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Note

Separation of 1-naphthylamine from the five known impurities and sub-ppm level detection and quantitation of 2-naphthylamine by normal-phase high-performance liquid chromatography[a]

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The free base 1-naphthylamine and certain of its sulphonic acid derivatives serve as diazo and coupling components in the preparation of azo dyes, including some permitted synthetic food colours. Technical 1-naphthylamine may contain some 1,1'-dinaphthylamine, 1,5-diaminonaphthalene, 1- and 2-naphthols and invariably a little of the 2-isomer (about 0.5%, w/w) as impurities (Fig. 1).

2-Naphthylamine has long been recognized as a human carcinogen and as a toxic chemical. Some azo synthetic food colours were actually found to contain

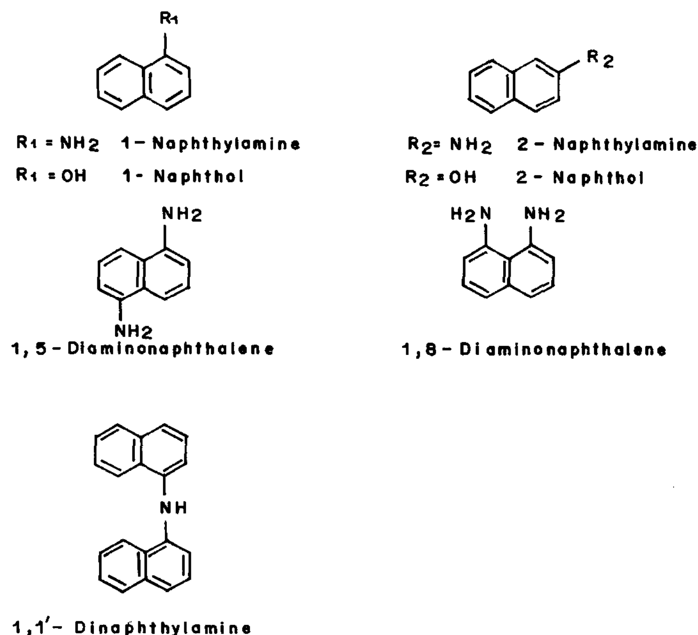

Fig. 1. Structures of 1-naphthylamine and the likely associated impurities.

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2-naphthylamine as an impurity[1,2], most likely a carry-over from the 1-naphthylamine intermediate used in their manufacture. Therefore, monitoring the 2-naphthylamine impurity in 1-naphthylamine should help to control the quality of such food colours.

High-performance liquid chromatography (HPLC), using a bonded strong cation exchanger[3], a bonded amino phase co-ordinated with Cu(II) as a ligand exchanger[4], a bonded β-cyclodextrin[5], a bonded octadecylsilane[6,7] and silica gel[8] as the stationary phases, has been applied for this isomer separation problem. Further, an open-tubular microcapillary column with β,β-oxydipropionitrile (BOP) as a stationary phase[9] and liquefied alkanes as the mobile phase resolved the isomers completely.

Unlike the previous attempt by Sliwiok and Szulik[8], we report in this paper an high resolution (R_s = 2.5) HPLC method using a silica gel column and 50% water-saturated diethyl ether–*n*-hexane (25:75) as the mobile phase with detection at 280 nm for the determination of underivatized 2-naphthylamine in technical 1-naphthylamine with sub-ppm as the lower detection limit in *n*-hexane solution. Moreover, the method simultaneously separated all the other four potential impurities mentioned earlier (Fig. 2).

EXPERIMENTAL

Liquid chromatography

The work was carried out on a Waters HPLC system (Millipore-Waters Chromatography Division, Milford, MA, U.S.A.) consisting of two Model 6000A dual-head reciprocating solvent-delivery systems controlled by a Model 660 solvent flow programmer, a Model U6K universal injector and a Model 440 dual-channel discrete multi-wavelength absorbance detector operating at 280 nm. The analogue output of the absorbance detector was recorded and processed with a Waters Model 730 data module (a printer, plotter and integrator).

A Waters μPorasil (irregular particles, 10 μm) stainless-steel pre-packed column (30 cm × 3.9 mm I.D.) was used.

Naphthylamine standards

A technical grade 1-naphthylamine sample was purified by repeated crystallizations from light petroleum (b.p. 60–80°C) in which 2-naphthylamine is more soluble. An old reagent grade 2-naphthylamine sample was similarly purified by recrystallizations from ethanol. The 1,5- and 1,8-diaminonaphthalenes ("Purum" grade) were obtained from Fluka (Buchs, Switzerland) and 1,1'-dinaphthylamine was prepared and purified according to a literature method[10].

Mobile phase

Diethyl ether and *n*-hexane were purified to HPLC quality in our laboratory.

A 50% water-saturated diethyl ether–*n*-hexane (25:75) mobile phase was prepared as directed in ref. 11 and was filtered through a 0.45-μm Millipore Fluoropore (PTFE) membrane filter (FHUP 047 00) using a Millipore all-glass filter apparatus (XX 15 047 00) before use.

About 50 ml of 50% water-saturated filtered mobile phase was passed through a Waters μPorasil column to ensure proper equilibration. The mobile phase flow-rate was 1.5 ml/min.

External standard calibration graph

Stock standard solution. About 10 mg of 2-naphthylamine were accurately weighed, dissolved and diluted to volume with *n*-hexane in a 100-ml volumetric flask. A 10.0-ml aliquot of this solution (A) was further diluted up to volume with *n*-hexane in a second 100-ml volumetric flask. This stock solution (B) corresponds to about 10 μg 2-naphthylamine per ml.

Working standard solutions. Aliquots of stock solution (B) ranging from 1 to 9 ml were diluted to volume with *n*-hexane in separate 10-ml volumetric flasks. An 100-μl volume from each working standard solution was injected into the liquid chromatograph to check the linearity of the detector (280 nm) response (peak area and height). This corresponds to *ca.* 100–900 ng 2-naphthylamine per injection.

Sample solution. About 100 mg of a technical sample were accurately weighed, dissolved and diluted to volume in a 100-ml volumetric flask and filtered through a 0.5-μm PTFE membrane filter. An 100-μl volume of this filtered solution was used for each injection.

RESULTS AND DISCUSSION

We were interested in developing a simple, sensitive, reliable and rapid HPLC method to determine 2-naphthylamine as an impurity in a 1-naphthylamine matrix without derivatization. The method was also expected simultaneously to separate other reported impurities such as 1,1′-dinaphthylamine, 1,5-diaminonaphthalene and 1- and 2-naphthols (Fig. 1). All of these compounds have a good solubility in *n*-hexane and other non-aqueous HPLC solvents and mainly differ in the position, number and nature (primary and secondary) of the nitrogen functionality. Therefore, HPLC in liquid–solid adsorption mode, using silica gel as a stationary phase, was expected to provide the selectivity necessary for the separation of these compounds. However, despite its potential, this HPLC mode has been used only sparingly in practice for this separation problem. Although Sliwiok and Szulik[8] achieved a partial resolution of the two naphthylamines on a silica gel column, they neither studied a simultaneous separation of other impurities nor attempted a determination of 2-naphthylamine in 1-naphthylamine.

Initially we performed some scouting experiments for the optimization of the mobile phase selectivity parameters, X_i, using a silica gel column. Thus, we blended *n*-hexane with modifiers like chloroform (proton donor activity, $X_d = 0.41$), dichloromethane (large dipole activity, $X_n = 0.53$) and diethyl ether (proton acceptor activity, $X_e = 0.53$)[12] to produce a series of binary and ternary mobile phases with different selectivities. A diethyl ether–*n*-hexane (20:75) (50% water-saturated) mixture offered one of the best selectivity factors ($\alpha = 1.29$) among various mobile phases tried (Table I). The use of 50% water-saturated mobile phase compositions helped to reduce tailing and offered better reproducibility of retention times. We also noted that, although acetonitrile–chloroform (10:90) offered the highest selectivity ($\alpha = 2.54$) for the separation of 1- and 2-naphthylamines, this mobile phase is not recommended for a routine use because such a mixture of two reactive organic solvents is known to form crystalline products on standing. A typical simultaneous separation of 1,1′-dinaphthylamine, 1- and 2-naphthols, 1- and 2-naphthylamines and 1,5-diaminonaphthalene is shown in Fig. 2 and the HPLC data for these compounds are given in Table II.

TABLE I

MOBILE PHASE SELECTIVITY FOR THE SEPARATION OF 1- AND 2-NAPHTHYLAMINES (NA)

Void volume time, t_0 = 3.15 min at the flow-rate 1.0 ml/min. All the mobile phases were 50% water-saturated.

Mobile phase No.	*Modifiers for n-hexane*	*Capacity factor, k'*		*Selectivity, α*
		1-NA	*2-NA*	
1	40% Diethyl ether	1.31	1.74	1.33
	20% Diethyl ether	2.49	3.21	1.29
2	60% Dichloromethane	1.35	1.54	1.14
3	20% Diethyl ether + 30% dichloromethane	1.09	1.28	1.17
4	45% Chloroform	1.10	1.35	1.22
5	22.5% Chloroform + 30% dichloromethane	1.27	1.53	1.20
6	35% Diethyl ether + 40% chloroform	0.67	0.85	1.27
7	10% Acetonitrile[a]	0.51	1.42	2.78

[a] In chloroform instead of *n*-hexane.

2-Naphthylamine is more basic and more polar than 1-naphthylamine (pK_a 4.14 and 3.92; dipole moment, in benzene at 25°C, 1.77 and 1.49 D, respectively). Further, the 2-isomer is the most linear of the two which means reduced steric hin-

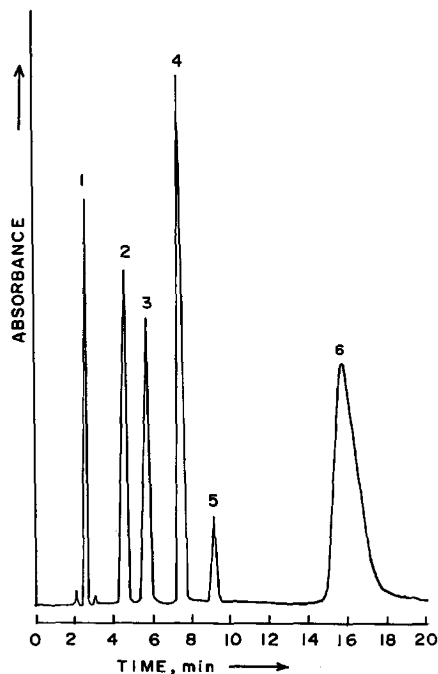

Fig. 2. Normal-phase HPLC separation of an artificial mixture of 1-naphthylamine and the five likely impurities. Column: Waters stainless-steel μPorasil (30 cm × 3.9 mm I.D.). Mobile phase: diethyl ether–*n*-hexane (25:75) (50% water-saturated); flow-rate 1.5 ml/min. UV detection: 280 nm. Peaks: 1 = 1,1'-dinaphthylamine; 2 = 1-naphthol; 3 = 2-naphthol; 4 = 1-naphthylamine; 5 = 2-naphthylamine; 6 = 1,5-diaminonaphthalene.

TABLE II

LIQUID–SOLID CHROMATOGRAPHY DATA FOR A SIMULTANEOUS SEPARATION OF AN ARTIFICIAL MIXTURE OF 1-NAPHTHYLAMINE AND THE POSSIBLE IMPURITIES

Void volume time, t_0 = 2.10 min at 1.46 ml/min.

No.	*Compound*	pK_a	*Retention time (min)*	*Capacity factor, k'*	*Selectivity, α*	*Resolution, R_s*
1	1,1'-Dinaphthylamine	<0.7	2.60	0.24		
					4.83	>5.00
2	1-Naphthol	9.30	4.54	1.16		
					1.50	1.86
3	2-Naphthol	9.57	5.75	1.74		
4	1-Naphthylamine	3.92	7.65	2.64		
					1.31	2.50
5	2-Naphthylamine	4.14	9.36	3.46		
6	1,5-Diaminonaphthalene	1.74	16.26	6.74		
		4.07			1.07	Poor
7	1,8-Diaminonaphthalene	4.29	17.22	7.19		

drance of the fused ring system towards the silica adsorbent. This enables 2-naphthylamine to interact more strongly with polar silanol groups on the surface of silica gel.

A strong and direct interaction of the polar primary amino functional group with a polar silanol adsorption site on the surface of silica (solute localization) demands the use of a modifier with a basic polar group such as diethyl ether (solvent strength parameter, ε^0 = 0.38; X_e = 0.53) which competes for a position directly over silanol by hydrogen bonding (solvent localization). The degree of solvent localization can be measured by a mobile phase parameter, *m*. Mobile phases containing alkyl ethers and acetonitrile are reported[13] to have large values of *m*. In the case of most liquid–solid chromatographic separations, localization effects in the stationary phase are of greater significance than interactions between solvent and solute molecules in the mobile phase. Thus, the use of a basic polar solvent such as diethyl ether yielded additional selectivity by solvent-specific localization effects leading to excellent resolution of 1- and 2-naphthylamine isomers in real samples too (Fig. 3).

External standard quantitation

A linear relationship was observed on plotting peak areas as well as peak heights against the corresponding injected mass (ng per 100 μl injected) at least up to 1000 ng 2-naphthylamine at the selected detection wavelength of 280 nm. This range covers the reported percentage range (up to 0.5%, w/w) of 2-naphthylamine in commercial 1-naphthylamine samples when the same volume of *ca.* 1 mg/ml sample solution is injected into the liquid chromatograph. We found that as the sample was dissolved in plain *n*-hexane, which is eluotropically much weaker than the mobile phase, there was no appreciable loss of resolution between the two naphthylamines at the sample loading used (100 μg in 100 μl). A regression analysis by the least-squares method (n = 8) for a plot of peak area (area units), y, or height (cm), y', against mass

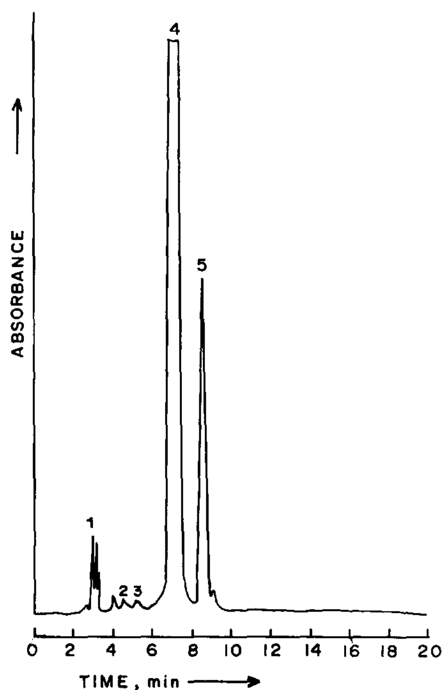

Fig. 3. HPLC chromatogram of a real technical sample of 1-naphthylamine dissolved in *n*-hexane. Details as in Fig. 2. 1,5-Diaminonaphthalene could not be detected in this particular sample.

injected (ng per 100 μl injected), x, for 2-naphthylamine yielded the following calibration equations with correlation coefficients, r, indicating excellent linearity:

Peak area: $y = 7259\,x + 23114$; $r = 0.99931$

Peak height: $y' = 0.015207\,x + 0.086667$; $r = 0.99925$

Using real samples of 1-naphthylamine, we studied the reproducibility of reten-

TABLE III

REPRODUCIBILITY OF RETENTION TIME, PEAK AREA AND PEAK HEIGHT OF 2-NAPHTHYLAMINE IN REAL SAMPLES OF 1-NAPHTHYLAMINE

Number of experiments for each sample, $n = 8$.

Sample	*Retention time*		*Peak area*		*Peak height*	
	Mean ± S.D. (min)	*R.S.D. (%)*	*Mean ± S.D. (area units)*	*R.S.D. (%)*	*Mean ± S.D. (cm)*	*R.S.D. (%)*
A	8.60 ± 0.042	0.49	9 925 519 ± 265 466	2.67	8.60 ± 0.042	0.49
B	8.58 ± 0.054	0.63	7 131 636 ± 148 399	2.08	6.39 ± 0.070	1.09

tion time, peak area and peak height for 2-naphthylamine (Table III). The reproducibility of peak heights was distinctly better than that of peak areas. Despite using a low-boiling mobile phase modifier like diethyl ether (b.p. 35°C), the reproducibility of retention time is good. Substitution of methyl *tert*-butyl ether (MTBE) (b.p. 55°C) for diethyl ether should improve the reproducibility further.

We have estimated that, under the chromatographic conditions employed, it is easy to detect at least 0.06 ppm (w/w) of 2-naphthylamine in *ca.* 1500 ppm (w/w) solution of 1-naphthylamine in *n*-hexane. This lowest detection limit corresponds to *ca.* 0.004% 2-naphthylamine in solid 1-naphthylamine samples and can be improved further by simply increasing the injection volume.

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