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Studies on Iodinated Compounds. III.[1] High-Performance Liquid Chromatographic Determination of Iodide (I^-)

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A simple and sensitive reversed-phase high-performance liquid chromatographic (HPLC) method was developed for the determination of iodide (I^-). Iodide was separated on a reversed-phase C_{18} column with 0.01 M dibutylamine phosphate in H_2O–CH_3OH (80:20) (pH 3.0) as a mobile phase, and detected with an ultraviolet (UV) detector at 225 nm. The assay can be practically performed in the range of 50 pmol (6.4 ng) to 15 nmol (1.9 μg) of iodide. The limit of detection was 500 pg (S/N = 3). The method was applied to the detection and assay of inorganic iodide contained in organic iodine compounds as an impurity.

Keywords——iodide; HPLC; dibutylamine phosphate; UV spectrum; UV detection; ion pair chromatography; monoiodocarnosine; monoiodotyrosine

Gas chromatography (GC)[2] and ion chromatography (IC)[3] have been used in the separation and quantitation of iodide ion (I^-). However, the sample must be derived to a suitable compound for GC, and an electrochemical detector is required for IC.

On the other hand, it was reported that inorganic iodide shows light absorption in the ultraviolet (UV) region.[4] We therefore tried to separate and analyze iodide ion quantitatively using a reversed-phase high-performance liquid chromatography (HPLC) equipped with a UV detector. This method was satisfactory to detect and assay inorganic iodide ion contained in organic iodide compounds as an impurity.

Experimental

Reagents——Potassium iodide (analytical reagent grade) was a product of Iwai Kagaku Co., Ltd. A 1.0 M dibutylamine phosphate (DBAP) solution (Reagent D4®) was obtained from Waters Associates. Other reagents were commercial ones of analytical reagent grade, unless otherwise stated. Monoiodocarnosine was prepared by the method previously reported.[1] Monoiodotyrosine (MIT) was a commercial preparation, obtained from Sigma Chem. Corp., labelled "contains approx. 5% tyrosine."

Apparatus——The HPLC equipment consisted of a 6000A pumping system, a U6K universal injector (Waters Assoc.) and a variable UV detector, model NS-310 (Nippon Seimitsu Kagaku). The column (3.9 mm i.d. × 30 cm) was packed by us with LiChrosorb RP-18, 7 μm (E. Merck). UV spectra were measured on a Hitachi type 100-50 double beam spectrophotometer with a type 200 recorder.

Determination of Iodide by HPLC——The mobile phase was prepared by dissolving 10 ml of 1.0 M DBAP (1 vial of Reagent D4) in 1 l of H_2O–CH_3OH (80:20, v/v); the solution was filtered through a 0.45 μm filter.[5] The final concentration of the prepared solution was 0.01 M and the pH was 3.0.[6] The flow rate of the mobile phase was set at 1 ml/min, and the wavelength of the detector at 225 nm. Calibration curves of iodide were prepared for 10^{-3}, 10^{-4} and 10^{-5} M levels of KI standard solution, by injecting 2.5—20 μl of sample into the HPLC instrument. Organic iodide samples were prepared as follows; monoiodotyrosine (MIT) was dissolved in 0.005 N HCl (0.5 mg/ml), while monoiodocarnosine (MIC) was dissolved in water (1.0 mg/ml), and the solution was filtered through a 0.45 μm filter, then subjected to HPLC.

Results and Discussion

Adsorption and tailing of basic samples on an HPLC column can be prevented by using acidic amine phosphate solution as the mobile phase in reversed phase HPLC on chemically bonded silica gel, from which residual silanol moieties have not been removed.[7] Iodide ion is not retained sufficiently on the reversed-phase HPLC column when ammonium phosphate buffer (pH 3.0) is used as the mobile phase, but it is retained on the column when DBAP is containing acetonitrile (λ_{max} nm (ε): 225 (14700), 0.01 M DBAP in H_2O–CH_3CN (75 : 25); 228 ion–pair formation of iodide and dibutylamine.

The UV spectra of iodide in a DBAP-containing solvent were measured. The maximum absorption of iodide in water was at 226 nm (ε 13400), and it was slightly shifted to the shorter wavelength region in DBAP solutions containing CH_3OH (λ_{max} nm (ε): 225 (12800), 0.01 M DBAP in H_2O; 224 (14200), 0.01 M DBAP in H_2O–CH_3OH (75 : 25); 222 (14400), 0.01 M DBAP in H_2O–CH_3OH (50 : 50)), and to the longer wavelength region in DBAP solutions containing acetonitrile (λ_{max} nm (ε): 225 (14700), 0.01 M DBAP in H_2O–CH_3CN (75 : 25); 228 (14500), 0.01 M DBAP in H_2O–CH_3CN (50 : 50)). The wavelength for detection was set at 225 nm, and iodide was detected with satisfactorily high sensitivity.

Calibration curves of iodide were prepared based on the peak height obtained with standard KI solutions. A good linear correlation ($r=0.9998$) of the peak height and concentration of iodide was obtained in the range of 0.25 nmol (31.8 ng) and 1.5 nmol (190 ng) of iodide at 0.1 AUFS. A good linear correlation was also obtained at other detector sensitivities: 50—200 pmol at 0.01 AUFS with $r=0.9992$, and 2.5 nmol—15.0 nmol at 1.0 AUFS with $r=0.9999$. Therefore, 50 pmol (6.4 ng) to 15 nmol (1.9 μg) of iodide may be assayed by the present method. The limit of detection of iodide was 500 pg (S/N=3, 0.005 AUFS).

The influence of other halogen ions on the determination of iodide ion by HPLC was examined. Bromide ion was detected with the UV detector used in the present study, but it was eluted at a different position (retention time) from that of iodide (retention times: Br^-,

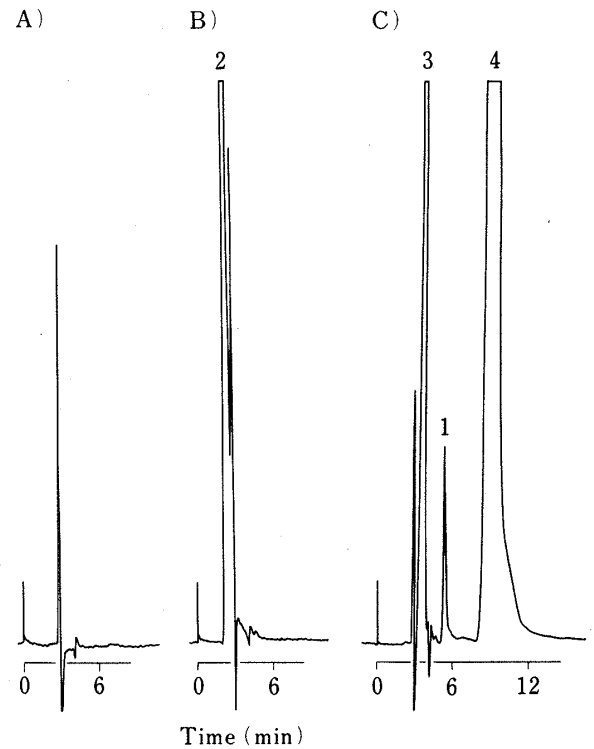

Fig. 1. HPLC Chromatograms

Sample, injection volume and sensitivity: A, H_2O (blank), 5 μl, 0.005 AUFS; B, MIC, 5 μl, 0.005 AUFS; C, MIT, 10 μl, 0.01 AUFS Peak identity: 1, iodide; 2, MIC; 3, tyrosine; 4, MIT.

4.4 min; I^-, 5.3 min (detected at 205 nm)). Fluoride and chloride, on the other hand, could not be detected with the UV detector at 205 nm, so that separation of fluoride and chloride from iodide could not be demonstrated under the present conditions of HPLC. The retention time and peak height of iodide on HPLC in the presence of fluoride and chloride were checked by adding 100 nmol KF, 100 nmol KCl or 500 nmol HCl to 1 nmol KI, but there was no significant change in these indices. Therefore, these halogen ions were considered not to disturb the assay of iodide by HPLC.

Reagent D4 (commercially compounded preparation) and a mixture of dibutylamine and phosphoric acid, prepared in our laboratory, gave the same results as the mobile phase for iodide assay.

This assay method for iodide was applied to detect and determine iodide ion possibly contained as an impurity in organic iodine compounds (MIC and MIT) (Fig. 1). MIC prepared in our laboratory did not contain any significant amount of iodide, while commercial MIT contained 11.7 ng of iodide in 5 μg of sample (0.23%). The recovery of iodide was found to be $96.4 \pm 3.44\%$ ($n=5$) when 12.7 ng iodide was added to 5 μg MIT.

In conclusion, iodide ion can be quantitatively and qualitatively determined by HPLC with the reversed-phase column and a UV detector by simple and sensitive procedures. The sensitivity of the present method is comparable to that of the electrochemical detector.[3] The present method permits simultaneous determination of iodide ion and an organic compound having absorption in the UV region.

References and Notes

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