Arch. Pharm. Res. **6**(2), 103~108 (1983)

Determination of Sulfur-containing Drugs by Gas Chromatography with Flame Photometric Detector

Kyoung Ho Kim, Yung Hyun Cho, Man Ki Park and Wang Kyu Lee
College of Pharmacy, Seoul National University, Seoul 151, Korea
(Received 26 September 1983)

Abstract □ Such sulfur-containing drugs as chlorpromazine, sulfonamides and thiazides were determined by gas chromatograghy with flame photometric detector (FPD) and flame ionization detector (FID). 0.6% QF-1 or 3% SE-30 on Chromosorb WAW-DMCS were found suitable for drugs listed above. In the experimental conditions given maximum response the FPD response and the response ratio of FPD increased with the number of sulfur atom per molecule. It was possible to obtain a linear calibration curve from 10^{-9} mole to 2×10^{-8} mole and to analyze variety of sulfur drugs by the calibration curve of one sulfur standard.
Keywords □ Flame photometric detector, Methylation of sulfonamides.

Several gas chromatographic methods of sulfur-containing drugs have been reported, and detection has been carried out by flame ionization detector (FID) or electron capture detector (ECD)[1-5]. Though the sulfur response is very selective and sensitive and is 10^4~10^5 more sensitive than the hydrocarbon response at 394 nm, flame photometric detector (FPD) has not been used to analysis of sulfur-contatining drugs[6]. FPD devised by Brody and Chaney[7] employed the chemiluminescense signals of the sulfur species (s_2*) and phosphorous species (HPO*) that are formed by combustion of smples in hydrogen-rich, hydrogen-air flame, has been modified to accomplish these detections simultaneously. FPD has been utilized in the detection of sulfur in gasolin[8-9] and natural gas[10-11], residual pesticides[12-14], atmospheric pollutants[15-17], and asothiocyanate in soils[18]. In this study, we investigated factors affecting the sulfur response of FPD and found that various types of sulfur-containing drugs could be determined by using one standard sulfur compound for intensity calibration.

EXPERIMENTAL METHODS

Apparatus

Pye-Unicam GCV Chromatograph was equipped with FID and FPD operated with 394 nm interference filter. The flow rate of carrier gas was 22ml/min, and the temperature of detector and injection port were 300°C and 290°C, respectively. The temperature of column oven and the flow rates of hydrogen and air were specified in "RESULTS AND DISCUSSION".

Column

A 4mm i.d.×1.5m coiled glass column was packed with 3% SE-30 or with 0.6% QF-1 on 80-100 mesh Chromosorb WAW-DMCS. The column packings were prepared by the solution coating method. The empty glass column was thoroughly rinsed with water, methanol and acetone, and dried.

Chemicals and Reagents

Chlorpromazine, sulfamethoxydiazine, polythiazide, hydrochlorothiazide were NF reference standards. Other sulfur-containing drugs were obtained from pharmaceutical industrial companies in Seoul.

Methylene chloride and dimethylsulfoxide were of guaranted reagent grade. Methyl iodide and tetrabutylammonium hydrogen sulfate were obtained from E. Merck. Tetrabutylammonium hydrogen sulfate was neutralized with sodium hydroxide and 0.1M solution was prepared in 0.5M sodium carbonate buffer (pH 10).

Preparation of Sample Solution

10mg of chlorpromazine was dissolved in 25ml of methylene chloride, and 0.1ml of the dimethylsulfoxide was dissolved in 25ml of dioxane and the solution was taken for GC. 10^{-2}M of sulfaguanol, sulfadiazine, sulfamoxol, sulfamerazine, sulfamethazine, hydrochlorothiazide were dissolved in 1.0ml of tetrabutylammonium sulfate (0.1M) in carbonate buffer of pH 10 (0.5M). After addition of 1.0ml of 0.32M methyl iodide (2%) in methylene chloride, the mixture was mechanically shaken for 90 minutes at 25°C. After centrifugation, 0.25ml of the organic phase was evaporated in a stream of nitrogen. Ethylacetate (0.05ml) was added and the solution was taken for analysis.

Hydrochlothiazide and polythiazide were analyzed with 3% SE-30, and the other sulfur-containing compounds were analyzed with 0.6% QF-1.

Determination of Flame Photometric Detectability

Chlorpromazine was prepared with concentration in 400μg/ml. Dilution in dioxane was determined by GC/FPD. The detectability was calculated from the amount that the signal to noise ratio was two.

RESULTS AND DISCUSSION

In FPD the square root of the response of sulfur is directly proportional to concentration, so the FPD response was calculated from the

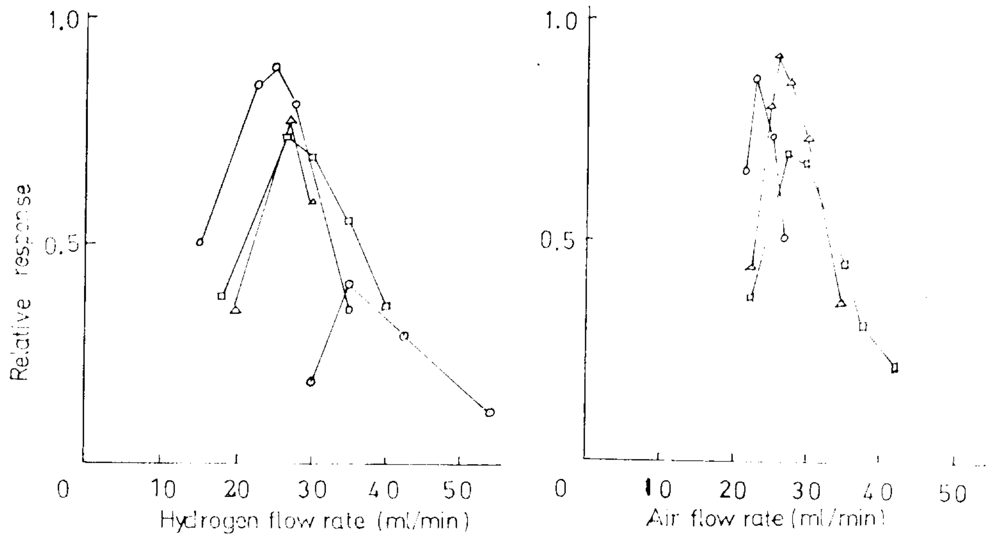

Fig. 1: Effect of hydrogen and air flow rates on the FPD response of chlorpromazine.
(Left) ○=27ml/min of air
□=30ml/min of air
△=25ml/min of air
⊙=38ml/min of air
(Right) ○=25ml/min of hydrogen
△=25ml/min of hydrogen
□=30ml/min of hydrogen

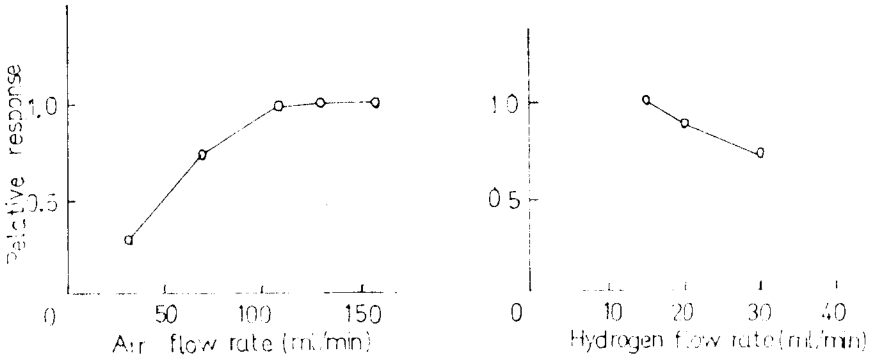

Fig. 2: Effect of air and hydrogen flow rates on the FID response of chlorpromazine.

corrected response equation (√H.W) proposed by Maruyama et al.[19], that is, the production of the half-height width and the square root of the peak height (H).

Effect of the Experimental Condition on the Response

1) Hydrogen and Air Flow Rates

Fig. 1 shows hydrogen and air flow rates were the important factors affecting the FPD response. Hydrogen flow rate 25ml/min and air flow rate 27ml/min gave the most optimum condition. This result was different from those that the response increased with decreasing hydrogen flow rate and increasing air flow rate or in vice versa with the different burner system and detector geometry reported by Mizany[20], Stevens et al.[21] and Maruyama et al.[19].

In FID hydrogen flow rate 15ml/min and air flow rate 100ml/min gave maximum response as shown in Fig. 2.

2) Carrier Gas Flow Rate

In general the peak is sharper and the peak height increases with increasing the carrier gas flow rate in gas chromatographic detectors. Of course Fig. 3 shows the peak height response increased with a increase in the carrier gas flow rate. Therefore it should be operated in the constant carrier gas flow rate.

The carrier gas flows through the jet nozzle, so the carrier gas flow rate below 5ml/min

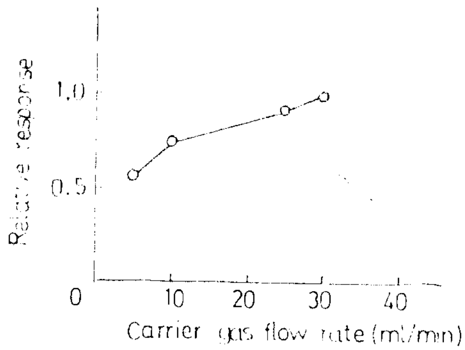

Fig. 3: Effect of carrier gas flow rate on the FPD response of chlorpromazine.

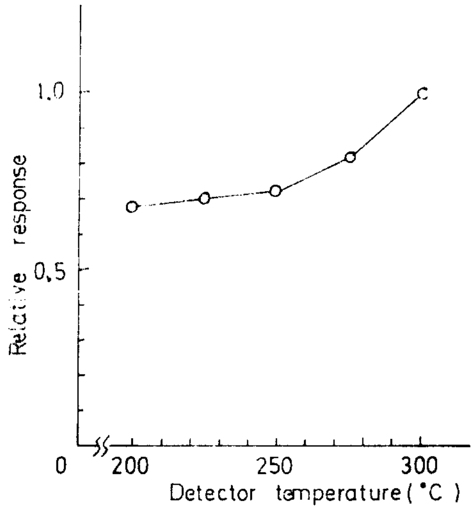

Fig. 4: Effect of detector temperature on the FPD response of chlorpromazine.

brought to flame out in the condition of hydrogen flow rate 25ml/min and air flow rate 27 ml/min.

3) Detector Temperature

The Effect of detector temperature on the FPD response was investigated from 200°C to 300°C maintaining a constant column temperature 170°C. The peak height response was good at high detector temperature. This result was reverse to that studied by Maruyama,[19] which was considered to result from the different detector geometry.

4) Column Temperature

Effect of the column temperature on the FPD response was studied in the range of 150°C to 250°C for three compounds of one sulfur atom in a molecule such as dimethylsulfoxide, chlorpromazine and sulfamethoxydiazine.

The corrected responses for three compounds were all the same regardless of column temperature as shown in Fig. 5. This result shows the column temperature is the factor with no influence on the FPD response.

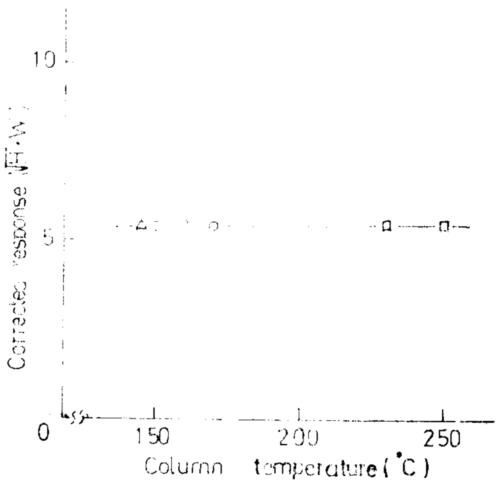

Fig. 5: Effect of column temperature on the FPD response.
△=Dimethylsulfoxide
○=Chlorpromazine
□=Sulfamethoxydiazine

Determination of Flame Photometric Detectability

Detectability is applied to specify detectors independent of column parameters and of sample size. The flame photometric detectability of chlorpromazine was 8.65×10^{-11} g/sec. This value considered to be corresponded with other compounds of one sulfur atom in a molecule.

Peak Height Response Ratio of FPD to FID

Table I shows that in the analytical consideration given maximum response the FPD response was superior to the FID response for sulfur-containing compounds. The peak height response ratio of FPD to FID (abbrev. FPD/FID ratio) for the compound with one sulfur atom per molecule was in 1.12 to 2.18 range, and the ratios of hydrochlorothiazide and polythiazide were 2.76 and 3.66 respectively. As previously reported, the response depends on the sum of effective carbon number in FID[22] and on the number of sulfur atom per molecule in FPD, and then the structure of compound has an effect on the FID response and little effect in the FPD response. Hence the FPD/FID ratio increases with the number of sulfur atom in a molecule.

Relationship between Response and Sample Size

The relationship between the flame photometric response and sample size in the range of 10^{-9} mole and 10^{-8} of sulfurcontaining compounds gave three straight lines as shown in Fig. 6. Calibration curves for chlorpromazine, sulfamethoxydiazine and dimethylsulfoxide containing one sulfur atom per molecule coincided with each other without regard to their oxidaition states and their slope was 5.31. The slope of hydrochlorothiazide with two sulfur atoms was 10.62 which doubled that of the compound with one sulfur atom in a molecule. The slope of polythiazide with three sulfur atoms was 15.93 which trebled. Therefore if all experime-

Table I: Peak height response ratio of FPD for some sulfur-containing compounds.

*Compound	Retention time (min.)	Number of sulfur atom	Peak height by FPD	Peak height by FID	FPD/FID response ratio
Sulfaguanole	0.95	1	19.9	16.4	1.12
Sulfamoxole	1.89	1	18.4	12.3	1.50
Sulfadiazine	1.96	1	16.8	8.8	1.91
Sulfamerazine	3.25	1	15.2	12.4	1.23
Sulfamethazine	3.38	1	14.4	8.2	1.76
Sulfamethoxydiazine	5.13	1	13.5	6.2	2.18
Sulfadimethoxime	6.65	1	12.9	7.1	1.82
Chlorpromazine	4.12	1	14.1	9.7	1.45
Hydrochlorothiazide	4.51	2	19.7	7.1	2.77
Polythiazide	6.23	3	21.5	5.9	3.64

* Hydrochlorothiazide and polythiazide were taken for GC with 3% SE-30 at 270°C, chlorpromazine with 0.6% QF-1 at 170°C and the other compounds with 0.6% QF-1 at 230°C.

ntal conditions are kept constant and the corrected response is used, it is possible to determine various sulfur compounds with the calibration curve of one standard sulfur compound by FPD. On the other hand, in case of the analysis of sulfur compounds by such detectors as FID, electron capture detector and TCD etc., it is necessary to prepare a calibration curve per compound.

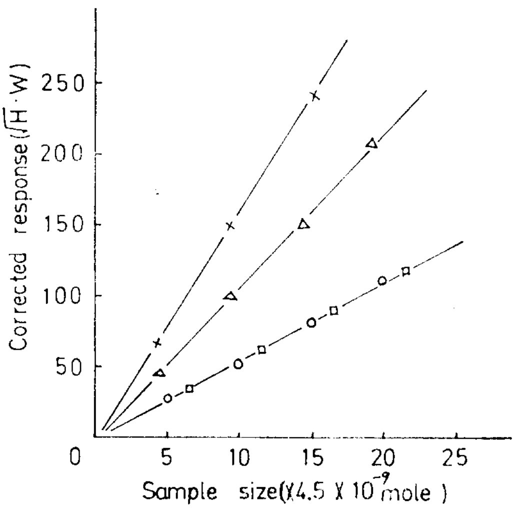

Fig. 6: Relationship between corrected response and sample size by FPD.
○=Chlorpromazine
□=Sulfamethoydiazine
△=Hydrocholrothiazide
×=Polythiazide

CONCLUSION

In the analytical conditions given maximum response the FPD response is superior to the FID response for sulfur-containing compounds. It is possible to determine various sulfur-containing drugs with the calibration curve of one standard sulfur compound by FPD.

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