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SEPARATION AND DETERMINATION OF XYLIDINE ISOMERS

COMPARISON OF GAS AND LIQUID CHROMATOGRAPHIC METHODS*

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SUMMARY

Gas and liquid chromatographic methods for the resolution and determination of xylidine isomers are described. The gas chromatographic method of choice involves a derivatization of xylidines with N-methylbistrifluoroacetamide, followed by immediate injection onto an Apiezon–Carbowax column. 3,4-Xylidine, the major compound of concern, is clearly separated from its isomers. Modification of this procedure allows for the determination of 3,4-xylenol as well. The latter can occur in low levels in samples of 3,4-xylidine. The optimum liquid chromatographic method involves the use of a normal-phase column and a solvent system consisting of methylene chloride–hexane–ethyl acetate (74:25:1). Detection is at 254 nm. This method resolves the six xylidine isomers in underivatized form.

INTRODUCTION

Xylidines are important industrial compounds, and therefore a rapid, reliable assay of these materials is needed. This report deals primarily with 3,4-xylidine.

The determination of 3,4-xylidine should consider two points. First, any method should resolve 3,4-xylidine from its isomers, of which there are six. Second, 3,4-xylidine and its isomers should be separated from process impurities. These could include xylenes, bromoxylenes, and/or xylenols.

There are a number of papers in the literature that describe gas chromatographic (GC)[1–3] and liquid chromatographic (LC)[4,5] approaches to the determination of xylidines. However, these are not entirely satisfactory with regard to resolution of isomers, quantitation, and/or ease of application.

This paper presents alternative methods, GC and LC, to those described in the literature. The GC method, which utilizes N-methylbistrifluoroacetamide (MBTFA) as derivatizing agent, and the LC method described herein are both reliable, rapid, and easy to use.

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EXPERIMENTAL

Apparatus

For the GC studies, a Hewlett-Packard gas chromatograph, Model 5840A, equipped with a flame ionization detector, and a 18850A data terminal were used. Columns used were:

(1) 3 m × 2 mm I.D., glass, packed with 3% Bentone-34 and 3% XE-60 on Gas-Chrom Q, 80–100 mesh, for underivatized xylidines;

(2) 3 m × 2 mm I.D., glass, packed with 4% Carbowax 20M and 10% Apiezon N on Chromosorb W, 60–80 mesh, for the MBTFA-derivatized xylidines;

(3) 50 m × 0.05 mm I.D., glass capillary wall-coated open tubular, WCOT, packed with Carbowax 20M, for underivatized xylidines.

For the LC studies, the following equipment was used: LDC Constametric III pump; LDC Spectromonitor III UV detector, set at 254 nm; Rheodyne Model 7125 injector with a 15-μl sample loop; LiChrosorb Si 60 column (24 cm × 4.6 mm I.D.). The mobile phase consisted of methylene chloride–hexane–ethyl acetate (74:25:1).

Chemicals and reagents

All chemicals were reagent grade and obtained commercially. LC solvents were glass-distilled, chromatographic grade, obtained from Burdick & Jackson (Muskegon, MI, U.S.A.). MBTFA was obtained from Pierce (Rockford, IL, U.S.A.).

Preparation of trifluoroacetylated xylidines for GC

About 20 mg of sample (containing xylidines) is dissolved in 2 ml of dry pyridine. To this solution is added 0.25 ml of MBTFA. The solution is warmed for 2–5 min at 80°C, and then diluted to 10 ml with additional dry pyridine. A 2-μl volume of this solution is injected into the gas chromatograph.

Operating conditions

For GC:

Column 1: helium flow-rate, 30 ml/min; column temperature, 150°C; injector temperature, 250°C; detector temperature, 300°C;

Column 2: helium flow-rate, 30 ml/min; column temperature, 170°C; injector temperature, 200°C; detector temperature, 300°C;

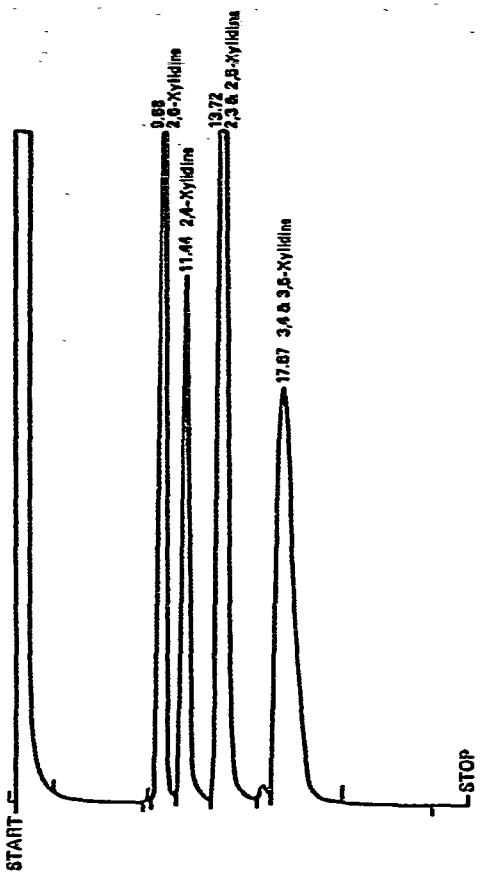
Column 3: nitrogen flow-rate, 1.0 ml/min; column temperature, 180°C; injector (splitless) temperature, 250°C; detector temperature, 300°C.

For LC: solvent flow-rate, 1.0 ml/min; effluent monitored at 254 nm.

RESULTS AND DISCUSSION

GC determination of xylidines

Without derivatization. In the initial attempts to determine 3,4-xylidine by GC, a 3 m × 2 mm I.D. column packed with 3% Bentone-34 and 3% XE-60 was used, in conjunction with a flame ionization detector. In this case, low levels of 3,4-xylidine in production samples of ribityl xylidine were determined. Also present in these samples were 2,3-xylidine, methanol, and water. For quantitation, benzyl alcohol was used as the internal standard. For samples known to contain only the 2,3- and 3,4-isomers,



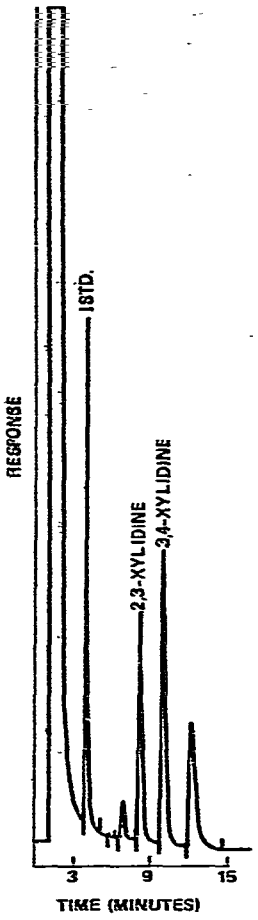


Fig. 1. GC of xylidine isomers on Bentone-34–XE-60.

Fig. 2. GC of xylidine sample on Bentone-34–XE-60. ISTD = Internal standard.

this method worked well. Xylidines of interest could be determined down to the 10-ppm level. However, the 2,5- and 3,5-isomers, if present, would elute together with the 2,3- and 3,4-xylidines (Fig. 1). A chromatogram of an actual sample is shown in Fig. 2.

With derivatization. To achieve better resolution of the xylidine isomers, a method described by Dove[1] was adapted. The primary interest was to determine the purity of production samples of 3,4-xylidine. MBTFA was used as the acetylating agent for the xylidines instead of trifluoroacetic anhydride as used by Dove. Samples were injected immediately following reaction with MBTFA onto a column (3 m × 2 mm I.D.) packed with 4% Carbowax 20M and 10% Apiezon N. Sample cleanup was unnecessary, and the 2,3- and 3,4-isomers were clearly separated from the other xylidines (Fig. 3). For quantitation, *n*-hexadecane was used as the internal standard.

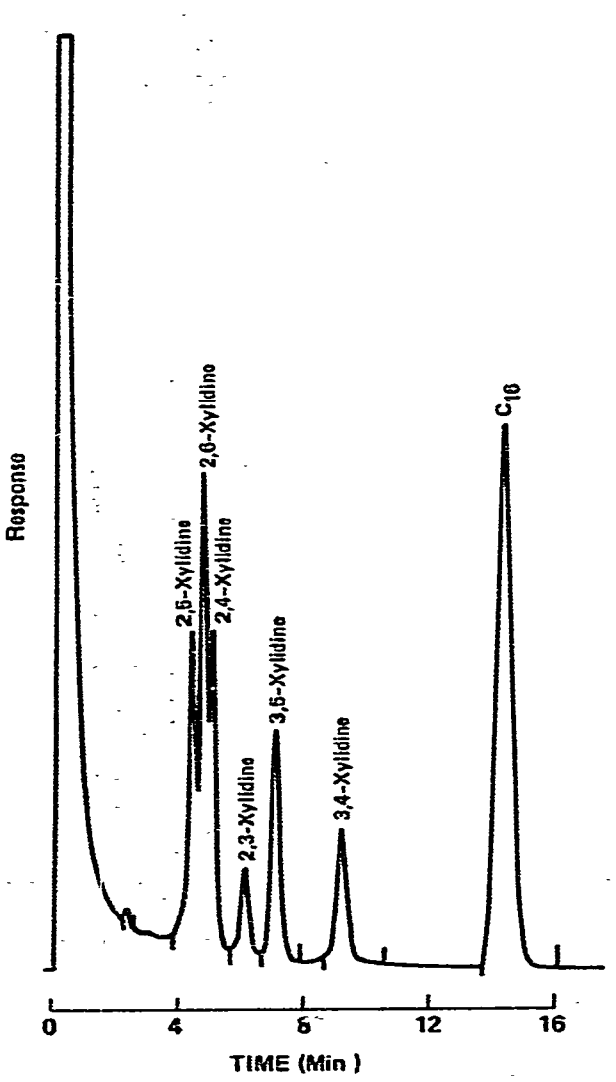


Fig. 3. GC of xylidine isomers on Carbowax 20M–Apiezon N.

To test the completeness of reaction of xylidine with MBTFA as a function of time, the constancy of response ratio (R_R, xylidine response: C_{16} response) was determined. Over a reaction time range of 1–150 min, the response ratio remained constant for the 2,3-, 3,5- and 3,4-isomers (Table I).

Experimental samples containing known amounts of xylidines were also analyzed. The GC assays were well in line with the expected results (Table II). That the method was reproducible was demonstrated by repetitive analysis of an actual production sample (Table III).

In production samples of 3,4-xylidine, a troublesome impurity proved to be 3,4-xylenol. In MBTFA-treated samples, it eluted, as the acetylated derivative, just after the solvent peak (Fig. 4a). However, on treatment of the derivatized sample with

TABLE I

COMPLETENESS OF ACYLATION WITH MBTFA AS A FUNCTION OF TIME

Time (min)	*R_R* values*		
	Xylidines		
	2,3-	*3,5-*	*3,4-*
1.0	0.995	0.954	1.00
25.0	0.990	0.948	0.995
55.0	0.999	0.958	1.00
150.0	0.997	0.957	1.00

* R_R = response ratio = $\frac{\text{peak area}_{\text{ref.}}}{\text{peak area}_{\text{int.std.}}} \times \frac{\text{wt.}_{\text{int.std.}}}{\text{wt.}_{\text{ref.}}}$

TABLE II

GC ANALYSIS OF SYNTHETIC XYLIDINE MIXTURES: RECOVERY DATA

Mixture designation	*Isomers present*	*Composition formulated (%, w/w)*	*Composition by analysis (%, w/w)*	*Recovery (%)*
A	3,4-	99.3	97.8	98.4
	2,3-	0.6*	0.6	100.0
B	3,4-	95.8	95.4	99.6
	2,3-	4.1*	4.1	100.0
C	3,4-	34.9	33.7	96.6
	2.3-	64.6*	63.4	98.1
D	3,4-	33.8	33.7	99.7
	2,3-	34.4	34.4	100.0
	3,5-	31.8	31.7	99.7

* The remainder of these mixtures consisted of small amounts of other isomers.

TABLE III

REPETITIVE GC ANALYSIS OF A PRODUCTION SAMPLE OF 3,4-XYLIDINE. STATISTICAL EVALUATION OF METHOD

Run No.	*Weight (mg)*		*3,4-Xylidine (%)*
	Sample	*C_{16}**	
1	24.6	31.2	97.7
2	33.4	31.7	98.3
3	23.6	28.4	99.3
4	24.6	31.3	99.2
5	24.1	30.3	98.7
6	25.1	31.8	98.9
7	26.5	32.0	97.9
Mean			98.6
Standard deviation (S.D.)			0.62
Coefficient of variation (C.V.) (%)			0.63

* Hexadecane was used as internal standard. Pyridine (10 ml) was the solvent, and MBTFA was the derivatization reagent.

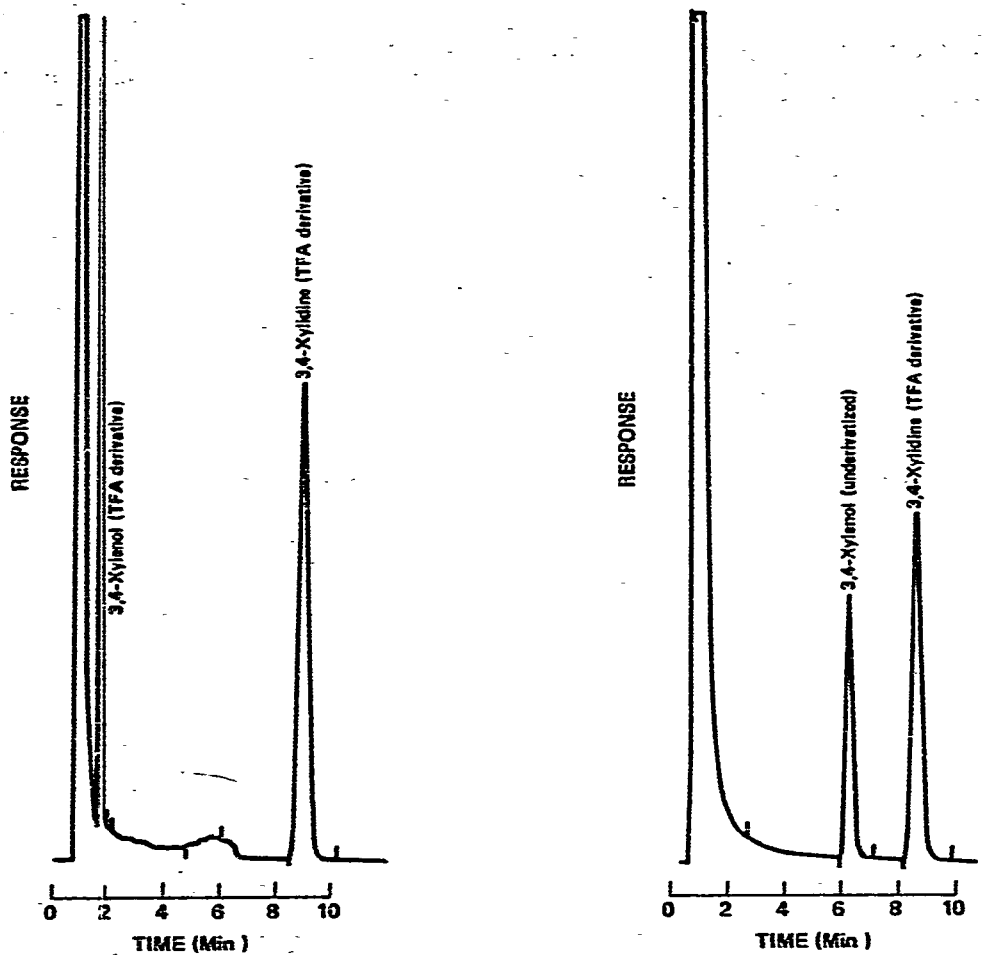


Fig. 4. GC of a mixture of 3,4-xylidine and 3,4-xylenol. (a), Immediately after reaction with MBTFA; (b), after treatment with $NaHCO_3$ and extraction with diethyl ether.

aqueous $NaHCO_3$, the acetylated 3,4-xylenol underwent hydrolysis, whereas the acetylated 3,4-xylidine did not, as indicated in the resultant chromatogram (Fig. 4b). The new peak corresponded to underivatized 3,4-xylenol and could be amenable to quantitation.

As a result of the foregoing manipulations, it was possible to develop a scheme (Fig. 5) for first determining 3,4-xylidine, after reaction with MBTFA, and subsequently 3,4-xylenol, following treatment of the sample with $NaHCO_3$. Peak assignment for the 3,4-xylenol was made by GC–mass spectrometry.

Other GC studies. For separating other impurities in xylidine samples by GC, a packing containing three phases was used: 5% SP-1200–1.75% Bentone 34, 5% OV-210 and 3% Versamid 900. The chromatogram obtained is shown in Fig. 6.

Materials that could be present in production samples of 3,4-xylidine are in-

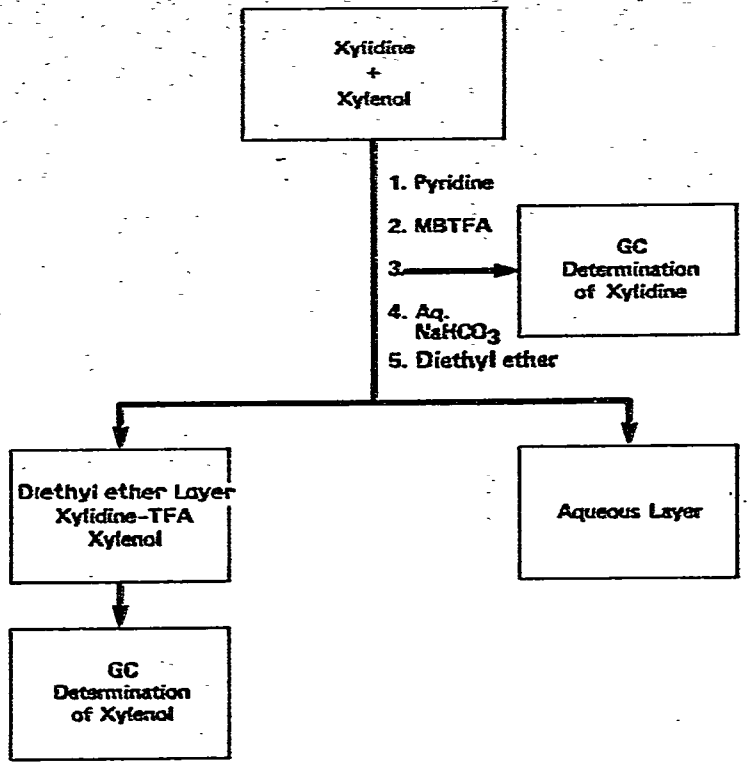


Fig. 5. Scheme for analyses of samples containing 3,4-xylidine and 3,4-xylenol.

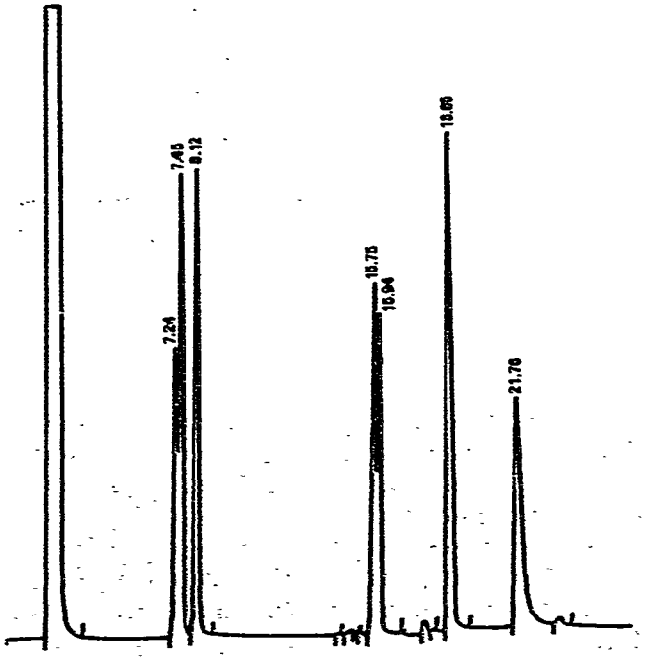
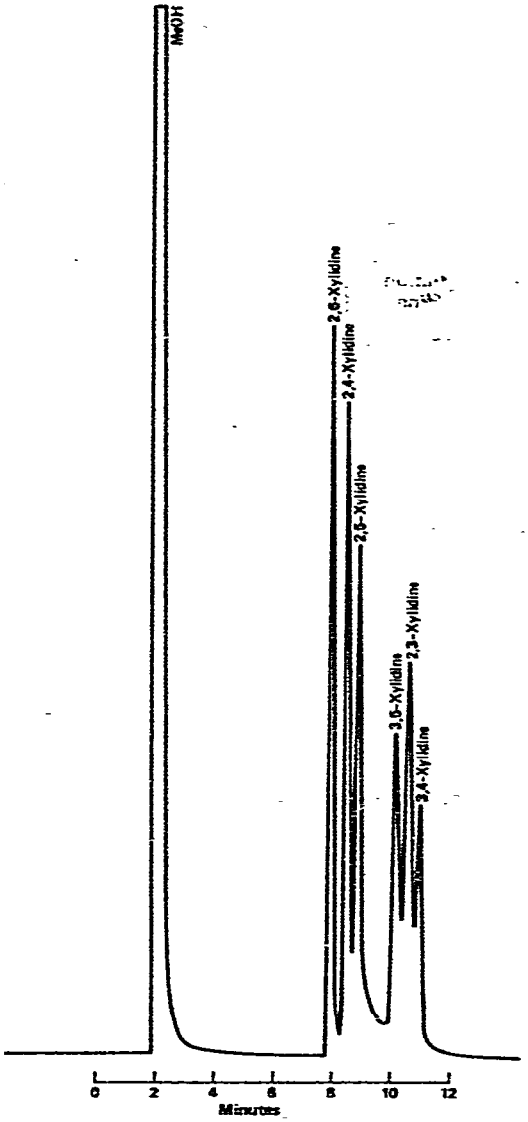


Fig. 6. GC of a mixture of xylenes, bromoxylenes, xylidine and xylenol. Compounds eluted: *p*-xylene (retention time 7.24 min); *m*-xylene (7.45 min); *o*-xylene (8.12 min); 3-bromo-*o*-xylene (15.75 min); 4-bromo-*o*-xylene (15.94 min); 3,4-xylidine (18.85 min); 3,4-xylenol (21.76 min). Conditions: glass column (4 m × 2 mm I.D.); column temperature, programmed from 70°C (after 4 min) to 185°C at 8°C/min; injector temperature, 250°C; detector (FID) temperature, 300°C; carrier gas, helium; flow-rate, 33 ml/min.



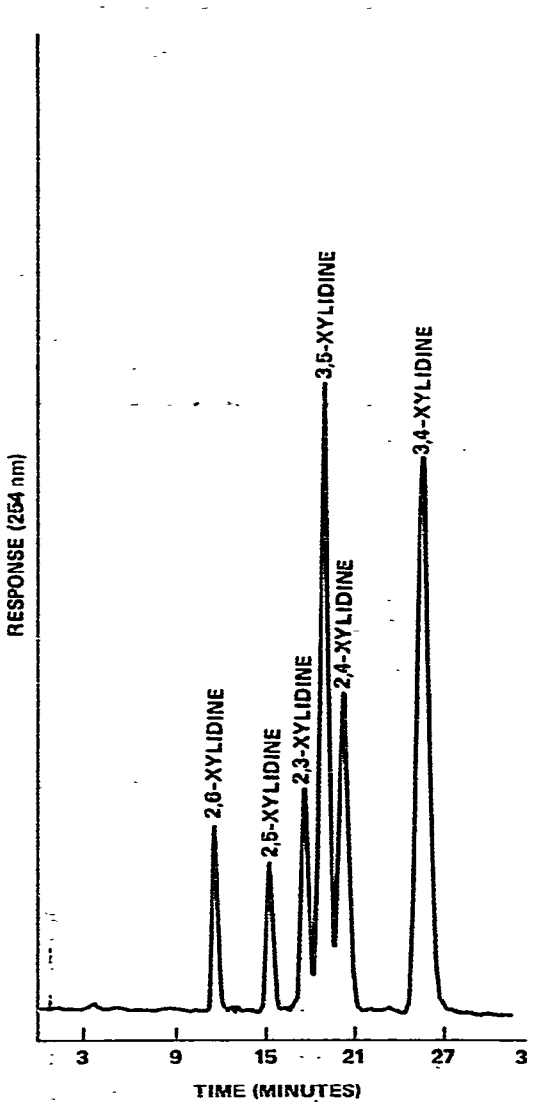


Fig. 7. Capillary column GC of xylidines.

Fig. 8. LC of xylidine isomers.

dicated, including isomers of xylene and bromoxylene. The xylidine and xylenol shown are in underivatized form. Xylenes and bromoxylenes could also be identified using the column packing suggested by Dove; *i.e.*, Carbowax 20M and Apiezon N[1], although in this case each set of isomers would elute in a single peak.

Finally, the use of capillary columns for resolving the isomers of xylidine was also investigated. A 20% Carbowax 20M, glass capillary WCOT column, 50 m long, separated the six xylidine isomers (Fig. 7). Although this resolution had definite possibilities, it was difficult to reproduce.

Overall, only the GC method requiring derivatization with MBTFA, followed

by chromatography on a Carbowax–Apiezon column, provided suitable separation of isomers in addition to reproducible quantitation.

LC determination of xylidines

The advantages of the GC method for determination of xylidines notwithstanding, it was decided to examine the use of LC for the same determination. This was based on the premise that LC would *not* require derivatization and would provide a more rapid determination of the xylidines.

In using LC, the problem of resolving the six xylidine isomers had to be addressed. Chow and Grushka[4] reported the separation of three xylidine isomers in an LC system using a copper amino complex on a normal-phase column, and Little *et al.*[5] reported the separation of two xylidine isomers on a similar column.

The possibility of separating the six isomers on a normal-phase column, using methylene chloride as the mobile phase, was examined. Separation was achieved initially for five of the xylidine isomers, with the 3,4-dimethyl isomer eluting as a broad, final peak. The elution profile appeared to reflect the interaction of the basic solutes with the slightly acid stationary phase, elution time being a function of solute basicity[6,7]. To improve the elution characteristics of the xylidines in general and of 3,4-xylidine specifically, the mobile phase was modified, first by the addition of ethyl acetate, and then by the addition of hexane. By keeping the concentration of ethyl acetate to a minimum and adjusting the methylene chloride–hexane ratio, it was possible to achieve separation of the six xylidine isomers (Fig. 8).

It is interesting to note that the capacity factors, k', of the xylidines are related to their basicity, the pK_a value being directly related to $\log k'$ (Fig. 9). In the course of

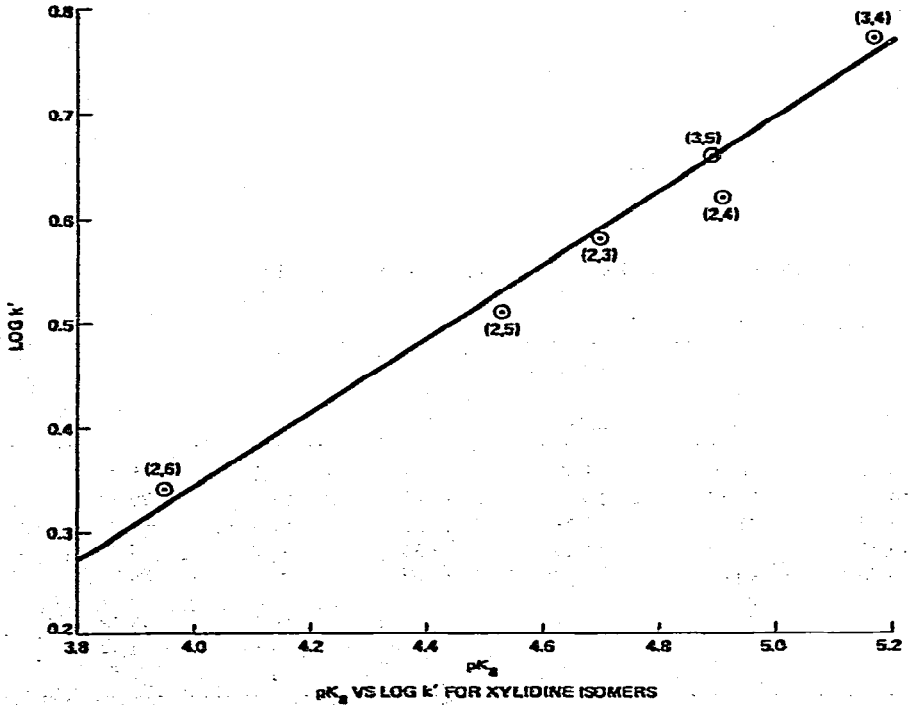


Fig. 9. Plot showing relationship between pK_a and capacity factor ($\log k'$) of xylidines. pK_a values are from ref. 9.

trying different variations in solvent ratios, a shift was noted in the elution order of the 2,4- and 3,5-dimethyl isomers. Depending on the ratio of methylene chloride to hexane in the mobile phase, one or the other of these isomers would elute first. This is not completely unexpected inasmuch as their pK_a values are very close.

The LC procedure, as ultimately adapted for determination of xylidines (*i.e.*, using a normal-phase column eluted with methylene chloride–hexane–ethyl acetate, 74:25:1), was evaluated for reproducibility of response factors, using *m*-chloroaniline as the internal standard (Table IV). Additionally, assays were performed on experimentally prepared mixtures containing 3,4- and 2,3-xylidines (Table V). Recoveries of xylidines exceeded 96%. This LC method was also used to determine xylidines in actual production samples (Table VI). The values compared favorably with those obtained by the GC method applied to the samples.

TABLE IV

REPRODUCIBILITIES OF RESPONSE FACTORS IN THE LC OF XYLIDINES

	*Response factor (amt./ht.)**		
	m-Chloroaniline	*Xylidines*	
		2,3-	*3,4-*
	0.583	1.22	1.26
	0.583	1.22	1.26
	0.587	1.23	1.27
	0.616	1.20	1.27
	0.564	1.16	1.22
$\bar{x}$	0.590	1.21	1.26
S.D.	0.02	0.03	0.02
C.V. (%)	2.1	2.3	1.65

* Amt. is in mg, ht. is in integrator units.

TABLE V

RECOVERIES OF 2,3- AND 3,4-XYLIDINE IN SYNTHETIC MIXTURES BY LC

	Composition (mg)				*Recovery (%)*	
	Actual		*Observed*		*2,3-*	*3,4-*
	2,3-	*3,4-*	*2,3-*	*3,4-*		
	23.74	26.99	23.62	26.70	99.5	98.9
	14.22	23.17	14.20	23.10	99.9	99.7
	3.17	21.05	3.23	20.61	101.9	97.9
	13.47	19.44	13.20	19.30	98.0	99.3
	5.23	16.05	5.20	15.50	98.1	96.1
$\bar{x}$					99.5	98.38
S.D.					1.59	1.44
C.V. (%)					1.6	1.5

TABLE VI

LEVELS OF 3,4- AND 2,3-XYLIDINE IN PRODUCTION SAMPLES. LC *VS.* GC

Sample identification	*Weight (%)*			
	3,4-Xylidine		*2,3-Xylidine*	
	LC	*GC**	*LC*	*GC*
A	93.5**	94.4	3.8**	3.2
B	96.2	97.3		
C	99.5	100.0		
D	97.6**	99.6		
E	97.6	98.7	1.2	1.2
F	99.2	98.4	1.6	1.4
G	97.3	99.4	2.1	1.2
Commercial grade	99.6	100.4	0.3	—

* The GC method used was the one described herein using MBTFA and the Carbowax–Apiezon column.

** By external standard method; all other LC determinations used *m*-chloroaniline as the internal standard.

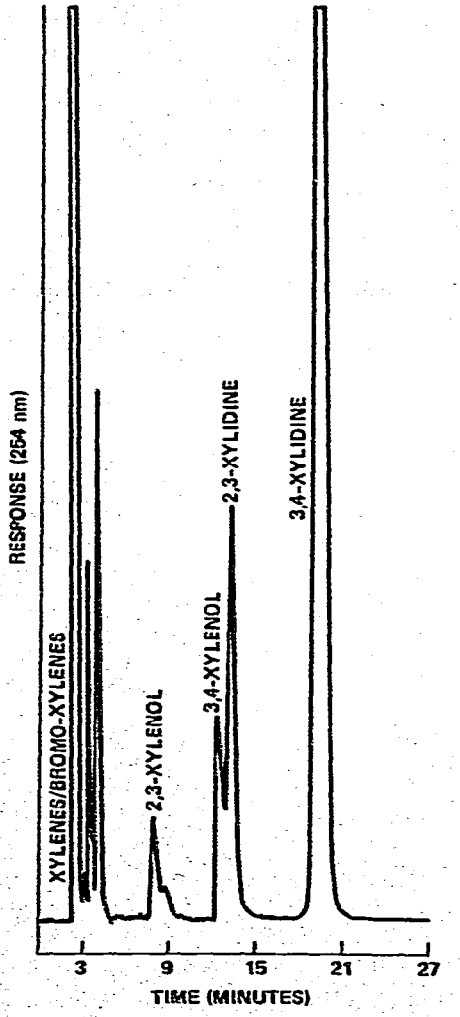


Fig. 10. LC of xylidines and impurities.

As noted previously, samples of 3,4-xylidine could contain, as impurities, xylenes, 3- and 4-bromo-*o*-xylenes, 3,4-xylenol, 2,3-xylidine, and other xylidine isomers. 3,4-Xylenol is occasionally present in production samples in relatively low levels (<1.0%). At a detector wavelength of 254 nm, it is not possible to quantitate these levels, although at 274 nm or by the use of fluorescent techniques, such quantitation may be effected[8]. In the present method, 3,4-xylenol elutes just prior to 2,3-xylidine (Fig. 10).

CONCLUSION

Several approaches to the determination of 3,4-xylidine and related materials have been explored. The GC assay, incorporating a derivatization step with MBTFA, has been shown to be reproducible, rapid, and amenable to routine analyses. The LC method, which requires no derivatization step, shows promise although it has not been used as extensively as the GC method. It, too, should be applicable to repetitive, routine assays of xylidines and related compounds.

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