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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF NAPHTHOLS AS 4-AMINOANTIPYRINE DERIVATIVES

APPLICATION TO CARBARYL

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SUMMARY

The high-performance liquid chromatographic determination of 1- and 2-naphthol after derivatization with 4-aminoantipyrine is described. The chromatographic analysis of the reaction mixtures showed two main coloured products for each naphthol. In order to investigate the nature of the reaction products a spectroscopic study (UV–VIS, IR, NMR) of the products separated by chromatography was performed. On the more stable 1-naphthol reaction product the quantitative determination of 1-naphthol was performed in the 0.05–10 ppm concentration range. Instability of the 2-naphthol products made their quantitative determination difficult; detection at two different wavelengths (460 and 412 nm) gave a very reliable criterion of qualitative identification. As 1-naphthol is the major degradation product of the insecticide carbaryl, this determination reaction of 1-naphthol was applied to carbaryl. The applicability and performance of the method was checked by determining the carbaryl content in commercial powder formulations and residues in apples.

INTRODUCTION

The determination of monohydric phenols by high-performance liquid chromatography (HPLC) after derivatization with 4-aminoantipyrine (4-AAP) has been discussed previously[1]. The aim of this study was to examine the derivatization reaction and its analytical utility when the method is applied to 1-and 2-naphthol. The direct determination of naphthols by HPLC with UV detection has been reported[2–4], and the reaction of naphthols with 4-AAP is known[5–9]. As for monohydric phenols[1], the combination of the specificity of the derivatization reaction with the sensitivity and separation power of HPLC seemed to be promising.

1-Naphthol is a degradation product of carbaryl (1-naphthylmethylcarbamate); in fact, both the metabolic processes and alkaline hydrolysis yield 1-naphthol. Carbaryl is a broad-spectrum insecticide, extensively used because of its effectiveness and low mammalian toxicity. Carbaryl may contain 2-naphthylcarbamate as a contaminant, derived from impure 1-naphthol containing 2-naphthol[10]. The determination of trace amounts of 2-naphthol is very important, as it has been found to produce cancerous tumours in rats[11].

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Numerous HPLC methods have been developed for the analysis of carbaryl and 1-naphthol with either UV[12–14] or fluorescence detection[15–17]. In this work, the determination of 1-naphthol as the 4-AAP derivative was employed for carbaryl analysis. The applicability and performance of this method was checked by determining carbaryl in powder formulations and residues in apples.

EXPERIMENTAL

The analyses were performed with a Spectra-Physics SP 8700 solvent delivery system with an SP 8750 organizer module. A Model 770 spectrophotometric detector at wavelengths variable from 200 to 600 nm and an SP 4270 computing integrator were used. A μBondapak-phenyl column (300 × 3.9 mm I.D.; Waters Assoc.) with methanol–water (65:35) as the mobile phase was chosen for HPLC determinations. Unless otherwise specified, the experimental conditions and derivatization procedures were as previously described[1].

RESULTS AND DISCUSSION

Chromatographic study of reaction variables in derivatization

The reaction variables in the previously described procedure[1] were investigated in order to select the optimum conditions for the naphthol derivatization reaction. The pH range 8.5–10.5 was studied; 1-naphthol gave the maximum yield at pH 9.5 and 2-naphthol at pH 8.5, so a compromise value of 9 was chosen. The Britton–Robinson buffer was then replaced with $NaHCO_3$–Na_2CO_3 buffer because of the blank interference at the detection wavelength of the 2-naphthol derivative (412 nm). According to Svobodova and Gasparic[18], the buffer capacity and the reaction yield were the same with the carbonate buffer. A suitable proportion of 4-AAP to oxidizing agent seemed to be 1:3, as previously determined; a greater proportion of 4-AAP increased both the naphthol response and the blank interference.

Chromatographic study of the reaction mixtures

Fig. 1 illustrates the chromatographic analysis of the chloroform extracts from reaction mixtures and shows two derivative products for each naphthol under the chromatographic conditions reported above. Other reaction by-products were negligible. Table I reports the capacity factors, k', for naphthol derivatives.

In order to investigate the nature of these coloured products, the chloroform extracts were separated by thin-layer chromatography (TLC). Concentrated solutions containing 5 mg of each naphthol were examined. According to Aly[7], mixtures of methylene chloride and ethyl acetate and of chloroform and ethyl acetate were investigated on silica gel plates. Methylene chloride–ethyl acetate (70:30) gave the best separation of the reaction products. The 2-naphthol chloroform extracts were not sufficiently stable for TLC analysis. Therefore, only the 1-naphthol products were examined.

A red band with R_F 0.3 and an orange band with R_F 0.5 were evident. Their HPLC analysis confirmed the two chromatographic peaks with k' values of 1.96 and 2.59, respectively. The stability of 1-naphthol products was determined. The chromatographic response of the product at $k' = 2.59$ was stable for three days, whereas

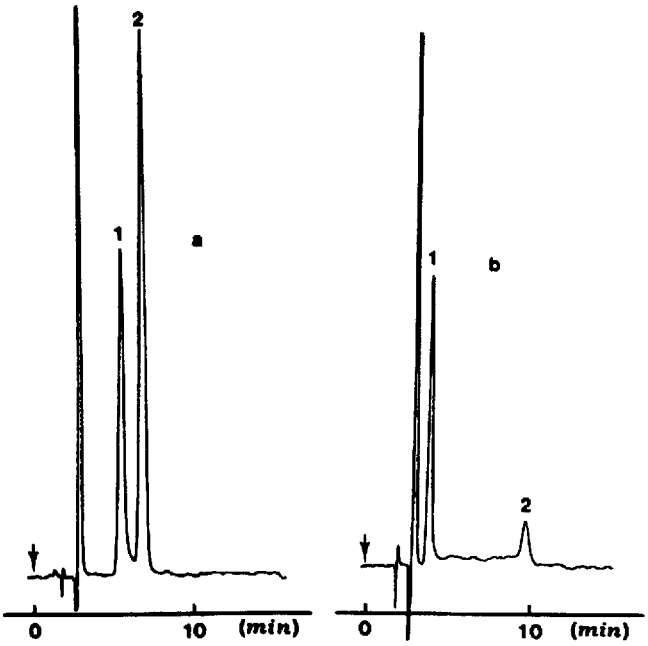

Fig. 1. Chromatographic analysis of (a) 1-naphthol and (b) 2-naphthol derivatives. Conditions as under Experimental; flow-rate, 1.5 ml/min; detector, visible, 460 nm, 1.6 × 0.04 a.u.f.s. Sample: (a) 1-naphthol, 3.30 ppm; (b) 2-naphthol, 10 ppm.

TABLE I

CAPACITY FACTORS (k') FOR NAPHTHOL DERIVATIVES

Compound	*Peak*	k'
1-Naphthol	First	1.96
	Second	2.59
2-Naphthol	First	1.09
	Second	4.06

at the same time that at $k' = 1.96$ was halved. Therefore, the coloured product at $k' = 2.59$ was selected for the quantitative determination of 1-naphthol by HPLC. However, the first peak area, was constant for 3 h after the reaction.

The low stability of the 2-naphthol derivatives allowed their identification but not an accurate quantitative determination by HPLC.

Spectroscopic study of the reaction products

The chloroform extracts from the naphthol reaction mixtures, red for 1-naphthol and faint green for 2-naphthol, showed maximum absorption at 460 and 412 nm, respectively. Previous spectrophotometric determinations[5–9] had been carried out at the single wavelength of 500 nm. UV–VIS spectra of both the 1- and 2-naphthol coloured products, separated by HPLC, were recorded.

The 1-naphthol derivative corresponding to the first chromatographic peak (k' = 1.96 and R_F = 0.3) showed maximum absorption at 515 nm, whereas the more stable derivative corresponding to the second peak (k' = 2.59 and R_F = 0.5) showed maximum absorption at 460 nm. For 2-naphthol derivatives, the first eluate (k' = 1.09) had a maximum at 460 nm and the second (k' = 4.06) at 412 nm. Therefore, a wavelength of 460 nm was selected for the chromatographic detection of naphthols.

After separation by TLC, the more stable 1-naphthol derivative (k' = 2.59) was investigated by IR and NMR spectroscopy. The IR spectrum of a chloroform solution showed only one significant band at 1660 cm^{-1} relating to the amide C=O group. A study of the NMR spectrum permitted the identification of the 4-AAP coupling product in the *para* position of 1-naphthol[19].

Detectability and linearity

Under the above chromatographic conditions, the detection limit at 460 nm, *i.e.*, the amount injected that gives a peak height equivalent to twice the noise level, was calculated to be 2 ng for 1-naphthol. The calibration graph of peak area *versus* concentration of standard samples was determined following the experimental procedure described previously[1]. The calibration graph was linear in the concentration range 0.05–10 ppm.

For 2-naphthol, the detection limit was calculated to be about 30 ng, while the low stability of its derivatives provided an inaccurate calibration graph. It was possible to detect the presence of 2-naphthol up to a 500:1 ratio between 1- and 2-naphthol. The chromatographic detection at two different wavelengths (460 and 412

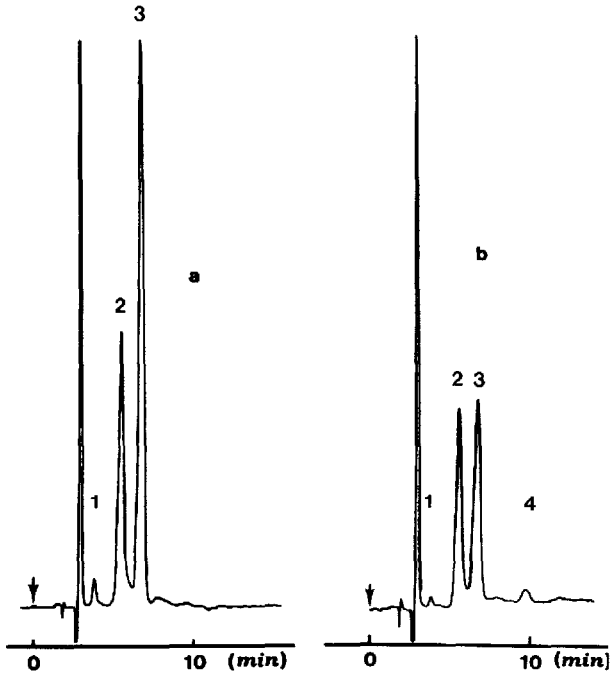

Fig. 2. Wavelength shifts for 4-aminoantipyrine naphthol derivatives. Conditions as in Fig. 1; (a) 460 nm, 1.6 × 0.04 a.u.f.s.; (b) 412 nm, 3.2 × 0.04 a.u.f.s. Sample: 1-naphthol, 3.9 ppm (peaks 2 and 3); 2-naphthol, 1.4 ppm (peaks 1 and 4).

nm) and the measurement of wavelength shifts further confirmed the presence of 2-naphthol. The bathochromic and hypsochromic shifts are shown in Fig. 2 for naphthol derivatives.

Carbaryl determination

A spectrophotometric method for carbaryl determination, based on the 4-AAP derivatization of 1-naphthol, has already been developed[9]. Here the same carbaryl hydrolysis conditions were applied. Complete hydrolysis was confirmed by HPLC. The detection limit was calculated to be 3 ng as carbaryl, consistent with the 1-naphthol detection limit. Following the derivatization procedure[1] and the separation conditions described above, carbaryl was determined in powder formulations and as residues in apples.

Formulation analysis. According to Appaiah *et al.*[9], 1–4 mg of powder formulation were dissolved in 10 ml of methanol, shaken and filtered. Hydrolysis, the derivatization reaction and chromatographic analysis were carried out as described previously. The features of the chromatograms, recorded at 460 and 412 nm, made it possible to exclude the presence of 2-naphthol in all of the formulations examined. An example is shown in Fig. 3.

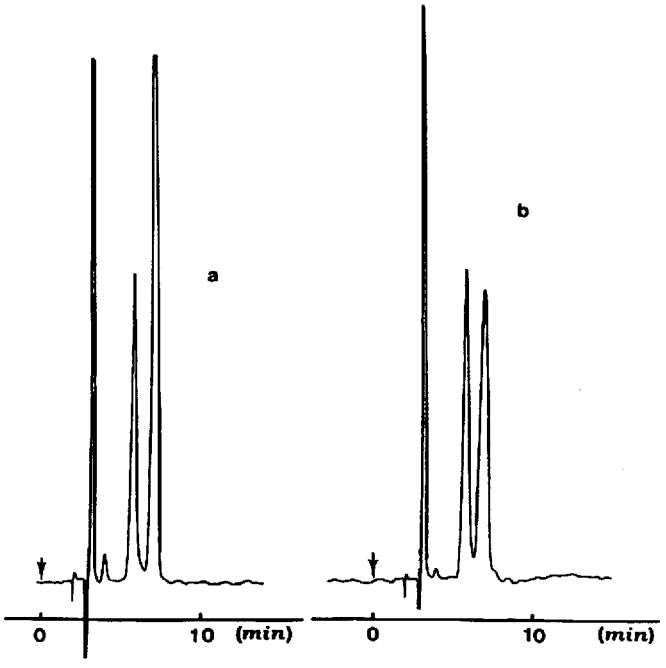

Fig. 3. Use of wavelength shifts of 2-naphthol derivatives for their identification in a carbaryl formulation. Conditions as in Fig. 1; (a) 460 nm, 1.6 × 0.04 a.u.f.s.; (b) 412 nm, 16 × 0.04 a.u.f.s.

Recovery of residues in apples. Apples (25 g), blended for 2 min, were extracted with two 50-ml portions of chloroform. The chloroform extracts, combined and filtered, were concentrated to 10 ml in a rotary vacuum evaporator. Chromatographic clean-up on a silica gel-Na_2SO_4 column[9] and on a C_{18} Sep-Pak cartridge[3,14] allowed

TABLE II

RECOVERY OF CARBARYL FROM APPLES

Amount added (ppm)	*Amount found* (ppm)*	*Difference (%)*
0.20	0.19	−5
0.50	0.52	+4
0.60	0.60	0
1.00	0.95	−5
2.00	2.03	+2

* Average of three analyses. Repeatability: ± 12–15%.

"clean" apple blank chromatograms to be obtained for different samples. Because it is rapid, clean-up on the cartridge was preferred.

Apple samples were spiked with known amounts of carbaryl (0.2–2 ppm) and then analysed. Recoveries varied from 95 to 104%, as reported in Table II. The detection limit (0.1 ppm) makes this method useful for the determination of carbaryl residues in apples.

CONCLUSIONS

The HPLC determination of 1-naphthol as 4-AAP derivative is a very selective and sensitive method. This procedure can be applied to the determination of carbaryl. Owing to its high selectivity and sensitivity, it is comparable to the other known methods. The low stability of 2-naphthol products permits only qualitative analysis. Wavelength shifts represent a reliable criterion for identification purposes.

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