Determining Method of Sulfites in Foods by Ion Chromatography

Myung-Hee Kim and Young-Zoo Chae

Seoul Metropolitan Government Institute of Health and Environment, Seoul, 140-212 Korea

ABSTRACT—An ion chromatographic (IC) method was developed for the determination of sulfites in foods. Sulfites refer to sulfur dioxide that was separated from a food sample by addition of acid and heating, and carried into a trapping solution by distillation. The trapping solution was applied to IC system. Sulfites was separated on an anionic separator column, HPIC-AS4A with 0.75mM $NAHCO_3$/2.25mM Na_2CO_3 as the eluent and determined by the use of conductivity detector.

The recoveries of sulfites added to water, carrot and apple at level of 1 ppm were 99.8%, 91.6% and 83.5%, respectively.

The detection limit was 0.2 ppm in the case of a 10 g sample size. All experiment could be finished within 20 minutes

Keywords□Ion chromatography, Food additive, sulfite

Sulfiting agents are one of the most widely used food preservatives. The term of sulfiting agents refers to sulfur dioxide and several forms of inorganic sulfites that liberate sulfur dioxide under the conditions of use. Sulfiting agents are used in food for many purposes: the inhibition of nonenzymatic and enzymatic browning, inhibition of microorganism growth, serving as an antioxidant, and stabilizing agent.

Six sulfiting agents are allowed for use in food: sulfur dioxide(SO_2), sodium sulfite($Na_2SO_3 \cdot 7H_2O$), potassium metabisulfate($K_2S_2O_5$), sodium sulfite anhydrous(Na_2SO_3), sodium bisulfite($NaHSO_3$) and sodium hydrosulfite($Na_2S_2O_4$).[1,2] But many foods contain a variety of sulfur containing compound, including the sulfur amino acid, sulfate, sulfites and sulfides. Therefore, it is too difficult to decide whether the agent was used intentionally or that was the natural level.

Sulfiting agents have been used widely since the early 19th century. But the analytical methods have undergone only minor modification. At present, several methods for measurement of sulfite residue level have been reported, but none of them is suitable for the measurement of all forms of combined sulfite.

Recently, several improved methods for the analysis of total SO_2 have been developed including colorimetric, titrimetric, indirect atomic absorption spectrometric, gravimetric, spectrophotometric, fluorimetric, gas chromatographic, and enzymatic techniques.[3-9]

But some of these methods require considerable time, and these are relatively insensitive and potentially inaccurate. So we tried to find out an accurate and sensitive analytical procedure to measure sulfites in food with a minimum amount of time and analytical effort. Then, we developed the ion chromatography for the determination of sulfur dioxide in food. This IC method offered a fast, more sensitive and effective techniques for the determination of sulfite in food.

MATERIALS AND METHODS

Equipment and Chemicals—A Dionex model 4000i ion chromatograph equipped with standard HPIC-AG4A guard column and HPIC-AS4A separator column. Eluent was 0.75 mM sodium hydrogen carbonate($NaHCO_3$)/2.25mM sodium carbonate, anhydrous(Na_2CO_3) at flow rate 1.7 ml/min. Dionex conductivity detector output set between 1 and 30 μs. Supressor was used anion micromemb-

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Reprint request; Dr. M.H. Kim at the above adress.

rane supressor regenerating with 0.025N-H_2SO_4. 50 μl sample loop was used for all experiment. Dionex (model 4290) computing integrator was used for calculation. All solutions were prepared in deionized water from guaranteed grade chemicals

Procedures—Homogenized sample(5-10g) was transferred to 500 m*l* flask and added 25 m*l*, 25% H_3PO_4 and 100 m*l*, H_2O. The flask was assembled to steam distillation apparatus and all glass joints were tightly sealed with stopcock grease. Placed 100 m*l* volumetric flask containing 20 m*l* trapping solution (3%-H_2O_2) under condenser outlet. When the volume of distillate was about 100 m*l*, the reaction was stopped and the distillate was volumed up to 100 m*l* accurately with H_2O. 50 μl distillate was injected into IC system. Standard solution (0.2-10 ppm) and blank(H_2O) were carried out with same as previous procedures.

RESULT AND DISCUSSION

Fig. 1 and Fig. 2 showed the IC chromatogram

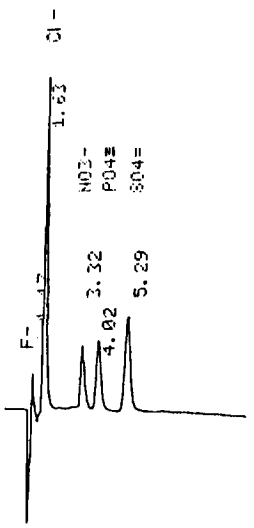

Fig. 1. ILC chromatogram of anion standards.

PEAK#	AREA%	RT	AREA	BC
1	17.838	1.17	933004	02
2	45.894	1.63	2400517	03
3	8.384	3.32	438511	01
4	10.424	4.02	545246	01
5	17.46	5.29	913267	01
TOTAL	100.		5230545	

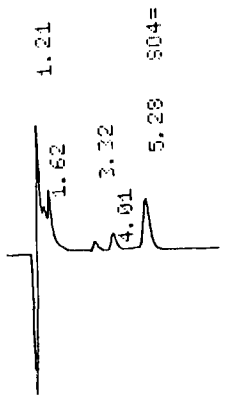

Fig. 2. ILC chromatogram of sample (Carrot).

PEAK#	AREA%	RT	AREA	BC
1	48.801	1.21	2206313	02
2	38.375	1.62	1734955	03
3	1.129	3.32	51060	01
4	2.502	4.01	113128	01
5	9.193	5.28	415605	01
TOTAL	100.		4521061	

of distillated sulfite standard solution and sample.

Retention time of sulfate ion peak (1 ppm as SO_2) was 5.29 min and the area count of the peak was 9,132.67. Samples was shown the same chromatogram; sulfate ion peak showed the 4,156.05 count at 5.28 min without interference by any other compositions in sample.

The analyzing time was finished within 10 min.

Standard calibration curve was linear over the concentration of 5 ppm(Fig. 3) and the detection li-

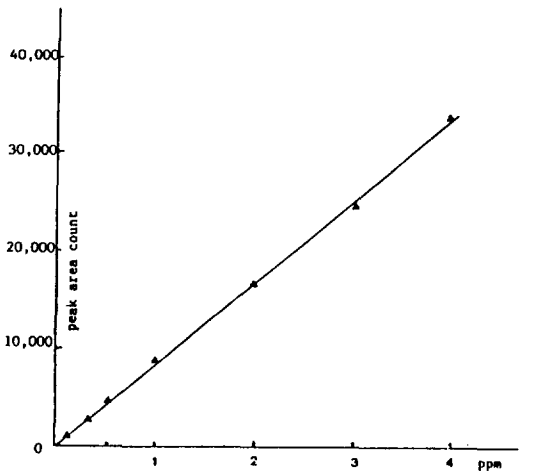

Fig. 3. Standard calibration curve.

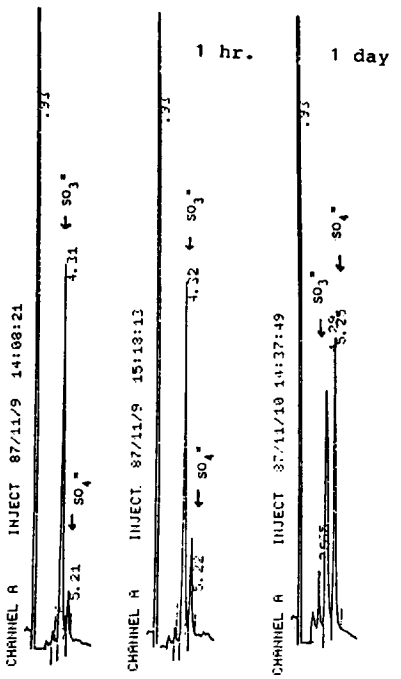

Fig. 4. ILC chromatogram on the sulfite ion unstability in alkaline trapping solution.

mit was 0.2ppm as SO_2.

Fig. 4 was shown the unstability of sulfite ion in sodium hydroxide + glycerin trapping solution. Retention time of the peaks of sulfite and sulfate ion were 4.31, 5.22 min. After 1 hr, sulfite ion peak was a little reduced and the sulfate ion peak was increased. After 1 day, this phenomenon showed remarkably that sulfite ion was changed to sulfate ion by oxidation. So we tried to used 3% hydrogen peroxide as a trapping solution converts SO_2^{-2} or SO_3^{-2} to SO_4^{-2} and the sulfate level provides an indication of the original sulfite evolved.[10]

Recoveries of 1 ppm standard solution with distilled water, 10 g of carrot and 10 g of apple were shown in Table 1. Recovery rate was determined by distillation and followed by IC method. Distilled water added 1 ppm standard presented excellent recovery rate, 99.8%. Whereas carrot added 1 ppm standard was 91.6 and apple was 83.5%. These result showed that the recovery rate was different by food matrics.[11]

Table 2 showed the recovery rate affected by trapping solution volume. At the volume of 15 m*l* and 30 m*l*, the recovery was shown considerably good but 20 m*l* of trapping solution presented very high recovery rate, 99.8 ± 0.53 %. So we used 20 m*l* trapping solution in this experiment.

This method combined the chemical approach of the distillation technique with the superior detection system available with ion chromatography.

At the present time, method for sulfur dioxide are differentiated by whether they measure free or free and bound sulfur dioxide-releasing chemicals.[12] The terms 'free' and 'bound' are defined by the methods. Bound sulfur dioxide means those compounds that release sulfur dioxide in a strong acid or base medium. Free sulfur dioxide means those sulfite species not combined with food constituents. Fujita *et al.*,[13] have demonstrated that the free sulfite is released on addition of the phosphoric acid to the test portion at 0 °C. Heating releases to bound sulfite. Also, increasing the concentration of phosphoric acid to 50 % leads to quantitative recovery of tightly bound sulfite. But we could analyzed only total sulfite. We think more studies are needed on this point.

We have three official methods for the determination of sulfites in food. First method is using titration with sodium hydroxide for quantitation. Food sample was transferred to a distillation flask

Table 1. Recoveries of standard and standard added samples

	No. of experiment	Area count	Recovery rate (%)
Direct injection (1 ppm STD)	10	9,132.7±11.5	-
Distillation (H_2O+ 1 ppm STD)	10	9,112.8±13.7	99.8±0.15
Distilla tion (carrot+ 1 ppm STD)	5	8,365.5±36.3	91.6±0.43
Distillation (apple 10g+ 1 ppm STD)	5	7,625.8±39.5	83.5±0.51

Mean±SE

Table 2. Effect of the volume of trapping solution

Volume	No. of experiment	Area count	Recovery rate (%)
10ml	5	8,101.6±40.7	88.7±0.51
15ml	5	8,938.7±39.2	97.9±0.44
20ml	5	9,110.2±48.3	99.8±0.53
30ml	5	8,998.3±50.7	98.5±0.50
40ml	5	8,401.8±50.7	92.0±0.60

Mean ±SE

containing 180 m*l* water that had been deoxigenated with a stream of carbon dioxide. Although this method (modified Monier-Williams technique) is the most frequently used for the sulfite determination, it has recently been reported to be unreliable at sulfite levels below 50 ppm. Also, it is not applicable to food that contains other volatile acids or organic sulfur compounds.

In method 2, its principle is almost same as that of method 1, except using other trapping solution, no deoxigenation step and titration with iodine solution. So this method gives a high detection limit, requires considerable time, and it is relatively insensitive and inaccurate in certain instance. The presence of other reducing substances in a food may produce an over estimation of sulfite.

In method 3, sulfite reacts with Fuchsin-formaldehyde solution and produces a reddish purple product, and it is determined colorimatically. The major problem of this method are lack of specificity and high detection limit. Especially at the high concentration of sulfite, the standard calibration curve by method 3 was shown a considerable quenching effect. And absorbance between distillated and directly developed standard solution was shown a quite differences (Fig. 5).

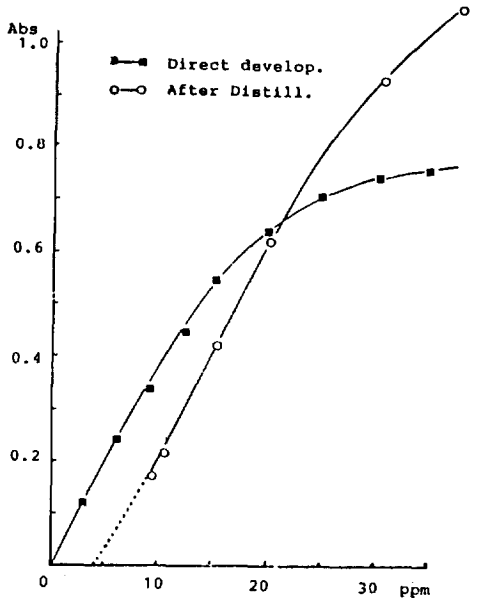

Fig. 5. Absorbance of distillated and directly developed standard solution with Fuchsin-formaldehyde.

None of them is suitable for the measurement of sulfite in food. So we tried to develop a new method for determination of sulfite by using a ion chromatography. We have arrived at a fast, accurate and reproducible procedure to monitor sulfite in a wide variety of food products.

국문요약

아황산염류는 표백, 살균, 갈변방지 및 항산화 등의 목적으로 식품첨가물로 많이 사용되고 있다. 이러한 아황산염류의 시험방법은 우리나라 보사부의 공정시험법으로 지정된 적정법과 비색법 이외에도 GLC, HPLC, AAS 등을 이용한 많은 분석방법들이 연구되어 왔으나 그 정밀도, 검출한계, 안정성 등에 많은 문제가 있다. 이에 저자 등은 IC를 이용하여 아황산염류를 신속하고 정확하게 정량할 수 있는 방법을 모색하여 다음과 같은 결과를 얻었다.

1) I.C를 이용하여 SO_3^{-2}로서 0.2ppm까지 검출할 수 있었으며 분석시간은 20분 이내였다.

2) 흡수액은 아황산이온의 안정성을 고려하여 3% H_2O_2을 사용함으로써 안정한 황산이온으로 산화시켜 정량하였다.

3) 증류수에 표준액을 첨가한 후 증류하여 측정한 회수율은 99.8%로 양호하였으나 실제 식품에 첨가한 경우는 식품의 종류에 따라 회수율의 차이가 있었다.

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