ELSEVIER

Journal of Chromatography A, 695 (1995) 142–148

JOURNAL OF CHROMATOGRAPHY A

Short communication

Determination of secondary amines in various foods by gas chromatography with flame photometric detection

Hiroyuki Kataoka*, Seiko Shindoh, Masami Makita

Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700, Japan

First received 20 September 1994; revised manuscript received 19 December 1994; accepted 20 December 1994

Abstract

A selective and sensitive gas chromatographic method for the determination of secondary amines in foods has been developed. After extraction of the sample with hydrochloric acid, secondary amines were converted into their N-diethylthiophosphoryl derivatives and then measured by gas chromatography using a DB-1701 capillary column and a flame photometric detector. Primary amines were eliminated through the reaction with *o*-phthaldialdehyde prior to the N-diethylthiophosphorylation. The calibration curves for secondary amines in the range 0.2–50 nmol were linear and sufficiently reproducible for quantitative determination. The detection limits of secondary amines, at a signal-to-noise ratio of 3, were ca. 0.05–0.2 pmol injected. Using this method, secondary amines in food samples could be accurately and precisely determined without any interference from coexisting substances. Analytical results for the determination of secondary amines in various food samples are presented.

1. Introduction

Secondary amines are nitrosated by nitrite in conditions of defined pH and in other conditions similar to those in the mammalian stomach or small intestine [1–4], and some of nitrosamines produced have strong mutagenic and oncogenic activities [5–7]. It is well known that nitrite is widely present in nature and is also produced in human saliva, and the possibility that nitrosation may occur in vivo, particularly in the gastrointestinal tract, upon ingestion of foods containing secondary amines has been reported [8,9]. Therefore, measurement of secondary amines in foodstuff is very important.

The determination of secondary amines has been carried out by high-performance liquid chromatography (HPLC) or gas chromatography (GC), but these methods have some inherent problems related to the difficulty in handling low-molecular-mass amines because of their high water solubility and volatility. HPLC analyses of secondary amines by using ultraviolet [10–13], fluorescence [14–16], electrochemical [17] and chemiluminescence excitation detection [18] require derivatization in order to increase the detection sensitivity. Although some of them are very sensitive, their selectivities for food samples with complicated matrices cannot be guaranteed. GC analyses of underivatized secondary amines result in adsorption and decomposition on the column and readily give tailed peaks [19–21]. In order to solve these problems, many derivatization reagents, such as 2,4-dinitrofluorobenzene [22,23], 2,4-dinitrobenzenesulphonate [24], alkyl chloroformate [25,26], pentafluorobenzoyl chlo-

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)01271-7

ride [27], flophemesyl chloride [28] and benzenesulphonyl chloride [29–32], for GC analyses of secondary amines by flame ionization detection (FID) [24,25], electron-capture detection [27,28], flame photometric detection (FPD) [29,30], thermospecific detection [26], chemiluminescence detection with a thermal energy analyser [32] and GC–mass spectrometry with selected ion monitoring (GC–MS-SIM) [23,31] have been reported. However, GC–FID methods lack sensitivity and selectivity. Other GC methods are highly sensitive, but these methods are not specific for secondary amines, except for the GC–MS-SIM methods which require expensive equipment. On the other hand, GC–FPD methods [29,30] based on the Hinsberg procedure [33] have been reported to be selective for secondary amines, but it was found that these amines overlapped with some primary amines in our experiments [34].

Recently, we have developed a selective and sensitive method for the determination of secondary amines by GC–FPD, in which these compounds are analysed as their N-diethylthiophosphoryl (DETP) derivatives after primary amines were eliminated through the reaction with *o*-phthaldialdehyde (OPA) [35]. By using this method, urinary secondary amines could be accurately and precisely determined without any influence from other constituent substances [35]. This paper reports the extension of this work to the determination of secondary amines in food samples.

2. Experimental

2.1. Reagents

Dimethylamine (DMA) and diethylamine (DEA) as the hydrochlorides, di-*n*-propylamine (DPA), di-*n*-butylamine (DBA), pyrrolidine (PYR), piperidine (PIP), morpholine (MOR), hexamethyleneimine (HMI), N-methylbenzylamine (NMBzA) and N-methylcyclohexylamine (NMCHA) as an internal standard (I.S.) were purchased from Nacalai Tesque (Kyoto, Japan). N-Methylethylamine (MEA) was purchased from Fluka (Buchs, Switzerland). Each amine was dissolved in 0.05 *M* hydrochloric acid containing 50% acetonitrile to make a stock solution at a concentration of 0.2 *M* and used after dilution with 0.05 *M* hydrochloric acid to the required concentration (0.01–0.1 m*M*). OPA (Nacalai Tesque) was used as a 0.2 *M* solution in acetonitrile. Diethylchlorothiophosphate (DECTP) (Tokyo Kasei Kogyo, Tokyo, Japan) was used as a 1% solution in acetonitrile after distillation. All other chemicals were of analytical-reagent grade.

2.2. Preparation of samples

Food samples were purchased at local retail markets and were treated for analyses on the same day without drying. For liquid samples, an aliquot (0.05–0.2 ml) was directly used as the sample for derivatization. For solid samples, an aliquot (0.2–1.6 g) was homogenized in 4 ml of 0.05 *M* hydrochloric acid with a Model LK-21 ultra-disperser (Yamato Kagaku, Tokyo, Japan). After centrifugation at 2000 *g* for 10 min, the precipitate was re-extracted with 2 ml of 0.05 *M* hydrochloric acid. The supernatants were combined and 0.1–0.4 ml of the combined solution was used as the sample for derivatization.

2.3. Derivatization procedure

To the standard solution containing 0.2–50 nmol of secondary amines or the sample prepared by above method were added 0.1 ml of 10 μM I.S., and the total volume was made up to 1.6 ml with distilled water after neutralization with 2 *M* sodium hydroxide. To the mixture was added 0.2 ml of 0.5 *M* phosphate buffer (pH 8) and 0.2 ml of 0.2 *M* OPA, and the mixture allowed to stand for 2 min at room temperature. To the reaction mixture was added 0.2 ml of 10% sodium carbonate and 0.2 ml of 1% DECTP, and then the mixture was incubated at 60°C for 10 min after tightly capping. In order to remove the excess of reagent, the reaction mixture was incubated again at 60°C for 5 min after addition of 0.2 ml of 50 m*M* cysteic acid. The reaction mixture was extracted with 0.2–0.4 ml

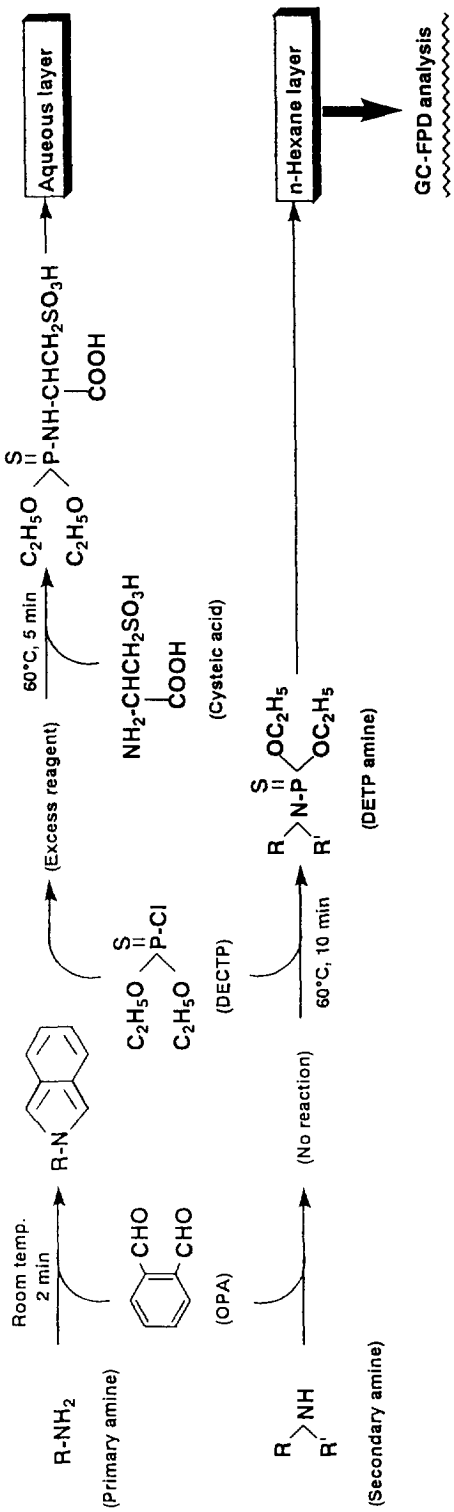

Fig. 1. Outline of selective derivatization of secondary amines. R, R′ = Alkyl or aryl group.

of *n*-hexane and 1 μl of this extract was injected into the GC–FPD system. The derivatization process is summarized in Fig. 1.

2.4. Gas chromatography

GC analysis was carried out with a Shimadzu 14A gas chromatograph equipped with a flame photometric detector (P-filter). A fused-silica capillary column (15 m × 0.53 mm I.D., 1.0 μm film thickness) of cross-linked DB-1701 (J&W, Folsom, CA, USA) was used. The operating conditions were as follows: column temperature, programmed from 100 to 260°C at 10°C/min; injection and detector temperature, 280°C; nitrogen flow-rate, 10 ml/min. The peak heights of secondary amines and the I.S. were measured and the peak height ratios against the I.S. were calculated.

3. Results and discussion

It is well known that OPA reacts only with primary amino groups. Therefore, it is considered that secondary amines might be selectively detected as suitable derivatives if a sample was previously treated with OPA. On the other hand, FPD with 526-nm interference filter inserted in the optical path is sensitive and selective for

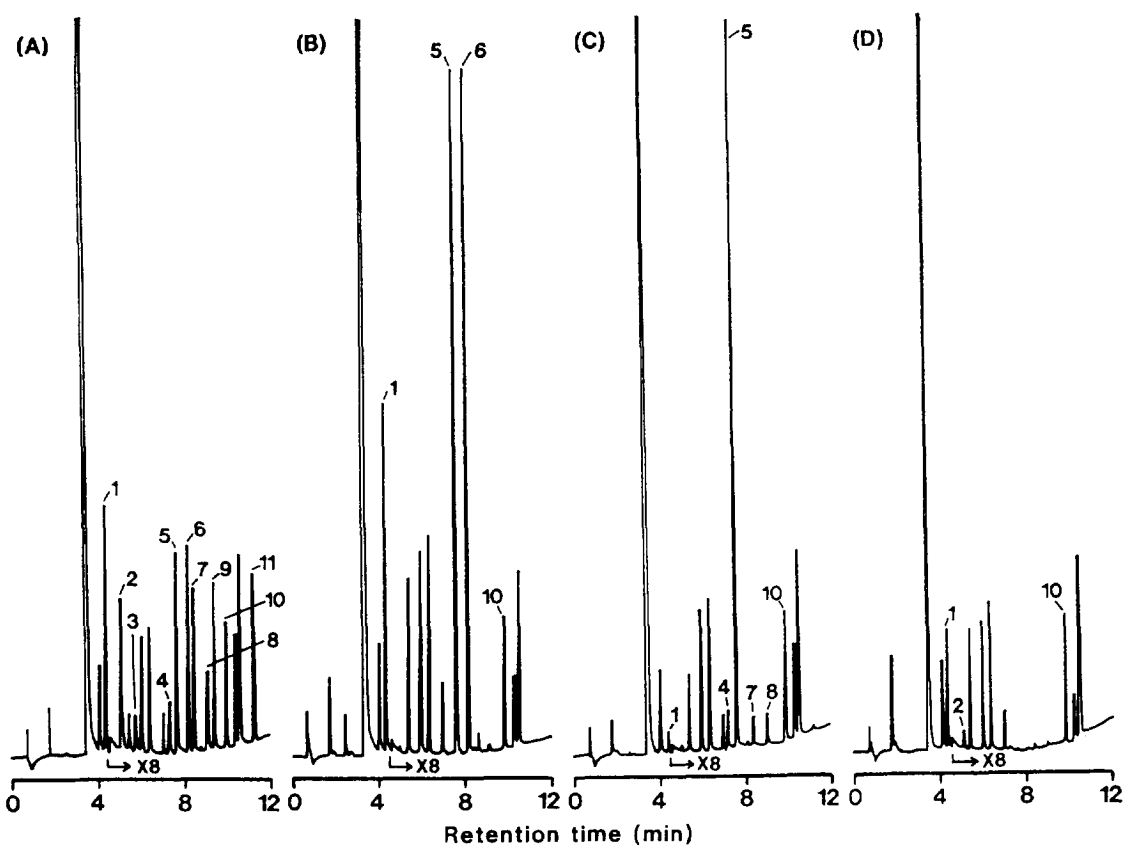

Fig. 2. Typical gas chromatograms obtained from (A) standard (containing 10 nmol of dimethylamine and 1 nmol of other secondary amines), (B) milk (0.1 ml), (C) red pepper (6.4 mg) and (D) oyster (52 mg). GC conditions are given under Experimental. The arrows show that the recorder response was raised up to 8-fold after ca. 4.5 min from sample injection. Peaks: 1 = dimethylamine; 2 = N-methylethylamine; 3 = diethylamine; 4 = di-*n*-propylamine; 5 = pyrrolidine; 6 = piperidine; 7 = morphorine; 8 = di-*n*-butylamine; 9 = hexamethyleneimine; 10 = N-methylcyclohexylamine (I.S.); 11 = N-methylbenzylamine.

phosphorous compounds. Therefore, secondary amines might be sensitively and selectively detected by GC–FPD if these amines were converted into volatile phosphorous-containing derivatives. On the basis of these ideas, we investigated a selective and sensitive method for the determination of secondary amines. The derivatization process of secondary amines is shown in Fig. 1. Primary amines in food samples were eliminated by the reaction with OPA as previously described [35]. Secondary amines did not react with OPA and therefore these compounds were selectively derivatized by the subsequent reaction with DECTP. Reaction conditions for diethylthiophosphorylation of secondary amines were established in the previous investigation [35]. This reaction was completed within 5 min at 60°C and excess DECTP was removed by reaction with cysteic acid. The DETP derivatives of cysteic acid and other amino acids in the samples were not extracted into *n*-hexane in alkaline media. On the other hand, the DETP derivatives of secondary amines were quantitatively extracted into *n*-hexane. These derivatives were volatile and stable, and eluted as separate symmetrical peaks although some peaks originated from reagents were observed (Fig. 2A). The derivatives provided an excellent FPD response and minimum detectable amounts of DMA, DEA, PYR, PIP, MOR and NMBzA to give a signal three times the noise under our instrumental conditions were ca. 0.08, 0.2, 0.06, 0.05, 0.07 and 0.08 pmol injected, respectively. NMCHA was chosen as an internal standard because it was well separated from the amines investigated. The calibration curves for DMA and the other amines were linear in the range 2–50 and 0.2–5 nmol, respectively, and the correlation coefficients were above 0.991.

For secondary amine analysis of foodstuff, hydrochloric acid was used to precipitate proteins. Secondary amines were quantitatively extracted from food samples by extraction twice with 0.05 *M* hydrochloric acid. Fig. 2B–D show the chromatograms obtained from several food samples. The recorder response was raised up to 8-fold at ca. 4.5 min from sample injection because of the large difference in concentration between DMA and the other amines. Secondary amines in these samples could be detected with-

Table 1
Recoveries of secondary amines added to several food samples

Sample	Amine[a]	Added	Amount found[b]		Recovery (%)
			Non-addition	Addition	
Wine	DMA	100 nmol/ml	26.4 ± 0.2 nmol/ml	125.0 ± 5.0 nmol/ml	99
	PYR	10	7.5 ± 0.6	17.4 ± 0.3	99
	PIP	10	7.9 ± 0.5	17.3 ± 0.3	94
	MOR	10	8.9 ± 0.04	18.5 ± 0.5	96
	DBA	10	ND[c]	10.1 ± 0.4	101
	NMBzA	10	29.8 ± 0.9	40.3 ± 0.8	105
Salted pollack roe	DMA	663 nmol/g	2086 ± 73 nmol/g	2792 ± 203 nmol/g	106
	PYR	66.3	15.0 ± 1.2	82.7 ± 1.2	102
	PIP	66.3	6.4 ± 0.3	77.4 ± 2.3	107
	MOR	66.3	4.9 ± 0.4	66.6 ± 1.2	93
	DBA	66.3	ND	60.6 ± 2.3	91
	NMBzA	66.3	6.7 ± 0.5	81.6 ± 1.3	113

[a] DMA = Dimethylamine; PYR = pyrrolidine; PIP = piperidine; MOR = morphorine; DBA = di-*n*-butylamine; NMBzA = N-methylbenzylamine.
[b] Mean ± S.D. ($n = 4$).
[c] Not detectable.

Table 2
Secondary amine contents in various foods obtained from commercial sources

Sample	Content (nmol/g or nmol/ml)[a]									
	DMA	MEA	DEA	DPA	PYR	PIP	MOR	DBA	HMI	NMBzA
Rice	ND[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bread	10.3 ± 0.4	ND	ND	ND	3.4 ± 0.3	3.5 ± 0.3	ND	4.2 ± 0.1	ND	ND
Red beans	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Soy beans	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Almond	38.1 ± 1.0	ND	ND	ND	ND	ND	ND	1.1 ± 0.05	ND	ND
Black pepper	165 ± 12	ND	ND	ND	453 ± 38	6351 ± 368	ND	9.0 ± 0.5	ND	ND
Red pepper	42.9 ± 2.5	ND	ND	120 ± 3	661 ± 36	ND	20.4 ± 0.1	21.6 ± 1.3	ND	ND
Wasabi	ND	ND	ND	ND	3.0 ± 0.03	ND	ND	4.2 ± 0.3	ND	2.0 ± 0.1
Garlic	96.1 ± 2.9	19.4 ± 0.01	ND	110 ± 0.4	277 ± 0.3	ND	ND	ND	ND	ND
Onion	10.2 ± 0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cabbage	14.3 ± 0.8	ND	ND	ND	ND	ND	ND	ND	ND	ND
Banana	4.8 ± 0.2	ND	ND	ND	1.6 ± 0.1	2.1 ± 0.05	ND	ND	ND	ND
Dried fungi	212 ± 10	ND	ND	ND	45.9 ± 5.3	ND	ND	ND	ND	ND
Salted algae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salted pollack roe	2086 ± 73	ND	ND	2.5 ± 0.2	15.0 ± 1.2	6.4 ± 0.3	4.9 ± 0.4	ND	ND	6.7 ± 0.5
Cod (dried)	1043 ± 38	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mackerel	2129 ± 390	ND	ND	ND	16.4 ± 1.9	15.9 ± 2.3	ND	ND	ND	ND
Oyster	39.3 ± 2.0	1.6 ± 0.1	ND	ND	ND	ND	ND	ND	ND	ND
Beef	26.4 ± 0.4	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pork	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chicken	18.9 ± 0.6	ND	ND	ND	ND	ND	ND	ND	ND	ND
Egg white	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Egg yolk	109 ± 3	ND	ND	ND	ND	ND	ND	ND	ND	ND
Yogurt	483 ± 50	ND	ND	ND	19.2 ± 1.9	18.0 ± 1.5	ND	ND	ND	ND
Cheese	29.8 ± 2.9	ND	ND	ND	17.4 ± 1.2	29.3 ± 3.1	ND	ND	ND	ND
Cow's milk	147 ± 7	ND	ND	ND	50.1 ± 0.4	62.4 ± 3.1	ND	ND	ND	ND
Wine	26.4 ± 0.2	ND	352 ± 25	52.9 ± 6.1	7.5 ± 0.6	7.9 ± 0.5	8.9 ± 0.04	ND	ND	29.8 ± 0.9
Beer	64.5 ± 5.7	2.5 ± 0.1	ND	ND	7.9 ± 1.1	22.2 ± 1.2	ND	31.2 ± 2.1	ND	ND
Sake	ND	ND	ND	ND	10.3 ± 0.1	ND	ND	14.5 ± 1.3	ND	ND
Bean jum	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] Mean ± S.D. ($n = 4$).
[b] Not detectable.

out any interference from coexisting substances. As shown in Table 1, the overall recoveries of secondary amines added to several food samples were 91–113% and the relative standard deviations were 0.4–8.2% ($n = 4$). The secondary amine contents in various food samples determined by this method are summarized in Table 2. It can readily be seen from our data that fish and fish products contained high concentrations of DMA (39–2129 nmol/g; literature value, 33–3556 nmol/g) and spices contained high concentrations of PYR (3–661 nmol/g; literature value, ND–1296 nmol/g) and PIP (ND-6351 nmol/g; literature value, ND–7553 nmol/g). On the other hand, secondary amine contents in grain, vegetable and meat were very low or not detectable. DMA was distributed in most of the foods investigated, but MEA, DEA, DPA, MOR, HMI and NMBzA were seldom detected in our study. These tendencies are similar to the results reported by Pfundstein et al. [9].

4. Conclusions

A convenient and reliable method for the determination of secondary amines in food samples has been established. This method is selective and sensitive, and food samples can be directly analysed without pre-treatment except for deproteinization and without any interference from other coexisting substances. We believe that this method provides a useful tool for routine analysis of foodstuffs.

References

[1] S.S. Miruish, *Toxicol. Appl. Pharmacol.*, 31 (1975) 325.
[2] G.B. Neurath, M. Duengar, F.G. Pein, D. Ambrosius and O. Schreiber, *Food Cosmet. Toxicol.*, 15 (1977) 275.
[3] B. Spiegelhalder and R. Preussmann, *Carcinogenesis*, 6 (1985) 545.
[4] W.R. Licht and W.M. Deen, *Carcinogenesis*, 9 (1988) 2227.
[5] R.C. Shank, *Toxicol. Appl. Pharmacol.*, 31 (1975) 361.
[6] W. Lijinsky, *Oncology*, 37 (1980) 223.
[7] A.R. Tricker and R. Preussmann, *Mutat. Res.*, 259 (1991) 277.
[8] S.E. Shephard, C.H. Schlatter and W.K. Lutz, *Food Chem. Toxicol.*, 25 (1987) 91.
[9] B. Pfundstein, A.R. Tricker, E. Theobald, B. Spiegelhalder and R. Preussmann, *Food Chem. Toxicol.*, 29 (1991) 733.
[10] E.S. Barreira, J.P. Parente and J.W. Alencar, *J. Chromatogr.*, 398 (1987) 381.
[11] J.R.L. Smith, A.U. Smart, F.E. Nancock and M.V. Twigg, *J.Chromatogr.*, 483 (1989) 341.
[12] C.X. Gao, I.S. Krull and T. Trainor, *J. Chromatogr. Sci.*, 28 (1990) 102.
[13] J.L. Rattay, E. Brandsteterova and D. Oktawec, *J. Liq. Chromatogr.*, 15 (1992) 307.
[14] S.C. Beale, J.C. Savage, D.W. Shawn, M. Wietstock and M. Novotny, *Anal. Chem.*, 60 (1988) 1765.
[15] A. Ibe, K. Saito, M. Nakazato, Y. Kikuchi, K. Fujinuma and J. Nishima, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 695.
[16] N.P.J. Price, J.L. Firmin and D.O. Gray, *J. Chromatogr.*, 598 (1992) 51.
[17] M. Maruyama and T. Nagayoshi, *J. Chromatogr.*, 594 (1992) 159.
[18] J.Y. Legender, J. Chalom, H. Kouwatli, M. Poulou, R. Farinotti and G. Mahuzier, *J. Chromatogr.*, 594 (1992) 386.
[19] A.D. Corcia, R. Samperi and C. Severini, *J. Chromatogr.*, 170 (1979) 325.
[20] M. Dalene, L. Mathiasson and J.A. Jonsson, *J. Chromatogr.*, 207 (1981) 37.
[21] L. Gronberg, P. Lovkvist and J.A. Jonsson, *Chromatographia*, 33 (1992) 77.
[22] E.W. Day, J. Golab and J.R. Koons, *Anal. Chem.*, 38 (1966) 1053.
[23] M. Koga, T. Akiyama and R. Shinohara, *Bunseki Kagaku*, 30 (1981) 745.
[24] S. Baba, I. Hashimoto and Y. Ishitoya, *J. Chromatogr.*, 88 (1974) 373.
[25] M. Makita, S. Yamamoto, K. Ikeda, T. Samejima and Y. Ohnishi, *Yakugaku Zasshi*, 97 (1977) 304.
[26] T. Lundh and B. Åkesson, *J. Chromatogr.*, 617 (1993) 191.
[27] A.C. Moffat, E.C. Horning, S.B. Matin and M. Rowland, *J. Chromatogr.*, 66 (1972) 255.
[28] A.J. Francis, E.D. Morgan and C.F. Poole, *J. Chromatogr.*, 161 (1978) 111.
[29] T. Hamano, A. Hasegawa, K. Tanaka and Y. Matsuki, *J. Chromatogr.*, 179 (1979) 346.
[30] T. Hamano, Y. Mitsuhashi and Y. Matsui, *Agric. Biol. Chem.*, 45 (1981) 2237.
[31] A. Terashi, Y. Hamada, A. Kido and R. Shinohara, *J. Chromatogr.*, 503 (1990) 369.
[32] B. Pfundstein, A.R. Ticker and R. Preussmann, *J. Chromatogr.*, 539 (1991) 141.
[33] O. Hinsberg, *Chem. Ber.*, 23 (1890) 2962.
[34] S. Ohrui, H. Kataoka, Y. Miyamoto, K. Ohtsuka and M. Makita, *Bunseki Kagaku*, 40 (1991) 119.
[35] H. Kataoka, M. Eda and M. Makita, *Biomed. Chromatogr.*, 7 (1993) 129.