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Note

Gas chromatographic quantification of a new antitumour agent, pentaziridinocyclophosphathiazene

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The insect chemosterilant, apholate, has been reported to have an antitumour activity on murine L 1210 and P 388 leukaemias and on B 16 melanoma[1]. A gas chromatographic (GC) detection of this compound has been reported by Bowman[2], who utilized a phosphorus flame detector and six different columns including Dexsil 300 and silicone OV-17. An analogue, pentaziridinocyclophosphathiazene (SOAz), has recently been shown to have a remarkable antitumour activity on the tumours described above[3,4]. However, we have been unable to find any report on the detection of this compound, which urged us to devise an analytical method. We have previously reported a high-performance liquid chromatographic (HPLC) determination of the compound in biological fluids[5]. In the present paper, a GC analysis is described.

EXPERIMENTAL

Chemical synthesis

SOAz was synthesized by Otsuka Chemical Co. (Tokushima, Japan) according to the procedure of Van de Grampel *et al.*[4].

Gas chromatography

A Shimadzu GC-R1A gas chromatograph equipped with a flame thermionic detector (Shimadzu FTD-R1A) was used to detect SOAz, which contains five aziridino groups. Chromatograms were recorded on a Shimadzu RPR-G1 processor. The glass columns (1 m or 2 m × 3 mm I.D.) were packed with 2% Dexsil 300 GC on Chromosorb W AW DMCS (80–100 mesh) (Wako, Osaka, Japan), 1% silicone OV-17 on Gas-Chrom Q (80–100 mesh) (Gasukuro Kogyo, Tokyo, Japan) or 1% silicone OV-17 on Chromosorb W AW DMCS (Wako). They were conditioned overnight before use at 340, 310 and 260°C, respectively.

The carrier gas (helium) flow-rate was 50 ml/min for the 1-m column and 60 ml/min for the 2-m column; hydrogen flow-rate 11 ml/min, air flow-rate 250 ml/min.

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TABLE I

GAS CHROMATOGRAPHIC ANALYSIS OF SMALL AMOUNTS OF SOAz USING A FLAME THERMIONIC DETECTOR

Columns	*Column length (M)*	*Temperatures (°C)*		*Retention time (min)*	*FTD output*	*Areas* ($\times 10^3$) at range 10*	
		Inj. port	*Oven*			*20 ng*	*5 ng*
2% Dexsil 300 GC on Chromosorb W AW DMCS	1	280	245	3.64	6.30	217.9 ± 39.3	51.7 ± 3.1
1% OV-17 on Gas-Chrom Q	2	300	270	7.18	6.00	101.5 ± 4.3	18.0 ± 3.7
	1	290	260	3.44	6.70	243.9 ± 10.0	52.7 ± 10.1
1% OV-17 on Chromosorb W AW DMCS	1	290	260	3.37	6.70	490.5 ± 11.0	103.3 ± 4.3

* Averages from three runs ± S.D.

The solvents dichloromethane and acetone, of analytical grade (Wako), were used to dissolve SOAz.

Standard solutions

SOAz (5 mg) was dissolved in acetone (25 ml) to produce a stock standard solution. Portions of this solution were diluted with acetone to obtain calibration solutions. The stock solution contained 200 ng/μl and could be used for at least 1 month if stored in a cool place.

Procedures

The operational parameters of the apparatus were as shown in Table I. The injection volume was 1 μl. Before analysis, several injections of 200–1000 ng of SOAz were required to produce constant FTD responses. Standards were preferably injected after each analysis. If the response sensitivity dropped after several successive analyses of amounts of less than 10 ng, 200 ng were injected to regain the initial response.

RESULTS AND DISCUSSION

Typical chromatograms are shown in Fig. 1, and a calibration curve is presented in Fig. 2. Although the peak area–concentration plot generally gave a smooth curve, especially at concentrations lower than 10 ng, the response of the FTD to SOAz was approximately proportional to concentration in the range of 0.5–200 ng/μl as shown in Fig. 2 and in Table II. Injections as large as 3 μl may be made for improvement of the detection limit.

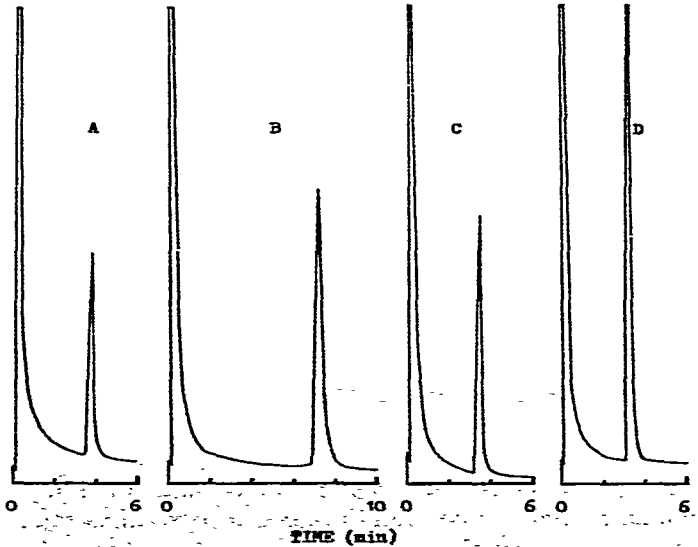

Fig. 1. Chromatograms of 20 ng of SOAz by GLC on different columns using a flame thermionic detector. A, 2% Dexsil (attenuation 32); B, 1% OV-17 on Gas-Chrom Q (2 m, attenuation 8); C, 1% OV-17 on Gas-Chrom Q (1 m, attenuation 32); D, 1% OV-17 on Chromosorb W AW DMCS (attenuation 32).

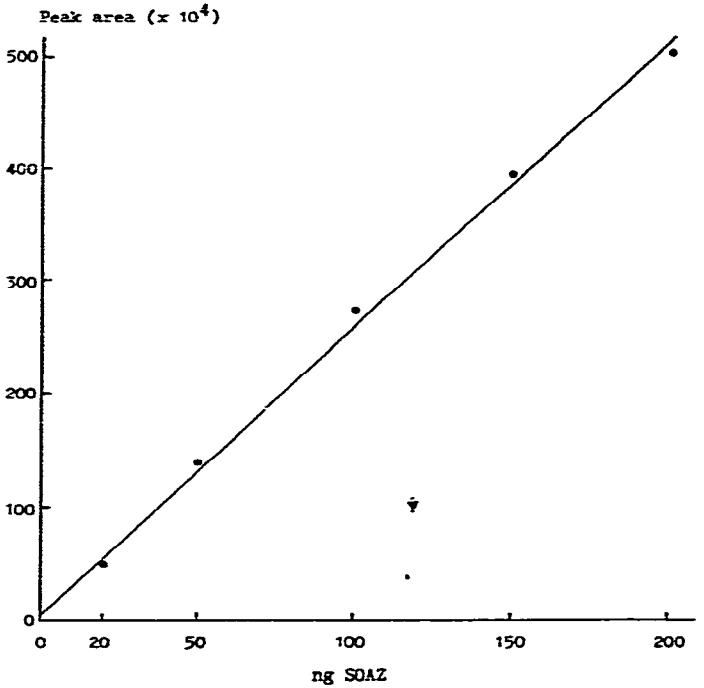

Fig. 2. Calibration curve for quantitation of SOAz obtained from 1% OV-17 on Chromosorb W AW DMCS.

TABLE II

PRECISION AND ACCURACY OF GC USING AN FTD FOR ANALYSIS OF SOAz

Injected amount (ng)	*Area counted* ($\times 10^3$) ± S.D.*	*Coefficient of variation (%)*
0.5	8.1 ± 1.1	13.6
5.0	103.3 ± 4.3	4.2
20.0	490.5 ± 11.0	2.2
50.0	1408.4 ± 43.2	3.1
100.0	2721.3 ± 113.4	4.2
150.0	3937.5 ± 52.2	1.3
200.0	5028.3 ± 102.1	2.0

* Values are averages from three runs, yielding a correlation coefficient of 0.999.

The column materials used were practically equivalent in their analytical function as shown in Table I. Low loading and proper conditioning by periodical injections of large amounts of SOAz seemed essential to obtain a constant reproducibility and a high sensitivity. Short columns with low loadings provided a higher sensitivity and were more time-saving than the long column tried. It seemed that the column of

1% OV-17 on Chromosorb W AW DMCS was least adsorptive and most suitable for the quantification of the compound.

The main problem is the column conditioning before and during analysis in order to maintain the sensitivity. This is considerably time-consuming and requires the injection of standards after several, preferably each, analyses to achieve precise quantitation. However, the present method requires no clean-up of biological samples such as plasma and urine, since the FTD is highly sensitive and specific for nitrogen-containing compounds and very insensitive to raw contaminants in extracts of such samples.

SOAz readily dissolves in dichloromethane, but this solvent should be avoided for it causes a severe negative response immediately after the injection into the FTD. Acetone is preferred because of the high solubility of the compound and the lack of a negative response.

We have previously reported the detection of SOAz in rat plasma and urine by HPLC[5]. The detection limit was 500 ng/ml of plasma, the injection volume being 40 μl. The detection limit by GC using an FTD was 0.5 ng/μl, identical to that by HPLC. However, the injection volume used was 40 times smaller. Thus, the present method should be superior to HPLC for the analysis of SOAz in biological samples if these contain a large amount of raw contaminants that might cause failure of HPLC columns. Moreover, the GC retention time is half as that of HPLC.

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