Simultaneous Analysis of Sulfa Drugs

Sulfa drugs are synthetic antibacterial agents that are widely used in the animal industry. On November 29, 2005, the Ministry of Health, Labor and Welfare of Japan issued the "Test methods for residual pesticides in foods, feed additives and veterinary drug components (partial revision)" (Shokuan No. 1129002). According to this document, many sulfa drugs are designated as compounds that require testing and standard values have been established.

Here, as a mobile phase, formic acid and methanol were used and different sulfa drugs were analyzed simultaneously. Using a TSKgel ODS-100V, 3µm column it is possible to obtain a chromatogram with favorable peaks for highly polar substances such as sulfaguanidine.

Figure 1. Chromatogram for sulfa drugs (each: 2.5mg/L)

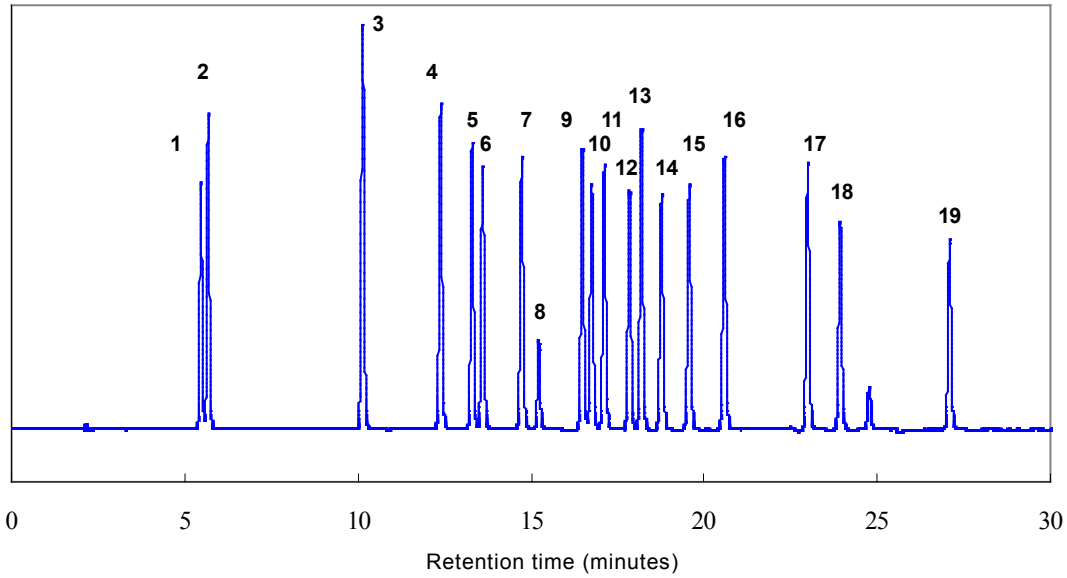


Table 1. Conditions

Column:	TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm	
Mobile phase:	A: 0.1% formic acid in water B: 0.1% formic acid in methanol	
Gradient:	0min (0%B)→ 30min (70%B) →32min (70%B)→ 33min (0%B)	
Flow rate:	1.0mL/min	
Temperature:	40℃	
Injection vol.:	10µL	
Samples:	1. sulfaguanidine	11. sulfamethoxypyridazine
	2. sulfanilamide	12. sulfachloropyridazine
	3. sulfacetamide	13. sulfamethoxazole
	4. sulfadiazine	14. sulfamonomethoxine
	5. sulfathiazole	15. sulfadoxine
	6. sulfapyridine	16. sulfabenzamide
	7. sulfamerazine	17. sulfadimethoxine
	8. trimethoprim	18. sulfaquinoxaline
	9. sulfamethizole	19. sulfanitran
	10. sulfadimidin	

Concurrent Analysis of Sulfa Drugs by LC/MS

Sulfa drugs are synthetic antibacterial agents that are widely used in the animal industry. A notice issued by the Ministry of Health, Labor and Welfare [Shokuan No. 1129002; "Concurrent test method I for veterinary drugs by HPLC (animal husbandry and fishery)"] lists 16 sulfa drugs and specifies that a quantitative test must be performed by HPLC and a confirmation test must be performed by LC/MS(/MS).

With regard to sulfa drug analysis, a past report (TSKgel Technical Information Sheet No. 118) introduced isolation using a UV detector. The present report measured the above-mentioned 16 sulfa drugs and trimethoprim (synthetic antibacterial agent often co-administered with sulfa drugs) using a MS detector. With all sulfa drugs, sharp peaks without tailing were obtained. For all sulfa drugs, quantification was confirmed within a concentration range of 0.02-1.0mg/L.

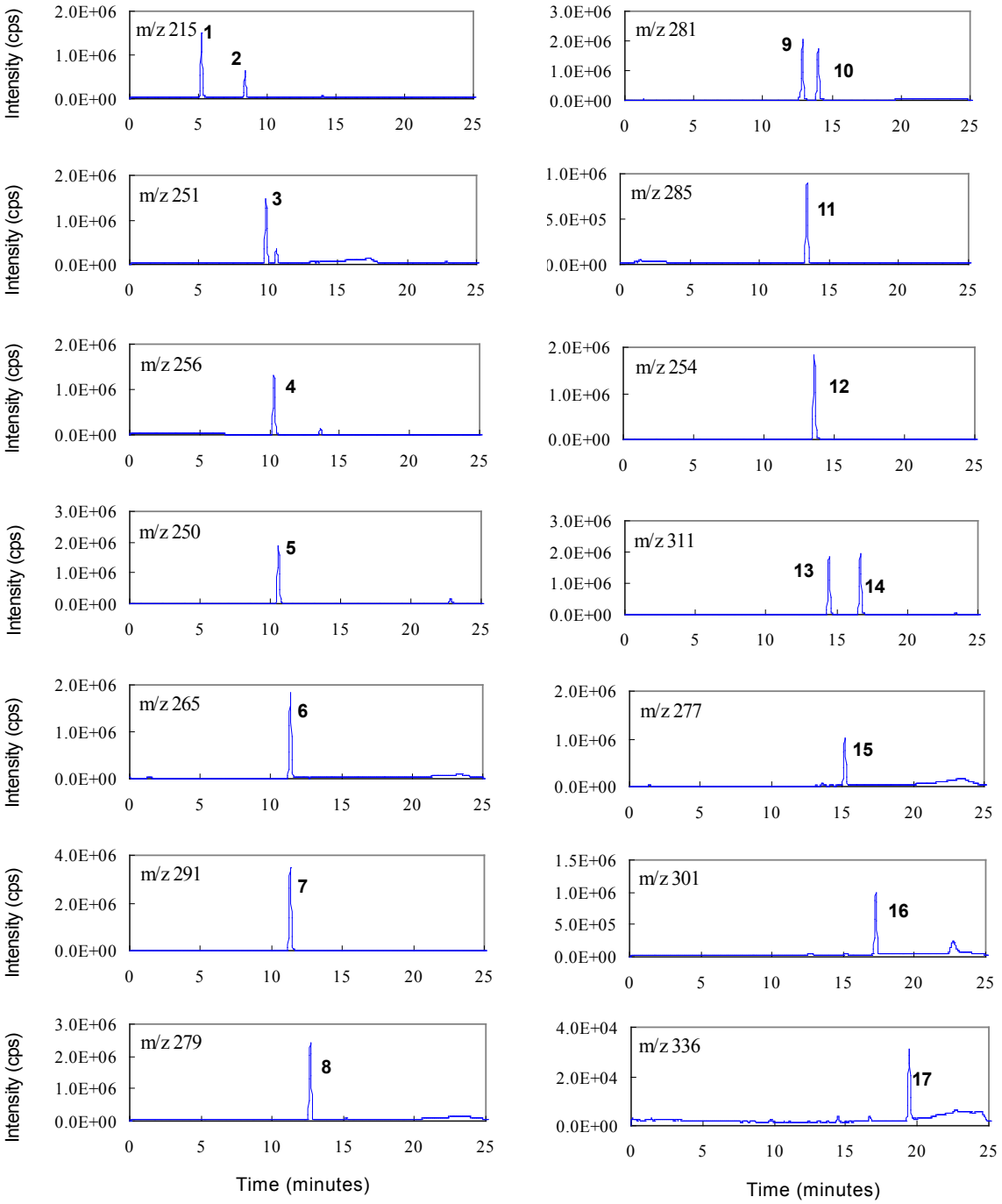
Table 1: Sulfa Drugs

Drug	m/z
sulfaguanidine	215
sulfacetamide	215
sulfadiazine	251
sulfathiazole	256
sulfapyridine	250
sulfamerazine	265
trimethoprim	291
sulfadimidin	279
sulfamethoxypyridazine	281
sulfamonomethoxine	281
sulfachloropyridazine	285
sulfamethoxazole	254
sulfadoxine	311
sulfadimethoxine	311
sulfabenzamide	277
sulfaquinoxaline	301
sulfanitran	336

Table 2. Measurement conditions

HPLC Conditions	
Column:	TSKgel ODS-100V, 3μm, 2.0mm ID x 15cm
Mobile phase:	A: 0.1% formic acid B: 0.1% formic acid in methanol
Gradient:	0min (0%B) → 20min (70%B) → 22min (70%B) → 23min (0% B)
Flow rate:	0.2mL/min
Temperature:	40℃
Injection vol.:	2μL
MS Conditions	
MS:	QTRAP® (Applied Biosystems)
Ion Source:	ESI positive
Mode:	SIM
Temperature:	500℃
Ionspray voltage:	5000 V

Figure 1. SIM chromatogram of each sulfa drug (0.5mg/L)



Samples:	1. sulfaguanidine	10. sulfamonomethoxine
	2. sulfacetamide	11. sulfachloropyridazine
	3. sulfadiazine	12. sulfamethoxazole
	4. sulfathiazole	13. sulfadoxine
	5. sulfapyridine	14. sulfadimethoxine
	6. sulfamerazine	15. sulfabenzamide
	7. trimethoprim	16. sulfaquinoxaline
	8. sulfadimidin	17. sulfanitran
	9. sulfamethoxypyridazine	