

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

No. L217

HPLC Determination of Sulfurous Acid Using Post-column OPA Derivatization with Fluorescence Detection

Sulfurous acid, which does not exist as the free acid [H₂SO₃], but as the ion [HSO₃⁻] in forms such as the sulfites [SO₃²⁻], appears widely in many antioxidants and measurement of the concentration in foods is important from the point of quality controls to ensure that acceptable levels are not exceeded. As sulfurous acid ion is contained in the exhaust gases from factories and automobiles, etc., which burn fossil fuels with high sulfur content, measurement of the concentration in environmental samples such as rain water should not be neglected.

For the determination of sulfurous acid ion, the Rankine method is often applied. In this method, the sample is distilled under strongly acidic conditions and the generated sulfurous acid is then oxidized by hydrogen peroxide to sulfuric acid which is then titrated with a standard reagent such as sodium hydroxide. The use of ion chromatography (IC) has been reported for the determination of sulfuric acid. Also, the direct determination of [SO₃²⁻] by IC is also possible. In both of these methods, however, there are some problems with respect to operability, sensitivity and accuracy of measurement.

In order to avoid the above problems, a method of high accuracy determination of sulfurous compounds using post-column OPA derivatization with fluorescence detection was developed for the high-sensitivity determination of sulfurous compounds.

In this method, sulfurous acid ion is made to react with formaldehyde to form the stable hydroxymethanesulfonic acid in order to prevent decomposition during sample preparation and separation. After separation on the analytical column, it is made to react with *o*-phthalaldehyde (OPA) and an amine which results in a fluorescent isoindole derivative, for detection. In this way, it has become possible to determine unstable sulfurous compounds with high sensitivity and high accuracy.

Instrument and Methods

The flow path diagram of the instrument used for the above analysis is shown in Fig. 1.

A spectrofluorophotometric detector with variable wavelength is used. Analytical conditions are shown in Table 1.

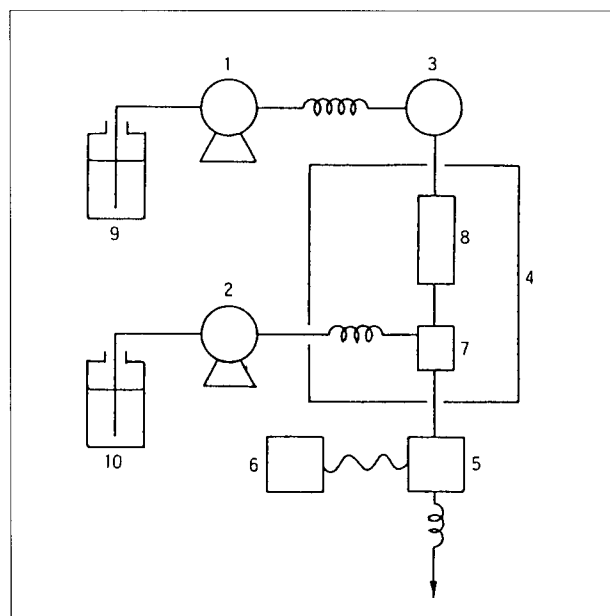


Fig. 1 Flow Diagram of HPLC system for the Determination of Sulfurous Acid.

Apparatus:

1, 2: pumps, 3: injector, 4: column oven, 5: fluorescence detector, 6: data processor, 7: reactor, 8: column, 9: mobile phase, 10: reagent.

Table 1 Analytical Conditions

	{for separation}
Column	: Shim-pack IC-A 1 (4.6mm I.D. × 100mm L.)
Mobile Phase	: 10mM (ammonium) citrate <pH = 3.2>
Flow Rate	: 1.0mL/min
Temperature	: 50°C
	{for detection}
Reagent	: 10mM <i>o</i> -phthalaldehyde methanolic solution and 500mM (sodium) borate <pH=9.2> (1 : 4, v/v)
Flow Rate	: 0.5mL/min
Reactor	: piping part J
Temperature	: 50°C
Detection	: fluorescence at Ex = 320nm Em = 390nm

■ Analysis of Standard Sample

Figure 2 shows the chromatogram of a standard solution of sulfurous acid analyzed by using the above mentioned method. In this analysis, the absolute amount of sulfurous acid injected was 1 pmol. The detection limit for sulfurous acid by this method was calculated to be 100 pmol. The calibration curve was linear over a wide range above 100 pmol and passed through the origin. Furthermore, the reproducibility for 5 repeat measurements was 0.54% (RSD). It was concluded that this method proved to be excellent in both sensitivity and accuracy.

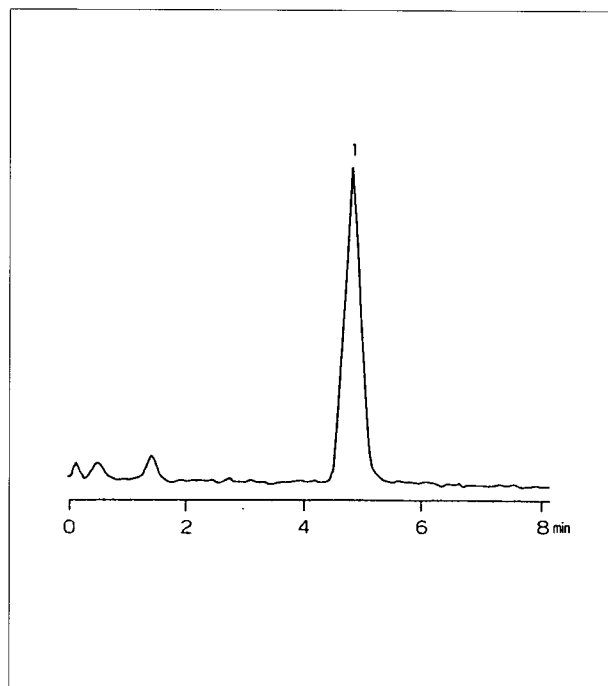


Fig. 2 Chromatogram of a Standard Solution of Sulfurous Acid.
Peak; 1: sulfurous acid (1 pmol).

■ Analysis of Sulfurous Acid in Rain Water

An example of analysis of sulfurous acid in rain using the above method, is shown in Fig. 3.

Just after a sample was taken from rain water, an equal volume of 10 mM citric acid buffer solution <pH4> containing 10 mM formaldehyde was added to the sample. From the results of the analysis, the concentration in the sample was calculated to be 2.1 pmol/ml. Because of the extremely high selectivity, it seems that this method is probably applicable to a broad variety of samples without the need for extensive pretreatment.

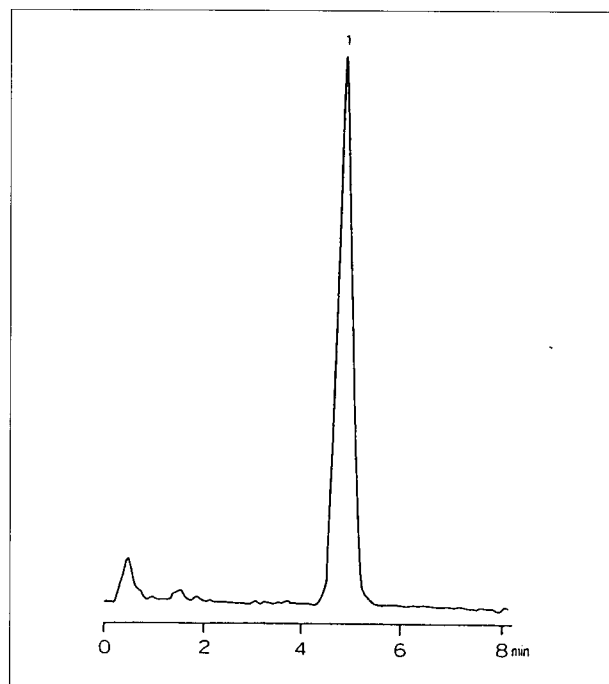


Fig. 3 Chromatogram of Rain Water.
Peak; 1: sulfurous acid (2.1 pmol/ml).



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