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Field Desorption Mass Spectral Analysis of Some Nonmutagenic Benzidine-Based Pigments

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ABSTRACT

This paper is concerned with field desorption mass spectral (FDMS) analysis of novel disazomethine, disazoacetoacetanilide (diarylide) and disazopyrazolone pigments containing nonmutagenic benzidine homologs. The FD spectra of the aforementioned types of pigments are characterized by intense parent ion peaks $[M^{+\cdot}]$ *and relatively intense* $[M^{+}]/2$ *daughter ions, a pattern which serves as a fingerprint for these molecular structures.* © *1998 Elsevier Science Ltd. All rights reserved*

Keywords: nonmutagenic, benzidine, FD spectra.

INTRODUCTION

In recent years, significant progress has been made in the characterization of involatile organic dyes and pigments using mass spectrometry. Papers published in this area include data from our laboratories in which desorption chemical ionization [1] (DCI) and fast atom bombardment [1,2] (FAB) were used in the analysis of high molecular weight disperse dyes and sulfonated azo and anthraquinone dyes. Whereas the use of ionization methods such electron impact (EI), chemical ionization (CI), DCI and FAB have been widely described in the characterization of organic compounds, field desorption mass spectrometry (FDMS) has received much less attention. Unlike CI, EI and DCI, ionization in FDMS takes place simultaneously with desorption. In FDMS analysis, the compound is dissolved in a solvent and applied to an emitter covered with graphite or silicon microneedles. After

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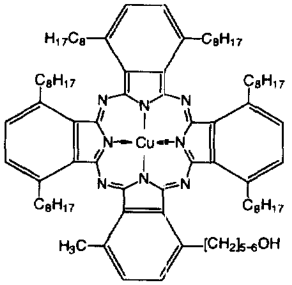
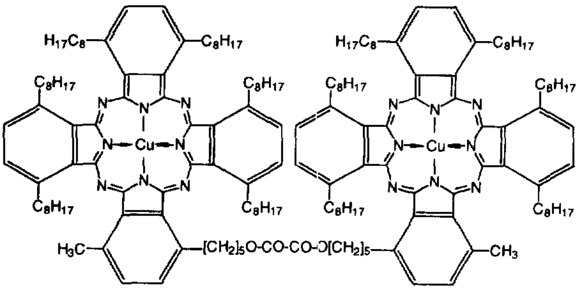
solvent evaporation, the sample left on emitter is subjected to a powerful electric field (10^7-10^8 V cm^{-1}) which ejects an electron from the analyte molecule to form molecular ions such as $[M^{+\bullet}]$ or $[M + H]^+$. In some cases, sodium adducts are also observed as minor components. Since little fragmentation occurs during FDMS analysis, however, molecular ions are often the only ions detected.

Field desorption is reported to produce mass spectra of organic [3,4] and inorganic [5] acids as well as organic [6–8] and inorganic [9] salts. This technique is also suitable for intermediates such as naphthalene and anthraquinone sulfonic acids or sulfates and organic dyes [10]. FDMS appears to be more useful than EI in confirming the structure of some cationic dyes (e.g. Crystal Violet, Brilliant Green, Methylene Blue), in cases where problems with Hofmann degradation or molecular rearrangements are observed [11]. Similarly, FDMS was reported to be the method of choice for confirming the structure of mesogenic octa-substituted phthalocyanines **1** and **2** [12], and was the only ionization method suitable for establishing the structure and purity of insoluble colorants such as indigo, zinc phthalocyanine, copper naphthalocyanine, indanthrone and quinacridone [13]. FDMS was also found to be a very useful analytical method in biochemistry where only sub-milligram quantities of dyes isolated from tissue cells were available [14].

In the present paper, FDMS has been used for the analysis of three types of organic pigments containing a nonmutagenic benzidine moiety.



1 (m/z 1348-1362)



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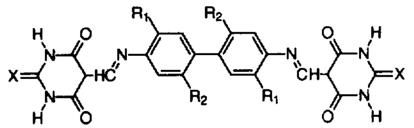


3

	R_1	R_2	X	MW
a	$OCH_2CH_2OCH_3$	H	O	608.5
b	CH_3	H	O	488.4
c	$OCH_2CH_2CH_3$	CH_3	O	604.6
d	$OCH_2CH_2CH_3$	CH_3	S	636.8

EXPERIMENTAL

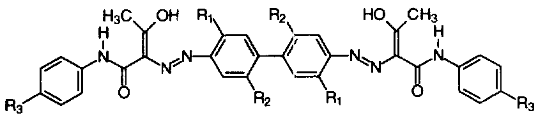
Pigment **3b** was obtained from Dianippon Ink and Chemicals, Inc. All others were synthesized in our laboratory. Mass spectra were obtained on a JEOL HX-110 (Tokyo, Japan) double focusing mass spectrometer. The instrument was operated at 1000 resolution and 10 KeV for all experiments. The pigments were dissolved in acetone and positive ions were detected. For FDMS analysis, 1 μl of solution was deposited on a silicon emitter and the solvent was allowed to evaporate. The current was set at 0 mA initially and increased at 4 mA min^{-1} until the sample was desorbed generally around 25 mA. The instrument was scanned from 40–1800 daltons using 20 s scans. The data presented was averaged and background subtracted.

For FAB analysis, 2 μl of the pigment dissolved in acetone was mixed with 3-nitrobenzyl alcohol on the probe tip. The xenon gun was operated at 6 KeV and the instrument was scanned from 200–2000 daltons using 20 s scans. The spectra shown in the present paper are 2 to 3 scans averaged without background subtraction.

For DCI analysis, 1 μl of solution was deposited on the DCI probe wire and the solvent was evaporated. The initial current was set at 0 A and ramped at a rate of 0.25 A min^{-1} to 0.85 A until the pigment was desorbed. The instrument was scanned from 80–1000 daltons using 5 s scans. The data shown were averaged and background subtracted.

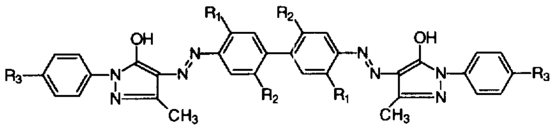
RESULTS AND DISCUSSION

The disazomethine (**3a,c,d**), diarylide (**4**) and disazopyrazolone (**5**) pigments evaluated in this paper were developed as alternatives to yellow, orange and red colorants derived from genotoxic benzidines. They were synthesized either by condensing the appropriate benzidines with barbituric or thiobarbituric acid and triethylorthoformate in DMF or by tetrazotization of the corresponding benzidines followed by coupling with acetoacetanilides or phenylpyrazolones. The methods used to synthesize the required benzidines were similar to a previously described procedure [15].



4

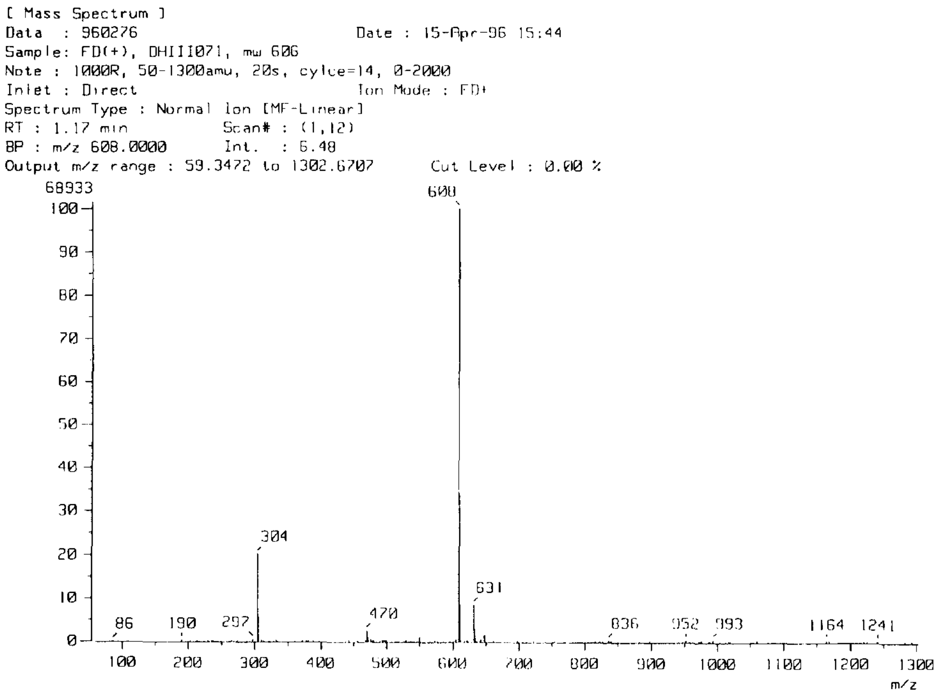
	R_1	R_2	R_3	MW
a	$OCH_2CH_2CH_3$	CH_3	H	704.8
b	$OCH_2CH_2CH_3$	CH_3	OCH_3	764.8
c	$OCH_2CH_2CH_3$	OCH_3	H	736.8



5

	R_1	R_2	R_3	MW
a	$OCH_2CH_2CH_3$	CH_3	H	698.8
b	$OCH_2CH_2CH_3$	CH_3	Cl	767.7
c	$OCH_2CH_2CH_3$	OCH_3	H	730.8

While the most important issue in the synthesis of **3–5** was the generation of products in high yield and purity, confirmation of the structures produced was also important. Although methods such as NMR could not be employed in all cases due to limited solubility of the pigments in suitable solvents, we found FDMS to be an effective method for providing the desired structural evidence. FDMS was also useful in establishing the purity of these pigments, as may be seen from inspecting the spectra provided in Figs 1–4. Since FDMS is a soft ionization process and there are no matrix ions, the spectrum of each pigment was characterized by an intense parent ion peak $[M^{+\cdot}]$ and a



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[ Mass Spectrum ]
Data  : 960276                          Date : 15-Apr-96 15:44
Sample: FD(+), DHIII071, mw 606
Note : 1000R, 50-1300amu, 20s, cylce=14, 0-2000
Inlet : Direct                          Ion Mode : FD+
Spectrum Type : Normal Ion [MF-Linear]
RT : 1.17 min           Scan# : (1,12)
BP : m/z 608.0000       Int.   : 6.48
Output m/z range : 59.3472 to 1302.6707      Cut Level : 0.00 %
```

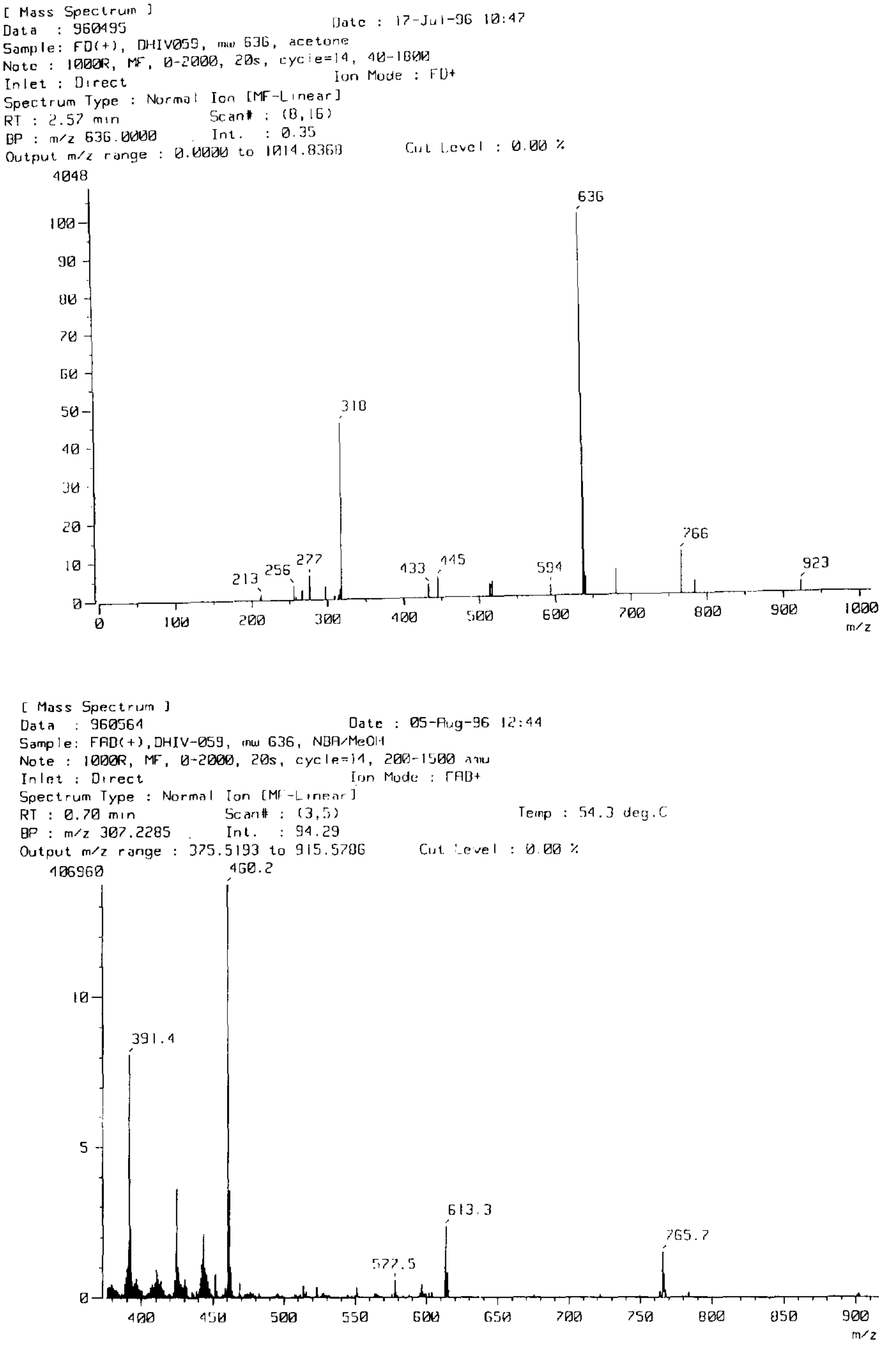
Fig. 1. FD mass spectrum of **3a**.

peak arising from fragmentation at the biphenyl linkage, the latter corresponding to $[M^+]/2$ (cf. Table 1).

As a basis for comparing the utility of FD, FAB and DCI mass spectrometric methods in the analysis of organic pigments, **3d**, **4a** and **5a** were also analyzed by FAB and DCI mass spectrometry. For pigments **4a** and **5a**, which are soluble in the FAB matrix (3-nitrobenzyl alcohol) employed, FAB spectra characterized by protonated molecular ions were obtained (cf. Figs 3 and 4). For **3d**, however, only ions associated with the matrix were observed (Fig. 2). For all FAB spectra, no mass information below m/z 400 was useful due to the intense ions produced by the matrix in this region. This means that it was not possible to confirm the formation of $[M^+]/2$ ions.

When DCI was used, all three pigments gave protonated molecular ions. Since this technique is more energetic than either FAB or FDMS, several fragments ions were also observed. None, however, corresponded to the $[M^+]/2$ ion.

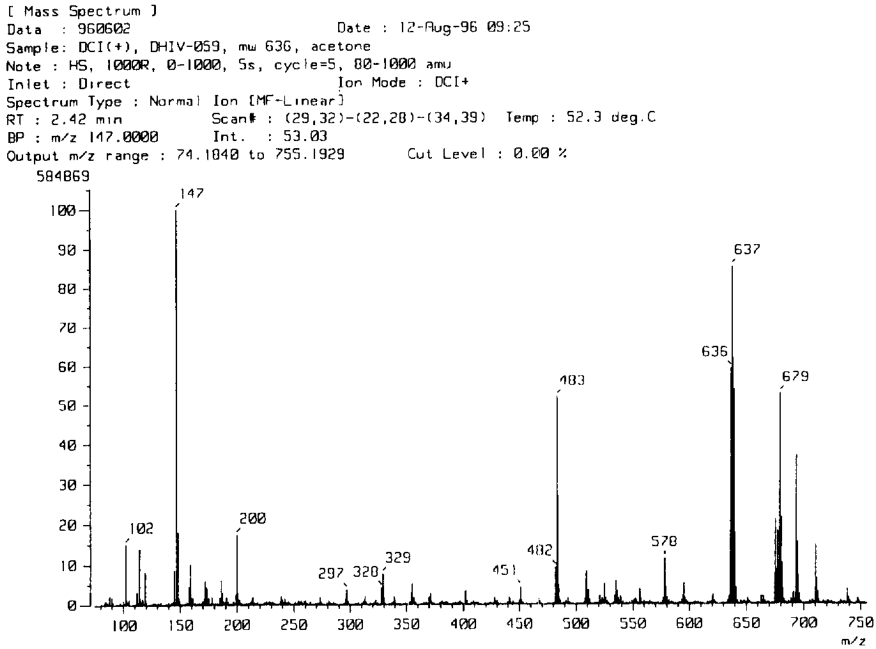
FDMS was also useful in helping us characterize products from the attempted synthesis of pigment **6** (Fig. 5). In this case, the molecular ion corresponding to the target structure could not be confirmed, as the intensity of the peak at m/z 575 is not above the background noise level. Interestingly, the peaks in the FDMS spectrum of the reaction products reveal that the



```
[ Mass Spectrum ]
Data  : 960495                                   Date : 17-Jul-96 10:47
Sample: FD(+), DHIV059, mw 636, acetone
Note : 1000R, MF, 0-2000, 20s, cycle=14, 40-1000
Inlet : Direct                                   Ion Mode : FD+
Spectrum Type : Normal Ion [MF-Linear]
RT : 2.57 min            Scan# : (8,16)
BP : m/z 636.0000        Int.   : 0.35
Output m/z range : 0.0000 to 1014.8368          Cut Level : 0.00 %
```

```
[ Mass Spectrum ]
Data  : 960564                        Date : 05-Aug-96 12:44
Sample: FAB(+),DHIV-059, mw 636, NBA/MeOH
Note : 1000R, MF, 0-2000, 20s, cycle=14, 200-1500 amu
Inlet : Direct                        Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.70 min          Scan# : (3,5)                    Temp : 54.3 deg.C
BP : m/z 307.2285      Int.   : 94.29
Output m/z range : 375.5193 to 915.5706     Cut Level : 0.00 %
```

Fig. 2. (a) FD, (b) FAB and (c) DCI mass spectra of **3d**.



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[ Mass Spectrum ]
Data   : 960602                        Date : 12-Aug-96 09:25
Sample: DCI(+), DHIV-059, mw 636, acetone
Note : HS, 1000R, 0-1000, 5s, cycle=5, 80-1000 amu
Inlet : Direct                         Ion Mode : DCI+
Spectrum Type : Normal Ion [MF-Linear]
RT : 2.42 min          Scan# : (29,32)-(22,28)-(34,39)  Temp : 52.3 deg.C
BP : m/z 147.0000      Int.   : 53.03
Output m/z range : 74.1040 to 755.1929        Cut Level : 0.00 %
```
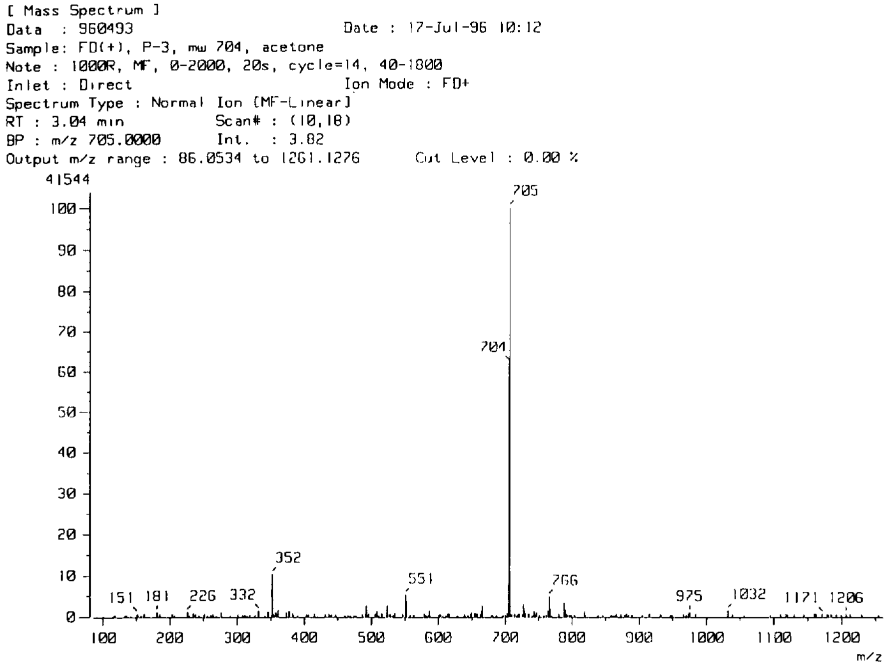
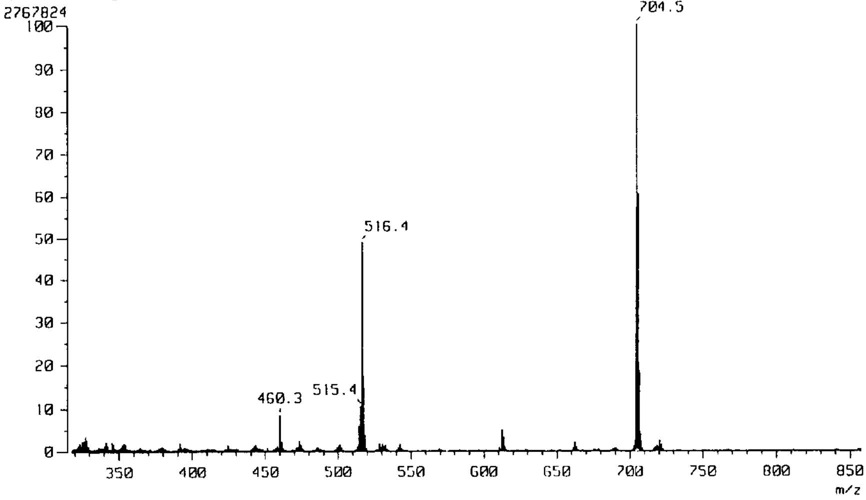

Fig. 2. cont.



```
[ Mass Spectrum ]
Data   : 960493                        Date : 17-Jul-96 10:12
Sample: FD(+), P-3, mw 704, acetone
Note : 1000R, MF, 0-2000, 20s, cycle=14, 40-1800
Inlet : Direct                         Ion Mode : FD+
Spectrum Type : Normal Ion [MF-Linear]
RT : 3.04 min          Scan# : (10,18)
BP : m/z 705.0000      Int.   : 3.82
Output m/z range : 86.0534 to 1261.1276       Cut Level : 0.00 %
```

Fig. 3 (a) FD, (b) FAB and (c) DCI mass spectra of **4a**.

```
[ Mass Spectrum ]
Data   : 960561                          Date : 05-Aug-96 12:03
Sample: FAB(+),P-3, mw 703, NBA/acetone
Note  : 1000R, MF, 0-2000, 20s, cycle=14, 200-1500 amu
Inlet : Direct                           Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.70 min           Scan# : (3,5)                  Temp : 54.3 deg.C
BP : m/z 704.4655       Int.   : 87.99
Output m/z range : 317.6558 to 857.7151        Cut Level : 0.00 %
```



```
[ Mass Spectrum ]
Data   : 960G01                          Date : 12-Aug-96 09:11
Sample: DCI(+), P-3, mw 704, acetone
Note : HS, 1000R, 0-1000, 5s, cycle=5, 80-1000 amu
Inlet : Direct                           Ion Mode : DCI+
Spectrum Type : Normal Ion [MF-Linear]
RT : 2.09 min           Scan# : (24,28)-(18,20)-(31,34)  Temp : 52.4 deg.C
BP : m/z 705.0000       Int.   : 26.04
Output m/z range : 75.6677 to 777.4481        Cut Level : 0.00 %
```

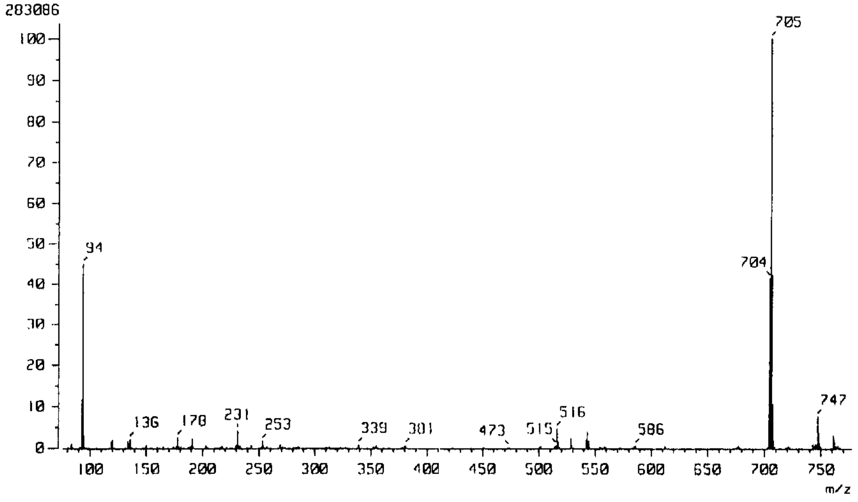
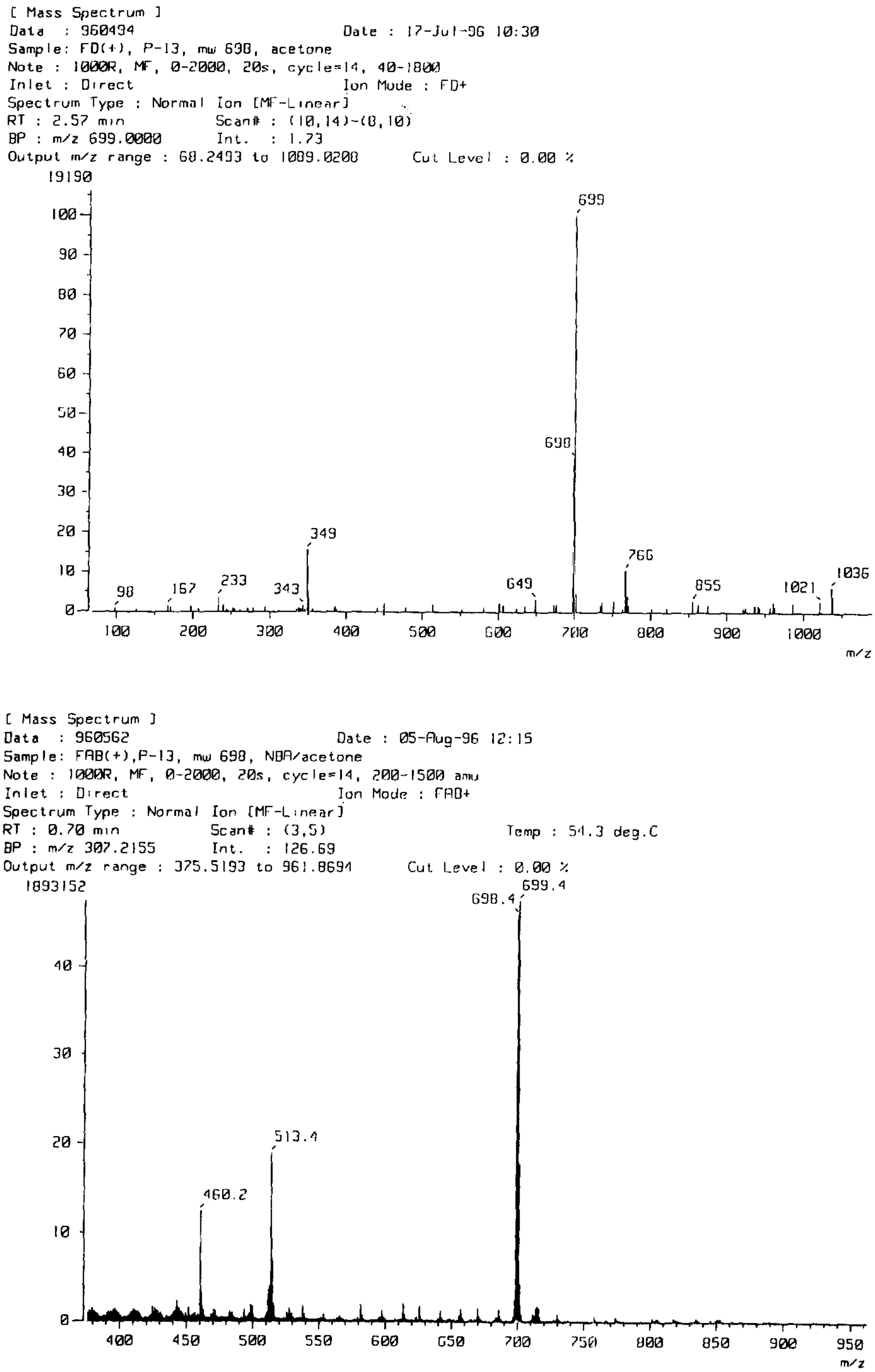


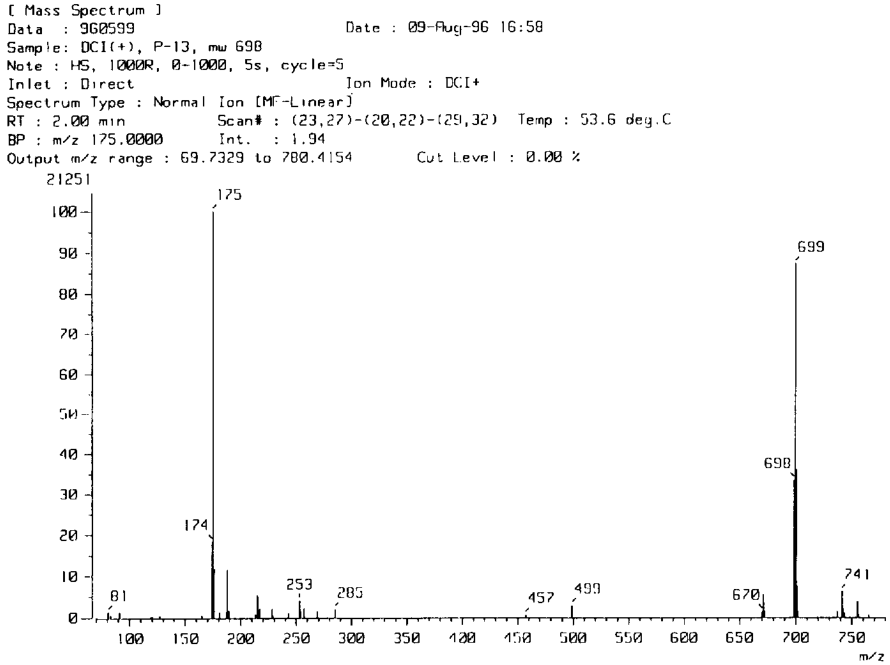
Fig. 3 cont.



```
[ Mass Spectrum ]
Data   : 960494                          Date : 17-Jul-96 10:30
Sample: FD(+), P-13, mw 698, acetone
Note : 1000R, MF, 0-2000, 20s, cycle=14, 40-1800
Inlet : Direct                           Ion Mode : FD+
Spectrum Type : Normal Ion [MF-Linear]
RT : 2.57 min           Scan# : (10,14)-(8,10)
BP : m/z 699.0000       Int.   : 1.73
Output m/z range : 68.2493 to 1089.0200      Cut Level : 0.00 %
```

```
[ Mass Spectrum ]
Data   : 960562                          Date : 05-Aug-96 12:15
Sample: FAB(+),P-13, mw 698, NBA/acetone
Note : 1000R, MF, 0-2000, 20s, cycle=14, 200-1500 amu
Inlet : Direct                           Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.70 min           Scan# : (3,5)                     Temp : 54.3 deg.C
BP : m/z 307.2155       Int.   : 126.69
Output m/z range : 375.5193 to 961.8694      Cut Level : 0.00 %
```

Fig. 4 (a) FD, (b) FAB and (c) DCI mass spectra of **5a**.

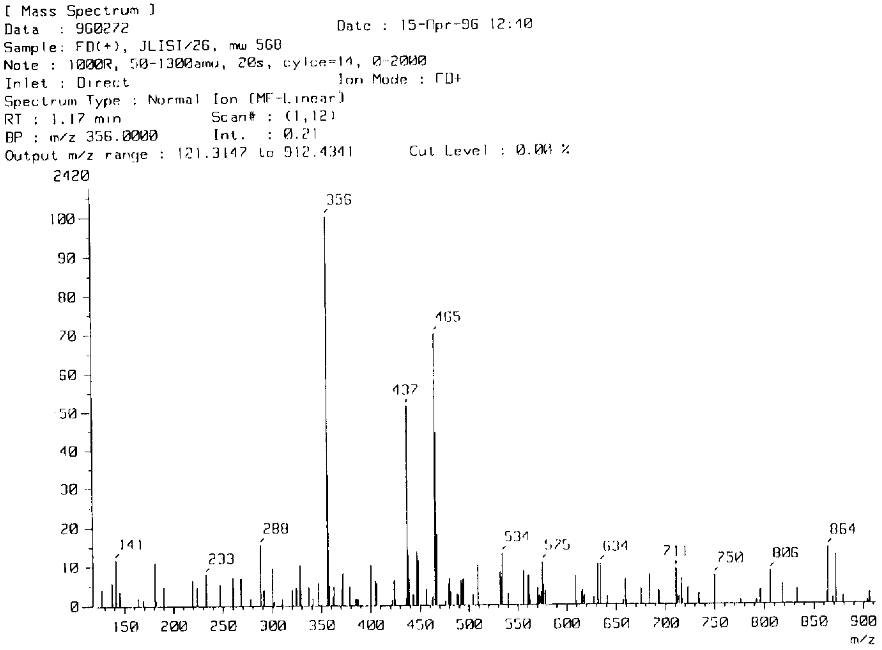


```
[ Mass Spectrum ]
Data   : 960599                           Date : 09-Aug-96 16:58
Sample: DCI(+), P-13, mw 698
Note : HS, 1000R, 0-1000, 5s, cycle=5
Inlet : Direct                            Ion Mode : DCI+
Spectrum Type : Normal Ion [MF-Linear]
RT : 2.00 min           Scan# : (23,27)-(20,22)-(29,32)  Temp : 53.6 deg.C
BP : m/z 175.0000       Int.   : 1.94
Output m/z range : 69.7329 to 780.4154       Cut Level : 0.00 %
```

Fig. 4 cont.

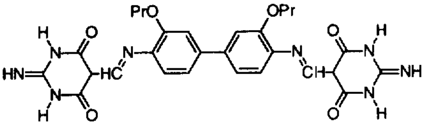
TABLE 1
Major Peaks Present in FDMS Spectra of Pigments **3**–**5**

Pigment	*m/z*	*Relative abundance (%)*
3a	608	100
	304	24
3b	488	100
	244	5
3c	604	100
	302	14
3d	636	100
	318	47
4a	705	100
	352	15
4b	764	100
	382	10
4c	736	100
	368	17
5a	699	100
	349	17
5b	767	100
	383	14
5c	730	100
	365	5



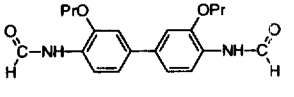
```
[ Mass Spectrum ]
Data   : 960272                              Date : 15-Apr-96 12:10
Sample: FD(+), JLISI/26, mw 568
Note : 1000R, 50-1300amu, 20s, cylce=14, 0-2000
Inlet : Direct                               Ion Mode : FD+
Spectrum Type : Normal Ion [MF-Linear]
RT : 1.17 min              Scan# : (1,12)
BP : m/z 356.0000          Int.   : 0.21
Output m/z range : 121.3147 to 912.4341        Cut Level : 0.00 %
```

Fig. 5. FD mass spectrum obtained following the attempted synthesis of **6**.

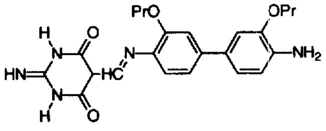


6 (m/z 574)

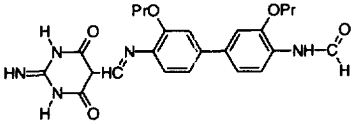
starting amine (3,3′-dipropoxybenzidine) reacted with triethylorthoformate to produce diformylated benzidine **7** and mono azomethines **8** and **9**. These results were consistent with the combustion analysis of this sample.



7 (m/z 356)



8 (m/z 437)



9 (m/z 465)

CONCLUSIONS

The results of this investigation demonstrate that FDMS merits preference over FAB and DCI mass spectrometry in the characterization of pigments derived from benzidines of the type employed in our study. The spectrum of such pigments is characterized not only by an intense parent molecular ion peak but also by a fragment ion corresponding to $[M^+]/2$. As a consequence of minimal fragmentation, FDMS is also useful for characterizing mixtures that may arise from pigment synthesis employing benzidines bearing bulkyl substituents *ortho* to the amino groups.

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