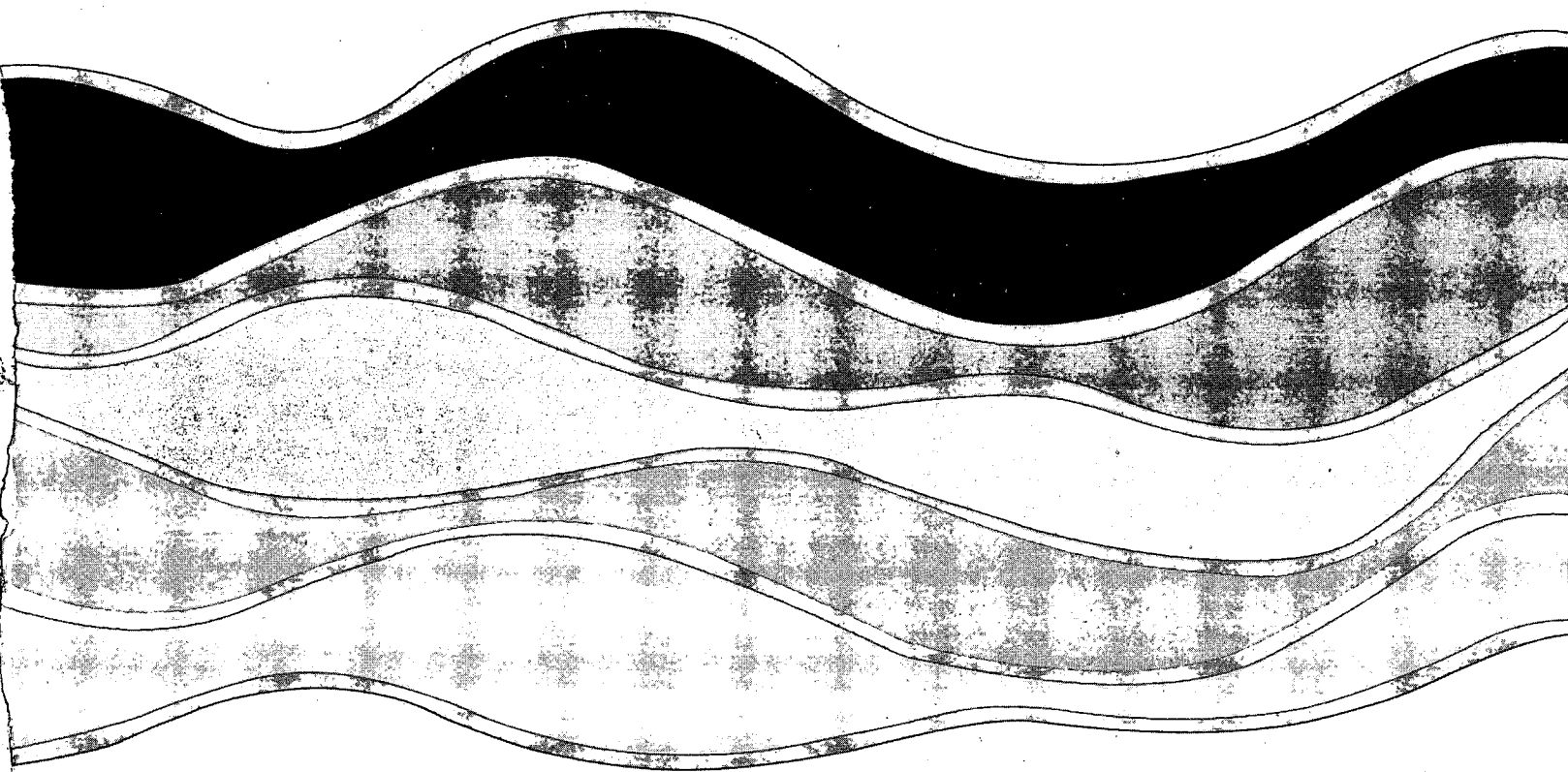
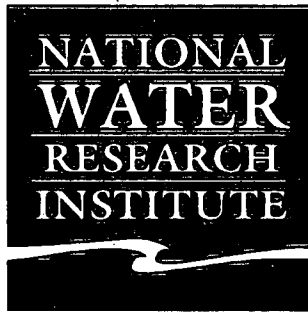


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**HIGH RESOLUTION GAS CHROMATOGRAPHY -  
MASS SPECTROMETRY OF SOME BENZIDINES  
IN INDUSTRIAL WASTE WATER**

**F.J. Onuska, K.A. Terry and R.J. Maguire**

**NWRI Contribution No. 97-202**

**High Resolution Gas Chromatography - Mass Spectrometry of  
Some Benzidines in Industrial Waste Water**

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## Management Perspective

Some aromatic amines used industrially are highly toxic and carcinogenic, and little is known of their occurrence in the Canadian environment. 3,3'-Dichlorobenzidine and benzidine, in particular, are carcinogenic substances that were declared toxic in CEPA PSL1 assessments under Section 11(c). At the time of the assessment there were no data on the occurrence of these two substances in Canada. It is important for Environment Canada to satisfy itself that substances found toxic under Section 11(c) but not 11(a) because there were no data on environmental occurrence are not present in the Canadian environment in concentrations or amounts that would pose a hazard to the environment. In the course of the Strategic Options Process deliberations on 3,3'-dichlorobenzidine after the finding that it was "CEPA-toxic", one company in Toronto identified itself as possibly the only user of 3,3'-dichlorobenzidine in the Canadian pigment industry. The purpose of this work was to determine whether this company was discharging 3,3'-dichlorobenzidine in its waste water. 3,3'-Dichlorobenzidine, benzidine, aniline (another PSL1 substance) and some substituted anilines were identified in four 24-hour composite samples of industrial waste water from the company during 1996. Further work will be aimed at determining the temporal nature of the discharge, and of the effectiveness of the sewage treatment plant to which it discharges in removing these substances from waste water.

## Sommaire à l'intention de la direction

Certaines amines aromatiques utilisées par l'industrie sont fortement toxiques et cancérigènes; en outre, on sait peu de chose de leur présence dans l'environnement canadien. En particulier, la 3,3'-dichlorobenzidine et la benzidine sont des substances cancérigènes qui ont été déclarées toxiques au terme des évaluations des substances paraissant sur la première liste des substances d'intérêt prioritaire (LSIP 1) en vertu de l'alinéa 11(c) de la *Loi canadienne sur la protection de l'environnement* (LCPE). Il n'existait aucune donnée sur la présence de ces deux substances dans l'environnement canadien au moment de leur évaluation. Environnement Canada doit s'assurer que les substances jugées toxiques aux termes de l'alinéa 11(c), mais pas en vertu de l'alinéa 11(a) parce qu'il n'existait pas de données sur leur présence dans le milieu, ne sont pas trouvées en concentration ou en quantité dangereuse pour l'environnement. Pendant les délibérations portant sur la 3,3'-dichlorobenzidine, dans le cadre du processus des options stratégiques, après que cette substance ait été déclarée toxique au sens de la LCPE, une entreprise de Toronto a déclaré qu'elle pouvait être la seule utilisatrice de cette substance au sein du secteur canadien des pigments. Cette recherche avait pour but de déterminer si cette entreprise déversait de cette substance avec ses eaux usées. En 1996, la 3,3'-dichlorobenzidine, la benzidine, l'aniline (une autre substance figurant sur la LSIP1) et certaines anilines à substituant ont été identifiées dans des échantillons composés de 24 heures des eaux usées industrielles de l'entreprise. Dans la suite des travaux, les chercheurs tâcheront de déterminer le caractère temporel des rejets et l'efficacité de l'usine de traitement des eaux d'égout pour éliminer ces substances.

## **Abstract**

The analysis of aromatic amines, particularly benzidines, at trace levels in environmental media has been difficult because of the lack of suitably deactivated capillary column stationary phases for gas chromatography. This report describes the use of a newer type of column, and it describes a method for the analysis of benzidines in waste water. The method was applied to the analysis of industrial effluent from a pigment-manufacturing company. 3,3'-Dichlorobenzidine and benzidine were found in four 24-hour composite waste water samples in 1996 at concentrations up to 650  $\mu\text{g/L}$ . Aniline and some substituted anilines were also found in one of the composite samples.

## Résumé

Faute de disposer de phases stationnaires adéquatement désactivées pour les colonnes capillaires servant à la chromatographie en phase gazeuse, il s'est révélé difficile de doser les amines aromatiques, particulièrement les benzidines, à l'état de traces dans les différents compartiments de l'environnement. Cet article décrit l'emploi d'un nouveau type de colonne ainsi qu'une méthode d'analyse de la benzidine dans des eaux usées. La méthode a été appliquée à celle d'effluents industriels produits par un fabricant de pigments. En 1996, les chercheurs ont trouvé de la 3,3'-dichlorobenzidine et de la benzidine dans des échantillons composés de 24 heures d'eaux usées à des concentrations pouvant atteindre 650 µg/L. L'aniline et des anilines à substituant ont aussi été identifiées dans l'un des échantillons composés.

## Introduction

Ionic and polar aromatic amines are used as intermediates for manufacturing synthetic dyes and in the synthesis of various herbicides. These compounds pose a challenge to environmental analytical chemists because they are very difficult to quantify, especially at low parts-per-billion (ppb) levels in industrial waste water and sewage. It is important to be able to quantify chemicals such as benzidine, dimethylbenzidine, dimethoxybenzidine (dianisidine) and 3,3'-dichlorobenzidine because they are suspected or demonstrated carcinogens (1). Various analytical methods have been employed for their quantitation, such as colorimetric methods based on diazotization and coupling reactions (2-5), and the spectrofluorimetric methods developed by Rindle *et al.* (6). None of these methods, however, provides the sensitivity, specificity, accuracy and precision required for environmental trace analysis.

The U.S. Environmental Protection Agency (EPA) proposed high performance liquid chromatography (HPLC) and HPLC-mass spectrometry (MS) methods (7, 8) such as Method 8321 for phenoxy acid herbicides (7) and Method 553 for benzidines (8). Applications of capillary HPLC (9) and micellar electrokinetic chromatography (10) are not particularly suitable for environmental analysis due to their limitation with amount of injection volume and their detection limits. Some manufacturers of open tubular columns claim that it is possible to separate aromatic amines with their WCOT-amine columns without any pretreatment. However, we have found the analysis of benzidine from environmental samples to be difficult and not reproducible because of adsorption effects, especially with injected amounts less than 50 nanograms (unpublished observations).

Benzidine and 3,3'-dichlorobenzidine in the presence of other aromatic amines can be derivatized to trifluoroacetyl (TFA) derivatives by treatment with trifluoroacetic anhydride (11). The TFA derivatives can be analyzed by high resolution gas chromatography (HRGC) and mass spectrometry (MS). Significantly improved peak shapes are obtained and detection limits are also significantly better. This report describes the procedure and its preliminary application to industrial waste water.

## Experimental Methods

### Chemicals

Aniline, monochloroaniline, dichloroanilines (Aldrich Chemicals, Wisconsin, U.S.A.) and benzidine, (m.p. 127-129 °C), 3,3'-dimethoxybenzidine (3,3'-DMOB), (m.p. 137-139 °C), 3,3'-dimethylbenzidine (3,3'-DMB) (m.p. 129-

131 °C), 3,3'-diaminobenzidine and 3,3'-dichlorobenzidine (3,3'-DCB) (Ultra Chemicals) were used. Trifluoroacetyl derivatives were prepared according to the method of Irvine and Saxby (11). Organic solvents were pesticide grade, and all reagents were of the highest purity available.

### Gas chromatography

All experimental work was done with a Hewlett Packard Model 5890A gas chromatograph equipped with a pressure-programmed injection system and MS detector. The HP mass spectrometer Engine system was connected to the UNIX data system and both selected ion monitoring mode of operation (SIM) and full scan mass spectra of the total ion chromatogram were collected. The separation was carried out on a WCOT amine DB-LXB column (J&B Scientific Co.), 35 m x 0.2 mm i.d. and film thickness of 0.33  $\mu\text{m}$  with temperature programming from 80 °C to 280 °C at 5 °C per minute. Helium was used as the carrier gas.

### Extraction

The filtration/extraction concentration scheme shown in Figure 1 is a generalized procedure which allows for the analysis of aromatic amines in raw sewage, secondary effluents, river water or industrial waste water.

The pH of a 1 litre water sample is adjusted to 12 using NaOH. The solution should be filtered through a glass fiber filter. The sample is then extracted with methylene chloride (100 mL x 3). Fifty mL of benzene is then added to the combined extracts and the volume is reduced to 5 mL with a rotary evaporator. The sample extract is then fractionated using 10 g of basic alumina (BioRad, 100-200 mesh). A wet slurry is prepared using methylene chloride:benzene (1:1) and transferred to a 30 cm x 11 mm i.d. column equipped with a Teflon stopcock. The extract is applied to the column, which is then eluted with 50 mL of methylene chloride:benzene (1:1) to collect fraction A. The second fraction (B) is collected by eluting with 25 mL of methanol. The column is regenerated with a further 100 mL of methanol, which may be collected as fraction C for the analysis of other compounds, if desired. Anilines, aniline- $d_5$  as an internal standard, benzidine and 3,3'-dichlorobenzidine elute in fractions A and B. The combined fractions are concentrated to 5 mL, then 100 mL of acetonitrile is added, evaporated to 5 mL, transferred to a 15 mL screw top centrifuge tube and reduced in volume to 1 mL with a gentle stream of helium.

The extract is derivatized with 0.2 mL of trifluoroacetic anhydride. The reaction mixture is shaken for several minutes, then allowed to stand at room temperature for 1 hour. One mL of toluene is then added to the extract and excess trifluoroacetic anhydride is removed by the addition of 2 mL of 1 M  $\text{NaHCO}_3$ , with shaking for 1 minute. The water layer is removed with a disposable pipette and the extract is washed three times with 2 mL of water each



time. The organic layer is then dried with sodium sulfate, and 1-2  $\mu\text{L}$  samples are injected into the GC-MS. The organic extract was stored at  $-16\text{ }^{\circ}\text{C}$  and no degradation was observed in organic solvent even after 3 months storage at this temperature.

Extraction recovery experiments were done by spiking distilled water at 1 and 20  $\mu\text{g/L}$  by adding 1 mL of methanol containing the appropriate amount of the test compound. In addition, samples of an industrial waste water were analyzed to determine the presence of benzidines.

## Results and Discussion

Analytical standards of several benzidines were examined to establish optimum operating conditions for their detection. Table 1 shows their gas chromatographic retention times and the  $m/z$  number of the ions used in their quantitation by mass spectrometry. The electron-impact mass spectra of benzidine, 3,3'-dichlorobenzidine, 3,3'-dimethoxybenzidine and 3,3'-dimethylbenzidine are shown in Figs. 1-4. HRGC-MS-SIM responses for benzidine and 3,3'-dichlorobenzidine were measured using aniline- $\text{D}_5$  as an internal standard. Accuracy and precision data from triplicate determinations (see Table 2) indicate that the method is very reproducible. Detection limits for benzidine and 3,3'-dichlorobenzidine are about 200 ppt with a 1  $\mu\text{L}$  injection (assuming a 1 mL extract from a 1 litre sample). The linear range of the SIM detector extends over about three orders of magnitude.

Samples of tap-water fortified with benzidine and 3,3'-dichlorobenzidine at the 5 and 100 ppb levels were analyzed to obtain validation results for HRGC-MS-SIM. Mean recoveries and precision estimates for a single analyst are shown in Table 3. The recoveries averaged about 65% with a standard deviation between 9% and 14% for each analyte.

Four 24-hour composite industrial effluent samples were collected by the Toronto Metropolitan Works Department during 1996 close to a company that used 3,3'-dichlorobenzidine in the manufacture of pigments. The water samples were immediately transported to our laboratory, where they were extracted and analyzed within 48 hours. The results of the analyses are shown in Table 4. 3,3'-Dichlorobenzidine was found in the effluent at concentrations up to 654  $\mu\text{g/L}$ . Benzidine was also found in the effluent, at concentrations up to 374  $\mu\text{g/L}$ . In addition aniline and some substituted anilines were found.

Preliminary work has indicated that 3,3'-dichlorobenzidine was found in the influent of the Toronto Humber sewage treatment plant that services the company whose effluent contained the 3,3'-dichlorobenzidine. This work has also shown that 3,3'-dichlorobenzidine was below our limits of detection in the

sewage treatment plant effluent that is discharged to Lake Ontario. Sludge analyses are not yet available. Work is continuing with a more intensive temporal sampling of the industrial effluent, and of the influent, effluent and sludges from the Toronto Humber sewage treatment plant, in order to characterize the extent of contamination by benzidines and other aromatic amines, and to assess the effectiveness of the sewage treatment plant in the removal of these substances from waste water.

### Acknowledgments

We thank Mr. J. Chan and Mr. M.P. Shaw of the Metropolitan Works Department of the Municipality of Metropolitan Toronto for providing the samples of industrial effluent. This work was partially supported by the Great Lakes 2000 Program of the Department of the Environment.

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Table 1. Retention time and quantitation ions for selected analytes

Compound	Retention time (min)	Quantitation ions (m/z)
benzidine	20.409	184
3,3'-dichlorobenzidine	25.556	252
3,3'-dimethoxybenzidine	25.600	244
3,3'-dimethylbenzidine	23.391	212
benzidine-D <sub>8</sub>	NA	192
3,3'-dichlorobenzidine-D <sub>6</sub>	NA	258

**Table 2. Accuracy and Precision Data from Triplicate Determinations**

<b>Compound</b>	<b>True conc., ppb</b>	<b>Mean, ppb</b>	<b>S.D. ppb</b>	<b>%RSD</b>	<b>DL, ppb</b>
<b>benzidine</b>	<b>22.8</b>	<b>21.5</b>	<b>0.8</b>	<b>3.0</b>	<b>0.20</b>
<b>3,3'-DCB</b>	<b>25.0</b>	<b>22.7</b>	<b>0.7</b>	<b>2.9</b>	<b>0.21</b>
<b>3,3'-DMOB</b>	<b>35.0</b>	<b>33.0</b>	<b>1.3</b>	<b>3.7</b>	<b>0.60</b>
<b>3,3'-DMB</b>	<b>34.0</b>	<b>32.8</b>	<b>1.0</b>	<b>3.1</b>	<b>0.30</b>

Recovery was in the range 42-76 %.

**Table 3. Mean Recoveries, Precision and Estimates of Single Analyst (SA) for Measurements of Benzidine and 3,3'-Dichlorobenzidine**

Compound	5 µg/L		100 µg/L	
	% Recovery	%RSD-SA	%Recovery	%RSD-SA
Benzidine	46.0	12	74.0	11
3,3'-DCB	42.0	9	80.5	9

Table 4. Temporal variation of benzidines and anilines in industrial waste water

Date	Benzidine µg/L	3,3'-Dichlorobenzidine µg/L	Others µg/L
03-13-96	ND	15.4	nil
07-13-96	374.0	654.0	An 12.8
ditto	374.0	654.0	2,6-DMA 811.0
ditto	374.0	654.0	2-ClAn 6.9
ditto	374.0	654.0	4-Cl-2NAn 3.5
09-10-96	9.5	30.5	nil
12-10-96	13.4	145.0	nil

Note: An=aniline; 2,6-DMA 2,6-dimethylaniline; 2ClAn 2-chloroaniline; 4Cl-2NAn 4-chloro-2-aniline.

## **Figure Captions**

**Figure 1. Electron impact mass spectrum of benzidine-TFA.**

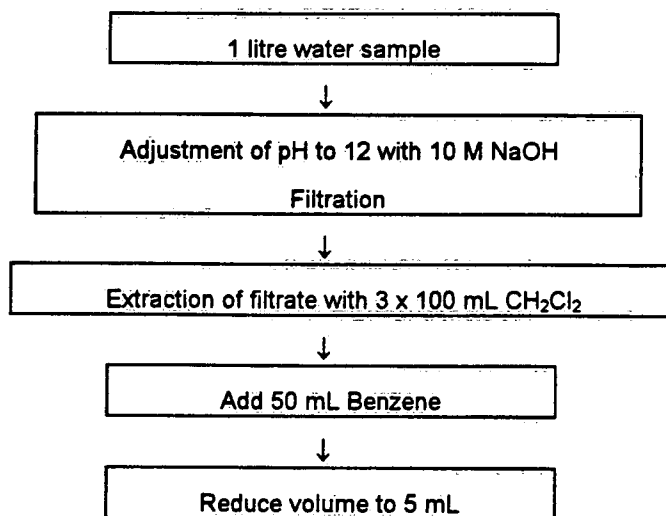
**Figure 2. Electron impact mass spectrum of 3,3'-dichlorobenzidine-TFA.**

**Figure 3. Electron impact mass spectrum of 3,3'-dimethoxybenzidine-TFA.**

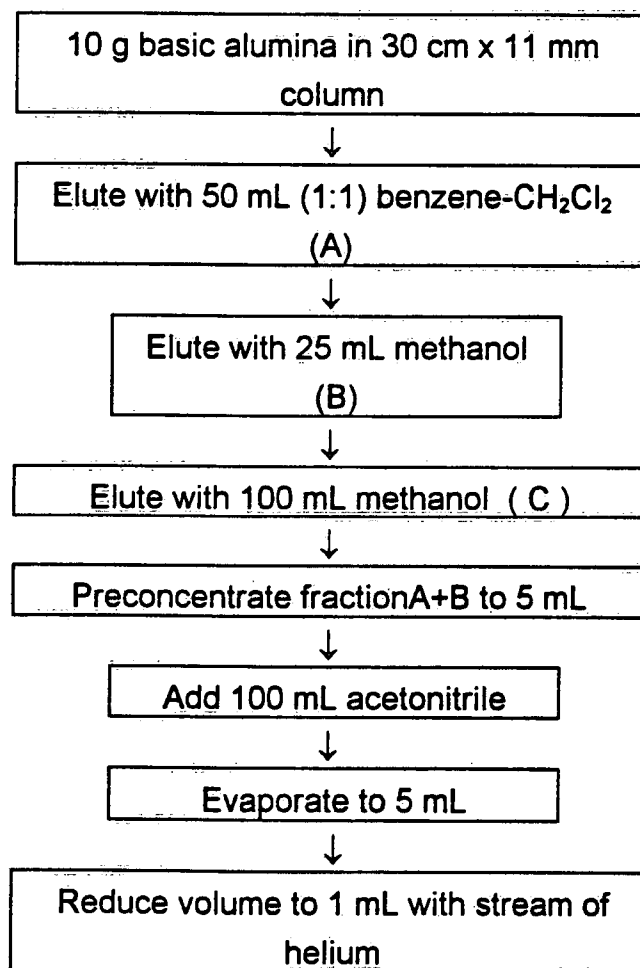
**Figure 4. Electron impact mass spectrum of 3,3'-dimethylbenzidine-TFA.**

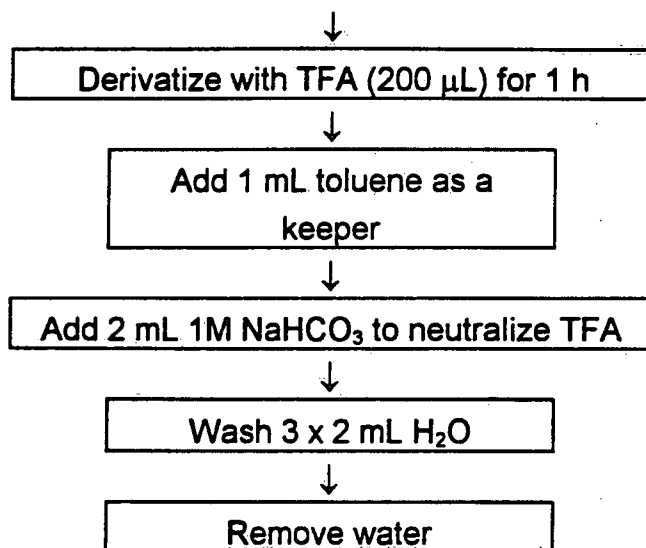


## Sample Cleanup Scheme



## Column Chromatography and Derivatization





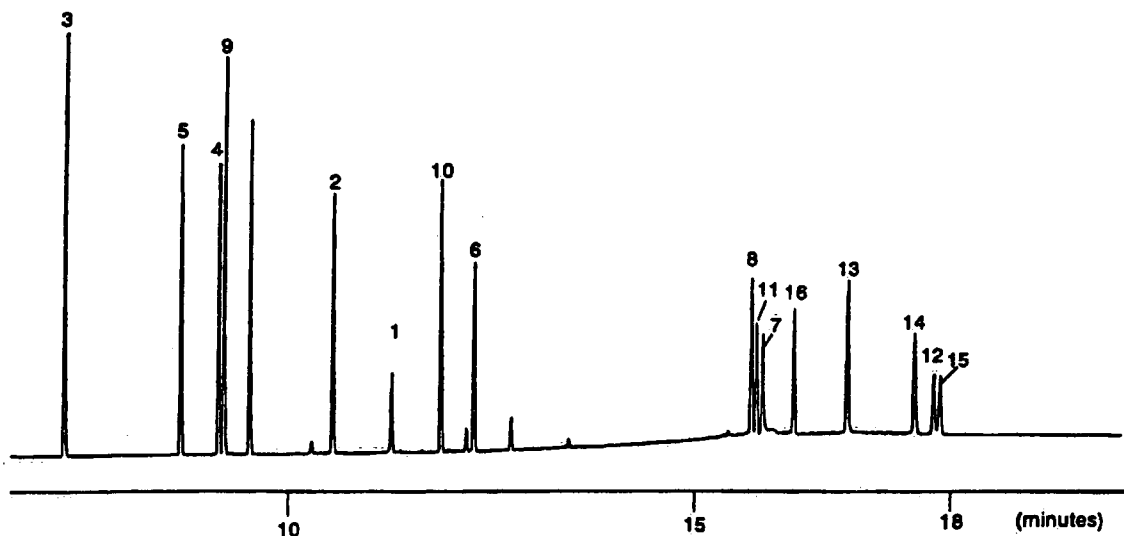
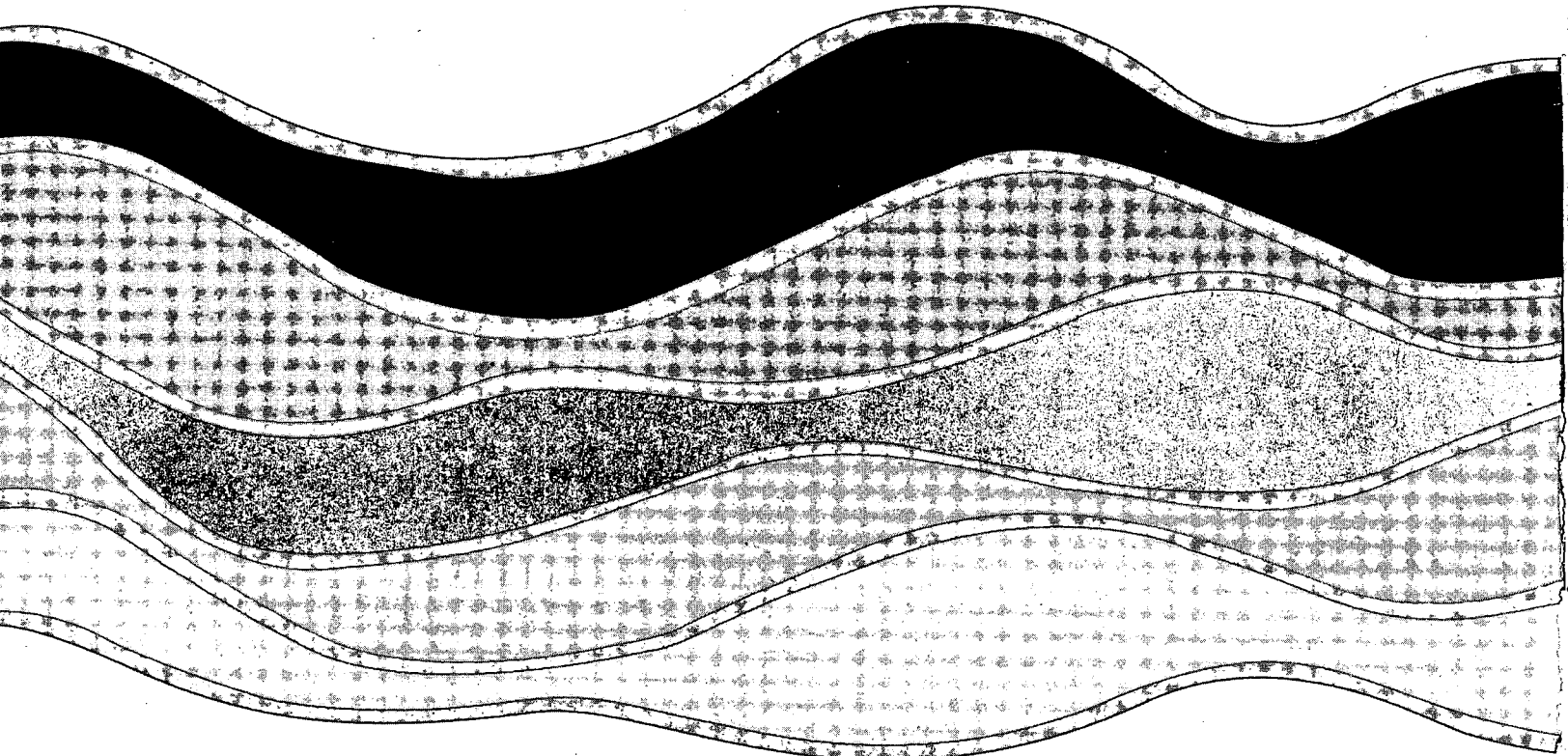


Figure 2: Benzidines and anilines by DB-LXB

Column: DB-LXB, 30m x 0.20mm I.D., x 0.33  $\mu$ m; w/ 1m x 0.32 mm I.D. retention gap; Oven: 50 °C for 2 min, 50-340 °C at 20'/min, hold at 340°C for 10 min; Carrier: Helium at 35 cm/sec at 50 °C; Injector: Splitless, purge on at 0.5min, 280 °C; Detector: FID, 320 °C; Nitrogen make up gas at 30 mL/min.

1. 2,4-Diaminoanisole ; 2. 2,4-Diaminotoluene; 3. o-Toluidine; 4. 2-Methoxy-5-methylaniline; 5. 4-Chloroaniline; 6. 2-Methyl-5-nitroaniline; 7. Benzidine; 8. 4,4'-Oxydianiline; 9. 2,4,5-Trimethylaniline; 10. 2-Aminonaphthalene; 11. 4,4'-Methylenedianiline; 12. 3,3'-Dimethoxybenzidine; 13. o-Tolidine; 14. 4,4'-Thioaniline; 15. 3,3'-Dichlorobenzidine; 16. 2-Aminoazotoluene.



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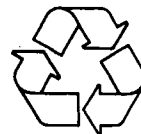


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