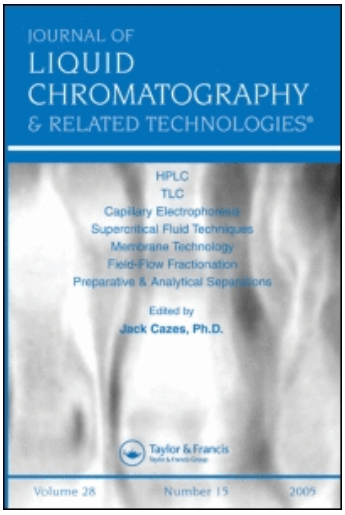

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THE ANALYSIS OF SOME SULPHONATED AZO DYES BY MIXED MODE HPLC

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ABSTRACT

A mixed mode (anion exchange/reverse phase) column for the analysis of some commercial sulphonated azo dyes was investigated as an alternative to simple reverse phase columns which require the use of either somewhat aggressive acidic elution solvents or the inclusion of ion pair reagents. Good separations of structural isomers were obtained using isocratic elution with solvents containing acetonitrile and near neutral phosphate buffers. A pattern of pH dependence for the retention times distinguished dyes according to the number of sulphonate groups present in their structure. Low levels of impurities in the commercial dyes could be observed by using a methanol/water solvent which did not elute the principal component.

INTRODUCTION

During investigations on the binding of acid azo dyes to wool it became necessary to establish that the commercial products being used consisted of a single organic colorant. Analysis by HPLC appeared to be the method of choice since the technique had the potential for high resolution and enabled quantitative measurements

through the use of modern electronic data acquisition systems. However, acid azo textile dyes contain one or more sulphonate groups in their structure which renders them highly polar so that the use of reverse phase column media and aqueous/organic eluents results in short retention times, particularly for the more highly sulphonated species, and poor resolution of structural isomers. The few recent papers concerned with the analysis of acid azo dyes by HPLC using reverse phase column media have described methods involving gradient elution with solvents which were either somewhat aggressively acidic (1) or which incorporated ion pair reagents (2,3).

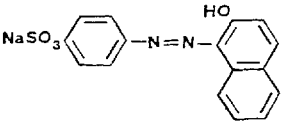
Multifunctional HPLC column packings have recently become available in which the supports, referred to as mixed mode, consist of a silica substrate bonded with both anionic (amine) as well as conventional reverse phase (C4-18) functionalities. These have been designed specifically for dealing with hydrophobic as well as ionic species and difficult applications such as the analysis of nucleotides, which have previously required special conditions such as ion pair reagents, can be developed by controlling the state of column media ionisation via eluent pH along with buffer strength and organic modifier composition. This paper describes the application of mixed mode HPLC to the analysis of some acid azo dyes.

MATERIALS AND METHODS

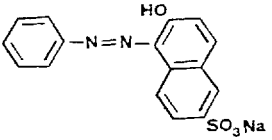
The dyes were obtained from Aldrich Chemical Co. Inc., and used as received. Their structures are shown in Table 1. The solvents were BDH HyperSolv HPLC grade. Aqueous buffers consisted of solutions of potassium dihydrogen phosphate adjusted to within 0.01 of the required pH with dilute potassium hydroxide using an Orion SA 720 pH meter and a BDH pH 7.00 reference buffer.

TABLE 1
Structure of the Dyes Used in this Study

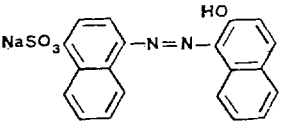
1. C.I. 15510
Acid Orange 7
(Orange II)



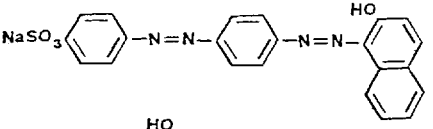
2. C.I. 15970
Acid Orange 12
(Crocein Orange G)



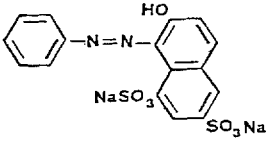
3. C.I. 15620
Acid Red 88



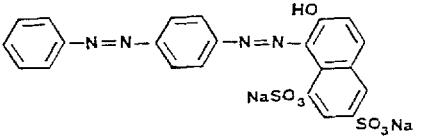
4. C.I 26900
Acid Red 151



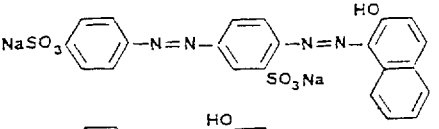
5. C.I. 16230
Acid Orange 10
(Orange G)



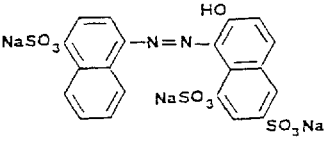
6. C.I. 27290
Acid Red 73
(Brilliant Crocein MOO)



7. C.I. 26905
Acid Red 66
(Biebrich Scarlet)



8. C.I. 16255
Acid Orange 18
(New Coccine)



HPLC was conducted with a Waters 500 pump with Valco C6W injection valve, a Waters 441 detector with 254 nm filter and a Shimadzu C-R3A Chromatopak recording integrator. The column was an Alltech (Alltech Associates,Inc., IL 60015) Mixed Mode RP-C8/Anion 150 x 4.6 mm stainless steel cartridge. All elutions were isocratic. Solvents A1, A2 and A3 consisted of 70:30 v/v acetonitrile: 0.067 M phosphate buffer at pH values of 6.7, 6.95 and 7.2 respectively. Solvents B1 and B2 consisted of 75:25 v/v acetonitrile: 0.05 M phosphate buffer at pH values of 6.6 and 6.8 respectively. Each of these five eluents were used at 2ml/min for the analysis of each dye individually and in admixture. Dye solutions were prepared in 50% methanol at concentrations such that a 5 µl injection contained either 2.5 µg of an individual product or 2.5 µg of each in admixture. The detector and integrator sensitivities were set to 1.

For the analysis of minor impurities detection sensitivity was increased by a factor of 10 and the column overloaded with the principle component by an injection of 100 µg of each product in 10 µl . The eluent used was 90:10 methanol:water at 1 ml/min. This retained the principal component indefinitely on the column and after the observation of the minor components the dyes were eluted and the column restored using solvent A3.

RESULTS AND DISCUSSION

Representative retention times observed with the A and B series of solvents are shown in Table 2. In all cases the commercial products appeared to consist largely of a single organic colorant except for dye 3 which under all conditions gave two peaks whose integrated areas were in the approximate ratio of 35:65.

The multifunctional character of the mixed mode column was clearly evident from the changes observed in

TABLE 2
Retention Times of Dyes [min]

Eluent: pH	A1 6.7	A2 6.95	A3 7.2	B1 6.6	B2 6.8
Dye					
1.	4.8	3.5	3.0	3.95	3.15
2.	6.1	4.3	3.6	4.85	3.8
3 (i).	12.9	8.5	6.7	9.4	7.0
(ii).	14.4	9.4	7.3	10.4	7.7
4.	10.9	7.5	6.3	7.7	6.0
5.	8.1	4.3	3.0	6.7	4.4
6.	21.4	10.1	6.5	15.9	9.6
7.	43	20	11.2	28.9	17.3
8.	60	20	8.9	47.5	22.7

response to differences in pH and the proportion of organic modifier in the eluting solvent.

Effect of pH

For any given dye the retention times with either the A or B solvents decreased as the pH was increased due to the anion exchange character of the column. For the particular group of dyes investigated the pH sensitivity was quite marked and increased with the number of sulphonate groups present and this appeared to be a useful diagnostic characteristic of the level of substitution. For example with the A series of solvents a change in pH from 6.7 to 7.2 resulted in decreases in retention time for the four monosulphonated dyes of between 51% and 63% The decreases for the disulphonated dyes were between 26% and 37% while for the trisulphonate it was 15% In other words for a pH increase 0.5 the retention time approximately halved for the monosulphonated dyes and halved again with each

additional sulphonate group. A change in pH from 6.7 to 6.95 gave smaller percentage reductions in the retention times but again the more highly substituted dyes showed the greatest proportional change. This pattern of behaviour was also observed with the B solvents at pH 6.6 and 6.8. The increase in pH of 0.2 reduced the retention times for the monosulphonated dyes 74 - 80%, for the disulphonated dyes 60 - 66% and with the trisulphonated dye the reduction was 48%

A mixture of all eight dyes could be separated with solvent A1 (pH 6.7) but dyes 7 and 8 had inconveniently long retention times and gave broad tailing peaks. At pH 6.95 their retention times were reduced but they coeluted as did dyes 5 and 2. At pH 7.2 dye 8 eluted before dye 7 due to its relatively greater pH sensitivity as noted above but dye 5 was not resolved from dye 1 for the same reason.

Effect of Solvent Composition

It was not possible to increase the organic component of the solvent and retain the same buffer concentration since phase separation occurred. For this reason the B series of solvents with a higher acetonitrile composition (75% c.f. 70%) also had a reduced buffer concentration (0.05M c.f. 0.067M). The effect of these changes was an increased speed of elution such that solvent B2 (pH 6.8) gave retention times roughly similar to those observed with solvent A2 (pH 6.95) even though within any one series an increase in pH gave a significant decrease in times. Solvent B2 gave a good separation of all eight dyes. Fig.1 shows a typical chromatogram under these conditions.

Effect of Dye Structure

Dyes 1 and 2 eluted early but were always baseline resolved. These structural isomers differed only in the position of the sulphonate substituent. The separation

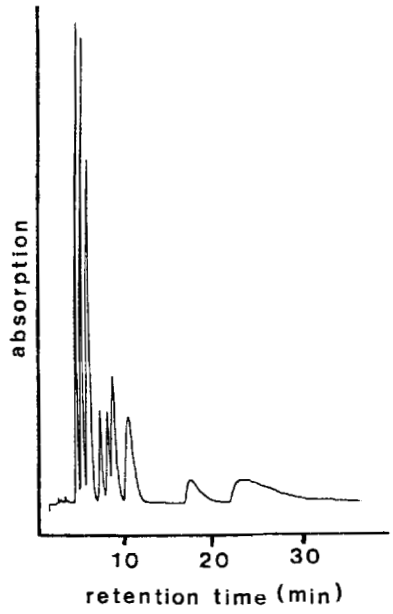


FIGURE 1. Chromatogram of the dye mixture on the 150 mm mixed mode (RP C8/anion) column with eluent B2. Flow rate: 2 ml/min. Sequence of dyes eluted: 1, 2, 5, 4, 3 (two component peaks), 6, 7, 8.

obtained in the present work was in contrast to a previously reported HPLC analysis (1) of these dyes on a conventional reverse phase column using gradient elution and acidic (pH 2.8 - 3.5) solvent mixtures which failed to resolve them even with a 250 mm column.

The components of dye 3 were retarded relative to dyes 1 and 2 under all conditions which was consistent with the greater hydrophobic character of the reported structure shown in Table 1. The two constituents were never fully resolved and this behaviour, including the pH dependence of the retention times, when compared with that of all the other dyes investigated, indicated strongly that they both had only one sulphonate

substituent and were of very similar hydrophobic character. They may therefore represent two structural isomers arising from the azo coupling of the dye precursors at different positions. Dye 4 eluted after dyes 1 and 2 which was consistent with its greater hydrophobic character, but it also always eluted before dye 3.

Dye 5 eluted after dyes 1 and 2 except at pH values greater than 6.8. It differed from these structures in having an extra sulphonate group and the anion exchange character of the column combined with the greater pH sensitivity of the disulphonates explained this aspect of its behaviour. Dyes 6 and 7 were similarly retarded relative to the monosulphonated dyes. These were also isomers which differed only in the position of the sulphonate substituents, but were well separated under nearly all conditions. They eluted more slowly than dye 5 at any particular pH which was consistent with their greater hydrophobic character and emphasised the utility of the multifunctional mixed mode column for this group of analytes. Dye 8, the only trisulphonated species investigated, eluted more slowly than the disulphonates at pH values of 6.8 or less, again in response to the ion exchange mode of the column.

Dye Impurities

All the dyes contained minor constituents but in the present study only those eluted by 90% methanol within 30 minutes of injection were observed. This did not preclude the presence of others since under the conditions used the dyes themselves, and therefore any similar constituents, were retained on the column. Therefore, the peaks observed were probably due to non-acidic substances such as dye precursors retained in the product during manufacture. Since these could well vary from batch to batch those observed in this study were not necessarily characteristic the dye types and may only

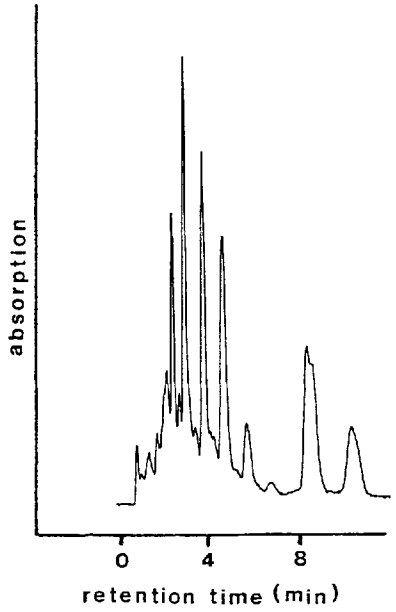


FIGURE 2. Chromatogram of impurities in dye 2 eluted with 90% methanol. Flow rate: 1 ml/min. Detection sensitivity 400 times that shown in Figure 1.

reflect the particular samples purchased. The total integrated area of the minor component peaks relative to that given by a known amount of dye enabled an estimate of the level of the observed impurities. The number and approximate amounts of observed impurities for each dye were as follows:- Dye 1 (3, 0.3%); Dye 2 (14, 1.1%); Dye 3 (10, 0.6%); Dye 4 (23, 5.5%); Dye 5 (16, 0.06%); Dye 6 (13, 1.8%); Dye 7 (8, 0.2%); Dye 8 (6, 0.04%). A typical chromatogram of the impurities in dye 2 is shown in Fig.2. The amount observed in most cases was probably insufficient to be of much significance in textile dyeing, but at least one product (dye 1) has been reported as a cosmetic colorant (2) and some of

these as well as food colorants are sulphonated azo dyes with structures similar to those investigated in the present study. The analysis of cosmetic and food dyes has been an important area of concern to public health authorities seeking to establish the nature of such additives and their minor constituents (2,3). Therefore, the results reported in the present paper form the basis for additional analytical methods in such applications.

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