

Analysis of Synthetic Antibacterials

Eggs, fishes and meat are being supplied from cultured livestock increasingly of late. Moreover, as the weight of imported foodstuffs in the total food we consume increases, survey of the actual conditions of these foodstuffs is being conducted at an increasing number of laboratories. Some of the feeds and veterinary pharmaceuticals for livestock are harmful to humans. It

has become necessary to conduct monitoring tests for harmful residual substances contained in livestock and marine foods. Introduced in this article are the determination of synthetic antibacterials, which are the objects of the above monitoring, as well as some useful drugs, using HPLC and thermospray LCMS.

1. Simultaneous Analysis of 13 Synthetic Antibacterials

Here is a simultaneous analysis of the designated 13 synthetic antibacterials, an application of high-sensitivity and rapid analysis utilizing the wavelength programming method with the optimum UV wavelengths for the

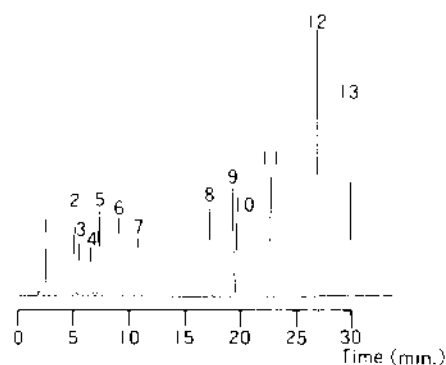
respective components, and the gradient elution method. The standard mixture sample was 20-500ppm of each component dissolved in 50% aqueous acetonitrile.

Table 1 Analytical Conditions

Apparatus	: LC-10A DGH-I
Column	: L-Column
Mobile Phase	: A → B Gradient Elution Method
	A : 10mM (Sodium) Phosphate (pH2.6)/Acetonitrile :
	85/15 (v/v)
	B : 100mM (Sodium) Phosphate (pH6.1)/Acetonitrile :
	40/60 (v/v)
Flow Rate	: 1.0mL/min.
Temperature	: 40°C
Detection	: SPD-10A 268nm
	(wavelength programmed with SCU-10A)

Table 2 Time Program

#	TIME	FUNC	VALUE	#	TIME	FUNC	VALUE
0	0.01	MARK A		10	24.00	WAVE A	283
1	4.00	WAVE A	285	11	25.00	B.CONC	100.0
2	5.00	B.CONC	0.0	12	28.00	WAVE A	350
3	6.00	WAVE A	227	13	30.00	B.CONC	100.0
4	6.85	WAVE A	267	14	30.10	B.CONC	0.0
5	8.00	WAVE A	366	15	45.00	STOP	
6	10.00	WAVE A	275				
7	16.00	WAVE A	265				
8	20.00	B.CONC	42.0				
9	21.00	WAVE A	258				



1. Oloquinox
2. Carbadox
3. Sulfamerazine
4. Thiamphenicol
5. Sulfadimidine
6. Furazolidone
7. Sulfamonomethoxine
8. Oxolinic acid
9. Sulfadimethoxine
10. Sulfaquinoxaline
11. Nalidixic acid
12. Piromidic acid
13. Nicarbazin

Fig.1 Chromatogram of Standard Mixture

2. Analysis of Synthetic Antibacterials by LCMS-QP1000EX

Introduced here is an example of analysis of 12 sulfa drugs by LCMS which features excellent quantitation capability. Sulfa drugs is the general term for compounds made up from sulfamine (paminobenzenesulfoamide). The structures of the 12 sulfa drugs are shown in Fig.2.

Although there are overlapping peaks on the TIC of sulfathiazole (STZ) and sulfadiazine (SDZ), sulfamethoxypyridazine (SMPD) and sulfadimidine (SDD), sulfadimethoxine (SDMX) and sulfaquinoxaline (SQ), in the mass chromatograms obtained with $(MH)^+$ ions, they are observed as independent peaks. As a result, determination of components which overlap is possible without any difficulty. Fig.4 shows mass spectra of some of the sulfa drugs that are often used as typical antibacterials. The mass spectra obtained were of the protonated species.

Table 3 Analytical Conditions

Instrument	: Simadzu LCMS-QP1000EX
Pump	: Simadzu LC-9A
Column	: ODS (4.6mm I.D.×150mm L.)
Mobile Phase	: A : 0.05M CH_3COONH_4 aq (pH : 3.5) B : CH_3CN A : B=3 : 1 (v:v)
Flow Rate	: 0.8mL/min.
Column Temp.	: 40°C
Block Temp.	: 280°C
Mass Range	: m/z 150-400
Scanning Interval	: 3 sec.

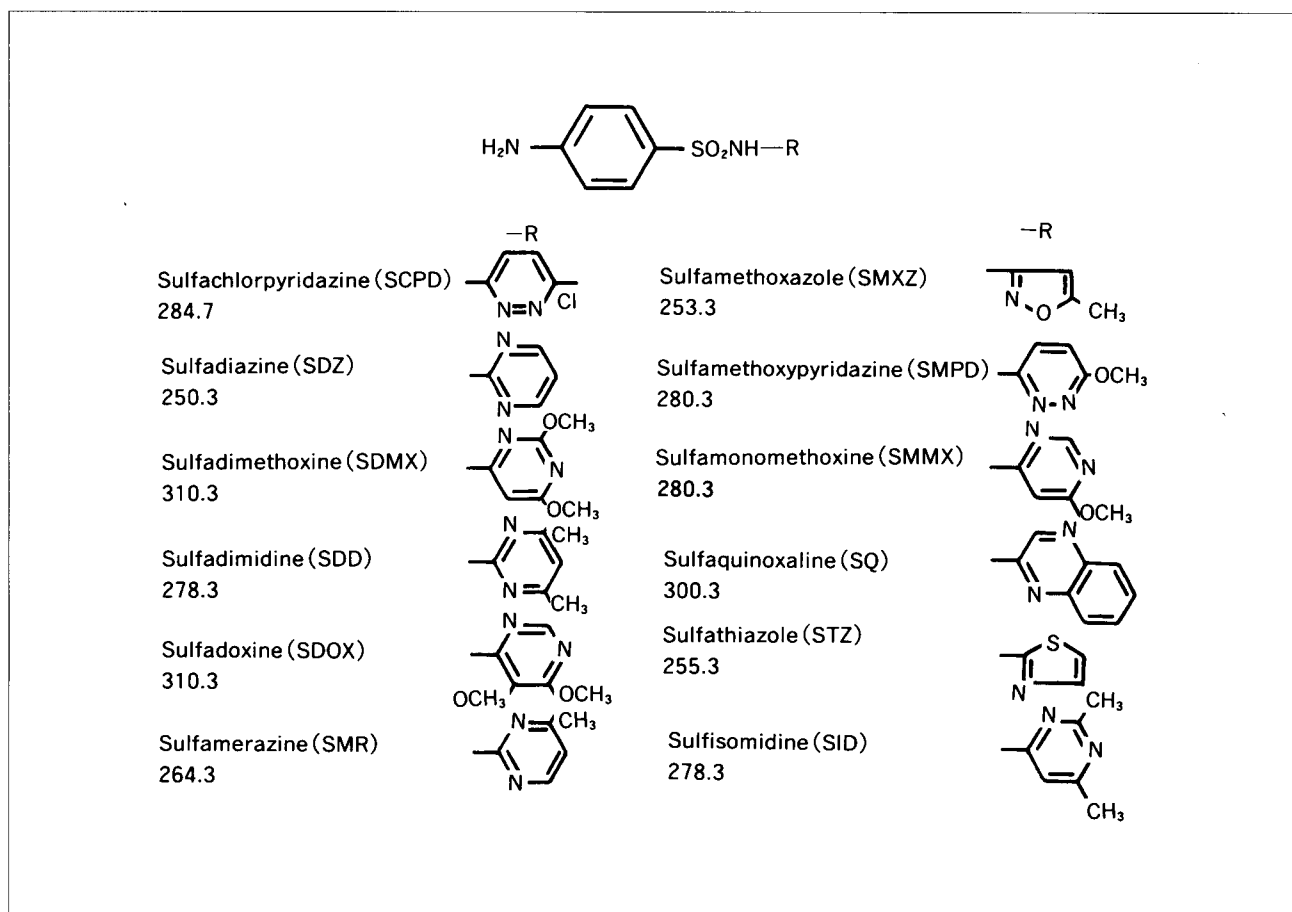


Fig.2 Structures of Sulfa Drugs

Fig. 4 Mass Spectra of Individual Sulfonamide Drugs (1)

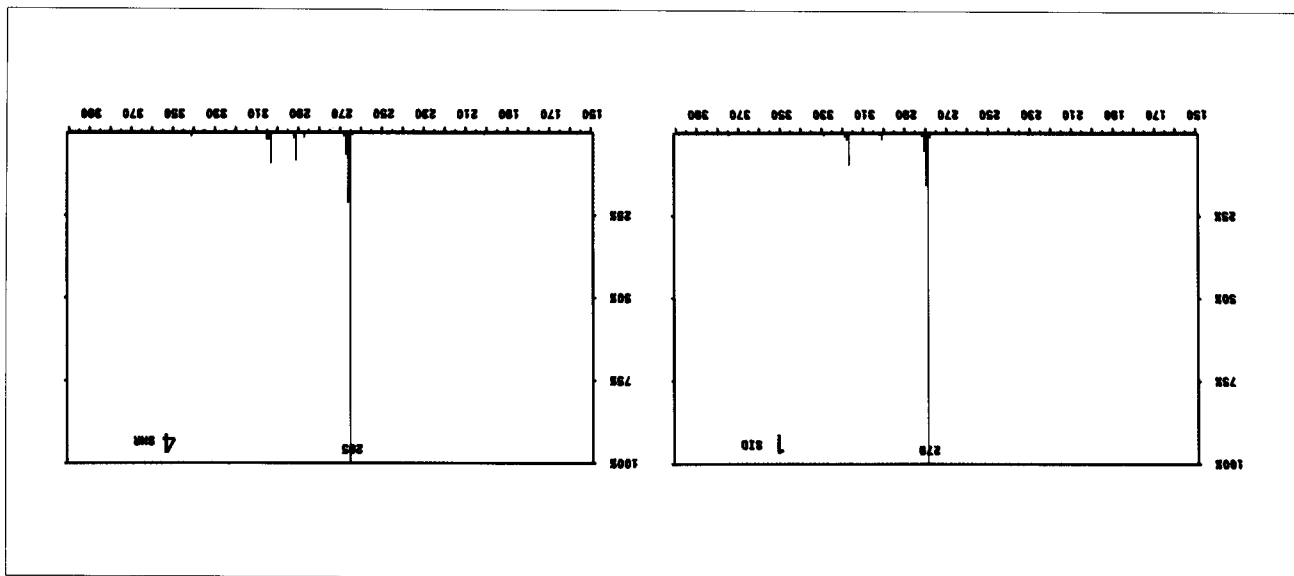
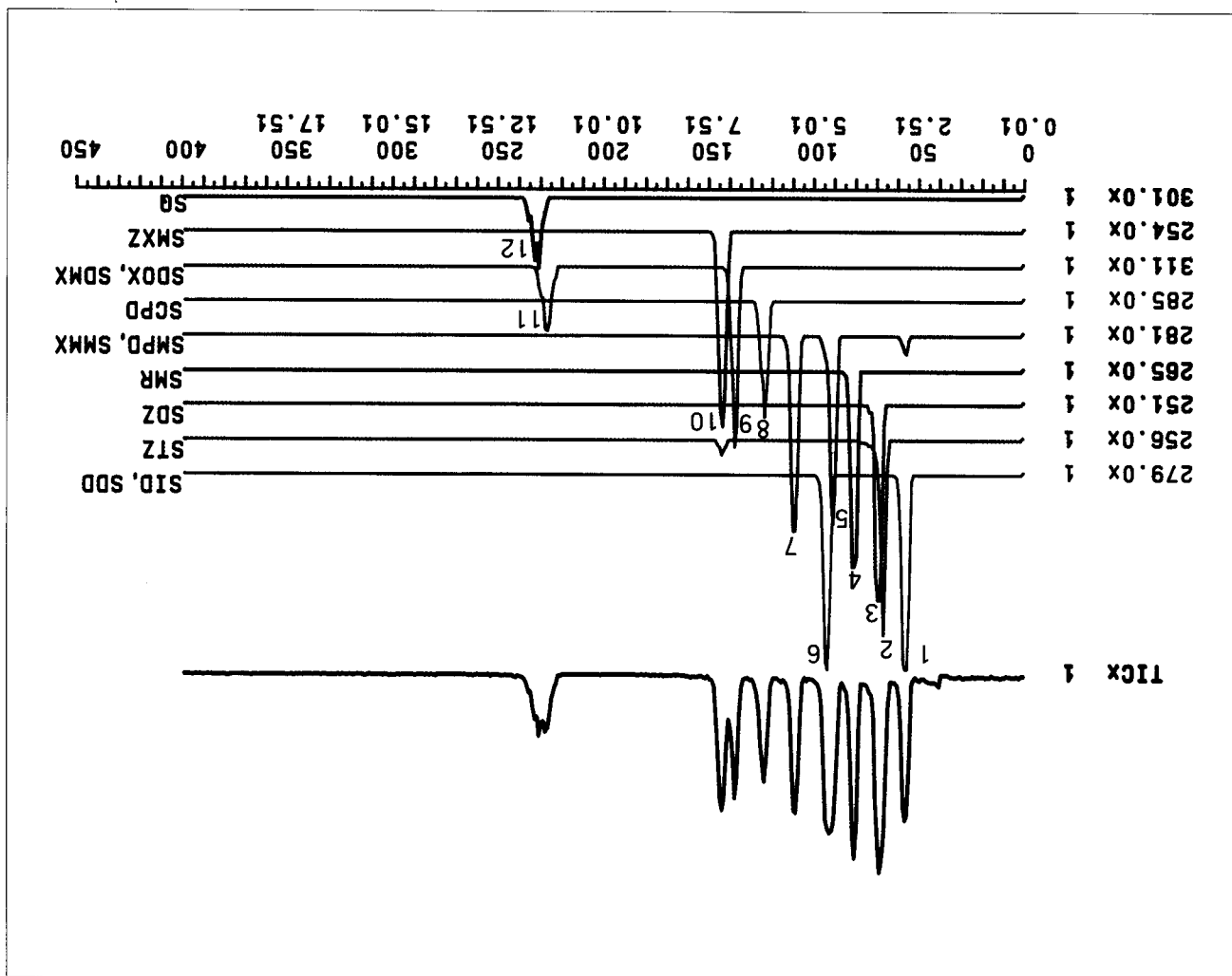


Fig. 3 Mass Chromatogram of Sulfonamide Drug Mixture



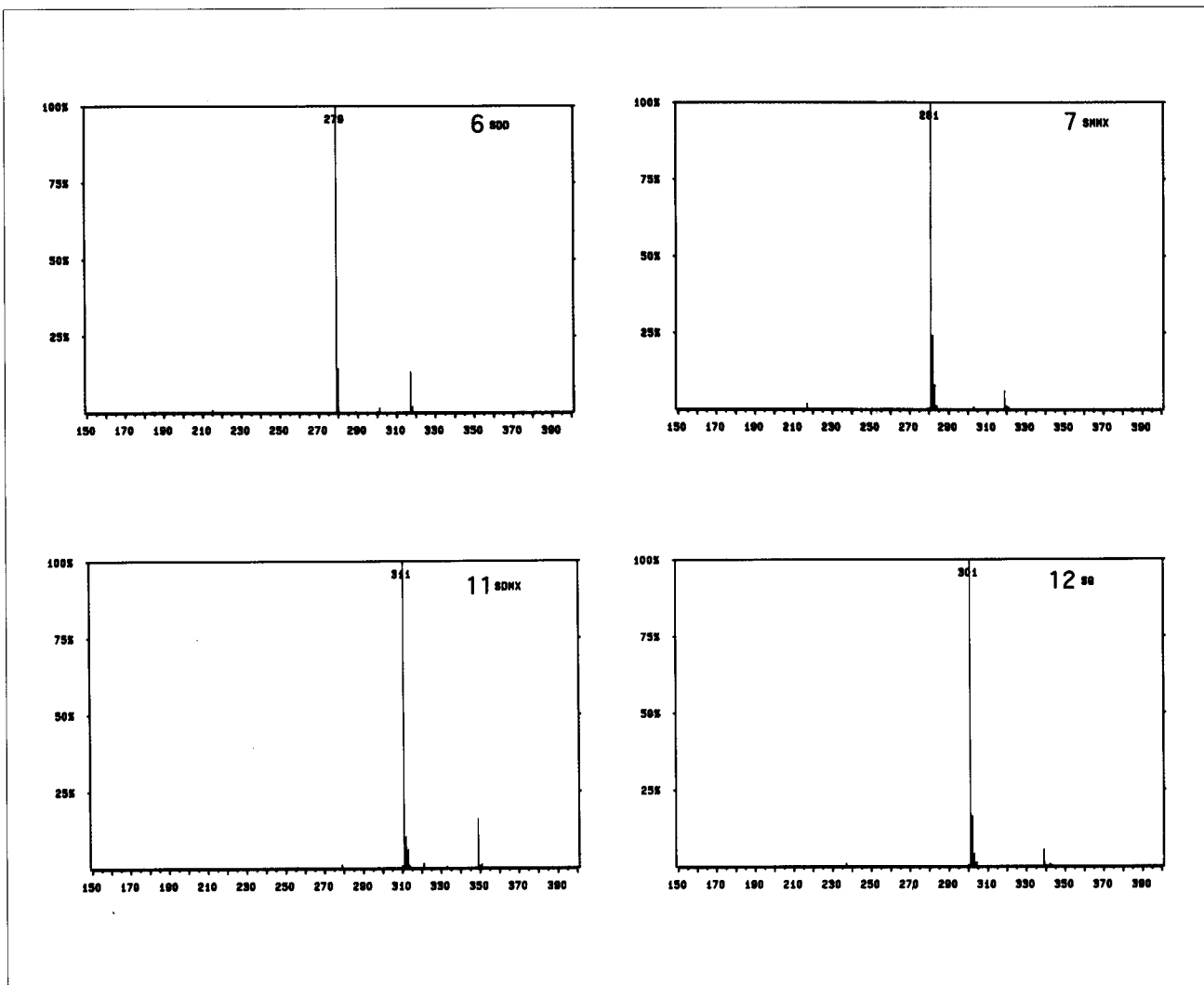


Fig.4 Mass Spectra of Individual Sulfa Drugs (II)

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