CHROM. 4176

Determination of thiamphenicol in biological fluids by gas chromatography

For the determination of thiamphenicol in biological studies the usual methods are bacteriological assay[1,2] and colorimetry[3] based on alkaline hydrolysis. However, the bacteriological method is inaccurate when other antibacterial agents are administered concurrently with thiamphenicol. In the case of the colorimetric method, it is necessary to separate the unaltered thiamphenicol from its major metabolites prior to alkaline hydrolysis; therefore the procedure is tedious and time consuming. To overcome these difficulties, a gas–liquid chromatographic procedure has been devised and described for thiamphenicol as trimethylsilyl ether[4]; however, the past report is not adequate enough to determine thiamphenicol in biological studies.

In this report an investigation was conducted to establish a more convenient method for the determination of thiamphenicol, using an electron capture detector, especially for biological studies.

Experimental

Apparatus and conditions. A Shimadzu Model GC-2C gas chromatograph equipped with electron capture attachment (300 mC Tritum Source) (ECD-1A) was used. Packing was (1) 1.5% DEGS (diethylene glycol succinate polyester) on Chromosorb W (60–80 mesh) in an U-shaped borosilicate glass column (150 × 0.4 cm I.D.) for the direct method, and (2) 1.5% OV-17 (Ohio Valley Chemical Company) on Shimalite W (80–100 mesh) (Shimadzu, diatomaceous earth) in an U-shaped stainless-steel column (75 × 0.4 cm I.D.) for the trimethylsilyl ether method.

Operating conditions were as follows. (1) For the direct method: column and detector temperatures, 185°; injection port temperature, 220°; nitrogen as carrier gas, 50 ml/min (0.5 kg/cm^2) at the inlet; for quantitative determination, nitrogen flow rate was increased to 150 ml/min (1.5 kg/cm^2) at the inlet; sensitivity, 100 MΩ; range, 0.4 V; applied voltage, 15 V. (2) For the trimethylsilyl ether method: column and detector temperatures, 215°; injection port temperature, 240°; nitrogen flow rate, 130 ml/min (1.4 kg/cm^2) at the inlet; sensitivity, 100 MΩ; range, 1.6 V; applied voltage, 10 V.

Sample preparation. Samples of 0.5 or 1.0 ml of biological fluid containing thiamphenicol were accurately added to 5.0 ml of ethyl acetate, and the mixture was vigorously shaken for 5 min. Na_2SO_4 was added in ethyl acetate solution for dehydration.

Of this ethyl acetate solution 3 μl were accurately injected directly into the gas chromatograph without prior concentration, using the direct method. When using the trimethylsilyl ether method, 0.5 or 1.0 ml of the ethyl acetate solution was pipetted into a test tube and was evaporated in a vacuum desiccator. Then 0.2 ml of trimethylsilyl reagent was added. The test tube was stoppered and was left at room temperature for 10 min. Then the solution was evaporated to dryness in a vacuum desiccator. After the suitable volume of pyridine was added to the residue, 2 μl of the supernatant of this solution was accurately injected into the gas chromatograph.

Results and discussion

As reported in the past[4], we can easily isolate thiamphenicol and chloramphe-

nicol as trimethylsilyl derivatives (Fig. 1). They can also be determined, however, without any pretreatment as illustrated in Fig. 2.

To identify the peak corresponding to thiamphenicol in the direct method, U.V. spectra of original and of effluent materials were compared, as illustrated in Fig. 3. From the result, it is assumed that the peak, as in the case of chloramphenicol in our previous paper[5], is not a thermally degradated product but is thiamphenicol itself. The retention time of thiamphenicol for quantitative analysis was 4.5 min under the con-

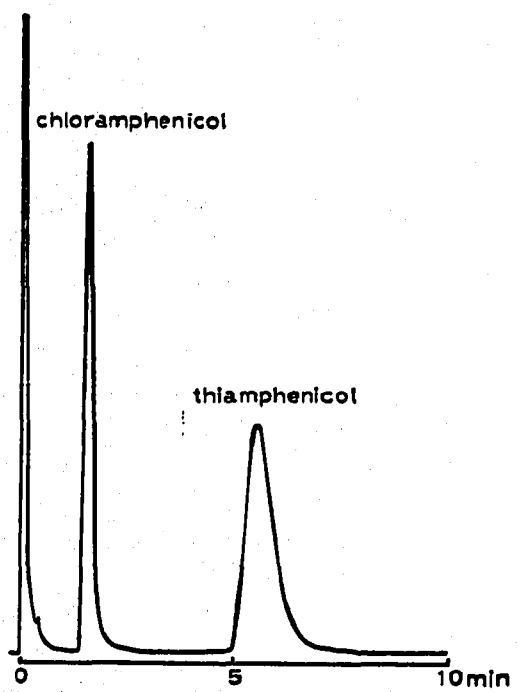

Fig. 1. Chromatogram of a mixture of trimethylsilyl ether derivatives of thiamphenicol and chloramphenicol. Conditions: 75 × 0.4 cm I.D. stainless-steel column packed with 1.5% OV-17 on 80–100 mesh Shimalite W, 215°, 130 ml/min (1.4 kg/cm²), electron capture detection system.

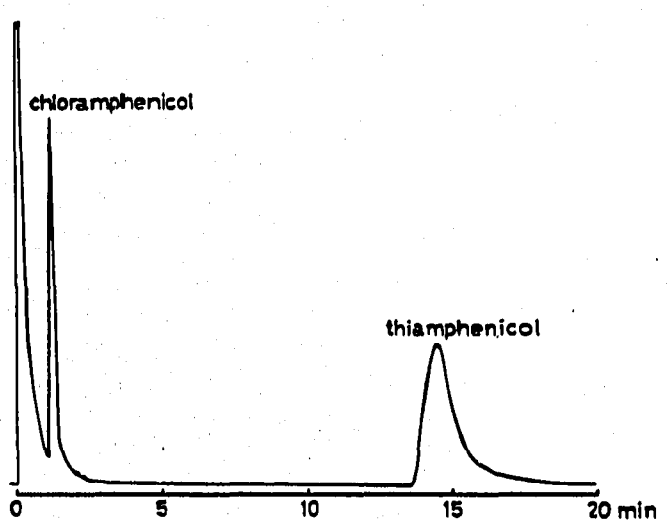

Fig. 2. Chromatogram of a mixture of thiamphenicol and chloramphenicol. Conditions: 150 × 0.4 cm I.D. glass column packed with 1.5% DEGS on 60–80 mesh Chromosorb W, 185°, 50 ml/min (0.5 kg/cm²) nitrogen, electron capture detection system.

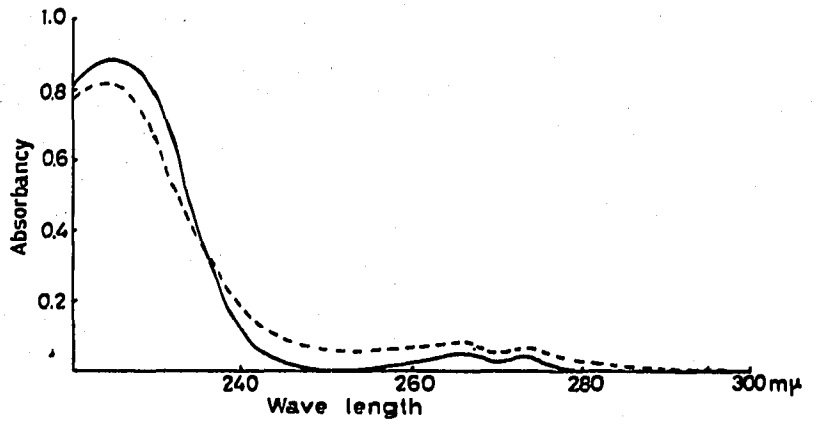

Fig. 3. U.V. spectra of thiamphenicol before and after chromatography. ————, original; – – – – – – –, effluent.

TABLE I

RECOVERY OF THIAMPHENICOL IN PLASMA

Added (*μg/ml*)	*Direct method*		*Trimethylsilyl ether method*	
	Found (*μg/ml*)	*Recovery* (%)	*Found* (*μg/ml*)	*Recovery* (%)
50	49.0	98.0	48.0	96.0
50	49.5	99.0	49.5	99.0
40	39.0	97.5	39.0	97.5
40	39.0	97.5	39.0	97.5
30	29.4	98.0	28.5	95.0
30	29.0	96.7	29.1	97.0
20	19.0	95.0	19.8	99.0
20	19.0	95.0	19.5	97.5
10	9.2	92.0	10.1	101.0
10	9.2	92.0	9.9	99.0
Average		96.1		97.9
St. dev.		2.4		1.7

TABLE II

RECOVERY OF THIAMPHENICOL IN URINE

Added (*μg/ml*)	*Direct method*		*Trimethylsilyl ether method*	
	Found (*μg/ml*)	*Recovery* (%)	*Found* *μg/ml*)	*Recovery* (%)
100	98.0	98.0	97.0	97.0
100	98.0	98.0	99.0	99.0
80	77.0	98.5	78.0	97.5
80	81.5	102.0	78.0	97.5
60	54.0	90.0	58.0	96.6
60	62.0	103.2	58.8	97.0
40	38.0	95.0	39.6	99.0
40	37.0	92.6	39.0	97.5
20	19.4	97.0	20.0	100.0
20	19.4	97.0	19.8	99.0
Average		97.1		98.0
St. dev.		3.9		1.1

ditions described above for the direct method. Calibration curves for thiamphenicol and for its trimethylsilyl ether, based on measurement of peak area by planimeter, were linear throughout the range of 0.05 to 0.2 μg and the range of 0 to 0.02 μg, respectively. The results of recovery experiments of thiamphenicol added to human heparinized plasma and urine are presented in Tables I and II.

The direct method is recommended when the concentration is over 10 μg/ml, but below this concentration, the trimethylsilyl ether method seems to be superior.

Faculty of Pharmaceutical Science, Kyushu University, Fukuoka (Japan)

TOSHINOBU AOYAMA
SADAO IGUCHI

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A new sampling apparatus for amino acid analysis

The method of SPACKMAN *et al.*[1] for amino acid analysis has been speeded up[2,3] and automated[4–9]. In addition, several methods for automatic sampling have been used. These methods are based on the use of either a number of columns and the elution of these columns, one after another, with the sample[5] or on the use of a number of sample loops or sample tubes as was done by MURDOCK *et al.*[5], DUS *et al.*[7], ALONZO AND HIRS[8] and by SLUMP AND VERBEEK[9]. A sample loop is a teflon or nylon tube in which a sample can be stored. Both ends of the loop are connected to two corresponding openings of a double rotary valve. The difficulty with this system is the necessity of connecting and disconnecting the sample loop for every new sample. A special valve has been designed by SLUMP AND VERBEEK[9] in order to overcome this problem.

A different system was developed by EVELEIGH AND THOMPSON[4] in which samples are loaded into small tubes, and are adsorbed on an ion-exchange resin.

The automatic sample loader described here is a simplification of the system as reported by SLUMP AND VERBEEK[9]. An extra valve is not neccessary, and furthermore, the problem of connecting and disconnecting the sample tube (DUS *et al.*[7]) is solved. The whole apparatus can easily be connected to a commercial amino acid analyzer provided it is coupled to a suitable timing system.