

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

No. L215

High Sensitivity and High Selectivity Analysis
of Ammonia with HPLC

Ammonia is generated by the corruption or decomposition of compounds containing nitrogen. Also ammonia, which is utilized as the main component related to the fixation of nitrogen, widely exists in nature. Industrially, it is generated from various production processes such as fertilizers, synthetic polymers, carbide, and pharmaceuticals. Quantitative measurement of ammonia is being performed in a wide field including quality control, measurement in working environment, monitoring of environmental atmosphere, as well as in the field of biochemistry.

Introduced here are a method that permits unequally high sensitive and selective analysis of ammonia (patent pending), and examples of analyses by this method applied for the analyses of sea water, rain water, and ammonia in a reagent.

For the purpose of selective analysis of ammonia by HPLC, post column derivatization is generally applied as is the case of analysis of amino acid, but derivatization reagents often applied conventionally such as ninhydrin and *o*-phthalaldehyde (OPA) are not so sensitive for ammonia as is the case of amines, and, in the case of complicated samples, are apt to be influenced by foreign substances. It has been found that by using sulfurous acid together with OPA, in place of mercaptan conventionally applied, ammonia can be detected with an unprecedentedly high selectivity and sensitivity. Table 1 shows comparison of fluorescence intensity OPA compounds in the presence of auxochromes. The optimum excitation wavelength and emission wavelength were obtained by the rapid scanning function of the RF-550 multifunction fluorescence detector. Figure 1 shows a flow diagram of HPLC for ammonia analysis and Figure 2, a chromatogram of ammonia standard (100 pmol).

Table 1 Comparison of Fluorescence Intensity between *o*-phthalaldehyde-sulfurous Acid and *o*-phthalaldehyde-2-mercaptoethanol

Compounds	OPA-Na ₂ SO ₃	OPA-2-ME
Ammonia	100	30
Glycine	56	496
Arginine	13	249
Leucine	25	350
Ethanolamine	16	516
Methylamine	28	225

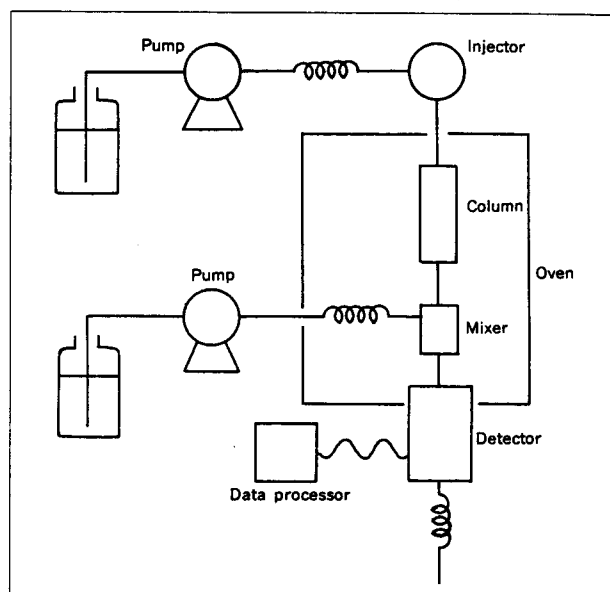


Fig.1 Flow Diagram of HPLC for Ammonia Analysis

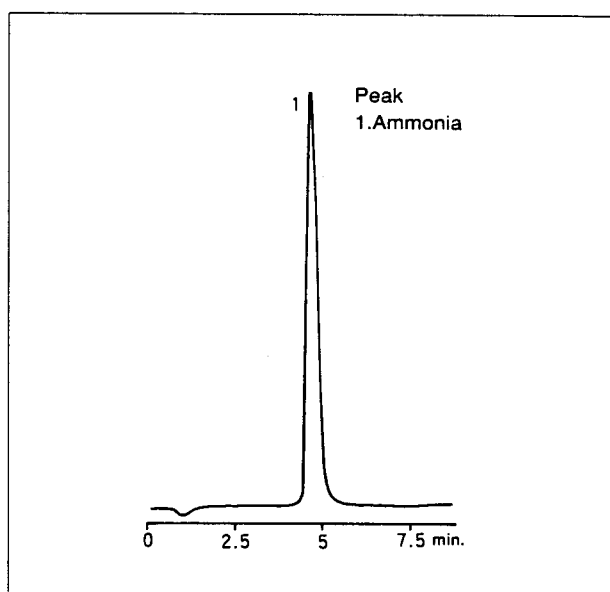


Fig.2 Chromatogram of Ammonia Standard (100pmol)

Table 2 shows the analytical conditions for the system, and in Figure 3, a calibration curve for ammonia. In the analysis introduced here, a good linearity was obtained within the range of 100 pmol-10 nmol.

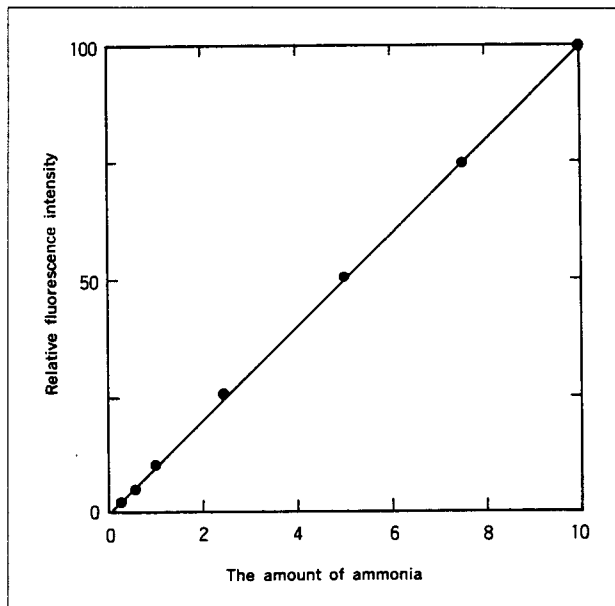


Fig.3 Relationship Between the Amount of Ammonia and Fluorescence Intensity

■ Application to Practical Samples

Figures 4, 5 and 6 show chromatograms of rain water (5 μ L), sea water (2 μ L), and commercially available Glutamin standard (500 μ g/mL, 2 μ L) as examples of application to practical samples. In each of the analyses, high sensitivity detection was possible without being hindered by foreign substances.

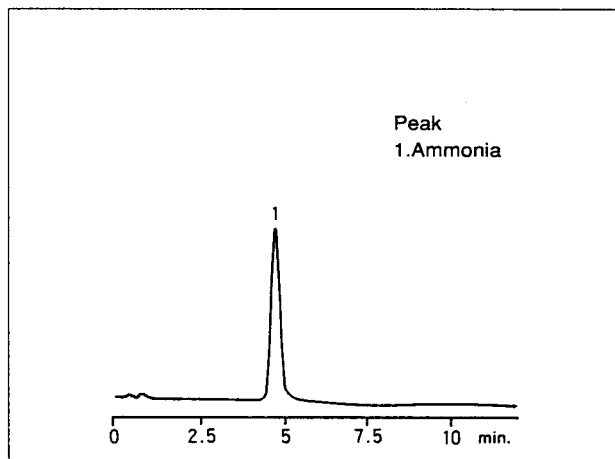


Fig.4 Chromatogram of Rain Water (5 μ L)

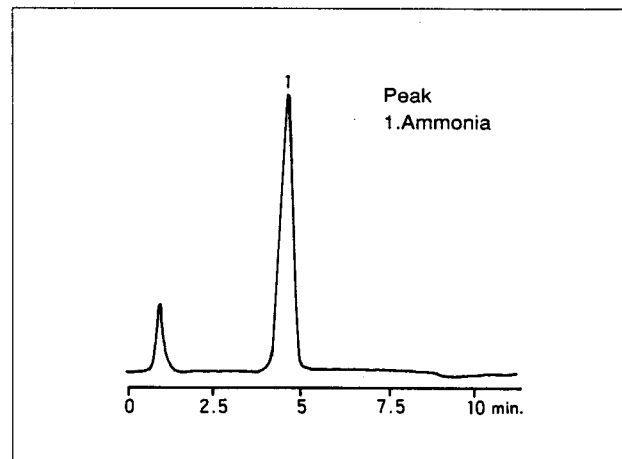


Fig.5 Chromatogram of Sea Water (2 μ L)

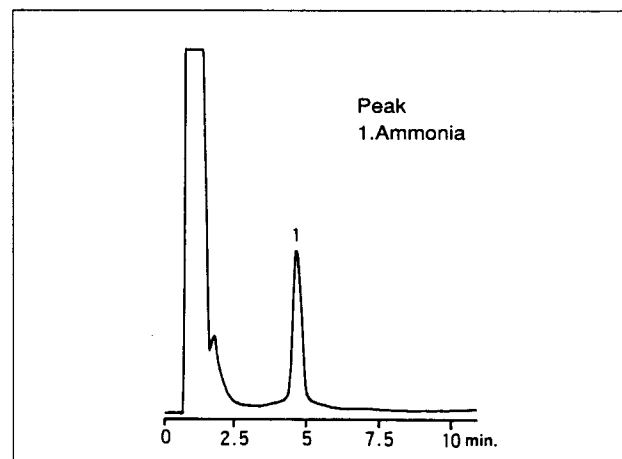


Fig.6 Chromatogram of Glutamin Standard (500 μ g/mL, 2 μ L)

Table 2 Analytical Conditions

	[for separation]
Column	: Shim-pack WCX-1 (4mm ϕ ×50mmL)
Mobile Phase	: 5mM (sodium) citrate <pH=6.2>
Flow Rate	: 1.0mL/min.
Temperature	: 50°C
	[for detection]
Reagent	: 20mM o-phthalaldehyde (methanol solution)/200mM (sodium) borate <pH=9.2> containing 2mM (sodium) sulfite=1 : 4 (v/v)
Flow Rate	: 0.5m/min.
Mixer	: piping part J
Temperature	: 50°C
Detector	: RF-550 at Ex=320nm Ex=390nm