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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF ALDEHYDES AT TRACE LEVEL AS THEIR 3-METHYLBENZOTHI-AZOLONE AZINE DERIVATIVES

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SUMMARY

The high-performance liquid chromatographic analysis of some simple carbonyl compounds as their 3-methylbenzothiazolone derivatives was studied. The detection was performed by means of an ultraviolet-visible spectrophotometric detector. All compounds except formaldehyde gave two isomers of the type *E* and *Z*. The derivatization was quantitative only for formaldehyde, acetaldehyde and benzaldehyde. The chromatographic conditions were optimized to solve the problem due to the presence of two isomers. The low detection limits obtained for these compounds make this method a competitive alternative to dinitrophenylhydrazine and lutidine methods.

INTRODUCTION

Carbonyl compounds are a major class of air pollutants. In particular, formaldehyde is the most abundant carbonyl compound in the atmosphere, while other aldehydes, such as acetaldehyde and benzaldehyde, have also been detected, and their concentrations may be comparable to that of formaldehyde¹.

Formaldehyde is a combustion product originating from many sources^{2,3} and is used as a raw material in numerous industries. It has been identified as a suspected carcinogen of class A2⁴, and for this reason in the U.S.A. the maximum tolerable limit in air has recently been reduced to 1.2 mg/m³. Carbonyls are also considered as an important class of compounds in atmospheric chemistry because of their role in gas and liquid phase reactions⁵.

Of particular interest in the dispersed liquid phase (fog, clouds, rain) is the reaction of carbonyl compounds with S(IV), leading to the formation of highly acidic compounds like hydroxyalkanesulphonic acids.

Concern about environmental pollution and occupational hazards due to the presence of formaldehyde has led to the development of analytical methods for the determination of aldehydic compounds at trace level. The two commonly used spectrophotometric methods are the Nash method (involving lutidine derivatives)⁶ and the methylbenzothiazolone hydrazone (MBTH) method⁷. The Nash method is

specific for formaldehyde, while the MBTH method is non-selective because it measures total aldehydes as their formaldehyde equivalent. Both methods show no response to ketones. Several other techniques have been reported for the separation and/or determination of various aldehydes or their derivatives. They include thin-layer chromatography (TLC)^{8,9}, gas chromatography¹⁰ and especially high-performance liquid chromatography (HPLC)¹¹⁻¹⁹.

The best known method for the separation and quantitation of carbonyl compounds at trace levels by HPLC is that proposed by Selim¹¹ which uses 2,4-dinitrophenylhydrazones as reactants. Several applications of this method have been reported involving both spectrometric¹²⁻¹⁴ and electrochemical detection¹⁵.

In previous papers we studied the possibility of using lutidine derivatization¹⁷ and the first step of the classical method with MBTH, *i.e.*, azine formation, see Fig. 1¹⁸ for the analysis of carbonyl compounds by HPLC using electrochemical detection. In both cases picogram quantities were detected. The advantage of the revised MBTH method is due to a more simple reaction and the possibility of analysing ketones. However the reaction can lead to the formation of two isomers (*Z* and *E*) with the possibility, in HPLC, of peak splitting and consequent problems when a quantitative analysis is sought.

The presence of two isomers was verified in a previous paper¹⁸ using a C₁₈ bonded phase. The two isomers have been further investigated by UV analysis and TLC. Moreover the HPLC behaviour of these compounds has been studied using different columns. Finally, microscale derivatization experiments have been carried out using standard mixtures in order to determine the yield and the detection limit of the method under conditions similar to those encountered with real samples.

EXPERIMENTAL

The LC equipment consisted of a quaternary solvent Perkin-Elmer (Norwalk, CT, U.S.A.) Model 410 system with a 6- μ l Rheodyne 7125S injector was employed. Detection was performed with a Perkin-Elmer dual beam LC95 variable wavelength detector and with a Coulchem electrochemical detector, ESA Model 5100A (Bedford,

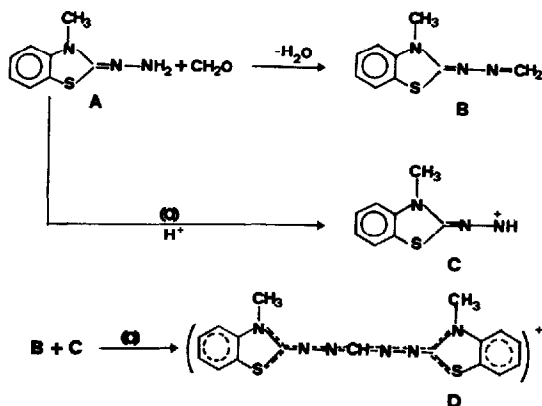


Fig. 1. Mechanism of the MBTH derivatization reaction with and without oxidant. The azine is compound B. (O) = (Oxygen).

MA, U.S.A.). The UV spectra (Fig. 2) were obtained with an Hewlett-Packard Model 1090 (Palo Alto, CA, U.S.A.) with a diode array detector, an HP integrator Model 79995-6A and a colour plotter Model 7440A. The UV spectra of MBTH derivatives were obtained with a Perkin-Elmer 551 UV-VIS spectrometer.

The following columns were tested: (a) silica gel Hypersil (HPLC Technology, Macclesfield, U.K.), 5 μm , 150 mm \times 4.6 mm, fully porous spherical adsorbent; (b) Hypersil ODS (HPLC Technology), 5 μm , 150 mm \times 4.6 mm reversed-phase material with a monolayer coverage of dimethyloctadecylsilyl groups; (c) Hypersil C₁ (HPLC Technology), 5 μm , 150 mm \times 4.6 mm reversed phase with a trimethyl chain; (d) Spherisorb C₆ (Phase Separation, Queensferry, U.K.), 5 μm , 150 mm \times 4.6 mm, bonded phase resulting from the highest possible coverage with an hexyl carbon chain.

A solution of 0.02 M potassium dihydrogenphosphate containing various amounts of methanol (HPLC grade; Carlo Erba, Milan, Italy) was used as the mobile phase. The pH was adjusted to the desired value by adding sulphuric acid. The mobile phase was first filtered with a 0.2- μm nylon membrane and then degassed. The flow-rate was held at 1 ml/min and all experiments were carried out at room temperature.

The TLC measurements were carried out by applying 3 μl of methanolic solutions (3 mg/ml) of the MBTH azine derivatives to a start line 1 cm from the bottom of the plate. The plates used were: (a) Merck 60, F₂₅₄ precoated TLC plates, 10 cm \times 5 cm, layer thickness 0.25 cm, developed with ethyl acetate-cyclohexane (20:80, v/v), spots of the separated azines developed by exposure to iodine vapours; (b) RP C₁₈, Merck precoated TLC plates, 10 cm \times 5 cm, layer thickness 0.25 mm, developing system 0.02 M potassium dihydrogenphosphate solution-methanol (90:10, v/v), spot visualization by exposure to air.

The microscale derivatization was carried out by adding 1 ml of MBTH hydrochloride, 0.5% in water (Fluka, Buchs, Switzerland) to a known volume of a solution of the carbonyl compounds of known concentration. The solution was kept for 1 h at 95°C in a closed vessel and after cooling it was extracted with 4 ml of isooctane. The organic phase was then directly injected into the column.

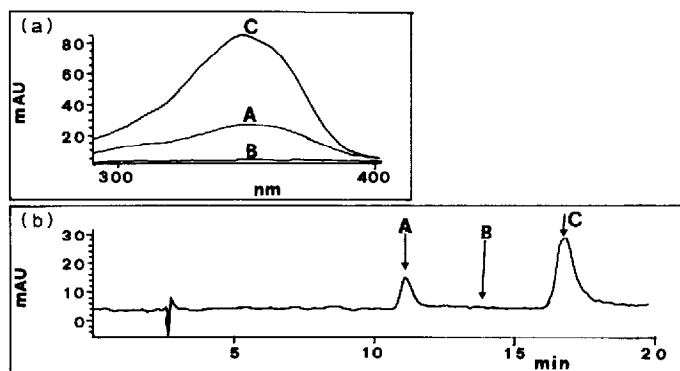
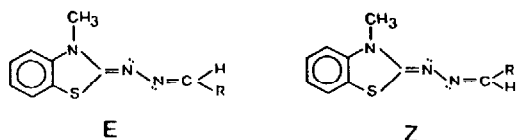


Fig. 2. HPLC separation of the *E* and *Z* isomers of the benzaldehyde derivative at $\lambda = 350$ nm (a), and UV spectra of the isomers (b). Chromatographic conditions: column, Hypersil ODS; eluent, 0.02 M KH_2PO_4 in water-methanol (30:70).

RESULTS AND DISCUSSION

In a previous paper the peak splitting of the compounds in reversed-phase (RP) chromatography was ascribed to the formation of isomers *Z* and *E* during the derivatization¹⁷. Evidence in support of this hypothesis was provided by the results obtained with an electrochemical detector (equal potential vs. current curves) as well as by the interconversion of one isomer into the other¹⁸.



Another experimental result which suggests the presence of isomers has been obtained by analysing the spectra of the two peaks with a multi-diode array detector (see Fig. 2 for the spectrum of the benzaldehyde derivative). The λ_{\max} and ϵ values of the most important azines so obtained are summarized in Table I.

The presence of double peaks, as already mentioned, is however not desired in analytical measurements since there is always a chance of peak overlapping. Moreover, for quantitation, both peaks must be considered. A survey of azine determination by TLC^{8,9} gave no indication of peak splitting. For this reason the TLC measurements were repeated both with silica gel and RP C₁₈ plates. The most significant results are reported in Table II. It is seen that, on RP plates, the isomers of aliphatic derivatives are not resolved, whereas the unsaturated ones are separated. The different compounds can easily be separated but there is overlapping when the number of compounds present is high. On the contrary, on silica gel plates, the isomers can easily be resolved with the exception of methyl ethyl ketone.

The same general trend is observed in HPLC when similar stationary phases are used. The chromatographic parameters relevant to the three RP columns are reported in Table III. No significant difference is observed. The peak splitting increases with the

TABLE I
VALUES OF λ_{\max} AND ϵ FOR THE MOST IMPORTANT MBTH CARBONYL DERIVATIVES

<i>MBTH derivative</i>	λ_{\max} (nm)	$\epsilon \cdot 10^4$ ($l \text{ mol}^{-1} \text{ cm}^{-1}$)
MBTH reagent	290	0.10
(a) Formaldehyde	308	2.78
(b) Acetaldehyde	305	2.48
(c) Propionaldehyde	308	1.59
(d) <i>n</i> -Butyraldehyde	308	2.13
(e) <i>n</i> -Valeraldehyde	308	1.55
(f) Acrolein	334	3.56
(g) Crotonaldehyde	327	3.45
(h) Benzaldehyde	346	4.80
(i) Acetone	309	2.32
(j) Methyl ethyl ketone	308	2.73
(k) Acetophenone	343	3.25

TABLE II
TLC SEPARATION OF MBTH AZINES

<i>MBTH derivative</i>	<i>RP-18</i>		<i>Silica</i>	
	R_{F1}	R_{F2}	R_{F1}	R_{F2}
(a) Formaldehyde	0.44		0.26	
(b) Acetaldehyde	0.40		0.26	0.38
(c) Propionaldehyde	0.35		0.32	0.42
(d) <i>n</i> -Butyraldehyde	0.31		0.34	0.50
(e) <i>n</i> -Valeraldehyde	0.25		0.36	.54
(f) Acrolein	0.41	0.43	0.28	0.40
(g) Crotonaldehyde	0.34	0.39	0.26	0.36
(h) Benzaldehyde	0.21	0.32	0.22	0.40
(i) Acetone	0.48		0.32	
(j) Methyl ethyl ketone	0.32		0.44	
(k) Acetophenone	0.18	0.32	0.48	

aromaticity of the carbonyl compound and with the aliphatic content of the bonded phase.

Fig. 3 shows a chromatogram of a standard mixture in which a compromise as been made between good separation and low or no peak splitting. A good resolution of acetone, acrolein and propionaldehyde was not achieved. However, acrolein can be determined selectively at 350 nm where only unsaturated derivatives have a significant response (Fig. 4).

TABLE III
SEPARATION OF MBTH DERIVATIVES ON DIFFERENT COLUMNS

Eluents: 0.02 M KH_2PO_4 with 77% (1), 70% (2) and 65% methanol (3). k_1 and k_2 are the capacity factors of the isomers.

<i>MBTH derivative</i>	<i>Hypersil ODS (1)</i>		<i>Spherisorb (2)</i>		<i>Hypersil C₁ (3)</i>	
	k_1	k_2	k_1	k_2	k_1	k_2
(a) Formaldehyde	0.80		0.71		0.66	
(b) Acetaldehyde	1.11		0.94		0.94	
(c) Propionaldehyde	1.87		1.46		1.54	
(d) <i>n</i> -Butyraldehyde	2.79		1.99		2.25	
(e) <i>n</i> -Valeraldehyde	4.26		2.89		3.36	
(f) Acrolein	1.55	1.72	1.14	1.26	1.46	
(g) Crotonaldehyde	1.92	2.29	1.45	1.62	1.80	2.00
(h) Benzaldehyde	2.75	4.75	2.13	2.92	2.86	3.66
(i) Acetone	1.61		1.10		1.35	
(j) Methyl ethyl ketone	2.79		1.80		1.99	
(k) Acetophenone	3.73	7.13	2.36	3.50	3.24	5.18

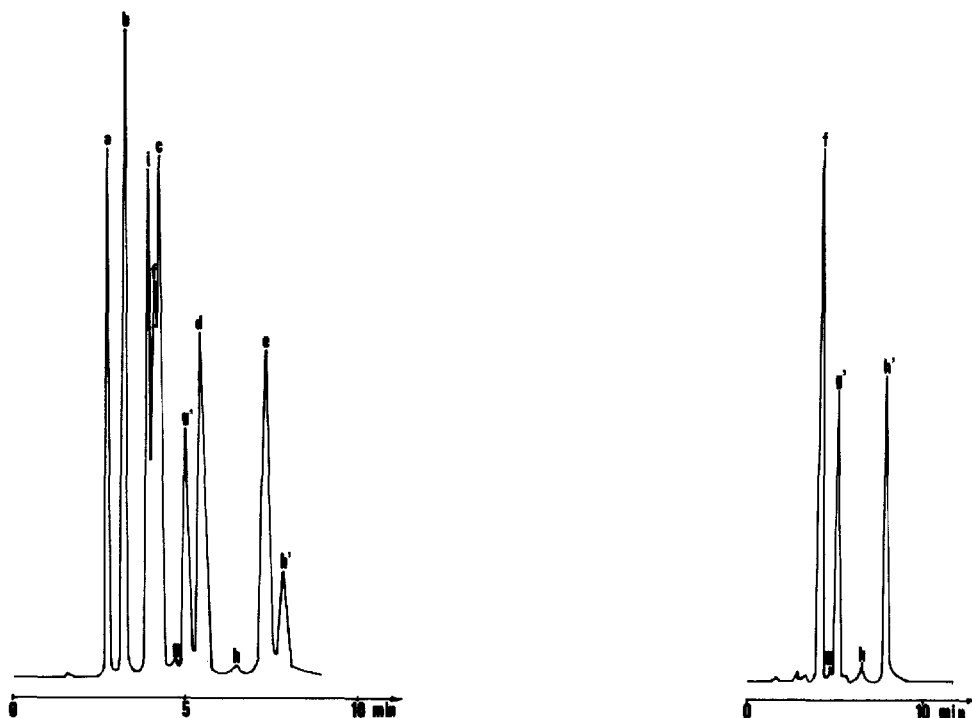


Fig. 3. Separation of azines on an Hypersil C₁ column. Chromatographic conditions: mobile phase, 0.02 M KH₂PO₄ in water-methanol (35:65); detection, $\lambda = 308$ nm. For peak identification, see Table I.

Fig. 4. As in Fig. 3, but with $\lambda = 350$ nm.

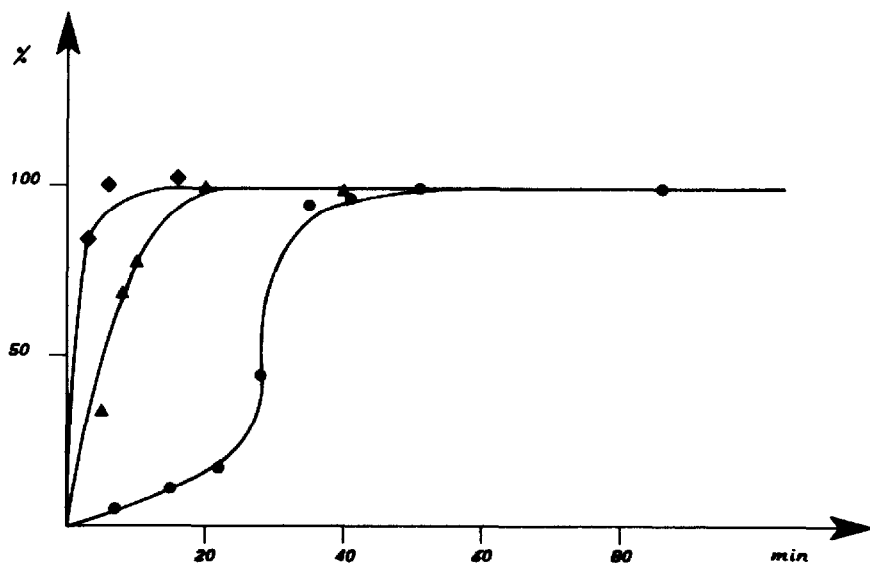


Fig. 5. Reaction yield vs. time of formaldehyde derivatization at different temperatures: (●) 25, (▲) 50 and (◆) 95°C.

TABLE IV

PERCENT YIELD OF DIFFERENT AZINES AFTER A REACTION TIME OF 1 h AT 95°C

<i>MBTH</i> derivative	Yield (%)
(a) Formaldehyde	100
(b) Acetaldehyde	100
(c) Propionaldehyde	45
(d) <i>n</i> -Butyraldehyde	45
(e) <i>n</i> -Valeraldehyde	40
(f) Acrolein	70
(g) Crotonaldehyde	70
(h) Benzaldehyde	100
(i) Acetone	20
(j) Methyl ethyl ketone	10
(k) Acetophenone	10

Derivatization results

To demonstrate the applicability of the proposed method to real samples such as fog and rain, we have evaluated the microscale derivatization yield in the concentration range 0–100 μM .

Fig. 5 shows the kinetics of azine formation from formaldehyde (10 μM) and a charge excess of MBTH at different temperatures. The percent yield of different azines after a reaction time of 1 h at 95°C is reported in Table IV. Since the extraction yield for all compounds is above 95%, the data of Table IV represent a rough measure of the reaction velocities of the different carbonyls with MBTH and therefore of the applicability of the method, if a reaction time of 1 h is considered a limit for the analysis. Based on the above argument, the proposed method is quite reliable for analysing formaldehyde, acetaldehyde and benzaldehyde. The calibration graph with microderivatization for formaldehyde is shown in Fig. 6 where the height of the

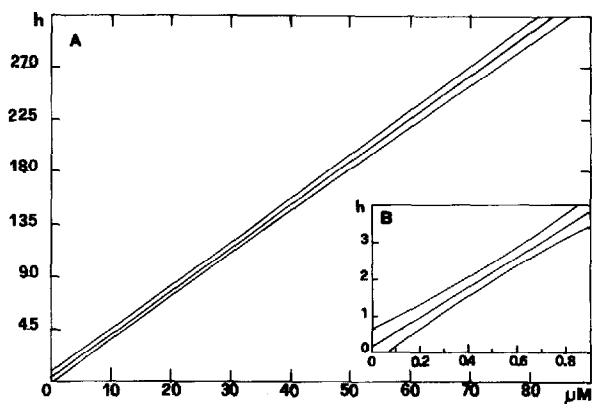


Fig. 6. Calibration graph for formaldehyde in the concentration range 0–100 μM obtained with microderivatization (A) and in the concentration range 0–1 μM (Insert B). The outer lines represent the 95% confidence limits.

TABLE V

COMPARISON OF DETECTION LIMITS (μM) FOR DIFFERENT CARBONYL DERIVATIVES OBTAINED WITH DIFFERENT METHODS

A = Spectrophotometric method; B = HPLC method with amperometric detector; MD = microscale derivatization; C = HPLC method with UV-VIS detector; D = HPLC method with coulometric detector.

Method		Formaldehyde	Acetaldehyde	Benzaldehyde
Nash	A ²⁰	5.5		
	B ¹⁷	1.5		
DNPH	B ¹⁵	0.19	0.48	0.15
	MD ¹⁵	0.48	0.48	0.36
MBTH	A ⁹	30.3	22.7	3.45
	B ¹⁸	0.04	0.13	0.47
	C ^a	0.07	0.08	0.19
	D ^a	0.13	0.14	1.60
	MD ^a	0.29	0.47	0.34

^a This work.

relevant peak is plotted vs. the concentration of the test solution. The limit of detection obtained from data at concentrations lower than 1 μM (insert of Fig. 6) calculated according to Miller and Miller²¹ is reported in Table V. In the same table the detection limits obtained with different methods given in literature are also presented for comparison. It is seen that the proposed method has the lower limit of detection for all the compounds studied.

Work is in progress to apply this method to real atmospheric samples.

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