JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Highly Sensitive Analysis of Iodide Anion in Seaweed as Pentafluorophenoxyethyl Derivative by Capillary Gas Chromatography

FENG-MIN LIN, HSIN-LUNG WU,* HWANG-SHANG KOU, AND SHUN-JIN LIN

Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan 807

A simple and sensitive gas chromatography (GC) method is described for the trace analysis of iodide anion (iodide) in processed seaweed as an organic derivative. The method is based on the derivatization of aqueous iodide extracted from seaweed with 2-(pentafluorophenoxy)ethyl 2-(piperidino)ethanesulfonate in toluene using tetra-*n*-hexylammonium bromide as a phase-transfer catalyst. The resulting pentafluorophenoxyethyl iodide is highly responsive to an electron-capture detector (ECD) and was analyzed by GC-ECD, giving a low detection limit of ~2.7 nM (2.7 fmol/μL injected). Interferences of some common anions in the analysis of iodide were studied and proved to be minimal. Application of the method to the analysis of iodide in processed seaweed was performed.

KEYWORDS: Iodide anion; processed seaweed; analytical derivatization; GC-ECD

INTRODUCTION

Iodide is an essential element for thyroid function and for the maintenance of normal human growth and development (*1*). Long-term deficiency in iodide intake is detrimental to the thyroid, leading to goiter (thyroid atrophy). The adult daily dietary allowance of iodide is very trace (~150 μg) (*1*), and highly sensitive methods are usually required for the analysis of iodide in various samples. Several methods including GC (*2*−*4*) and liquid chromatography (LC) (*5*) with sensitive detectors have been developed for monitoring iodide at trace levels. In GC, pentafluorobenzyl bromide (PFBBr) is widely used for the derivatization of organic nucleophiles for GC with electron-capture detection (ECD) (*6*, *7*) including carboxylic acids, phenols, mercaptans, and sulfonamides. We initially introduced PFBBr to the analytical derivatization of an inorganic anion (*8*) for ultrasensitive analysis of sulfide, but in the derivatization of iodide, the resulting derivative of iodide (pentafluorobenzyl iodide, PFBI) is not easily separated from the excess reagent (PFBBr) (*9*), due mainly to the close resemblance of both PFBI and PFBBr in chromatographic properties stemming from their only structural difference in the halogen substituent (i.e., bromine and iodine, respectively, for PFBBr and PFBI). Therefore, a chemically removable reagent, 2-(pentafluorophenoxy)ethyl 2-(piperidino)ethanesulfonate (PFPES), was devised (*10*) and used in the derivatization of iodide, resulting in better separation of the iodide derivative from the derivatizing reagent. In addition, PFPES is a nonlachrymatory solid and easily handled as compared to PFBBr, which is volatile and lachrymatory at room temperature. In this paper, a simple and practical method for the analysis of iodide in processed seaweed, based on a phase-transfer catalyzed reaction of iodide from seaweed with PFPES, was established. The resulting derivative (PFBI) has two moieties (iodo and pentafluorophenoxy) with high electron affinity, leading to the sensitive analysis of iodide by GC-ECD.

MATERIALS AND METHODS

Chemicals and Solutions. Potassium iodide (KI), potassium iodate (KIO_3), and sodium sulfite (Na_2SO_3) (E. Merck, Darmstadt, Germany), 1,2,4,5-tetrachlorobenzene (TCB) (internal standard, IS) (TCI, Tokyo, Japan), tetra-*n*-hexylammonium bromide (THAB) (Fluka, Buchs, Switzerland), iodine (I_2) (Shimakyu's, Osaka, Japan), toluene (Tedia, OH), and sulfuric acid (Fisher, NJ) were used without further treatment. PFPES was synthesized at our laboratory (*10*). All other chemicals were of analytical reagent grade. Solutions of PFPES and TCB were prepared by dissolving the appropriate amounts in toluene. Solutions of potassium iodide, THAB, and sulfuric acid were prepared in Milli-RQ (Waters) treated water (water).

Gas Chromatographic Conditions. A Shimadzu 14B gas chromatograph equipped with an ECD and an AOC-20i autoinjector was used. The capillary column was 50 m × 0.2 mm i.d. with 0.25 μm film of BP5 (Shimadzu, Japan). The operation temperatures were 170, 240, and 280 °C, respectively, for the column, injector, and detector. The pressures of nitrogen carrier and makeup gas were 250 and 60 kPa, respectively. The split injection with an inlet ratio of 1/20 was applied. The ratios of peak area of the iodide derivative to the IS used for evaluating the optimum conditions were computed by SISC data system (Taiwan) for derivatization and quantitation evaluation.

Extraction and Derivatization of the Iodine Species from Processed Seaweed. Processed seaweed samples were all from food companies in Taiwan. Seaweed (150 mg) was placed in a 10-mL glass-stoppered test tube containing 2.0 mL of water (or 2.0 mL of iodide reference solution for standard addition analysis) and triturated at room temperature with a homogenizer (Ultra-Turrax T8, S8N-8G) (Ika, Germany) at 25000 rpm for 10 min. The sample residue on the

* Corresponding author (telephone 886-7-312-1101, ext. 2316; fax 886-7-315-9597; e-mail m555001@cc.kmu.edu.tw).

10.1021/jf020861m CCC: $25.00
Published on Web 01/10/2003

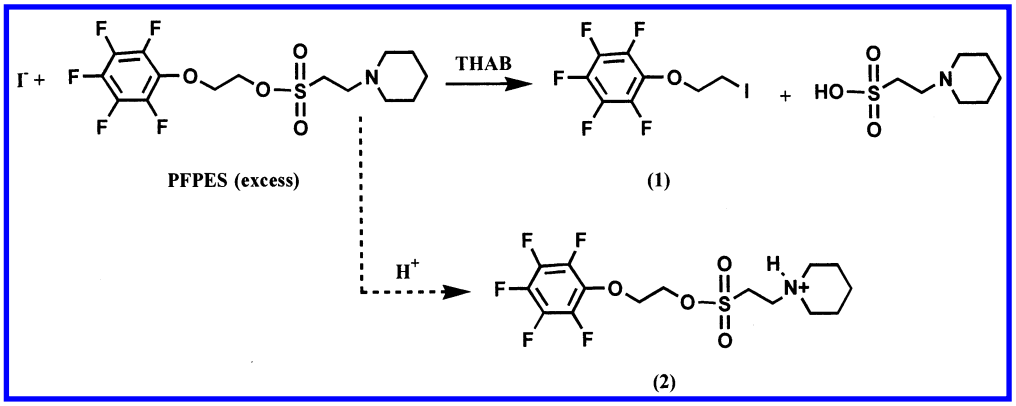

Figure 1. Phase-transfer catalyzed derivatization of iodide (I^-) (in water) with 2-(pentafluorophenoxy)ethyl 2-(piperidino)ethanesulfonate (PFPES) (in toluene) (solid arrow) and protonation of excess PFPES (dashed arrow) with aqueous acid for removal: tetra-*n*-hexylammonium bromide (THAB), a phase-transfer catalyst; compound **1**, a highly lipophilic iodide derivative for GC-ECD analysis; compound **2**, a water-soluble ammonium species from excess PFPES to be removed.

homogenizer was washed to a 10-mL test tube with 0.5 mL of water for free iodide analysis (or 0.5 mL of 5 mM Na_2SO_3 for total iodine analysis). The resulting solution was sonicated (Bransonic, Danbury, CT) for 20 min and then centrifuged for 10 min at 1800*g*. A 0.5-mL aliquot of the supernatant as sample solution was subjected to the following derivatization.

A 500-μL aliquot of the sample solution or reference iodide solutions (at various concentrations) was pipetted into a 20-mL screw-capped test tube containing 100 μL of THAB (5.0 mM) in water, 200 μL of TCB (30 μM) in toluene, and 400 μL of PFPES (20.0 mM) in toluene. The reaction mixture was mechanically shaken at 70 °C for 1 h in a thermostated water bath. After reaction, 1 mL of H_2SO_4 (0.5 M) was added and mixed by vortexing for 20 s. The separated toluene layer was further washed with 1 mL of water in a 2.0-mL microcentrifuge tube by vortexing for 20 s. The washed toluene layer was used for GC-ECD analysis by an autosampler (sample size of 1.0 μL).

RESULTS AND DISCUSSION

Figure 1 shows a reaction scheme for the derivatization of iodide with PFPES and the removal of excess PFPES after derivatization, leading to a cleaner chromatogram (**Figure 2**). Derivatization of iodide without subsequent acid treatment of the excess reagent (PFPES) resulted in a complicated chromatogram with a lengthy run time (**Figure 2**).

The trace element of iodine could exist as iodide, iodine, or iodate in various samples. In this method, the iodide anion can be easily derivatized with PFPES, but iodine and iodate must be reduced to iodide before derivatization. Several methods are available for the reduction of iodate or iodine to iodide with suitable reducing agents such as sodium sulfite (*11*, *12*) and ascorbic acid (*3*, *5*).

Ascorbic acid is an organic acid and could be derivatized by PFPES (*10*). Therefore, sodium sulfite was tried as the reducing agent. The effects of various concentrations of sodium sulfite (0.25−10 mM × 0.5 mL) on the reduction of iodine (2.5 μM × 500 μL) or iodate (2.5 μM × 500 μL) were studied. The optimized concentration of sodium sulfite for the reduction of iodine or iodate (evaluated by the peak area ratio of the resulting derivative) was found to be ≥2.5 mM, and 5 mM (500 μL) sodium sulfite was used for reduction. The average derivatization yields (percent, $n = 3$) of iodide at 70 °C for 2 and 1 h were 96.5 ± 1.9 and 84.6 ± 1.4, respectively, based on levels of 0.125, 0.25, and 0.2 μM of iodide tested. The iodide derivative is stable for 48 h (*10*). Because the difference in the yield is small and the resulting derivative is highly sensitive to an ECD, a shorter reaction time (1 h) is used for the derivatization of iodide at 70 °C. The optimized parameters were formulated to the protocol for the treatment and derivatization of iodine species.

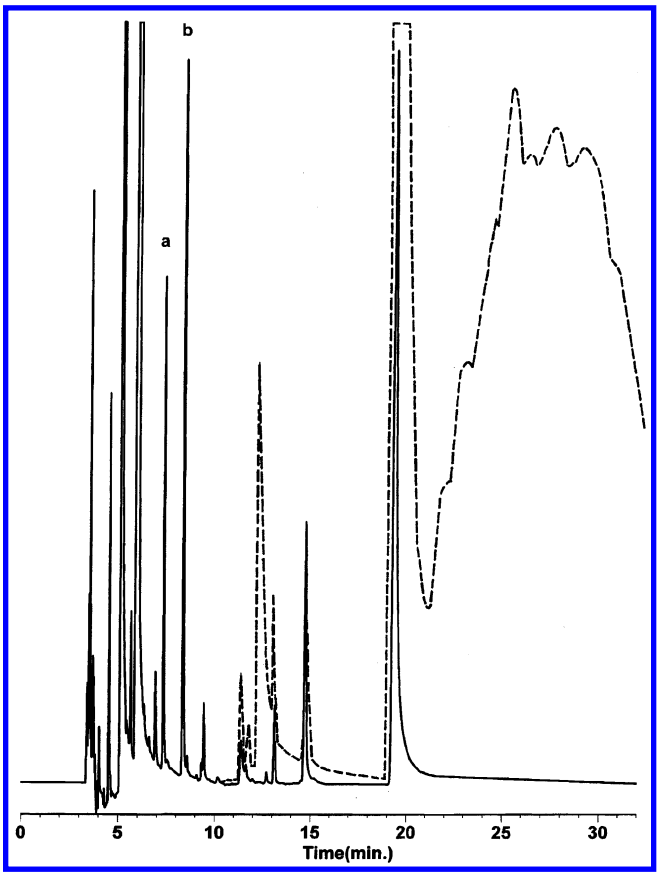

Figure 2. Composite GC chromatograms for the determination of reference iodide (2.5 μM × 500 μL): peak a, derivative of iodide; peak b, TCB (IS). Iodide was derivatized with PFPES with (solid line) and without acid treatment (dotted line). GC conditions: 50 m × 0.2 mm i.d. with 0.25 μm BP5 capillary column; carrier, nitrogen; inlet pressure, 250 kPa; makeup gas pressure, 60 kPa; operating temperatures for column, injector, and detector, 170, 240, and 280 °C, respectively; electron capture detector.

Interferences. Interferences from several common anions with the determination of iodide were examined. As shown in **Table 1**, the method is not affected by 500 μM each of F^-, Cl^-, Br^-, CO_3^{2-}, HCO_3^-, NO_2^-, NO_3^-, $H_2PO_4^-$, HPO_4^{2-}, or SO_4^{2-}, equivalent to a molar ratio of 200 of each tested anion to iodide. Sulfite was used as the reducing agent in this study,

Table 1. Results of Interference Study for Iodide Analysis

anion	concn (μM)	added as	recovery (%)[a]
none			100.0
F^-	500	KF	100.1 ± 0.7
Cl^-	500	NaCl	99.6 ± 1.1
Br^-	500	KBr	100.2 ± 0.5
CO_3^{2-}	500	K_2CO_3	101.2 ± 0.8
HCO_3^-	500	$KHCO_3$	100.8 ± 1.0
NO_2^-	500	KNO_2	100.9 ± 0.6
NO_3^-	500	KNO_3	101.4 ± 1.0
$H_2PO_4^-$	500	KH_2PO_4	100.2 ± 0.6
HPO_4^{2-}	500	K_2HPO_4	100.6 ± 0.7
SO_4^{2-}	500	K_2SO_4	100.9 ± 2.2
SO_3^{2-}	5000	Na_2SO_3	104.8 ± 3.1
$S_2O_3^{2-}$	50	$K_2S_2O_3$	101.8 ± 0.4
S^{2-}	50	K_2S	101.9 ± 4.6

[a] Peak area ratios (PAR) of the iodide derivative to IS in the absence of tested anion was arbitrarily set as 100, and the recovery (%) was calculated as the ratio of PAR from iodide with tested anion to PAR from iodide without test anion ($n = 3$); iodide concentration tested at 2.5 μM ($n = 3$).

and **Table 1** also indicates that sulfite at higher concentration of 5 mM does not interfere with the determination of iodide; thiosulfate ($S_2O_3^{2-}$) and sulfide (S^{2-}) at lower concentration of 50 μM do not interfere. The results indicate that the method does not interfere in the analysis of iodide coexisting with common anions at the levels tested.

Analytical Calibration. To examine the quantitative applicability of the method, six different concentrations of reference solution containing iodide anion in the range of 0.05−2.5 μM was evaluated. A calibration graph was established with the peak area ratios of the iodide derivative to IS as the ordinate (Y) versus the amount of iodide derivative (expressed as micromolar) as the abscissa (X). The linear regression equation obtained is $Y = (0.299 \pm 0.005)X - (0.008 \pm 0.001)$ with a correlation coefficient 0.999. The detection limit based on blank signal (*13*) of iodide is 2.7 nM (sample size of 1 μL). This indicates that the method is highly sensitive for the analysis of iodide anion.

Analysis of Free Iodide and Total Iodine in Processed Seaweed. Seaweed sample in the absence of iodide was unavailable as a sample matrix for the analysis of iodide; therefore, a standard addition approach (*13*) was used. The applicability of the method to the analysis of iodide in seaweed was evaluated by spiking samples with and without iodide. Four different concentrations of reference solution containing iodide spiked in the samples were prepared in the range of 0.1−2.0 μM. The linearity between the PAR (Y) and iodide (X, μM) was examined. Correlation coefficients ($n = 5$) of free iodide and total iodine obtained are all over 0.999 and 0.997, respectively. The precision based on the known amount of iodide in seaweed cannot be studied because of the coexistence of the endogenous iodide in seaweed. Therefore, the precisions (relative standard deviations, RSDs) based on the slope and intercept of the related regression equations for intraday ($n = 5$) and interday ($n = 6$) analysis were studied for free iodide and total iodine. **Table 2** shows that the RSDs for the slope and intercept are all below 4.9 and 5.5%, respectively. The method was applied to the analysis of iodide in seaweed. A typical chromatogram for the analysis of iodide in the sample is shown in **Figure 3**, indicating that no significant peaks from seaweed sample interfere with the IS. The reagent blank obtained in the analytical calibration of reference iodide solution also showed no peak interference with the iodide derivatives (data no shown). **Table 3** shows the analytical results of free iodide and total iodine in processed seaweed; RSDs ($n = 3$) for free iodide and total iodine analysis are all below 5.1 and 6.1%, respectively.

Table 2. Precisions for the Slope and Intercept of the Regression Equations from Free Iodide and Total Iodine Analysis in Processed Seaweed

sample	slope	RSD (%)	intercept	RSD (%)
intraday[a]				
free iodide	0.215 ± 0.006	3.0	0.203 ± 0.010	4.9
total iodine	0.211 ± 0.007	3.4	0.296 ± 0.012	4.1
interday[b]				
free iodide	0.215 ± 0.008	3.5	0.201 ± 0.011	5.5
total iodine	0.207 ± 0.010	4.9	0.299 ± 0.013	4.5

[a] Intraday assay variance was calculated from the triplicate analysis values of reference iodide at five intervals on a single day ($n = 5$). [b] Interday assay variance was calculated from the triplicate analysis values of reference iodide on six consecutive days ($n = 6$).

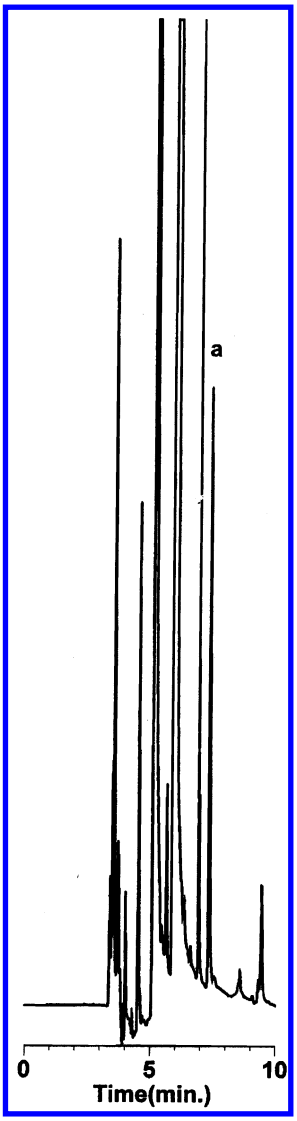

Figure 3. GC chromatogram for the analysis of free iodide in seaweed (from sample 4 in **Table 3**): peak: a, derivative of iodide. This sample was derivatizated without the addition of IS, indicating that no other peaks interfere with the IS.

In conclusion, a simple, sensitive, and selective method was developed for the trace analysis of iodide and total iodine in processed seaweed by derivatization and GC-ECD. The method could be useful for the analysis of iodide or iodine species in

Table 3. Results of Analysis of Free Iodide and Total Iodine in Processed Seaweed Samples

sample[a]	free iodide found[b] (μg g^{-1})	RSD (%)	total iodine found[b] (μg g^{-1})	RSD (%)
1	2.39 ± 0.05	2.3	3.65 ± 0.22	6.1
2	2.86 ± 0.15	5.1	4.31 ± 0.20	4.7
3	2.97 ± 0.09	3.1	4.37 ± 0.15	3.4
4	4.46 ± 0.07	1.5	6.26 ± 0.13	2.0
5	5.04 ± 0.18	3.6	7.91 ± 0.29	3.7
6	5.76 ± 0.17	2.9	9.35 ± 0.22	2.4

[a] Six different seaweed samples were all from food companies in Taiwan. [b] Free iodide and total iodine concentrations were averages of three replicate analyses.

foods, environmental samples, and biosamples, especially in cases requiring high sensitivity.

LITERATURE CITED

(1) (a) Farwell, A. P.; Braverman, L. E. Thyroid and antithyroid drugs. (b) Marcus, R.; Coulston, A. M. The vitamins intoduction. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 9th ed.; Hardman, J. G., Limbird, L. E., Eds.; McGraw-Hill: New York, 1996; (a) pp 1391−1394, (b) pp 1548−1549.

(2) Maros, L.; Kaldy, M.; Igaz, S. Simultaneous determination of bromide and iodide as acetone derivatizates by gas chromatography and electron capture detection in natural waters and biological fluids. *Anal. Chem.* **1989**, *61*, 733−735.

(3) Shin, H. S.; Oh-Shin, Y. S.; Kim, J. H.; Ryu, J. K. Trace level determination of iodide, iodine and iodate by gas chromatography−mass spectrometry. *J. Chromatogr. A* **1996**, *732*, 327−333.

(4) Mishra, S.; Singh, V.; Jain, A.; Verma, K. Determination of iodide by derivatization to 4-iodo-*N*,*N*-dimethylaniline and gas chromatography−mass spectrometry. *Analyst* **2000**, *125*, 459−464.

(5) Verma, K.; Jain, A.; Verma, A. Determination of iodide by high-performance liquid chromatography after precolumn derivatization. *Anal. Chem.* **1992**, *64*, 1484−1489.

(6) Poole, C. F.; Poole, S. K. Derivatization as an approach to trace analysis by gas chromatography with electron-capture detection. *J. Chromatogr. Sci.* **1987**, *25*, 434−443.

(7) Nicholson, J. D. Derivative formation in the quantitative gas chromatographic analysis of pharmaceuticals. *Analyst* **1978**, *103*, 1−28.

(8) Wu, H. L.; Funazo, K.; Tanaka, M.; Shono, T. Electron-capture gas chromatographic determination of sulphide as a new pentafluorobenzyl derivative. *J. Chromatogr.* **1981**, *219*, 312−316.

(9) Wu, H. L.; Lin, S. J.; Funazo, K.; Tanaka, M.; Shono, T. Trace determination of iodide by derivatization and electron-capture gas chromatography. *Fresenius' Z. Anal. Chem.* **1985**, *322*, 409−412.

(10) Lin, S. J.; Wu, H. L.; Lin, C. Y. A new derivatization reagent 2-(pentafluorophenoxy)ethyl 2-(piperidino)ethanesulfonate for gas chromatography. *Anal. Chim. Acta* **1999**, *386*, 113−122.

(11) Reifenhauser, C.; Heumann, K. G. Development of a definitive method for iodine speciation in aquatic systems *Fresenius' Z. Anal. Chem.* **1990**, *336*, 559−563.

(12) Hou, X.; Dahlgaard, H.; Rietz, B.; Jacobsen, U.; Nielsen, S. P.; Aarkrog, A. Determination of chemical species of iodine in seawater by radiochemical neutron activation analysis combined with ion exchange preseparttion. *Anal. Chem.* **1999**, *71*, 2745−2750.

(13) Skoog, D. A., Holler, F. J., Nieman, T. A., Eds. *Principles of Instrumental Analysis*, 5th ed.; Saunders College Publishing: Philadelphia, PA, 1998; pp 13, 15−18.

Received for review August 6, 2002. Revised manuscript received November 18, 2002. Accepted November 24, 2002. H.-L.W. is grateful to the National Science Council (Taiwan) for financial support of this work (NSC 90-2113-M-037-026 and NSC 91-2113-M037-022)

JF020861M