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Gas chromatographic determination of trace nitrite after derivatization with ethyl 3-oxobutanoate

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ABSTRACT

A gas chromatographic method was developed for the determination of nitrite using ethyl 3-oxobutanoate as a derivatization reagent. Nitrite was reacted with ethyl 3-oxobutanoate in the presence of hydrochloric acid to form ethyl 2-hydroxyimino-3-oxobutanoate (EHOB) quantitatively. The resulting EHOB was extracted with ethyl acetate and then determined sensitively by gas chromatography with electron-capture detection. This method has been applied successfully to the determination of nitrite in river water and human saliva, with a detection limit of 2 ng/ml and recoveries of 94–100%.

INTRODUCTION

Nitrite is widespread in nature and its level varies widely among foods and in the environment. Nitrite is generally regarded as a hazardous compound, because it may react with amines and amides to produce carcinogenic N-nitroso compounds [1]. Nitrite is also known to produce excess abnormal haemoglobin, causing methaemoglobinaemia [2]. The development of a method for the determination of nitrite in foods and the environments is, therefore, of great importance. Several techniques have been reported for the determination of nitrite, including spectrophotometry [3–5], polarography [6], fluorimetry [7], flow-injection analysis [8] and ion chromatography [9]. Gas chromatographic (GC) determinations of nitrite have also been reported using different derivatization reagents, *e.g.* hydralazine [10], *o*-phenylenediamine [11], aromatic primary amines [12–14] and pentafluorobenzyl bromide [15]. However, some of the GC methods have disadvantages such as instability of the reaction product, a high reaction temperature or a time-consuming reaction.

It is wel known that nitrite reacts readily with ethyl 3-oxobutanoate in acidic media to give ethyl 2-hydroxyimino-3-oxobutanoate (EHOB) [16,17]:

$$CH_3COCH_2CO_2C_2H_5 \xrightarrow[H^+]{NO_2} CH_3COCH(NO)CO_2C_2H_5 \longrightarrow \underset{\text{EHOB}}{CH_3COC(=NOH)CO_2C_2H_5}$$

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We have developed a GC method, using the above reaction, in which trace amounts of nitrite are converted into EHOB. The derivatization is very fast and proceeds quantitatively without temperature control. Owing to the high sensitivity of EHOB to electron-capture detection (ECD), nitrite at ppb levels can be successfully determined by this method.

EXPERIMENTAL

Apparatus

Chromatographic analyses were performed on a Yanako (Kyoto, Japan) G-2800 gas chromatograph equipped with a ^{63}Ni-source electron-capture detector. A glass column (2 m × 3 mm I.D.) was packed with 5% diethylene glycol succinate + 1% H_3PO_4 on 60–80-mesh Chromosorb W DMCS (Gasukuro Kogyo, Tokyo, Japan). Nitrogen was used as the carrier gas at a constant flow-rate of 45 ml/min. The detector, injection port and column temperatures were maintained at 260, 220 and 200°C, respectively. The peak heights were measured with a digital integrator (Yanako S-1200).

For mass spectral identification of the nitrite derivative, a Hewlett-Packard Model 5890 gas chromatograph coupled with a Hewlett-Packard Model 5970B mass spectrometer was used. An HP-1 (100% dimethylpolysiloxane) (Hewlett Packard) capillary column (12 m × 0.2 mm I.D.) was directly interfaced to the electron-impact ion source. The injection port and interface temperatures were maintained at 250 and 280°C, respectively. The initial column oven temperature was 60°C and was increased at 20°C/min to 200°C. The ionizing voltage was 70 eV. Helium was used as the carrier gas at 4 psi (27.6 kPa).

Reagents

Ethyl 3-oxobutanoate was obtained from Tokyo Kasei Kogyo (Tokyo, Japan) and used as received. All other reagents were of analytical-reagent grade. Distilled, deionized water was used throughout. A stock standard nitrite solution (1 mg/ml) was prepared by dissolving 1.500 g of sodium nitrite (dried at 110°C for 3 h) in 1 l of water. Working standard solutions were prepared by appropriate dilution of this stock standard solution.

Samples

River water samples were collected from the city of Kobe. Human saliva samples (1 g) were weighed into a 100-ml volumetric flask and diluted to volume with water. The sample solutions were centrifuged at 3000 rpm (*ca.* 1000 *g*) for 5 min to remove insoluble matter and the supernatants were subjected to GC analysis.

Procedure

A 10-ml volume of sample solution was placed in a 20-ml test-tube fitted with a ground-glass stopper, then 0.02 ml of ethyl 3-oxobutanoate and 1 ml of concentrated hydrochloric acid (*ca.* 11 *M*) were added successively. The mixture was allowed to stand for 5 min at room temperature, then 5 ml of ethyl acetate were added. After shaking for 1 min, the ethyl acetate layer was transferred into a 10-ml test-tube and dehydrated with a small amount of anhydrous sodium sulphate. A 2-μl aliquot of the ethyl acetate extract was injected into the gas chromatograph and the derivative peak height was measured.

A 10-ml volume of each standard solution (0.01–0.1 μg/ml) was treated in the same manner to prepare a calibration graph. The nitrite content in the sample solution was calculated from the calibration graph.

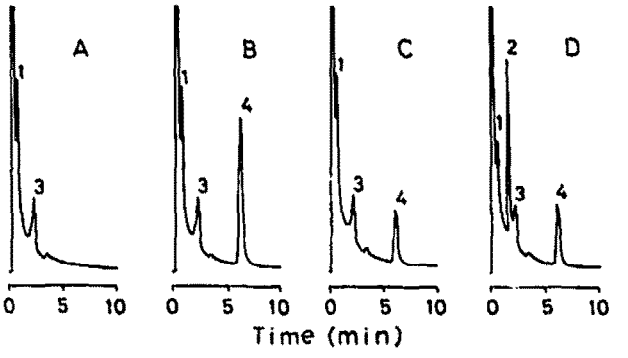

Fig. 1. Gas chromatograms obtained in the analyses of (A) reagent blank, (B) 0.1 μg/ml nitrite, (C) river water and (D) human saliva. Peaks: 1 = ethyl 3-oxobutanoate; 2 and 3 = unknowns; 4 = ethyl 2-hydroxyimino-3-oxobutanoate (EHOB). For GC conditions, see Experimental.

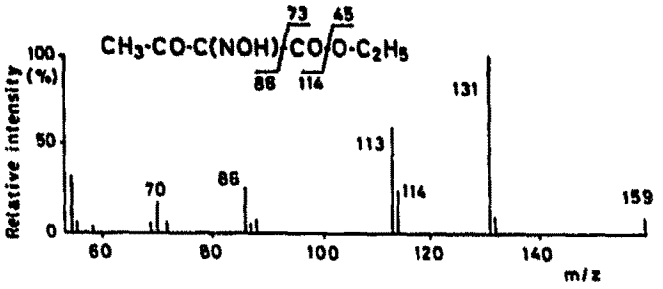

Fig. 2. Mass spectrum of nitrite derivative of ethyl 3-oxobutanoate.

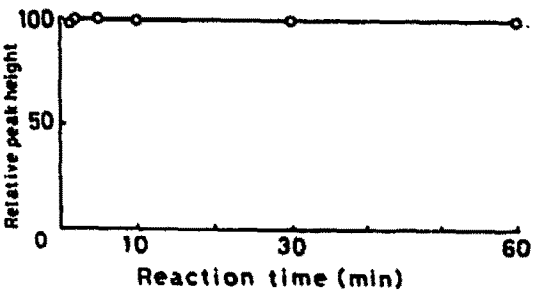

Fig. 4. Effect of reaction time on the formation of EHOB. For other conditions, see Experimental.

RESULTS AND DISCUSSION

Identification of the derivative

The application of the reaction of nitrite with ethyl 3-oxobutanoate to give EHOB [15,16] to the determination of nitrite has not been reported. Initially, we tried to confirm whether this reaction occurred even at low concentrations of nitrite: a 0.1 μg/ml nitrite standard solution was subjected to the derivatization. Fig. 1 shows gas chromatograms obtained for (A) reagent blank, (B) nitrite standard (0.1 μg/ml), (C) river water and (D) human saliva. Peaks 1 and 4 correspond to ethyl 3-oxobutanoate and its nitrite derivative, respectively. The identification of the derivative (peak 4) was carried out by GC–MS. The mass spectrum obtained (Fig. 2) exhibits the parent ion at *m/z* 159 and fragment ions at *m/z* 114 and 86. The mass spectrum shows the derivative to be EHOB. No change in the chromatograms was observed whether the chromatography was done immediately or after storage of the ethyl acetate extract at room temperature for a few days, indicating that EHOB is relatively stable.

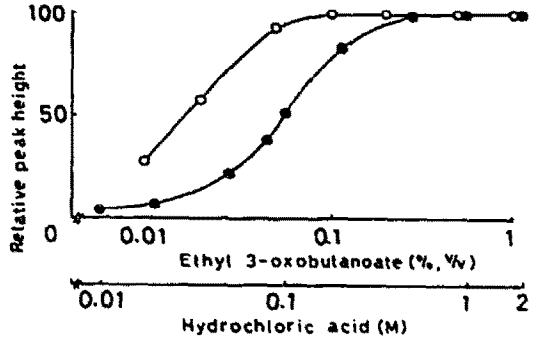

Fig. 3. Effects of (○) ethyl 3-oxobutanoate and (●) hydrochloric acid concentrations on the formation of EHOB. For other conditions, see Experimental.

Optimum derivatization conditions

The reaction of nitrite with ethyl 3-oxobutanoate proceeds in acidic media. We chose hydrochloric acid, which has been widely used in nitrite reactions [16]. In order to optimize the derivatization conditions, we examined the effects of the concentrations of ethyl 3-oxobutanoate and hydrochloric acid and the reaction time, using a 0.1 μg/ml nitrite standard solution. The derivatizations were carried out in almost the same manner as described under Experimental. The concentrations of ethyl 3-oxobutanoate and hydrochloric acid in the reaction mixture were varied separately and the peak heights of the resulting EHOB were measured.

Fig. 3 shows the effect of the concentration of ethyl 3-oxobutanoate on the formation of EHOB. When the ethyl 3-oxobutanoate concentration in the reaction mixture was higher than 0.1% (v/v), the peak height of the resulting EHOB reached a maximum. Fig. 3 also shows the effect of the concentration of hydrochloric acid. The EHOB peak height reached a maximum at hydrochloric acid concentrations above 0.5 *M*. From these results, final concentrations of *ca.* 0.2% (v/v) ethyl 3-oxobutanoate and *ca.* 1 *M* hydrochloric acid were adopted.

Fig. 4 shows the effect of reaction time on the formation of EHOB. The derivatization was completed rapidly. The peak height of EHOB became a maximum with reaction times longer than 2 min. Therefore, the reaction time was fixed at 5 min.

Several organic solvents were tested for extraction of the resulting EHOB. The distribution coeffi-

TABLE I

EFFECT OF FOREIGN ANIONS

The concentration of nitrite was 0.1 μg/ml.

Anion	Added as	Concentration (μg/ml)	Relative peak height[a]
None			100.0 ± 1.8
Cl^-	NaCl	100	101.4 ± 0.4
F^-	KF	100	99.5 ± 3.2
Br^-	KBr	100	99.4 ± 0.9
I^-	KI	100	69.5 ± 0.7
I^-	KI	10	93.8 ± 0.6
NO_3^-	KNO_3	100	100.9 ± 2.2
SO_4^{2-}	Na_2SO_4	100	98.9 ± 3.2
SO_3^{2-}	Na_2SO_3	100	69.0 ± 3.0
SO_3^{2-}	Na_2SO_3	10	96.1 ± 1.6
CO_3^{2-}	Na_2CO_3	100	98.4 ± 3.9
HCO_3^-	$NaHCO_3$	100	100.7 ± 3.4
HPO_4^{2-}	K_2HPO_4	100	96.8 ± 3.4
$H_2PO_4^-$	KH_2PO_4	100	99.9 ± 3.3
CH_3COO^-	CH_3COONa	100	101.3 ± 1.9
SCN^-	KSCN	100	87.9 ± 2.9
SCN^-	KSCN	10	94.3 ± 2.1

[a] Mean ± standard deviation for three determinations.

cient of EHOB, defined as the ratio of the concentration in the organic phase to that in the aqueous phase, were measured by a double extraction. The ratio of the concentration or peak response of the first to that of the second extract is equal to one plus the distribution coefficient (D) times the ratio of the volume of the organic phase (V_o) to that of the aqueous phase (V_a):

$$C_1/C_2 = 1 + DV_o/V_a$$

The distribution coefficients are 53 for ethyl acetate, 27 for diethyl ether, 0.27 for cyclohexane and 0.02 for *n*-hexane. When ethyl acetate was used as the extraction solvent, *ca.* 96% of the resulting EHOB in the aqueous phase was transferred into the ethyl acetate extract in this method.

Calibration

A calibration graph was constructed by plotting the peak height of EHOB (y) *versus* the concentration of nitrite (x). A good linear relationship, $y = 0.358x + 0.021$, with a correlation coefficient of 0.998, was obtained in the range 0.01–0.1 μg/ml. The relative standard deviations for five replicate derivatizations of nitrite were 3.4% at 0.01 μg/ml and 1.5% at 0.1 μg/ml. EHOB has a high ECD response, and the detection limit of the method was found to be 0.02 μg of nitrite in 10 ml of aqueous sample.

TABLE II

DETERMINATION OF NITRITE IN RIVER WATER AND HUMAN SALIVA WITH RECOVERY TEST

Sample	NO_2^- added[a]	NO_2^- found[a]		Recovery (%)
		Spectrophotometric method [4]	GC method (this work)[b]	
River water				
A	0	0.025	0.026 ± 0.001	
	0.05		0.073 ± 0.002	94
B	0	0.027	0.026 ± 0.001	
	0.05		0.076 ± 0.001	100
C	0	0.012	0.014 ± 0.001	
	0.05		0.063 ± 0.001	98
Human saliva				
A	0	4.67	4.56 ± 0.14	
	5		9.27 ± 0.42	94.2
B	0	5.41	5.20 ± 0.07	
	5		9.94 ± 0.36	94.8

[a] Units: river water, μg ml; human saliva, μg g.

[b] Mean ± standard deviation for three determinations.

Effect of foreign anions

The effect of foreign anions, which may co-exist in the sample, on the determination of nitrite was studied. Table I shows the results of interferences by Cl^-, F^-, Br^-, I^-, NO_3^-, SO_4^{2-}, SO_3^{2-}, CO_3^{2-}, HCO_3^-, HPO_4^{2-}, $H_2PO_4^-$, CH_3COO^- and SCN^-. Most of the anions examined, at a concentration of 100 μg/ml, did not interfere with the formation of EHOB. On the other hand, I^-, SO_3^{2-} and SCN^- interfered seriously. The interferences by I^- and SO_3^{2-} may be attributed to the reduction of nitrite by the anions. However, these three anions at a concentration of 10 μg/ml showed no significant interference. The levels of I^-, SO_3^{2-} and SCN^- in ordinary environmental samples are usually lower than the interfering concentrations shown in Table I. These results suggest that this method can be used for the determination of nitrite in environmental samples.

Applications

River water and human saliva samples were analysed by both this method and a spectrophotometric method [4]. Recovery tests with nitrite added to these samples were also carried out in order to assess the efficiency of the method. Fig. 1 shows typical gas chromatograms obtained for river water (C) and human saliva (D). Table II shows the results for the determination and recovery of nitrite in real samples. The nitrite concentrations for each sample determined by the two methods were in good agreement. The recoveries of the spiked nitrite were greater than 94%. The results in Table II indicate that there is no significant interference from the sample matrices in the derivatization step and in the GC determination.

CONCLUSION

A simple and sensitive GC method was developed for the determination of nitrite at trace levels. The reliability and selectively of the method are excellent, and it can be used to determine nitrite accurately at ppb levels in a variety of samples.

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