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Note

Electron-capture gas chromatographic determination of nitrite as the pentafluorobenzyl derivative

HSIN-LUNG WU* and SU-HWEI CHEN
School of Pharmacy, Kaohsiung Medical College, Kaohsiung 800 (Taiwan)
KOICHI FUNAZO
Department of Chemistry, Osaka Prefectural Technical College, Neyagawa, Osaka 572 (Japan)
and
MINORU TANAKA and TOSHIYUKI SHONO
Department of Applied Chemistry, Faculty of Engineering, Osaka University, Yamada-oka, Suita, Osaka 565 (Japan)
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Nitrite occurs ubiquitously in nature, and it is used widely in foods, fertilizers, detergents and various chemical processes. Owing to the possibility of its reaction with amines or amides to form carcinogenic N-nitroso compounds under conditions similar to those of the human stomach[1,2], the determination of nitrite in a variety of matrices is very important. Numerous approaches have been established for the determination of nitrite. Spectrophotometry is widely used, based on the diazotization of various aromatic amines with nitrite in acidic media and on subsequent coupling of the diazonium ions formed with Cleve's acid[3], N-(1-naphthyl)ethylenediamine[4], 8-quinolinol[5] or resorcinol[6,7], Generally, spectrophotometric methods are subject to various interferences and lack of specificity. Other methods less frequently used are polarography[8], enthalpimetry[9], chemiluminescence[10], which involves reduction of nitrite to nitric oxide and its subsequent chemiluminescent reaction with ozone, and non-specific volumetric titration[11] using *o*-iodosobenzoate as the titrant.

Recently, liquid chromatography with or without derivatization has been reported for the determination of nitrite/nitrate. Detection in liquid chromatography is mainly based on conductimetry[12,13], spectrophotometry[14], ultraviolet spectrophotometry[15–22] or amperometry[23]. Conductivity detection suffers from the inherent drawbacks of poor specificity and low sensitivity. In addition, nitrite is liable to be oxidized in acidic media during the liquid chromatographic process[24].

Several gas chromatographic (GC) techniques have been developed for the determination of nitrite/nitrate in various matrices. Derivatization of nitrite to achieve a suitable volatility before GC analysis in these methods is based on a variety of approaches, *e.g.*, nitration of benzene[25] with nitrate which is formed by oxidative pre-treatment of nitrite, conversion of nitrite into the trimethylsilyl derivative of 1*H*-benzotriazole[26] through multiple reactions and ring closure of hydralazine with nitrite to form tetrazolophthalazine[27], which is then partitioned to the organic layer. We have reported the derivatization of nitrite to 3,4-dichlorobromobenzene[28] or *p*-

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bromochlorobenzene[29], which is based on the Sandmeyer reaction. Generally, derivatization reactions in GC analysis are complicate. To reduce this complexity for the GC of anions, we have recently established a simple method for the determination of nitrite[30] or other anions by a one-step derivatization reaction with pentafluorobenzyl bromide, the derivative formed in the reaction solution being analysed directly by GC with flame-ionization detection without any further treatment after the reaction.

In this work, a further attempt was made to increase the sensitivity of the determination of nitrite by electron-capture GC in the light of the high electron affinity of the pentafluorobenzyl derivative of nitrite. The method was applied to the determination of nitrite in foods, saliva and river water. The results indicated that the method is simple, specific, sensitive and reliable.

EXPERIMENTAL

GC conditions

A Varian 3700 gas chromatograph equipped with a ^{63}Ni pulsed linearized electron-capture detector was used. The column was a coiled stainless-steel tube (3.6 m × 2 mm I.D.) packed with 10% OV-210 on Chromosorb W HP (80–100 mesh). The injection port and detector temperatures were kept at 180 and 250°C, respectively. The column was maintained isothermally at 150°C. Nitrogen was used as the carrier gas at a flow-rate of 50 ml/min. A Varian 9176 recorder with a chart speed of 2.5 mm/min was used.

Materials

α-Bromo-2,3,4,5,6-pentafluorotoluene (PFBBr) (Aldrich, Milwaukee, WI, U.S.A.), *p*-nitrobromobenzene (Tokyo Kasei, Tokyo, Japan), 10% silicone OV-210 on Chromosorb W HP (80–100 mesh) (Alltech, Deerfield, IL, U.S.A.) and pre-coated thin-layer chromatography (TLC) plates with a layer thickness of silica gel 60 of 2 mm (E. Merck, Darmstadt, F.R.G.) were used without further treatment. Sodium nitrite (Wako, Osaka, Japan), acetone, methanol and other reagents were of analytical-reagent grade. Deionized and distilled water was used to prepare aqueous solutions. A solution of the internal standard (I.S.) was prepared by dissolving a suitable amount of *p*-nitrobromobenzene (7.39 μM) in acetone. Solutions of the reference standard of various concentration were prepared by dissolving the appropriate amount of sodium nitrite in 10^{-5} M sodium hydroxide solution. PFBBr solution (0.66 mM) was prepared by diluting a suitable volume of PFBBr with acetone.

Solutions of samples were prepared as follows.

Foods. Samples of foods were prepared according to the reported procedure[31], and the extracts obtained were analysed after suitable dilution by both spectrophotometric[32] and GC methods. The other samples mentioned below were also treated identically.

Saliva. The saliva was expectorated into a tared beaker and was quantitatively transferred into a volumetric flask with the aid of a suitable amount of water. Further dilution was made before analysis.

River water. The collected river water was filtered and the pH of the filtrate obtained was checked. Suitable dilution was made before analysis.

Procedure

A 0.70-ml volume of PFBBr solution, which is equal to 0.07 μl of PFBBr, was placed in a 10-ml glass-stoppered test-tube containing 0.10 ml of reference standard solution or sample solution, then 0.20 ml of I.S. solution was added. The reaction solution was shaken for 2 h at 50°C in a thermostated water-bath. At the end of the reaction, an aliquot of the reaction solution was subjected to GC analysis.

RESULTS AND DISCUSSION

In order to establish the optimum derivatization conditions for pentafluorobenzylation of 0.87 nmol of nitrite, several parameters that affect the formation of the nitrite derivative were evaluated by procedures similar to those described previously[30].

The results led to the adoptation of the following conditions: 10^{-5} *M* sodium hydroxide solution for the alkaline sample solution, 0.07 μl of PFBBr for derivatization, acetone as the water-miscible organic solvent and 2 h at 50°C as the reaction time.

The temperature of electron-capture detector in some instances drastically affects the detection response[33]. Therefore, the detector temperature was checked in the range 190–280°C. No significant variation in the detector response was observed.

Interference

In order to apply the method to the determination of nitrite in a variety of matrices, interferences from several common anions that may coexist in the samples were studied. The results are shown in Table I. Halides anions such as Cl^-, Br^- and I^- at 100 ppm and F^- at 25 ppm do not interfere in the nitrite determination. The method also is not affected by 100 ppm of SO_4^{2-}, NO_3^- or $H_2PO_4^-$, 50 ppm of HPO_4^{2-} or 25 ppm of CH_3COO^-, $S_2O_3^{2-}$, HCO_3^- or CO_3^{2-}. SCN^- and S^{2-} at 20 and 10 ppm, respectively, do not interfere. CN^- has a negative interference at the concentrations examined down to 5 ppm. From the results in Table I, it is clear that the specificity of the method is satisfactory.

Analytical calibration

To evaluate the quantitative applicability of the method, six different concentrations of reference standard solutions containing nitrite in amounts of 2–40 ng were determined in order to construct a calibration graph of amount of nitrite anion as abscissa *versus* the peak-height ratio of the derivative to the I.S. as ordinate. A linear regression equation, $y = 0.0352x + 0.0258$, was obtained with a correlation coefficient of 0.999. This indicates the suitability of the method for the determination of nitrite. The detection limit of the method, defined as the concentration of nitrite that gives a signal twice the average noise, was found to be 0.46 ng of nitrite in 0.1 ml of aqueous sample. The derivatization yield of nitrite is about 80% compared with an authentic sample of nitrite derivative synthesized and isolated in our laboratory.

The typical gas chromatogram presented in Fig. 1 demonstrates the good chromatographic properties of the nitrite derivative, with a short retention and good resolution. Peak a in Fig. 1 was identified by comparison of its retention with that of the authentic nitrite derivative. For this purpose the derivative was first synthesized

TABLE I

RESULTS OF INTERFERENCE STUDY

Nitrite concentration: 0.4 μg/ml.

Anion	*Concentration (ppm)*	*Added as*	*Recovery (%)**
None			100.0 ± 1.47
Cl^-	100	NaCl	99.5 ± 1.65
Br^-	100	KBr	98.9 ± 2.88
I^-	100	KI	98.4 ± 2.35
SO_4^{-2}	100	Na_2SO_4	101.9 ± 2.66
NO_3^-	100	$NaNO_3$	100.0 ± 0.05
$H_2PO_4^-$	100	NaH_2PO_4	99.8 ± 2.63
HPO_4^{2-}	50	Na_2HPO_4	99.7 ± 1.85
CH_3COO^-	25	CH_3COONa	102.8 ± 0.86
$S_2O_3^{2-}$	25	$Na_2S_2O_3$	100.2 ± 2.41
F^-	25	NaF	100.7 ± 4.46
HCO_3^-	25	$NaHCO_3$	100.0 ± 2.43
CO_3^{2-}	25	K_2CO_3	100.3 ± 1.09
SCN^-	20	KSCN	98.8 ± 1.52
S^{2-}	10	$Na_2S \cdot 9H_2O$	98.6 ± 1.55
CN^-	5	KCN	94.1 ± 1.80

* Mean ± S.D. of triplicate analyses.

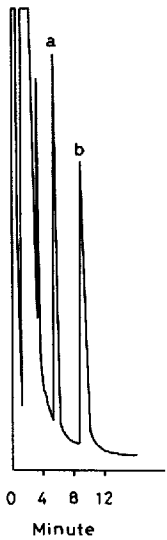

Fig. 1. Typical gas chromatogram for nitrite analysis. Peaks: a = nitrite derivative; b = *p*-nitrobromobenzene (I.S.).

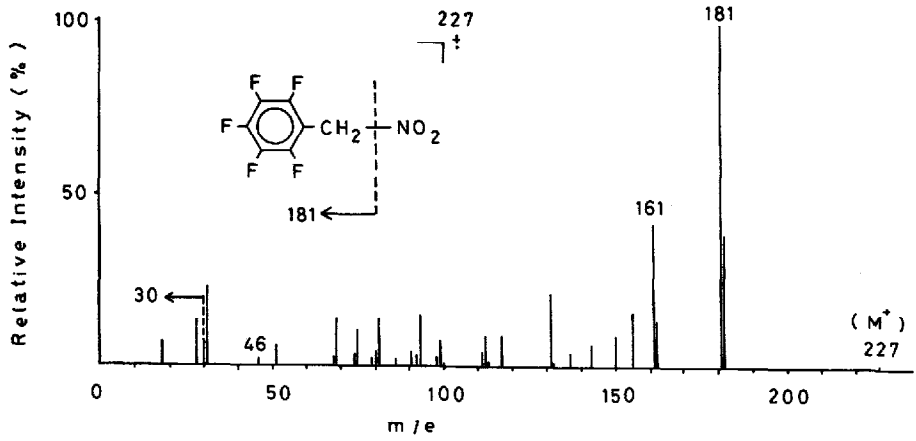

Fig. 2. Mass spectrum of nitrite derivative.

and separated on a TLC plate using as the solvent system cyclohexane–ethyl acetate (8:1). The structure of the isolated derivative was analysed by IR spectroscopy and mass spectrometry under the conditions indicated previously[30]. The mass spectrum obtained, as shown in Fig. 2, exhibited a molecular ion peak at $m/e = 227$. Other ion peaks at $m/e = 46$ (NO_2^+) and $m/e = 30$ (NO^+) indicate the presence of a nitro

TABLE II

RESULTS OF INTERCOMPARISON STUDY FOR THE DETERMINATION OF NITRITE

Sample	*NO_2^- added (ppm)*	*Nitrite concentration (ppm, w/w)*	
		*GC–ECD**	Spectrophotometry
Pork sausage	0	16.63 ± 0.01	16.56
	8	23.58 ± 0.32	23.70
Meat ham			
A	0	9.68 ± 0.15	9.78
	8	18.10 ± 0.24	18.18
B	0	7.47 ± 0.14	7.40
	8	15.36 ± 0.04	16.20
Saliva			
A	0	8.59 ± 0.01	8.95
	9	18.86 ± 0.29	17.99
B	0	18.88 ± 0.56	18.34
	9	26.92 ± 0.47	27.12
River water**			
A	0	1.47 ± 0.06	1.28
	9	10.92 ± 0.13	10.20
B	0	0.19 ± 0.00	0.14
	9	9.87 ± 0.16	9.53

* Mean ± S.D. of triplicate analyses.
** Results in ppm (w/v).

group[34]. The IR spectrum of the isolated nitrite derivative also supported the presence of a nitro group by giving absorption bands at 1560 and 1364 dm^{-1}, corresponding to its antisymmetric and symmetric vibrations. Therefore, the nitrite derivative synthesized is identified as α-nitro-2,3,4,5,6-pentafluorotoluene.

The retention time of peak a in Fig. 1 obtained under the present GC conditions was identical with that of the isolated derivative. Therefore, the two compounds are assumed to have the same structure.

Application

The proposed method was applied to the analysis of several real samples, with or without added nitrite and the results obtained were compared with those determined by the spectrophotometric method. As shown in Table II, the nitrite contents for each sample determined by the two methods are in good agreement, and the recovery of the nitrite added to the various samples is also satisfactory. Therefore, the reliability and specificity of the method are high, and the method can be used to determine nitrite in a variety of samples with good accuracy. The sample size used for the determination of nitrite in this method is small.

It will be attractive to modify the method for the determination of nitrite in plasma.

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