal standard were calculated.

Standard and reaction mixtures were analysed under identical conditions. The reaction mixture (25 mg) together with the internal standard (10 mg) was dissolved in 25 ml of the mobile phase and chromatographed. The percentage of 1-NAA and 2-NAA were calculated from the peak areas.

RESULTS AND DISCUSSION

The HPLC separation of 1-NAA, 2-NAA and 3-NBSS is shown in Fig. 1. The peaks were identified by injecting the individual compounds. It can be seen that 1-NAA is well resolved not only from its positional isomer 2-NAA but also from 3-NBSS used as an internal standard. The conditions for HPLC separation were optimized using three different stationary phases, *viz.*, μBondapak C_{18}, C_8 and CN, and eluents containing acetonitrile, methanol and 2-propanol. The μBondapak CN cyano-bonded reversed-phase column with 0.15 *M* ammonium sulphate–2-propanol (97.5:2.5, v/v) was selected over other systems not only because of the better separation obtained between 1-NAA and 2-NAA but also because the peak shapes were undistorted and reproducible.



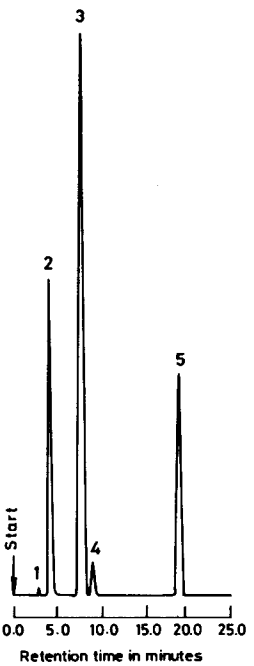


Fig. 1. Chromatogram of a typical mixture containing 1-NAA (1.2 μg), 2-NAA (0.1 μg) and 3-NBSS (6.1 μg). Peaks: 1 = 3-NBSS; 2 = 1-NAA; 3 = 2-NAA.

Fig. 2. Chromatogram of a reaction mixture (10 μg) together with internal standard (6.1 μg). Peaks: 1 = MCA; 2 = internal standard; 3 = 1-NAA; 4 = 2-NAA; 5 = naphthalene.

TABLE I

RETENTION DATA

Compound	Retention time (min)	k'	α	λ_{max} (nm)
1-Naphthalenecarboxylic acid	5.90	0.84	2.36	211
2-Naphthalenecarboxylic acid	9.54	1.98		222
1-Naphthaleneacetic acid	7.65	1.39	1.30	222
2-Naphthaleneacetic acid	9.00	1.81		223
1-Naphthalenesulphonic acid	7.90	1.47	1.53	225
2-Naphthalenesulphonic acid	10.39	2.25		226
1-Amino-naphthalene	12.09	2.78	1.12	236
2-Amino-naphthalene	13.19	3.12		235
Naphthalene 1-acetate	14.46	3.52	1.06	219
Naphthalene 2-acetate	14.92	3.72		219
1-Naphthalenol	22.35	5.98	1.05	219
2-Naphthalenol	23.26	6.27		221
Naphthalene	18.44	4.76		222
Sodium 3-nitrobenzene-sulphonate	4.02	0.26		260

A series of 1- and 2-substituted naphthalenes such as 1- and 2-naphthalenecarboxylic acid were also subjected to the HPLC separation under the same conditions. The wavelengths of maximum absorption and the retention data for all the compounds are given in Table I. A wavelength of 223 nm, where the compounds under investigation absorb UV radiation, was selected for detection. When the UV detector was set at 0.001 a.u.f.s., the limit of detection for 2-NAA in a large excess of 1-NAA was found to be $3 \cdot 10^{-9}$ g with a signal-to-noise ratio of 4.0. The response factors for 1-NAA and 2-NAA in the concentration ranges 90–100% and 1–10%, respectively, were determined and are given in Table II. They were found to remain constant throughout out these ranges.

Standard mixtures containing different amounts of 1-NAA and 2-NAA were prepared and analysed by HPLC. The results are given in Table III. The accuracy of the method was determined by the standard addition technique. Subsequent additions of 1-NAA and 2-NAA were accurately reflected in their peak heights. The measured amounts of 1-NAA and 2-NAA agreed well with the actual values, to within 1.59% and 2.34%, respectively. Linear regression analysis of the data with

TABLE II

RESPONSE FACTORS OF 1-NAA AND 2-NAA

Compound	Response factor[a] (mean, n = 10)	S.D.	R.S.D.[b] (%)
1-NAA	0.0865	0.0017	1.9653
2-NAA	0.0503	0.0014	2.7834

[a] With respect to 3-NBSS used as internal standard.

[b] Relative standard deviation.

TABLE III

ANALYTICAL DATA FOR STANDARD MIXTURES

Sample No.	1-NAA (%)		Error (%)	2-NAA (%)		Error (%)
	Taken	Found[a]		Taken	Found[a]	
1	90.18	90.64	+ 0.51	9.82	9.59	− 2.34
2	91.53	92.41	+ 0.96	8.47	8.63	+ 1.89
3	94.79	93.28	− 1.59	5.21	5.12	− 1.73
4	97.25	96.49	− 0.78	2.75	2.80	+ 1.82
5	98.97	99.32	+ 0.45	1.03	1.05	+ 1.94

[a] Average of three determinations.

x = percentage of the component taken and y = percentage of the component found yielded the equations $y = 0.9884x + 0.9387$ and $y = 0.9869x + 0.5361$ with correlation coefficients of 0.9359 and 0.9980 for 1-NAA and 2-NAA, respectively.

Reaction mixtures were collected during the course of condensation of naphthalene with MCA and analysed by the proposed method. A typical chromatogram of the reaction mixture is shown in Fig. 2. The unreacted naphthalene and MCA do not interfere as they elute at 18.44 and 3.26 min, respectively. These results show that the procedure is suitable not only for determining trace amounts of 2-NAA in 1-NAA but also for the separation and determination of several 1- and 2-substituted naphthalenes. The method is simple, rapid and convenient for the quality control naphthaleneacetic acids.

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