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Application of electrospray mass spectrometry in the detection and determination of Remazol textile dyes

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Abstract

The electrospray (ES) behaviour of selected Remazol textile dyes, their hydrolysis products and the latters' reaction, following elution from a strong anion-exchange cartridge, with 30% concentrated HCl in MeOH, is studied and applied to the direct analysis of dye containing effluent. For unambiguous identification and determination of these textile dyes in effluents, it is necessary to resort to ES utilising MS–MS and MS^3. Further, a tabular review of recent applications of HPLC–ES-MS and, to a lesser extent, CE–ES-MS with reference to drug and pesticide analysis is presented.

Keywords: Mass spectrometry; Electrospray ionization; Remazol dyes; Dyes

1. Introduction

Electrospray mass spectrometry (ES-MS) was introduced by Yamashita and Fenn [1] in 1984 and has made a significant commercial impact in the last decade [2]. The mechanism of the transformation of ions in solution to ions in the gas phase prior to their mass analysis in a mass spectrometer has been reviewed by, among others, Kebarle and Tang [3], Briuns [4] and Gaskell [5] with Cole [6] recently editing a book on the fundamentals, instrumentation and applications of ES-MS. Along with matrix-assisted laser desorption ionisation (MALDI), it has widened the scope of MS analysis of high-molecular-mass compounds such as proteins, nucleotides and synthetic polymers by virtue of the detailed information regarding molecular mass and structure from extremely small quantities of material that can be provided. ES-MS relies, in these cases, on the production of multiply charged ions whose m/z values can be analysed on virtually all types of mass spectrometer. Low-molecular-mass molecules such as drugs, dyes and pesticides have also been subject to investigation by ES-MS. Use of the BIDS ISI database reveals 26, 12 and 14 publications, respectively, in the period 1993–1998 for these classes of compounds. Detailed structural information on molecules of a wide range of molecular mass can be obtained by resort to cone voltage fragmentation with a single MS instrument, collisionally induced dissociation (CID) with triple quadrupole MS instruments, and MS^n techniques using quadrupole ion-trap instrumentation [6]. ES has become one of the most important ionisation techniques for the on-line coupling of liquid phase separation techniques such as high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) with mass spectrometry (MS). Perusal of the aforementioned database publications shows how HPLC–ES-MS and, to a lesser extent, CE–ES-MS, is being used for the

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Table 1
Recent applications of HPLC–ES-MS and CE–ES-MS in drug analysis

Sample	Analyte(s)	Analytical technique	MS information	Analytical information	Reference
Standard solutions	β-Lactam antibiotics	HPLC–ES-MS	$[M-H]^-$ and $[M+H]^+$ used	LOD 100 ppb	[7]
Milk	β-Lactam antiobiotic residues	HPLC–ES-MS		LOD 10 ppb	[8]
Human plasma	Iloperidone and its principal metabolite	HPLC–ES-MS	Selected ion monitoring (SIM)	SPE used. LOQ 250 pg ml^{-1} (for both) linear calibration in range 250 pg to 20 ng ml^{-1} recoveries 73–101% (for both) intra day precision up to 12.5% (for both)	[9]
Formulations	Over-the-counter cough/cold actives	HPLC–ES-MS			[10]
Standard solutions	13 Drugs of forensic significance	Capillary zone electrophoresis ES-MS			[11]
Fermentation broths	Antibiotics	HPLC–ES-MS			[12]
Standard solutions	17β Fatty acid esters of testosterone	Direct infusion ES-MS	$[M+H]^+$; fragmentation in source or in collision cell of triple quadrupole		[13]
Blood plasma	Girard hydrazone derivatives of testosterone enanthate and undecanoate	HPLC–ES-MS	M^+; source fragmentation gave major ions at M-59, M-87; these three ions were monitored using SIR		
Cough syrup	Basic drugs	CE–ES-MS	$[M+H]^+$ used; negative ion ES-MS used for alkyl sulphonates and food dyes		[14]
Adulterated traditional Chinese medicine	Synthetic chemical drugs	HPLC–ES-MS	Molecular ions and specific fragment ions from in source CID used to detect adulterants		[15]
Mammalian cell extracts	Adenine nucleotide-containing metabolites of bisphosphonate drugs	Ion pair HPLC ES-MS	Negative ion ES-MS; Tandem MS and CID were used for identification of metabolites		[16]
Blood	Immunosuppressants	HPLC–ES-MS		On-line SPE used prior to HPLC–ES-MS LOD 0.05 μg l^{-1} for both drugs	[17]

identification and determination of organic analytes such as drugs (Table 1) and pesticides (Table 2).

This paper will present results of our recent study on the ES-MS, ES-MS–MS and ES-MS^n behaviour of selected Remazol textile dyes and their hydrolysis products and its application to the detection and determination of Remazol textile dyes with particular reference to effluent samples.

2. Experimental

2.1. Apparatus

Direct infusion ES-MS was carried out for the Remazol dyes with a Finnigan MAT (CA, USA) LCQ quadrupole ion trap mass spectrometer. The mass range of the detector was m/z 50–2000. For direct injection/infusion ES-MS the interface to the mass spectrometer was operated with the heated capillary at a temperature of 220°C. The sheath and auxiliary gas flows [both nitrogen gas delivered from a Whatman nitrogen generator (Whatman, Haverhill, MA, USA)] were set at 50 and 0 arbitrary units, respectively. The source voltage and current were 5 kV and 100 μA.

Liquid chromatography in the dye studies was carried out with a ThermoQuest (Hemel Hempstead, UK) HPLC which consisted of a Spectra System P4000 pump, vacuum degassing with the SCM 1000, a Spectra System AS3000 autosampler, a 20 μl injection loop, a Spectra System UV2000 detector connected to a SN4000 signal processor and the PC1000 software system. The separation column used was the Galaxy Luna series 5 μm C_{18} which was 150×4.6 mm I.D. and supplied by Phenomenex

Table 2
Recent applications of HPLC–ES-MS and CE–ES-MS in pesticide analysis

Sample	Analyte(s)	Analytical technique	MS information	Analytical information	Ref.
Water	Organophosphorus pesticides	HPLC–ES-MS	SIM using $[M+Na]^+$	SPE used prior to HPLC–ES-MS, LOD 0.01 μg l^{-1}	[18]
Water	Acidic herbicides (e.g. 2,4-D, bentazones, MCPA, MCPP, MCPB etc.)	HPLC–ES-MS	SIM using $[M-H]^-$	SPE used prior to HPLC–ES-MS; LOD 0.01–0.03 μg l^{-1}	[19]
Water	Selected herbicides	HPLC–ES-MS	$[M+Na]^+$ and $[M+H]^+$ ions used	Liquid–liquid extraction used with dichloromethane prior to HPLC–ES-MS	[20]
Formulations	Quaternary ammonium herbicides	CE–ES-MS			[21]
Fruit+vegetables	Carbamates	HPLC–ES-MS		SPE used after homogenisation and extraction; overall recovery >80%; LODs of the order of several hundred pg per g of sample	[22]
Water	Polar pesticides (phenmediphan, ethofumesate and fenamiphos)	HPLC–ES-MS	SIM	SPE used prior to HPLC–ES-MS; LOD 10–20 pg	[23]
Water	Atrazine and its six major degradation products	HPLC–ES-MS		SPE used prior to HPLC–ES-MS; recoveries >80%; day-to-day precision 2.3–7.7% at 3 ng l^{-1} using two ion SIM	[24]
Water	Poly(ethylene glycols) and related mono- and dicarboxylated forms	HPLC–ES-MS	SIM	SPE used prior to HPLC–ES-MS; for molecules with ethoxy chain lengths ⩾4, analyte recoveries better than 83%; limit of quantitation 0.1–0.3 ng l^{-1}	[25]
Water	Imidazolinone herbicides	HPLC–ES-MS		SPE used prior to HPLC–ES-MS; linear calibration for 1–50 ng injected	[26]
Ground water	Acidic herbicides (phenoxy acids, sulphonylureas, phenols etc.)	HPLC–ES-MS	Negative ionisation ES-MS used. MS–MS also used	LODs for MS with SIM 1 μg l^{-1}; LODs for MS–MS three–four times higher	[27]
	Triazines	CE and reversed electroosmotic flow CE–ES-MS with cetyltrimethylammonium bromide		Reversed electroosmoticflow CE gave better separation	[28]
Water	Arylphenoxypropionic herbicides in water	HPLC–ES-MS		SPE used prior to HPLC–ES-MS; linear calibration for 1–200 ng injected; LOD 3–10 ng l^{-1}	[29]

(Macclesfield, UK). The same column protected with a 30-mm guard column of similar characteristics was used in the interfacing of the HPLC system with the LCQ system for the detection and determination of the dye compounds using MS–MS and MS^n techniques.

The mobile phase used for the HPLC–UV–Vis dye studies was 1% phosphoric acid–acetonitrile (80:20, v/v) at 0.75 ml min^{-1} flow-rate. Ten-microlitre samples were injected and 15 min running time allowed per sample. Detection was carried out at 500 and 600 nm.

For MS^n characterisation of the four Remazol dyes direct infusion via a syringe pump located on the mass spectrometer was used as the injection method. In all cases solid-phase extraction (SPE) processed dyes were investigated, the injected concentration being 5.0 ppm. The LCQ parameters were similar to those used for direct infusion ES-MS as above the flow-rate being 50 μl min^{-1} and the spray voltage 4.25 kV. After a suitable parent ion had been identified at the MS stage, its fragmentation was carried out. For each stage of fragmentation the major, or most persistent peak from the previous stage was chosen as the 'parent' and is underlined in Table 3. Collision energy was optimised for each stage of fragmentation using the LCQ software.

The HPLC equipment was used as an injection system for the MS–MS and MS^n detection and determination of the dyes. Injections of 20 μl were made of each standard into a mobile phase consisting of acetonitrile–0.01 g/100 ml ammonium acetate in water (90:10, v/v). In order to make the flow-rate 0.3 ml min^{-1}, the sheath and auxiliary gas flow-rates were raised to 65 and 30 units respectively, arbitrarily set by the software. No attempt was made to optimise the HPLC separation of the dyes since they co-eluted with the solvent front and were resolved by

Table 3
ES-MS^n data for Remazol dyes by syringe infusion with optimised collision energy values for each stage of fragmentation

Dye	MS peak	MS–MS		MS^3		MS^4		MS^5	
		Peak	CE	Peak	CE	Peak	CE	Peak	CE
Blue	801.9	738.1	69.9%	525.1	78.0%	468.1	66.0%	ND[a]	ND
				478.2		317.4			
				468.3					
				393.1					
				277.9					
Black	742.1	678.1	48.0%	465.06	40.0%	409.1	48.0%	299.1	ND
	528.5	542.0		50.1		408.1		364.1	
	397.5			586.1		436.1		344.1	
	227.2			570.2				316.1	
Red	399.5	368.4	20.0%	314.03	20.0%	259.9	20.0%	ND	ND
(using peak at m/z 399.5)	530.0	377.6		36.5		282.3			
	344.4	354.8				228.6			
	283.4	556.9				304.6			
	198.6								
Red	530.0	317.1	34.0%	253.1	22.0%	225.1	22.0%	197.1	20.0%
(using peak at	399.5	249.2		225.1		197.1			
m/z 530.0)	344.4	342.2				189.2			
	283.4								
	198.6								
Yellow	441.5	399.1	36.0%	290.1	36.0%	261.9	36.0%	ND	ND
	399.6	369.1		382.2		274.1			
	497.1	304.1		271.5		161.9			
	538.9	413.1		262.1					
	271.6								

[a] ND, not determined; CE, collision energy.

virtue of the MS–MS and MS^3 filters on the LCQ. This HPLC–MS LCQ configuration also facilitated the automated collection of analytical data and the determination of the dyes in an effluent sample.

2.2. Reagents/analytes

All chemicals used in the dye hydrolyses and chromatographic analyses were of analytical reagent quality. Solvents were of HPLC grade. Two dyes (Remazol Red RB, Remazol Golden Yellow RNL) were supplied as solid dyes while Remazol Black B and Remazol Blue GG were supplied in a 33% water–sulphuric acid solution by Fruit of the Loom (Buncrana, Ireland).

2.3. Techniques

First, 100-ml solutions of 1.0 m*M* NaOH containing 0.5 g l^{-1} final concentration of each dye were hydrolysed in a water bath at 70°C for 15 min. Then, 10-ml samples of the hydrolysed dye containing solutions were passed through 500 mg/3 ml SAX Bond Elut cartridges (strong anion-exchanging quaternary amine) that had been preconditioned with 3 ml methanol followed by 3 ml Milli-Q water. Effluent samples were similarly treated. All the dye components of the solutions were retained by the solid-phase and were subsequently eluted using 3 ml of 30% (v/v) HCl in methanol. The eluted dyes and treated effluent samples were then evaporated to dryness at room temperature and then redissolved in 1 ml solution of water–acetonitrile (1:1, v/v) prior to the application of ES-MS, HPLC–UV–Vis, HPLC–MS–MS or MS^3.

3. Results

Sulphonated azo dyes are widely used in the

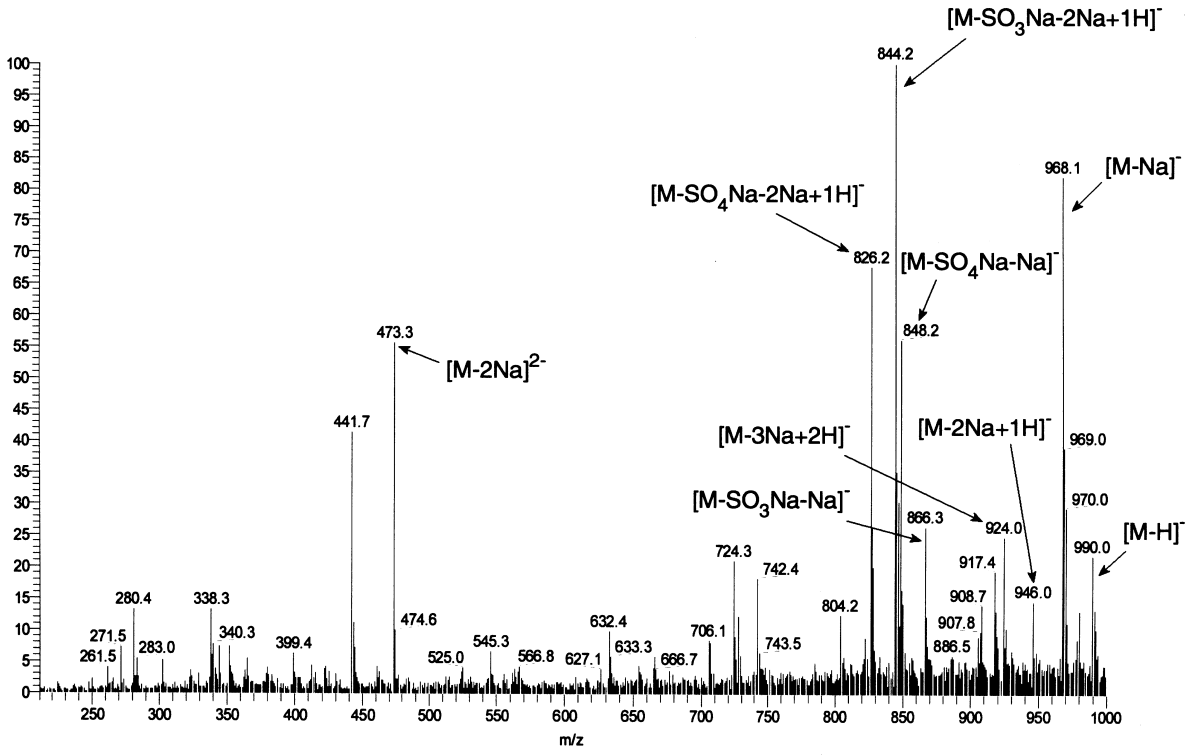
textile industry to colour natural fibres. Manufacture of these dyes can cause an environmental problem if the effluent is not carefully monitored and controlled. Azo dyes have been shown to undergo reduction in natural waterways and the degradation products include amines which are known to be carcinogenic. There is therefore an interest in sensitive techniques to monitor and identify low levels of azo dyes and their degradation products. Although the sulphonic acid group(s) provide these dyestuffs with essential water solubility they also render them involatile and hence GLC cannot be used for their determination. Oxspring et al. [30] have reported the separation and determination of reactive textile dyes, which included sulphonated azo dyes, by HPLC and CE using visible spectrometric detection. Limits of detection (LODs) by HPLC were generally one to two orders of magnitude superior to CE and of the order of 10^{-7} mol l^{-1}. CE, however, showed a higher efficiency of separation with up to 4.25×10^{5} theoretical plates and could satisfactorily resolve impurities in the dye preparations and also the hydrolysis products of these sulphonated azo dyes which are what are discharged in the textile effluent. Farry et al. [31] have developed an off line method using SAX Bond Elut cartridges to concentrate these hydrolysis products from environmental samples prior to the application of separation techniques such as CE and HPLC. The LOD for the CE determination of Remazol Black B (I) following such concentration from a 1-l sample of hydrolysed dye was lowered to 10^{-7} mol l^{-1}. A drawback of this analytical method was that on elution from the cartridge with 30% conc. HCl in MeOH, this and other hydrolysed Remazol dyes were subject to some chemical degradation.

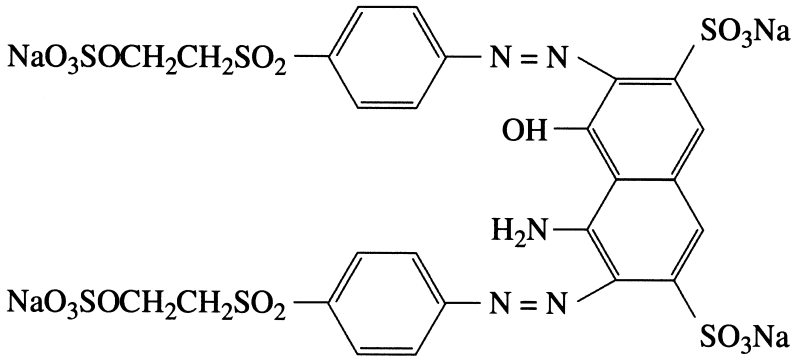
The interfacing of HPLC and CE with a sensitive mass spectrometric detection system such as ES-MS are therefore seen as viable hyphenated techniques for the identification and quantification of such sulphonated azo dyes, their degradation and metabolic products in samples such as effluents, river waters etc. The first step is, however, to examine the ES-MS behaviour of the sulphonated azo textile dyes, their hydrolysis products and the latters' reaction, following elution from a SAX cartridge, with 30% conc. HCl in MeOH. A study of the ES-MS–MS and ES-MS^n behaviour is also necessary at this stage.

Four sulphonated azo dyes were investigated by negative ion ES-MS, Remazol Black B (I), and three other sulphonated azo dyes (Remazol Navy Blue GG, Remazol Red RB and Remazol Golden Yellow RNL) whose precise chemical structures are not in the public domain. These four dyes were also hydrolysed in 1 m*M* NaOH to give their hydrolysis products which were subjected to negative ion ES-MS. The hydrolysis products were subsequently concentrated on SAX cartridges and then eluted with 30% conc. HCl in MeOH to give the hydrolysed dyes and degradation products, each mixture being investigated by negative ion ES-MS. Figs. 1–3 show the negative ion ES-MS of Remazol Black B, its hydrolysis product and the product of the latter's treatment with 30% conc. HCl in MeOH respectively. Remazol Black B has a molecular mass of 991 as the uncharged tetrasodium salt and, as such, does not give rise to a molecular ion in ES-MS (Fig. 1). A signal is, however, observed at m/z 990 which would appear to be due the monoanion, formed after loss of $1H^{+}$ from the phenolic OH group. Further examination of the ES-MS reveals that a group of monoanions give signals at 968.1, 946.0 and 924.0 corresponding to species $[M\text{-}Na]^{-}$, $[M\text{-}2Na+1H]^{-}$ and $[M\text{-}3Na+2H]^{-}$, respectively. (M is the molecular mass of the tetrasodium salt, i.e. 991). The m/z signal at 902 corresponding to $[M\text{-}4Na+3H]^{-}$ is not observed. The 968.1 signal is confirmed as due to a singly charged species since isotope peaks are observed at 969.0 and 970.0. Ballantine et al. [32] have also observed that polysulphonated azo dyes can produce such ion series, each being the result of varied amounts of cation–proton exchange during the ionisation process. In particular, Ballantine et al. [32] have observed a strong dianion series for a hexasulphonated diazo dye, Reactive Red 120, on direct infusion in MeOH–water (50:50, v/v) into an ES source. Remazol Black B does not, however, give such a dianion series corresponding to

$$\frac{[M\text{-}4Na+2H]^{2-}}{2},\quad \frac{[M\text{-}3Na+1H]^{2-}}{2},\quad \frac{\{M\text{-}2Na]^{2-}}{2},\quad \frac{[M\text{-}Na\text{-}H]^{2-}}{2},\quad \frac{[M-2H]^{2-}}{2}$$

which would be observable at m/z values 450, 461, 472.5, 483.5 and 494.5, respectively. The signal at 473.3 could be due to $([M\text{-}2Na]^{2-})/2$. Triply charged species such as $[M\text{-}4Na+1H]^{3-}$, $[M\text{-}3Na]^{3-}$



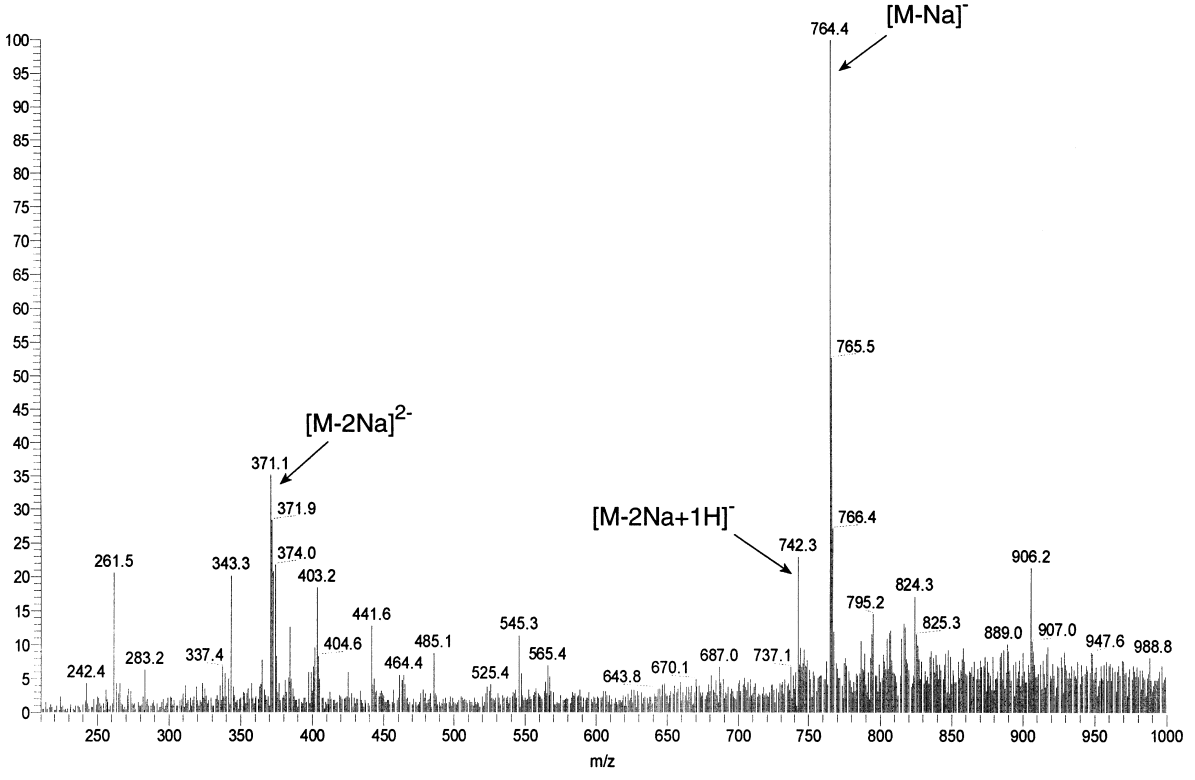


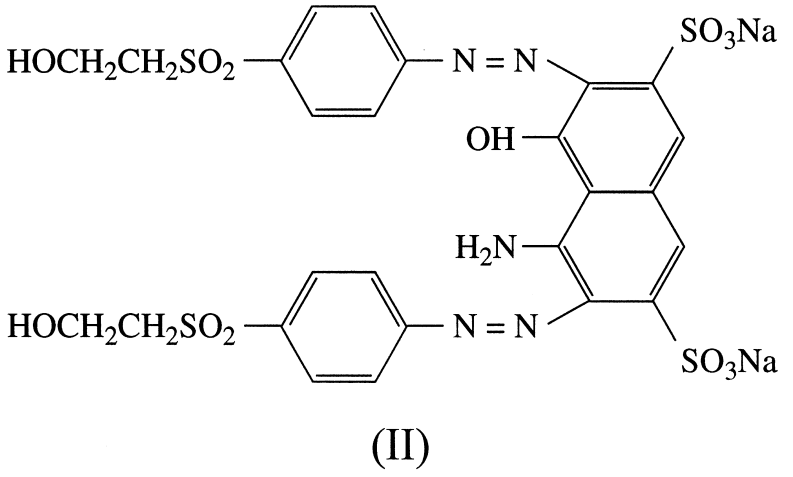
(I)

Fig. 1. ES-MS for the unhydrolysed Remazol Black B (I) (molecular mass 991).

at m/z values 300 and 307, respectively, and quadruply charged species such as $[M\text{-}4Na]^{4-}$, $[M\text{-}3Na-H]^{4-}$ at m/z values 225 and 230, respectively, are also not observed.

Relatively intense signals are also observed at m/z 866.3, 848.2, 844.2 and 826.2, with the signal at 844.2 being the most intense. These signals can be explained by invoking the loss of SO_3 and SO_4 groups from Remazol Black B as well as the cation–proton exchange processes already discussed. For example, the singly charged anions given by $[M\text{-}SO_4Na-Na]^-$, $[M\text{-}SO_4Na-2Na+1H]^-$,





(II)

Fig. 2. ES-MS for the hydrolysed Remazol Black B (II) (molecular mass 787).

$[M\text{-}SO_3Na\text{-}Na]^-$ and $[M\text{-}SO_3Na\text{-}2Na+1H]^-$ would be expected to give signals at m/z values of 849, 827, 865 and 843, respectively.

The negative ion ES-MS of the hydrolysed Remazol Black B, (II), in which the two $-CH_2-O-SO_3Na$ groups have been converted to $-CH_2-OH$ groups to give a molecular mass of 787 for the disodium salt is shown in Fig. 2. The $[M\text{-}1]^-$ species is not evident this time at m/z 786 but the mono-anion series corresponding to $[M\text{-}Na]^-$ and [M-

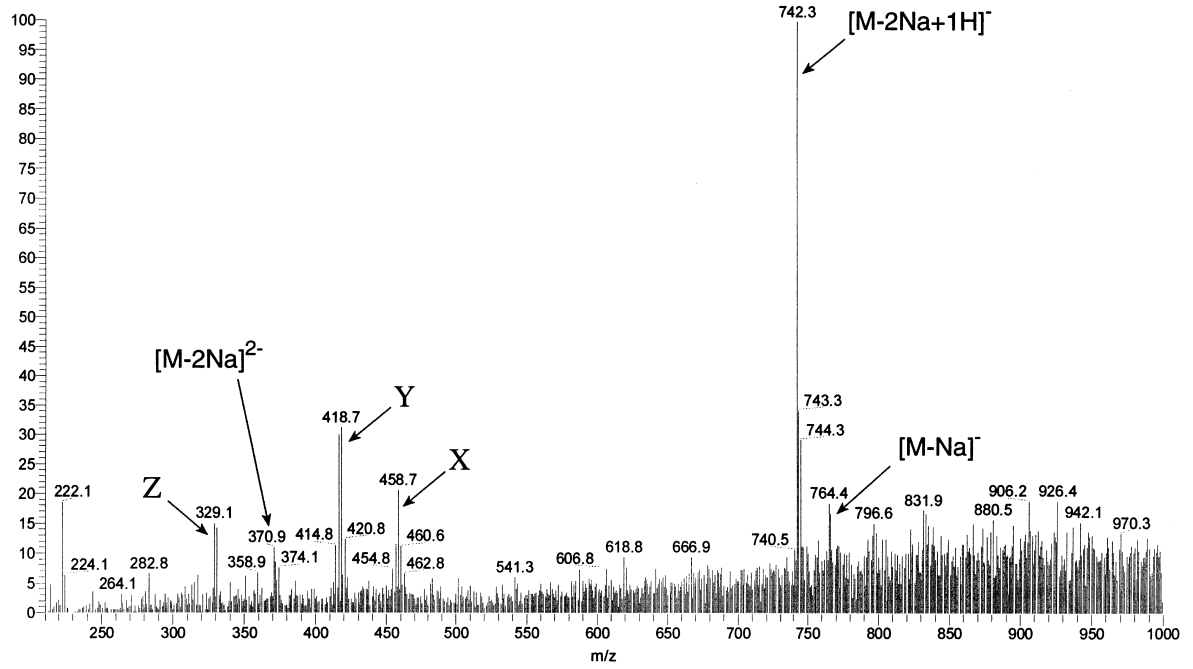


Fig. 3. ES-MS for the hydrolysed and SPE processed Remazol Black B.

$2Na+1H]^-$ is again observed at m/z 764.4 and 742.3 respectively. The isotope pattern on the most intense 764.4 signal with further signals at 765.5 and 766.4 confirms the species' monocharged nature.

The dianion corresponding to $[M\text{-}2Na]^{2-}$ is observed at m/z 371.1 and there are no signals this time due to the loss of SO_3 and SO_4 groups which are, in any case, absent in the hydrolysed molecule.

Fig. 3 shows the ES-MS of the hydrolysed dye following its reaction with 30% conc. HCl in MeOH, as is utilised in elution from the SPE anion-exchange cartridge. The signal at m/z 764.4 has now significantly diminished leaving the 742.3 signal with an isotope pattern at 743.3 and 744.3 suggesting that it is singly negatively charged. It is believed that these two signals are still due to the hydrolysed dye but that their relative proportions is now due to infusion of a methanolic solution into the ES source instead of an alkaline solution (pH 11), as was the case with the hydrolysed dye before SPE. This would suggest that the infusion of a methanolic solution gives rise to predominance of the $[M\text{-}2Na+1H]^-$ species over $[M\text{-}Na]^-$ whereas the latter is favoured on infusion of an alkaline solution of the hydrolysed dye. Studies by Farry et al. [31] have shown that, following this SPE procedure, two CE peaks are observed for Remazol Black B, both absorbing at 600 nm. This would suggest that the harsh conditions of elution from the SAX cartridge resulted in some degradation of the hydrolysed Remazol Black B but that the chromophoric centre of the molecule was not disrupted. Further examination of Fig. 3 in the m/z 300–500 region shows a residual 370.9 signal corresponding to $[M\text{-}2Na]^{2-}$ for the hydrolysed dye and the appearance of signals at m/z values 458.7, 418.7 and 329.1 which probably correspond to multiply charged species of the degraded hydrolysed dye.

Remazol Navy Blue GG, whose complete molecular structure is not in the public domain, is quoted by the manufacturers to be very similar in structure to Remazol Black B with a molecular mass of 847.3 (hydrolysed dye) and containing two azo groups. If it is assumed that this dye has also two $-CH_2-O-SO_3Na$ groups which are converted to $-CH_2-OH$ on hydrolysis and that it also has a phenolic group, then a $[M\text{-}1]^-$ signal could be expected at m/z $847.3+204-1$, i.e. 1050.3. As was the case for Remazol

Black B, a relatively small signal was indeed observed, in this case at m/z 1050.0 (Fig. 4). Again, if Remazol Navy Blue GG is a tetrasodium salt then a singly charged ion series should be observable for

$$\frac{[\text{M-Na}]^-}{1}, \quad \frac{[\text{M-2Na} + 1\text{H}]^-}{1}, \quad \frac{[\text{M-3Na} + 2\text{H}]^-}{1}$$

i.e. at m/z values of 1028.3, 1006.3 and 984.3. The first two are observed at values of 1028.2 and 1006.1.

Again, as with Remazol Black B, the $[\text{M-2Na}]^{2-}$ species is detected at 503.0 and the singly charged anions given by $[\text{M-SO}_4\text{Na-Na}]^-$, $[\text{M-SO}_4\text{Na-2Na} + 1\text{H}]^-$, $[\text{M-SO}_3\text{Na-Na}]^-$ and $[\text{M-SO}_3\text{Na-2Na} + 1\text{H}]^-$ would be expected to give signals at 909.3, 887.3, 925.3 and 903.3. It would therefore appear that Remazol Navy Blue GG has a molecular mass of 1051 which is 60 units in excess of Remazol Black B. The functional groups of the two molecules are likely to be identical and 60 units can be accounted for by a substituent that is not involved in the ionisation processes in the ES probe.

Hydrolysed Remazol Navy Blue GG has a similar ES-MS behaviour (Fig. 5) to hydrolysed Remazol Black B in that $[\text{M-Na}]^-$ is observed at 824.4 (with expected isotope pattern at 825.5) but this time $[\text{M-2Na} + 1\text{H}]^-$ expected at 847.3-46+1, i.e. 802.3 is lost in the background. $([\text{M-2Na}]^{2-})/2$ is again located at 401.3. Following SPE and elution with 30% conc. HCl in MeOH, the $[\text{M-2Na} + 1\text{H}]^-$ species rises from the background at 802.2 and predominates over the $[\text{M-Na}]^-$ signal at 824.1 (Fig. 6) as was the case with Remazol Black B. Further examination of Fig. 6 shows a residual $([\text{M-2Na}]^{2-})/2$ signal at 400.9 but no evidence of multiply charged species of the degraded hydrolysed dye.

Less chemical information was provided by the manufacturers on the other two Remazol dyes, namely Remazol Red RB (molecular mass of hydrolysed form 847.7) and Remazol Golden Yellow RNL

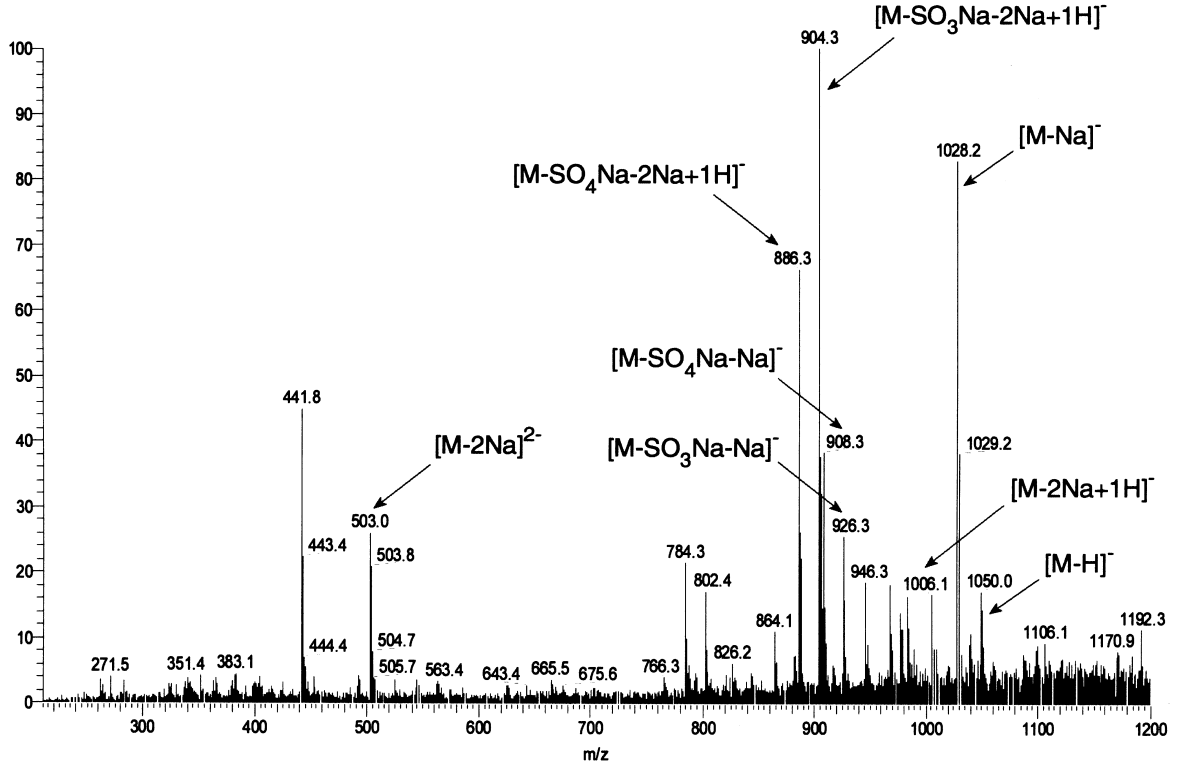


Fig. 4. ES-MS for the unhydrolysed Remazol Blue GG.

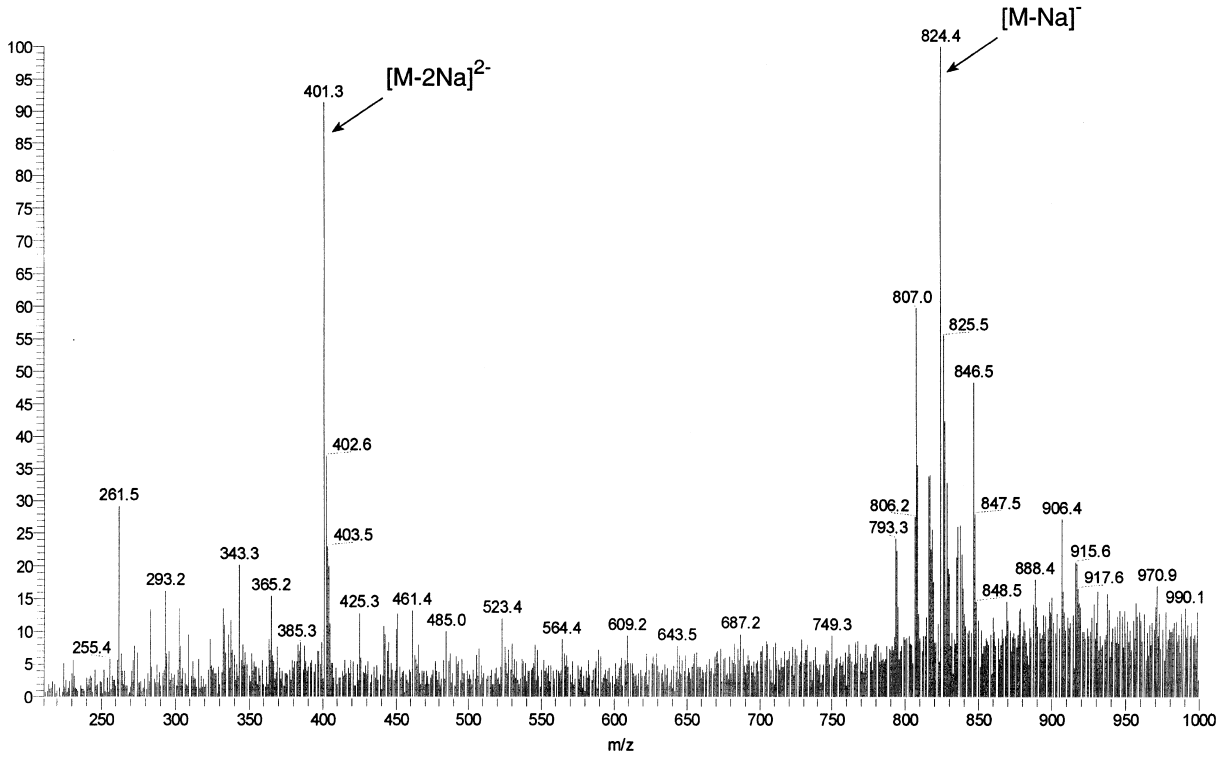


Fig. 5. ES-MS for the hydrolysed Remazol Blue GG.

(molecular mass of hydrolysed form 480.4). Attempts to locate patterns observed for the ES-MS of Remazol Black B, Remazol Navy Blue GG and their hydrolysis products, e.g. singly charged ion series etc were unsuccessful. The ES-MS of the hydrolysed dyes that have been subjected to SPE and elution with 30% conc. HCl in MeOH show predominant signals at m/z 530.2 for Remazol Red RB and at m/z 399.6 for Remazol Golden Yellow. The identity of these signals is not obvious although they are of analytical value in the monitoring of effluents by direct infusion negative ion ES-MS, ES-MS–MS, ES-MS^n and in hyphenated techniques involving HPLC and CE.

Direct injection ES-MS of a mixture of these four Remazol dyes, as the hydrolysed dyes that have been subjected to SPE and elution with 30% conc. HCl in MeOH shows that the main signals (e.g. at m/z 742.3 for Remazol Black B and m/z 802.2 for Remazol Blue GG) are observed for all four dyes. Effluent sample A from a textile plant, containing such hydrolysed dyes, was then subjected to direct injection ES-MS and Remazol Black B and Remazol Blue GG signals at m/z values 764.9 and 824.7, respectively, due to the $[M\text{-}Na]^-$ species, were just detected among signals due to other effluent constituents and high background signals. This effluent sample was then subjected to SPE with elution with 30% conc. HCl in MeOH followed by direct injection ES-MS (Fig. 7). Again, the presence of Remazol Black and Remazol Blue GG were just detected among other signals and high background signals at m/z values 742.3 and 802, respectively, corresponding to the species $[M\text{-}2Na+1H]^-$. The presence of one or both these Remazol dyes in the effluent sample A was confirmed by HPLC with visible detection at 600 nm after the SPE procedure (Fig. 8). HPLC also confirmed the presence of a relatively small concentration of hydrolysed Remazol Red RB detected at 500 nm, eluting at $\approx$9 min which was lost in the background of the mass spectrum in direct injection ES-MS. It is therefore

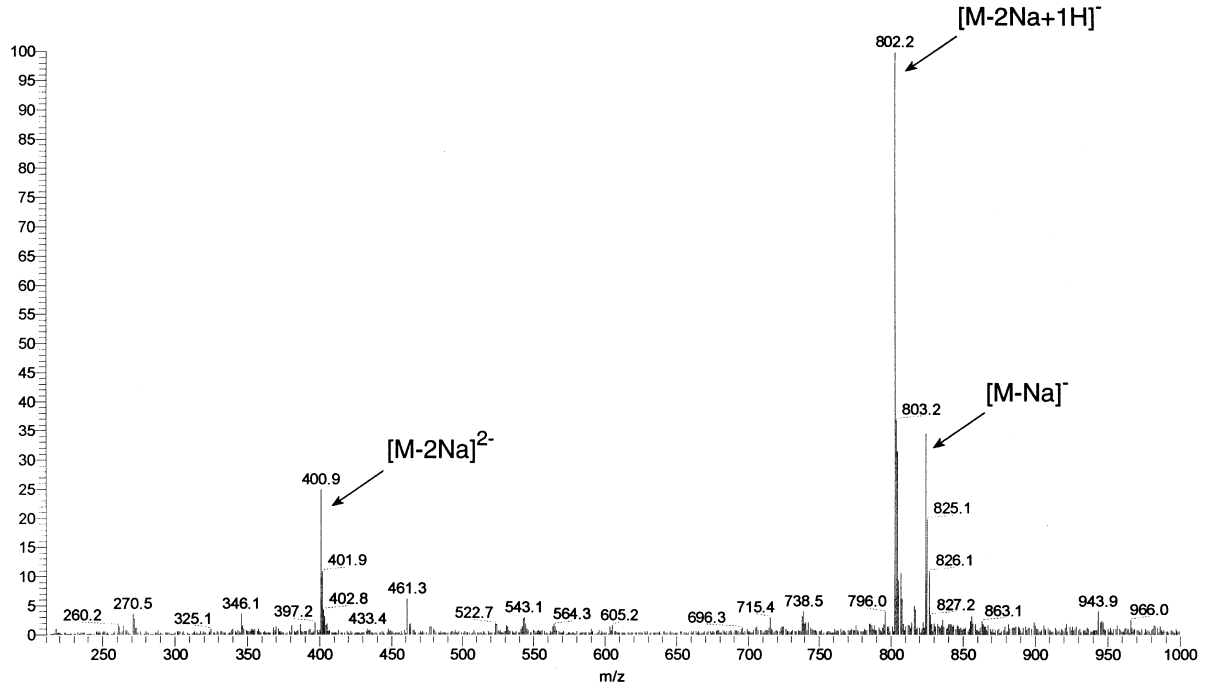


Fig. 6. ES-MS for the hydrolysed and SPE processed Remazol Blue GG.

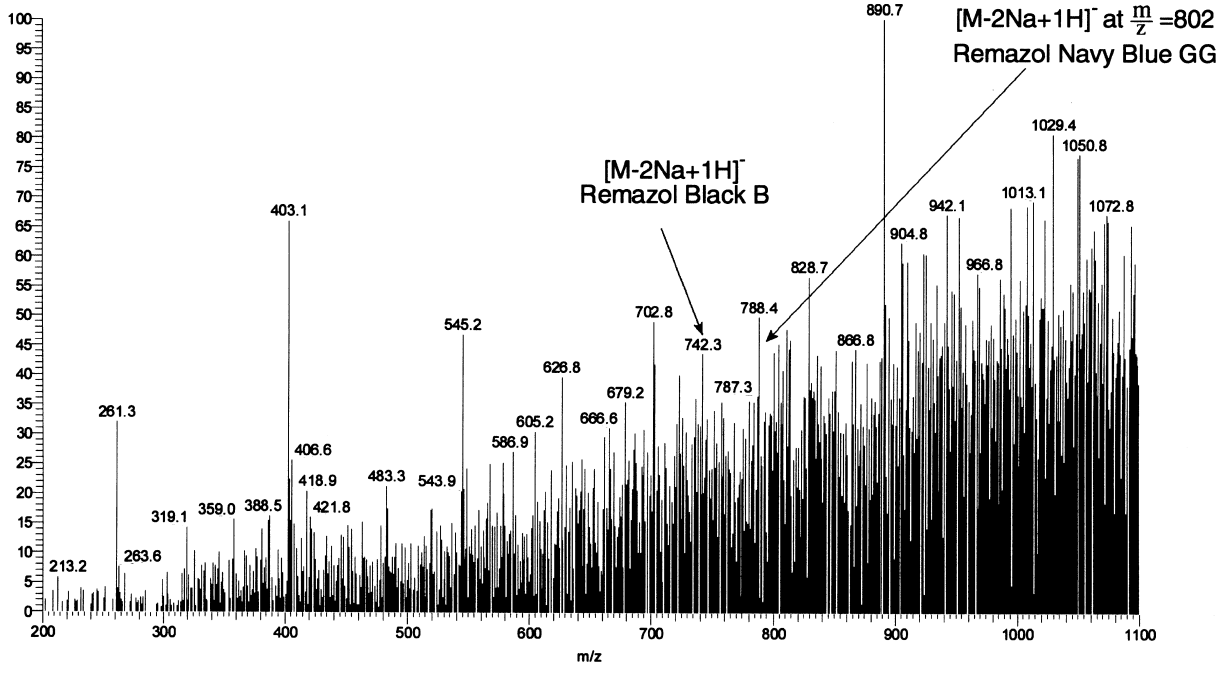


Fig. 7. Direct injection ES-MS of an effluent sample containing hydrolysed textile dyes that have been subjected to the SPE procedure using elution with 30% conc. HCl in MeOH.

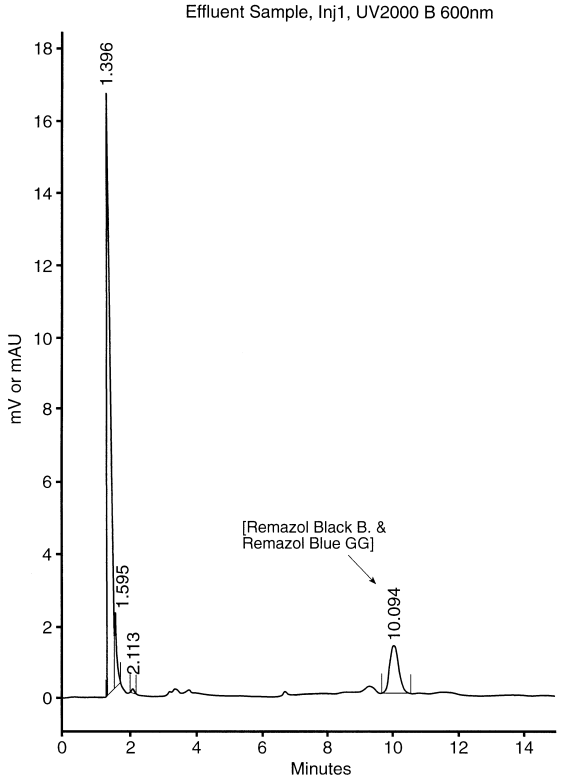


Fig. 8. HPLC–UV analysis of effluent sample taken through analytical method involving SPE. HPLC conditions: mobile phase 1% H_3PO_4–acetonitrile (80:20, v/v); flow-rate 0.75 ml min^{-1}; sample volume 10 μl; detection at 600 nm.

obvious that, for unambiguous identification of these textile dyes in effluent samples, it is necessary to resort to ES-MS^n and/or HPLC–ES-MS. The mobile phase in these HPLC studies contains phosphoric acid which is incompatible with ES-MS detection so further work is currently underway to efficiently separate the hydrolysed dyes and their SPE processed products by HPLC using a mobile phase compatible with ES-MS detection.

MS^n characterisation provides a unique fingerprint for each dye under investigation. This is of particular relevance in these dye studies given the relatively complex spectra produced in the MS mode. SPE processing is successful in removing interfering signals and a much cleaner MS trace is produced thereby making the choice of which peak to use for fragmentation purposes a relatively easy one.

Table 3 lists the MS^n data for each dye under investigation. A persistent peak of significant magnitude is chosen in each case from the MS spectrum. For Remazol Black and Blue this choice was relatively easy as large predominant peaks at m/z 742.1 and 801.9, respectively, were observed. Strong signals at m/z 441.5 and 399.6 were observed for Remazol Yellow and in this case the former was preferred. Remazol Red proved to be the most problematic dye in these studies due to low signal intensity and the fact that its major peaks at m/z 399.5 and 530.0 were observed in some of the other dye spectra. It was initially decided to choose the peak at m/z 399.5 and to rely on the fragmentation process to produce a dissimilar daughter ion to the yellow dye. However, since all the dyes elute with or close to the solvent front in HPLC a ‘shadow’ of the yellow m/z 399.5 ion is seen in the scan filter for the red dye but the fingerprint m/z 368.3 signal indicative of the red is not present. As the concentration of red dye is increased to around 5.0 ppm the m/z 368.3 ion is observed and a shoulder with the yellow ‘shadow’ peak forms indicating that the red is now being detected. In mixtures of the four Remazol dyes it is still possible to separate the red and the yellow dyes. It was realised, however, that this approach would cause problems for the determination of the red and yellow dyes in a complex matrix such as an effluent sample, especially if the yellow was present in excess. The MS^n fragmentation profile of the red dye using the major m/z peak at 530.0 was therefore studied. This provided a unique signal and fragmentation pattern for the red dye. The MS^3 stage, in particular, yielded a single and unique predominant ion at m/z 253.1.

Using these MS^n conditions, analytical data for the dyes was collected and is shown in Table 4. The LODs for the Remazol blue, black and yellow dyes was 100.0 ppb, S/N ratios being 2, 4 and 3, respectively. The LOD of Remazol Red RB using the m/z 399.5 signal was taken to be 5.0 ppm, the concentration at which a reproducible peak for the dye was first seen. However, as discussed above, this signal was of little practical use in the determination of the red dye in effluent. Using the m/z peak at 530.0 the LOD was 7.50 ppm (MS–MS; $S/N=2$) while MS^3 yielded an LOD of 1.25 ppm, the point at which an increase in concentration caused a corre-

Table 4
Calibration, RSD and LOD data for Remazol dyes by HPLC–MS–MS or MS^3

Dye	Calibration data			RSD data at 7.50 ppm		LOD	
	R^2	Range (ppm)	n	RSD (%)	n	LOD (ppm)	S/N
Blue	0.9991	0.75–50.00	11	6.40	6	0.10	2
Black	0.9936	0.75–50.00	11	9.13	6	0.10	4
Red (m/z 399.5)	0.9140	5.00–30.00	5	21.53	5	5.00	N/A
Red (m/z 530.0)	0.9595	7.50–50.00	5	16.65	6	7.50	2
Red (m/z 530.0, MS^3)	0.9677	2.50–50.00	8	19.96	6	1.25	N/A
Yellow	0.9944	0.75–50.00	11	4.08	6	0.10	3

sponding increase in signal magnitude from what could only be considered as background signals. Acceptable correlation coefficients for the dyes were achieved over the ranges shown in Table 4. Six replicate injections (five for the red dye at m/z 399.5) were used to calculate RSD values on peak area at the 7.50 ppm concentration level and were 6.40, 9.13 and 4.08% for the blue, black and yellow dyes, respectively. Remazol Red RB again proved problematic having a higher RSD at 21.53% for the m/z 399.5 signal as this concentration is just above the LOD for the compound. For the m/z 530.0 signal, the MS–MS RSD was 16.65% and the MS^3 RSD 19.96%. For this reason, MS–MS of the m/z 530.0 signal was used for the determination of red dye in the SPE-treated effluent sample.

Application of the HPLC–MS^n technique was made to the determination of these Remazol dyes in effluent sample B from a textile plant. Effluent was collected from a sample holding tank and a 60-ml aliquot treated using the SPE method. The final volume following elution from the solid-phase cartridge was 1.0 ml. A 1:10 dilution of this sample was made in methanol before injection onto the LC–MS–MS system. Three injections of a methanol blank were first carried out to ensure that the system was completely clear of any dye from previous analyses. Six replicate injections of the SPE processed effluent sample were then made and the resulting chromatograms recorded. From Fig. 9 it can be seen that the black, blue and yellow dyes are readily detected. Only when using the MS–MS and MS^3 peaks generated from the m/z 530.0 parent ion can the red dye be detected as shown in Fig. 10. The 'shadow' peak due the yellow dye is also shown when using the m/z 399.5 parent ion.

Dye concentration in the effluent was then calculated by means of a simple peak area:concentration ratio based on the known peak area of SPE processed dye standards at the 7.50 ppm level. It is possible to use such a method as the calibration signal is linear over the range in which the measured concentration falls. Also, as standards are treated in the same way as the sample, efficiency of SPE is not a factor that needs to be considered. The calculated levels of the four dyes as determined by HPLC–MS^n were 0.81, 1.77, 52.80, and 1.23 ppm for the blue, black, red and yellow dyes, respectively, which corresponded to 130 ppb, 300 ppb, 8.80 ppm and 210 ppb, respectively, in the effluent. Six or seven replicate injections were made to calculate RSD values based on peak area. These decreased in line with increasing amount of dye found in the effluent.They were: blue (36.96%),yellow (14.01%) and black (9.79%). Remazol Red RB gave an anomalous RSD value, despite its high concentration in the effluent, of 37.33%.

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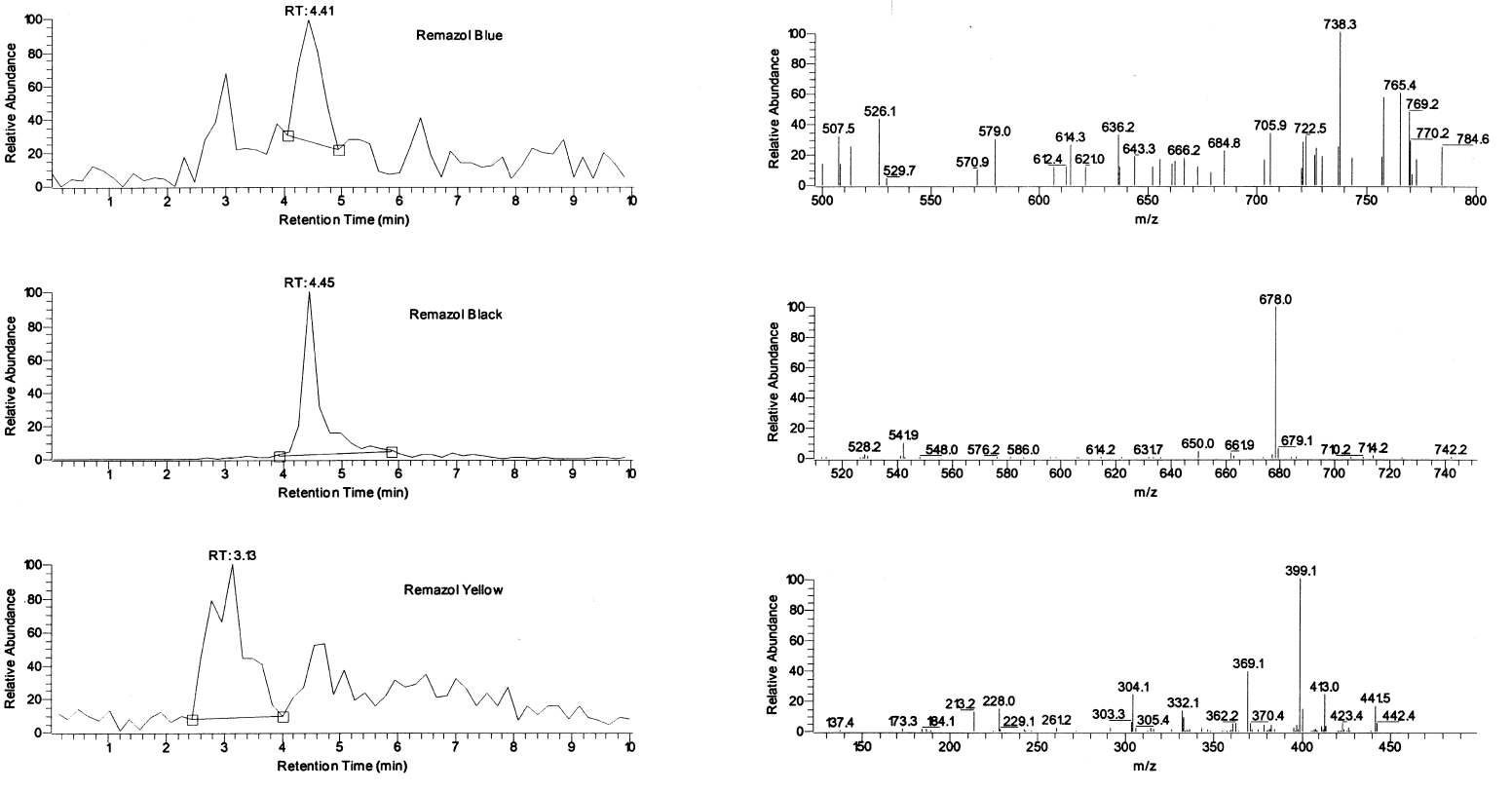

Fig. 9. LC–MS–MS analysis of an effluent sample taken through analytical method involving SPE. LC conditions: mobile phase acetonitrile–0.01 g/100 ml ammonium acetate in water (90:10, v/v); flow-rate 0.30 cm^3 min^{-1}; sample volume 20 μl. The left-hand traces show the MS–MS ion chromatograms, while the corresponding right-hand spectra show the presence of the daughter ion indicative of each dye as described in Table 3. RT, retention time.

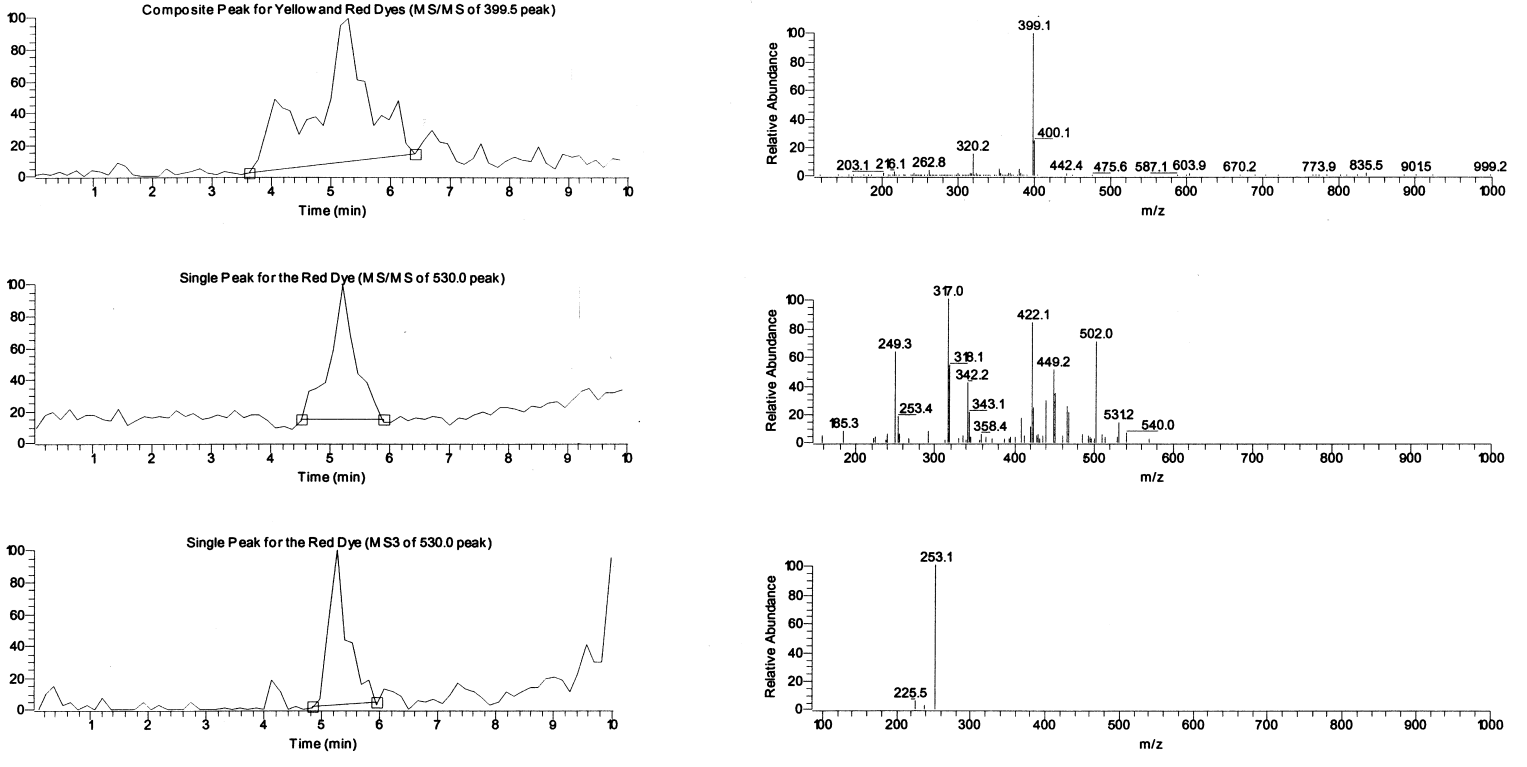

Fig. 10. LC–MS–MS and MS^3 analysis for the detection of Remazol Red RB in an SPE processed effluent. LC conditions: mobile phase acetonitrile–0.01 g/100 ml ammonium acetate in water (90:10, v/v); flow-rate 0.30 ml min^{-1}; sample volume 20 μl. The first left-hand trace shows the MS–MS ion chromatogram based on the m/z 399.5 parent ion to contain a 'shadow' of the yellow dye. The second and third left-hand traces refer to MS–MS and MS^3 ion chromatograms due to fragmentation of the m/z 530.0 parent ion. The corresponding right hand spectra show the presence of the daughter or granddaughter ions indicative of each dye as tabulated in Table 3.

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