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Note

Behaviour of 3-methyl-2-benzothiazolone azines of carbonyl compounds in high-performance liquid chromatography

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Formaldehyde and other carbonyl compounds are a major class of air pollutants produced by many industrial processes and combustion sources. Formaldehyde yields the carcinogenic bis(chloromethyl) ether upon photoreaction with hydrogen chloride[1] in moist air and acrolein is on the EPA Priority Pollutant List. Because of the environmental importance of these compounds, sensitive and rapid techniques for their determination are needed. The two commonly used colorimetric methods for the determination of formaldehyde and other aldehydes are the Nash procedure[2] and the methylbenzothiazolonehydrazone (MBTH) method[3]. The Nash spectrophotometric method is specific for formaldehyde, while the MBTH procedure is a nonselective colorimetric method for low molecular weight aliphatic aldehydes, measuring total aldehydes as their formaldehyde equivalent. Both methods show no re-

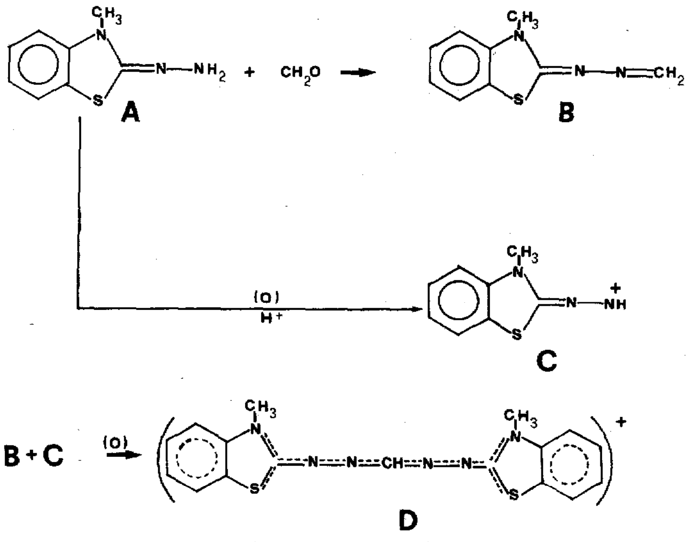

Fig. 1. Mechanism of the derivatization reaction.

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sponse for ketones. The analysis of a wide range of carbonyl compounds as 2,4-dinitrophenylhydrazone derivatives by high-performance liquid chromatography (HPLC) was extensively studied in the literature[4–10].

Recently the Nash derivatization procedure was also applied in our laboratory for measuring formaldehyde at trace levels by HPLC and electrochemical detection[11]. Two papers[12,13] have been published about the thin-layer chromatography (TLC) analysis of the MBTH derivatives of some aliphatic aldehydes.

The present paper reports on the possibility of utilizing the first step of the MBTH derivatization method (*i.e.* azine formation, see Fig. 1) for the speciation of a wide series of carbonyl compounds by HPLC with electrochemical detection. Because of its sensitivity electrochemical detection is frequently applied to trace analysis. The classical MBTH spectrophotometric procedure is based on the reaction of aldehydes with an acidic aqueous solution of MBTH reagent to form azines. These azines in turn form blue products by oxidative coupling with a second molecule of MBTH.

In this study we have utilized only the first step of the derivatization procedure, which, as opposed to the classical spectrophotometric method, would also permit the analysis of ketones.

EXPERIMENTAL

Apparatus and reagents

A Hewlett-Packard 1010 A liquid chromatograph equipped with a Rheodyne 7120 injector with a 20-μl sample loop was used. A Metrohm 656 electrochemical detector with a glassy carbon electrode was used. A silver/silver chloride electrode was used as the reference together with a Metrohm VA 641 potentiostat and a d.c. amplifier. The chromatographic column was 25 × 4.6 mm I.D. packed with Erbasil C_{18} (Carlo Erba). The mobile phase consisted of methanol (HPLC grade) and KH_2PO_4 (0.02 *M* in deionized water).

The mass spectra were recorded on a Finnigan Model 1020 spectrometer.

The MBTH hydrochloride was obtained from Fluka (Switzerland) and used without further purification. All other chemicals and the carbonyl compounds were the best available grade from various suppliers.

Preparation of azines

Our first step in the development of this procedure was to prepare gram quantities of a few azine carbonyl derivatives in order to examine their behaviour in HPLC. A few grams of the MBTH-derivatives of formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, acrolein, crotonaldehyde, benzaldehyde, acetone, methyl ethyl ketone and acetophenone were prepared according to the procedure of Hüng *et al.*[14]. The identity of the products was confirmed by mass spectrometry (MS) (see Table I). As shown in Table I, the parent peaks are the highest peaks in the spectra, except for the crotonaldehyde and the benzaldehyde derivatives. This is favourable for the application of HPLC–MS and GC–MS techniques.

The stock solutions were prepared by dissolving known amounts of pure recrystallized solids in ethanol. All other standard solutions were obtained by successive dilution.

TABLE I

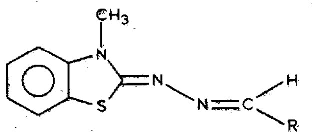
MASS SPECTRA (70 eV) OF MBTH CARBONYL DERIVATIVES OBTAINED USING A DIRECT INLET PROBE

Relative intensities of the parent ions (in italics) and of the five most abundant peaks are reported.

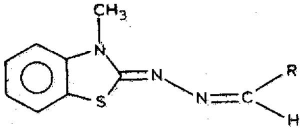
MBTH derivative	
Formaldehyde	*191*(100), 163(44), 149(43), 148(34), 136(36), 109(36)
Acetaldehyde	*205*(100), 190(15), 164(18), 163(47), 136(47), 109(39)
Propionaldehyde	*219*(100), 164(43), 163(26), 150(25), 136(52), 109(38)
Butyraldehyde	*233*(100), 205(23), 164(68), 150(47), 136(46), 109(42)
Acrolein	*216*(100), 150(33), 136(26), 109(49), 69(22), 39(40)
Crotonaldehyde	*231*(32), 216(100), 164(5), 150(16), 136(13), 109(18)
Benzaldehyde	*267*(87), 150(100), 109(42), 90(48), 77(40), 51(43)
Acetone	*219*(100), 163(61), 150(26), 136(67), 109(52), 39(23)
Methyl ethyl ketone	*233*(100), 164(51), 163(38), 150(19), 136(60), 109(51)
Acetophenone	*281*(100), 163(27), 150(35), 109(32), 104(45), 77(41)

RESULTS AND DISCUSSION

The separation of some carbonyl azine derivatives by reversed-phase HPLC is shown in Fig. 2. Double peaks appear in the chromatograms for all carbonyls except for formaldehyde and acetone which yield symmetrical derivatives. The double peaks observed for unsymmetrical carbonyl derivatives have to be assigned to the *Z* and *E* isomers.



E



Z

We cannot assign the *Z* and *E* structure to the two peaks without using other instrumental techniques, but based on the characteristics of the bonded phase (C_{18}), we can reasonably assign the less linear *Z* structure to the first eluted peak. Naulet *et al.*[15] have studied the NMR behaviour of MBTH-azine isomers, emphasizing the low activation energy of the isomerization process and the dependence on pH.

Similarly, we have noticed, the consistent dependence of k' and peak height ratio of the isomers on the eluent pH during attempts to find the optimum separation conditions.

In order to confirm that the two peaks represent two isomers, we have collected the two fractions (A and B in Fig. 3) corresponding to the retention times of the *Z* and *E* isomers of the crotonaldehyde derivative. Repeated HPLC analysis of the two collected fractions showed that the ratio of the heights of the *Z* and *E* peaks changed with time until the equilibrium was re-established after about 5 h.

Further evidence of the isomeric nature of the two peaks is given in Fig. 4 for

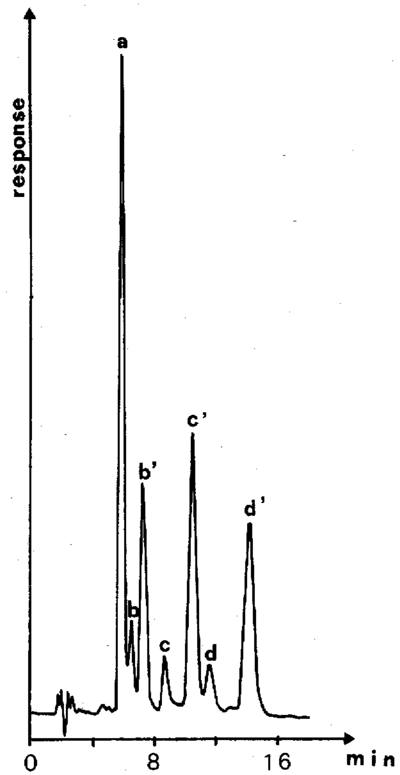


Fig. 2 HPLC separation of C_1–C_4 aliphatic aldehydes as MBTH azines derivatives. (a) Formaldehyde, (b) and (b') acetaldehyde, (c) and (c') propionaldehyde, (d) and (d') butyraldehyde. Column: Erbasil C_{18}. Eluent: methanol–KH_2PO_4 (0.02 *M* in water) (70:30, v/v); flow-rate: 1.2 ml/min; oxidation potential: + 1.05 V.

the crotonaldehyde derivative. The response of the electrochemical detector *vs.* the applied potential for the two fractions, shows the same trend and tend to zero at the same potential. Therefore, the two peaks that appear for each unsymmetrical derivative, can be reasonably attributed to substances which have the same oxidizable groups and not to interfering by-products.

Table II shows the capacity factors of the MBTH derivatives. The difference between the elution times for the *Z* and *E* peaks, expecially for aromatic carbonyl derivatives, is remarkable. The occurence of two peaks for each compound complicates the separation of the C_1–C_4 carbonyls. Under our chromatographic conditions only the formaldehyde peak does not have interfering peaks.

The electrochemical behaviour of the MBTH derivatives was examined by measuring the responses of the electrochemical detection (peak heights) at different potentials of the working electrode. Fig. 5 shows an example of this electrochemical behaviour. For all the examined azines the curves indicate no significant response at potential values lower than + 0.80 V. The optimum working potential is + 1.05 V.

Typical calibration curves of the azines derivatives of formaldehyde, acetal-

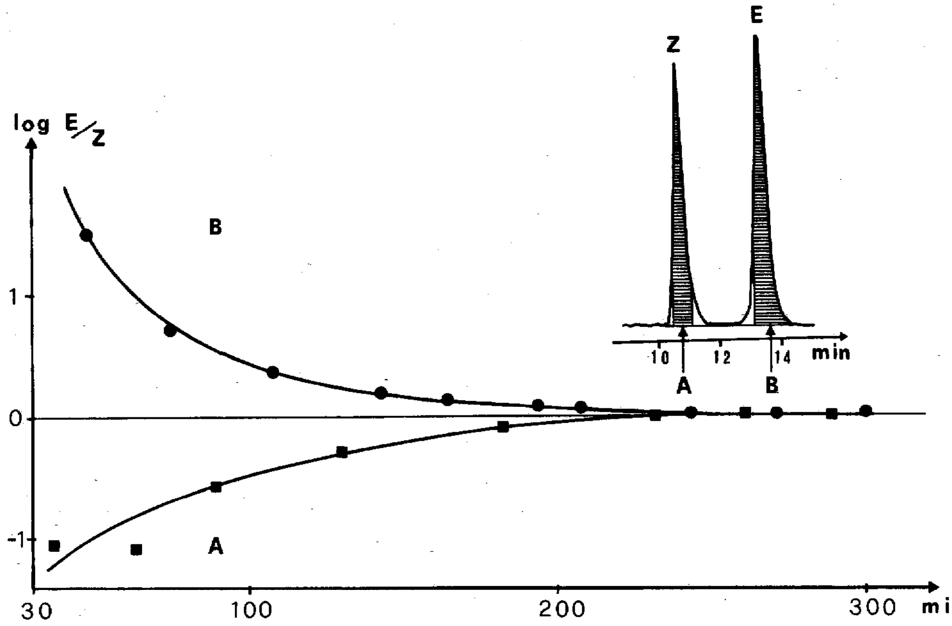


Fig. 3. Kinetics of the isomerization of the HPLC fraction of *Z*-MBTH-crotonaldehyde and *E*-MBTH-crotonaldehyde derivatives. A and B are the two collected fractions. *E*/*Z* is the ratio between the peak heights of the *E* and *Z* isomers.

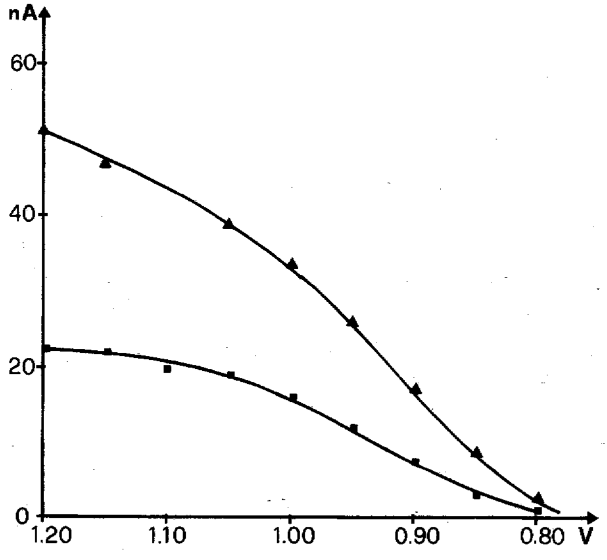


Fig. 4. Relationship between the response of the electrochemical detector and the applied potential for the crotonaldehyde *Z* (▲) and *E* (■) isomers. Experimental conditions as in Fig. 2.

TABLE II

HPLC CAPACITY FACTORS (k') OF THE MBTH DERIVATIVES AND THE RATIO BETWEEN THE k' OF THE Z AND E ISOMERS

Chromatographic conditions as in Fig. 2

MBTH derivative	k'	k'_E/k'_Z
Formaldehyde	2.22	
Acetaldehyde *Z*	2.67	
E	3.00	1.13
Propionaldehyde *Z*	3.89	
E	4.89	1.25
Butyraldehyde *Z*	5.44	
E	6.89	1.27
Acrolein *Z*	3.30	
E	4.10	1.24
Crotonaldehyde *Z*	4.56	
E	6.22	1.37
Benzaldehyde *Z*	7.4	
E	17.8	2.40
Acetone	3.55	
Methyl ethyl ketone *Z*	5.11	
E	6.22	1.21
Acetophenone *Z*	8.22	
E	19.11	2.32

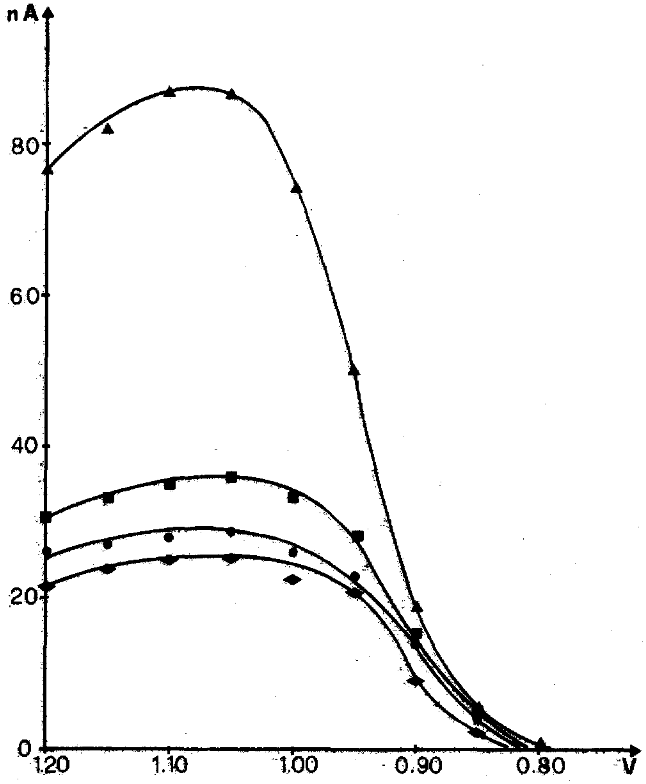


Fig. 5. Relationship between the electrochemical response and the applied potential for the MBTH derivatives of: formaldehyde (▲), acetaldehyde (●), propionaldehyde (■) and butyraldehyde (▲). For unsymmetrical carbonyls only the response of the major peak is shown. Chromatographic conditions as in Fig. 2.

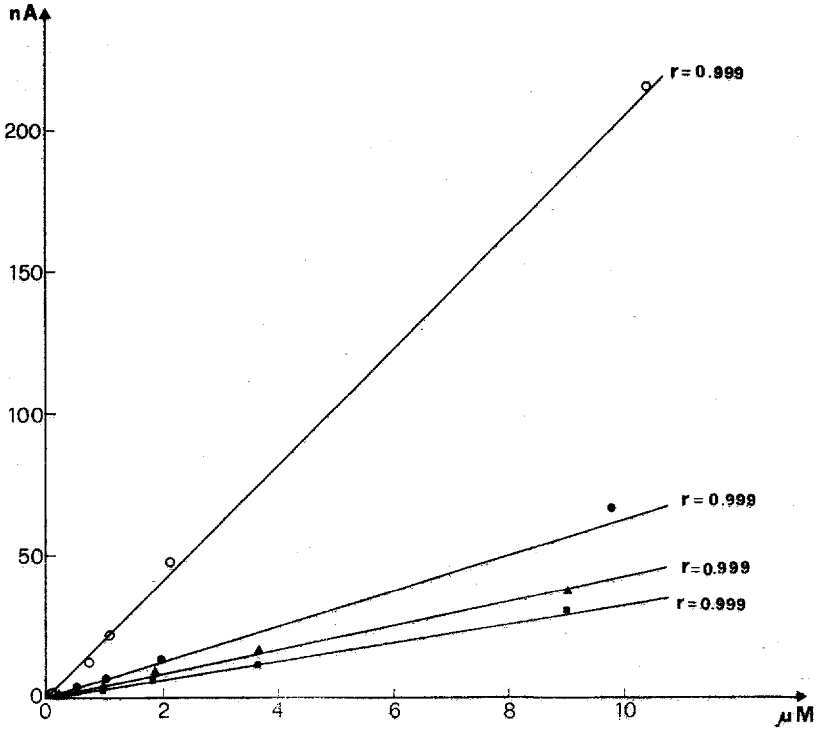


Fig. 6. Calibration curves for carbonyl azines: electrochemical response (nA) *vs.* concentration of carbonyls (*μM*). (○) Formaldehyde, (●) acetaldehyde, (▲) propionaldehyde, (■) butyraldehyde.

dehyde, propionaldehyde and butyraldehyde are shown in Fig. 6. Excellent linearity was obtained for all the examined azines in the range from 10^{-7} *M* to 10^{-5} *M*.

The reproducibility was also determined for quantitative analysis. Multiple injections of calibration mixtures yielded relative standard deviations in the range from 1.6% to 7.5%.

TABLE III

DETECTION LIMITS AT AN OXIDATION POTENTIAL OF + 1.05 V

The values are calculated for free carbonyls for a signal-to-noise ratio of 3.

Carbonyl compound	*Detection limit (picomoles)*
Formaldehyde	0.7
Acetaldehyde	2.5
Propionaldehyde	5.4
Butyraldehyde	4.8
Acrolein	7.2
Crotonaldehyde	8.4
Benzaldehyde	9.4
Aceton	0.3
Methyl ethyl ketone	8.5
Acetophenone	13.5

Table III shows the detection limits calculated as free carbonyl with a signal-to-noise ratio of 3.

The electrochemical response for this class of carbonyl derivatives is very high and the application to trace analysis is very promising. Work is in progress in our laboratory to improve the yields of microscale derivatization reactions in order to enable the application of the present method to the analysis of atmospheric samples for formaldehyde. Different chromatographic conditions might permit the speciation of a wider series of carbonyl compounds.

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