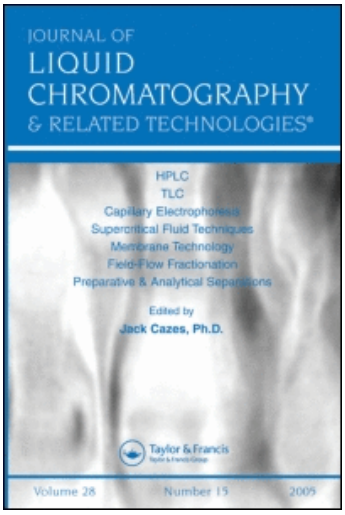

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THE PURIFICATION OF AMINO COUMARIN DYES BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A reliable method for the separation of amino coumarin dyes from associated impurities has been developed using reverse phase high performance liquid chromatography using an ODS-18 column and mixtures of methanol and water. Further, the relation between the retention times and structure of amino coumarines has also been studied.

INTRODUCTION

7-substituted amino Coumarins are known to lase (1,2) in the visible region of electromagnetic spectrum when their solutions are pumped by nitrogen laser at 337 nm. The lasing efficiency of these coumarin dyes depends upon the level of impurities (3). The presence of these impurities may quench the fluorescence, thereby affecting the laser output. Many conventional purification techniques like crystallisation, column chromatography (4), thin layer chromatography (5,6) are useful in the purification of these dyes, but they are time

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consuming and in some cases the purity we attain from these techniques is not sufficient for lasing purpose.

Reverse phase high performance liquid chromatography offers a simple and reliable method for the purification of these dyes and the purity we attain from this method is quite satisfactory for lasing purpose.

The following dyes are used in this study.

(1) $R = CH_3$; $R_1 = R_2 = H$; $R_3 = H$

(2) $R = CH_3$; $R_1 = R_2 = H$; $R_3 = CH_3$

(3) $R = CH_3$; $R_1 = H$; $R_2 = C_2H_5$; $R_3 = CH_3$

(4) $R = CH_3$; $R_1 = R_2 = C_2H_5$; $R_3 = H$

(5) $R = CH_3$; $R_1 = R_2 = C_3H_7$; $R_3 = H$

(6) $R = CH_3$; $R_1 = R_2 = C_4H_9$; $R_3 = H$

(7) $R = CF_3$; $R_1 = R_2 = C_2H_5$; $R_3 = H$

(8) $R = CF_3$; $R_1 = R_2 = C_3H_7$; $R_3 = H$

All the above dyes were prepared by Pechmann Condensation (7,8) between monohydric phenol and β-Keto ester.

Each dye was dissolved in methanol and introduced into a ODS - 18 column (25 cm x 4.6 mm I.D. particle size 10 μm) via a Rheodyne (loop volume 20 μl) injector. A mobile phase consisting of variable amounts of methanol and water were used at a flow rate of 2.0 ml/min. Other equipment for the study included two LDC pumps (constametric II G and Constametric III), a LDC gradient master, a LDC spectromonitor II and a LDC recorder.

RESULTS AND DISCUSSION

All the coumarin dyes listed above were monitored for their purity using HPLC. Excellent separation was obtained

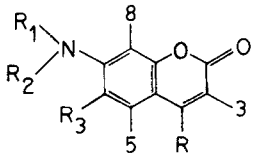

FIGURE 1
The basic structure of amino coumarin and all dyes that are related by structure. Each available position is numbered.

by varying the methanol concentration in the methanol-water elution solution for each dye. The range of methanol concentration varied from a minimum of 51% methanol used for 7-amino-4-methyl coumarin to a maximum of 80% used for 7-diethyl amino -4-methyl coumarin.

Fig (2) shows the HPLC traces of coumarin (3) and the impurity present monitored at five different wavelengths 230 nm, 254 nm, 281 nm, 315 nm and 367 nm. The absorbance maximum (λ max) for 7-ethylamino - 4, 6 - dimethyl coumarin is 367 nm. If the impurity is assumed to have the same λ max and extinction coefficient (ε), the sample is shown to be no more than 93% pure. This is an invalid assumption as evidenced by the variation in peak areas for the impurity observed at the other wavelengths. Therefore, the sample may be much less pure than 93%, but in the absence of isolation and characterization of the λ max and ε for the impurity, no more precise evaluation is possible. Corresponding data for the other coumarin dyes was also obtained.

Table 1 lists the dyes, the k^1 values. λ max, the optimum solvent system, and maximum purity of each dye based on the assumption of equal absorbance for each component at that wavelength.

With this knowledge, the necessity for further purification was obvious. Using optimum separation conditions, the presence

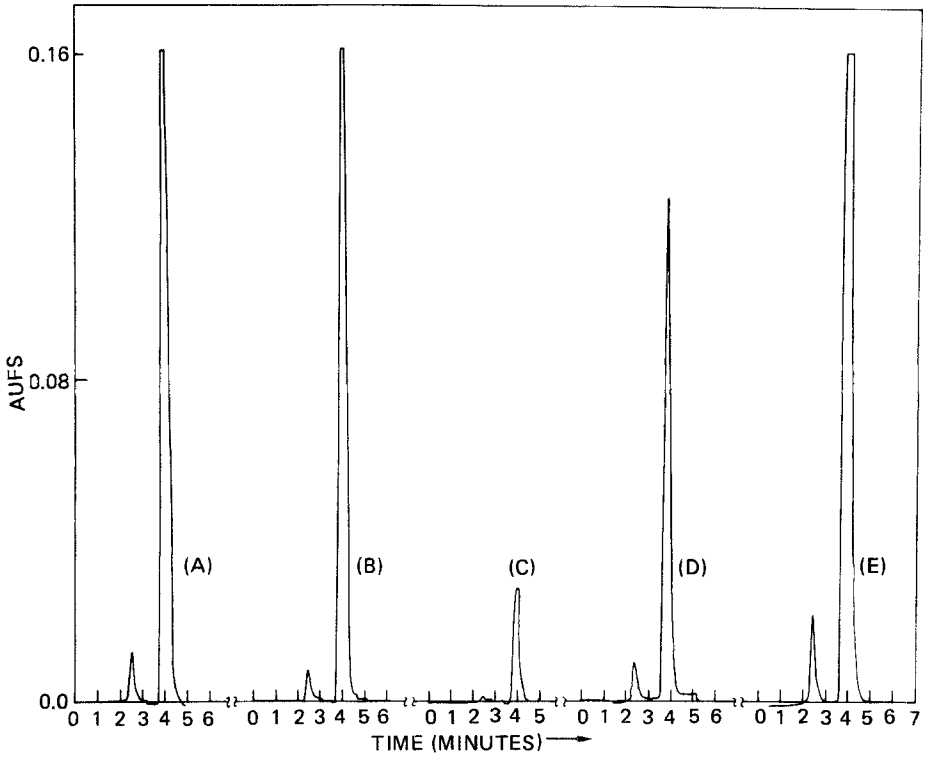

FIGURE 2
7-ethylamino-4,6 dimethyl coumarin chromatographed on reverse phase and detected at five specific wavelengths. Curve A, 230nm; curve B, 254nm; curve C, 281nm; curve D, 315nm; curve E, 367nm.

of each dye, its purity, and some insight concerning the necessary scale-up for preparative HPLC, may be obtained.

Relative Retention Times:

Values of t_R usually vary in a regular and predictable manner with repeated substitution of some group i into a sample molecule (e.g. as in the series of homologs, benzologs etc.). Often some function of t_R or k' will be linear with n, the number of repeating group i within the sample molecule (e.g. -

Table I

Coumarin	Solvent[1] composition	k^1	λ max(nm)[2]	Maximum purity (%)
Coumarin (1)	51/49	1.61	350	99
Coumarin (2)	51/49	2.78	350	99
Coumarin (3)	70/30	1.70	367	93
Coumarin (4)	80/20	1.22	379	98
Coumarin (5)	80/20	1.82	380	95
Coumarin (6)	80/20	3.48	380	90
Coumarin (7)	80/20	3.00	408	99
Coumarin (8)	80/20	2.00	408	99

1 Ratio of methanol to water in the solvent system

2 The λ max was determined spectrophotometrically in the corresponding solvent system.

CH_2 groups for a homologous series). For isocratic elution, a general relationship that is often obeyed in such cases is the Martin rule (9).

$$\log k^1 = A + Bn$$

Here A and B are constants for a given sample series and a specific LC system (same column, mobile phase and other conditions).

In all the derivatives of 7-amino-4-methyl coumarins, the number of carbon atoms(n)attached to the nitrogen are varying. The validity of the above equation to the derivatives of 7-amino -4-methyl coumarins was tested by plotting $\log k^1$ against the number of carbon atoms (Fig. 3). In the above graph, the number of carbon atoms attached to nitrogen in (1), (4), (5), & (6) are 0, 4, 6 and 8 respectively. The following equation was obtained between $\log k^1$ and n using the least square fit.

$$\log k^1 = - 0.36 + 0.185\ n$$

Table II

Dye	k
Coumarin (1)	0.39
Coumarin (2)	0.65
Coumarin (3)	1.70
Coumarin (4)	3.0
Coumarin (5)	5.52
Coumarin (6)	11.78
Coumarin (7)	10.87
Coumarin (8)	5.70

[1] The k^1 for each compound was calculated in a solvent system consisting of 70% methanol and 30% water.

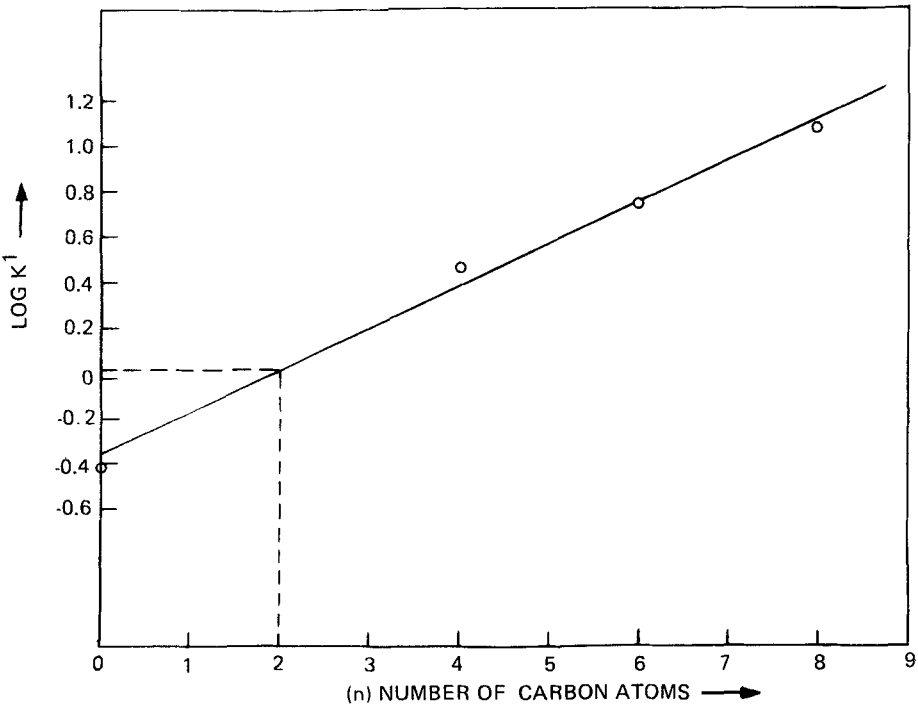

FIGURE 3
Plot between log k' and (n) the number of carbon atoms

Effect of $-CH_3$ group on k^1:

The introduction of given functional group i into the sample molecule will change k^1 for that compound by some constant factor α_i, in a given LC system. Between the coumarins (1) and (2), the latter contains a $-CH_3$ attached to the sixth position of the coumarin ring. The α_i value for the $-CH_3$ group was determined from the k' values of coumarins (1) and (2): $\alpha_i = 0.65/0.39 = 1.66$.

From the graph, for a compound containing two carbons attached to nitrogen the log k^1 value is 0.01. By taking into consideration α_i of $-CH_3$ group and log k' of the above compound, the log k^1 of coumarin (3) was calculated. The calculated log k^1 value of coumarin (3) was 0.23. The experimentally observed log k^1 value of coumarin (3) was 0.23. The observed and calculated values are in very good agreement.

In the case of 4-trifluoromethyl coumarins, 7-dipropylamino-4-trifluoromethyl coumarin was eluted earlier than the corresponding diethyl derivative. In the reverse phase BPC separations the more polar compound will elute earlier than the less polar one. The k^1 values indicate that the dipropylamino compound is more polar than the corresponding diethyl-amino compound.

CONCLUSION

A method for separation, purification, and quality control of coumarin dyes has been reported. This PHLC technique proved to be a simple, accurate and rapid method of monitoring the amino coumarins. The relation between the retention times and structure of amino coumarins was studied.

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