

図 58 ピロリジン (水 20  $\mu$ L 添加)

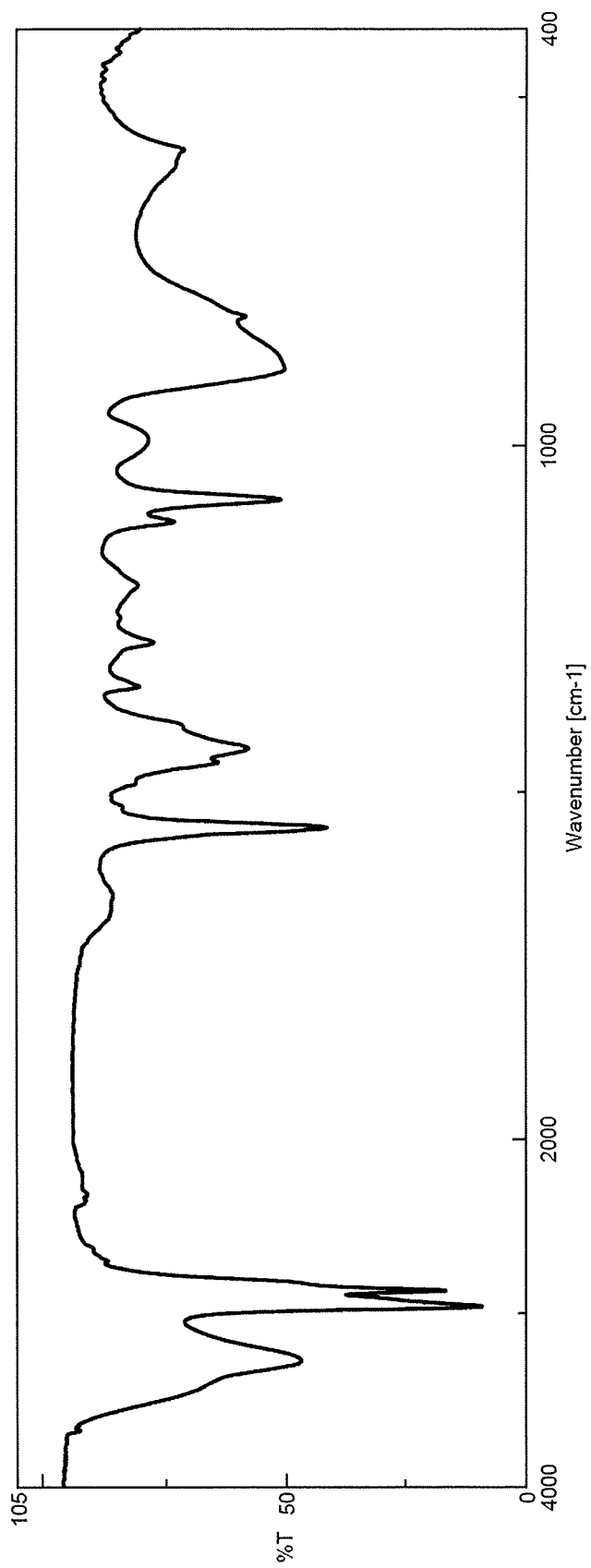


図 59 ピロリジン (水 40  $\mu$ L 添加)

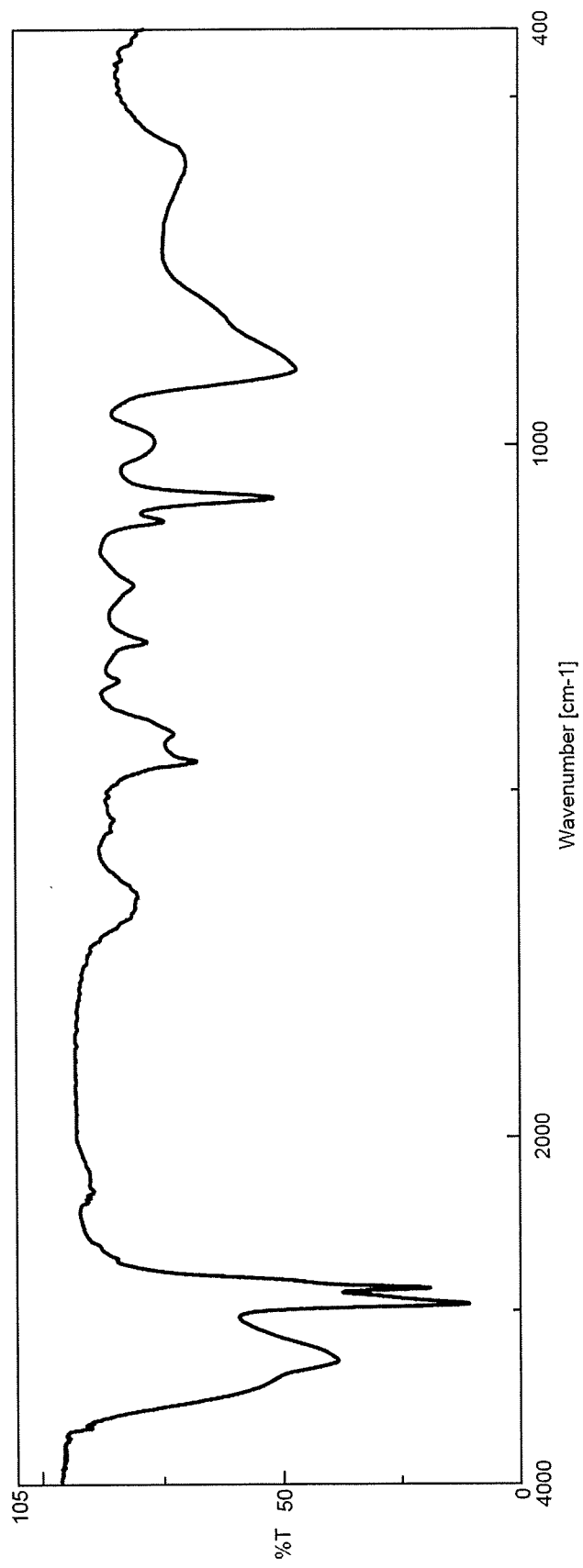


図 60 ピロリジン (水 80  $\mu$ L 添加)

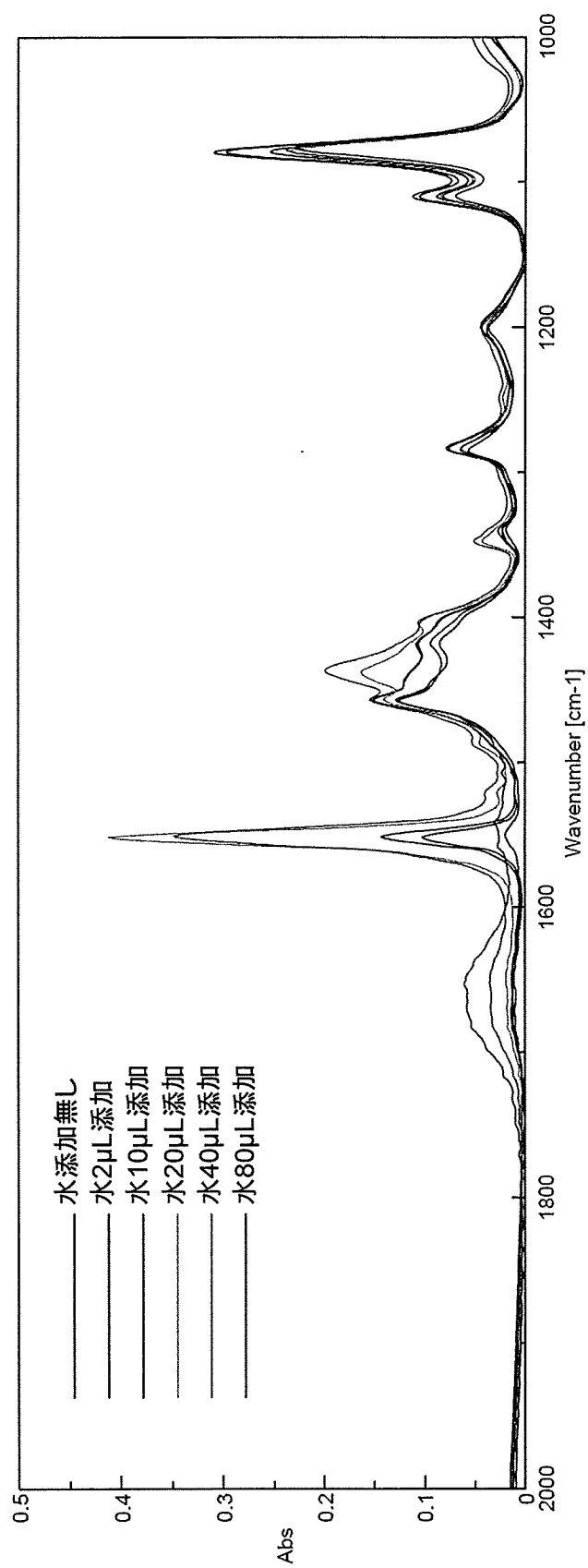


図 61 ピロリジン (水 0~80  $\mu$ L 添加 ; 縦軸 Abs 変換)

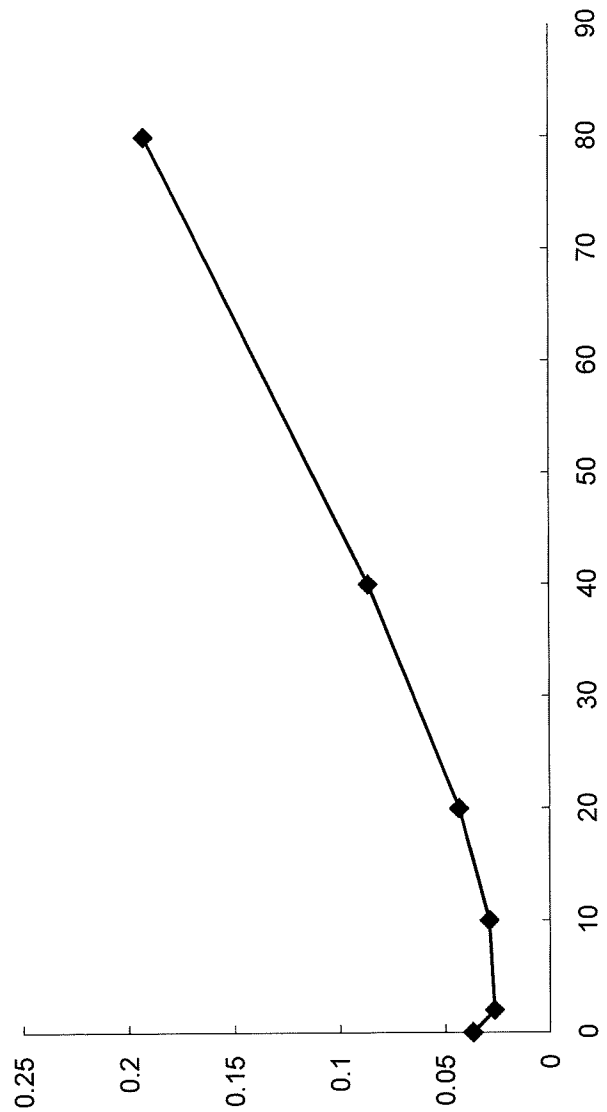


図 62 1650/1080  $\text{cm}^{-1}$  ピーク高さ比  
 (ピーク高さはベースライン法にて算出)



図 63 1550/1080  $\text{cm}^{-1}$  ピーク高さ比  
 (ピーク高さはベースライン法にて算出)

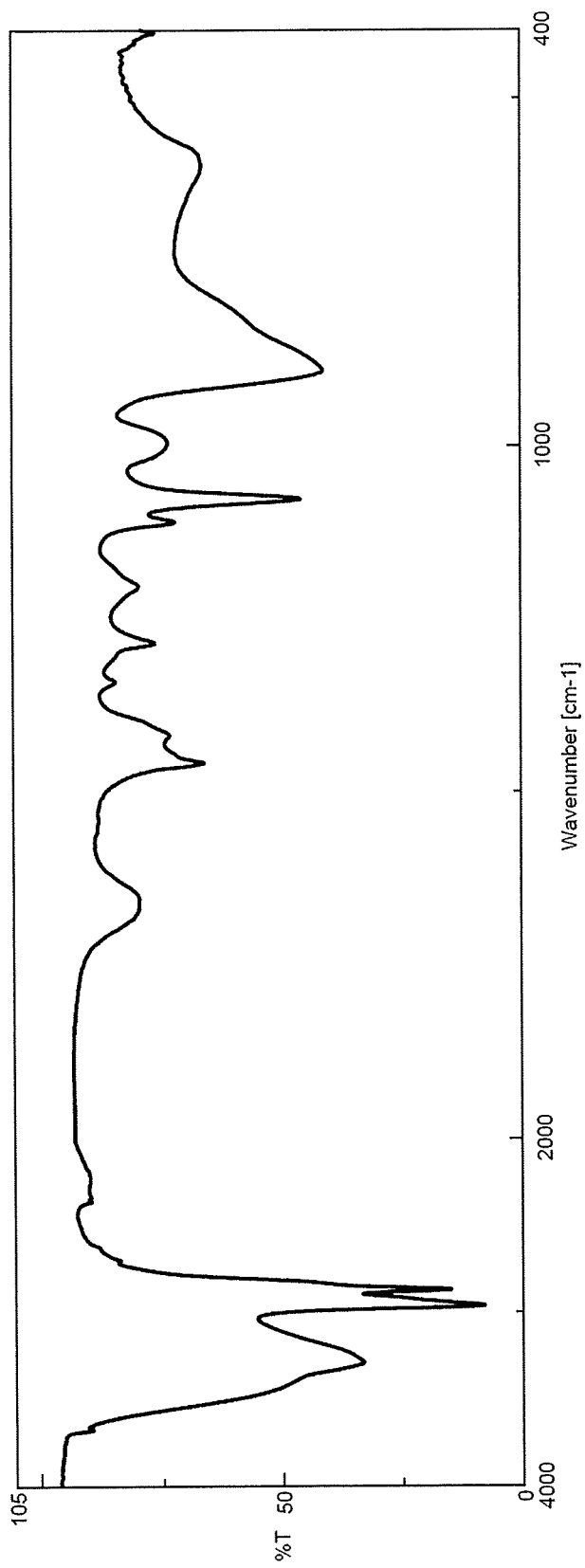


図 64 ピロリジン (水 100  $\mu$ L 添加)

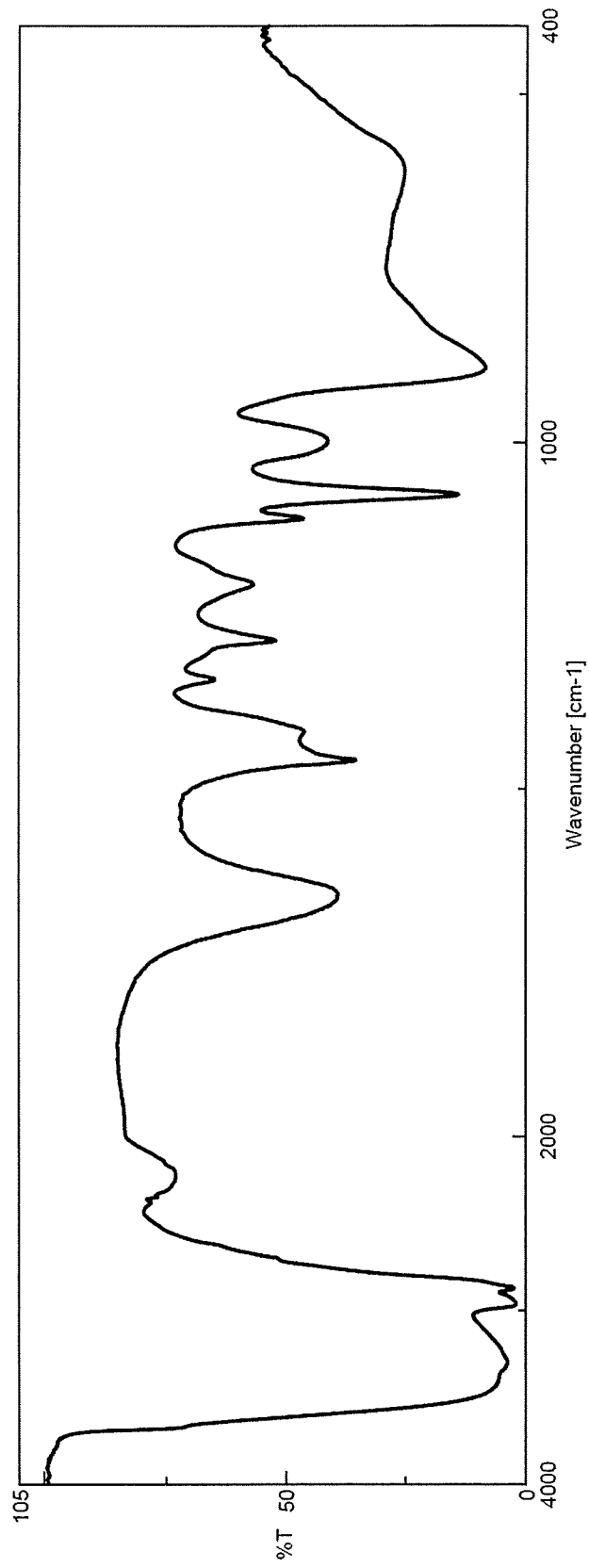


図 65 ピロリジン (水 200  $\mu$ L 添加)



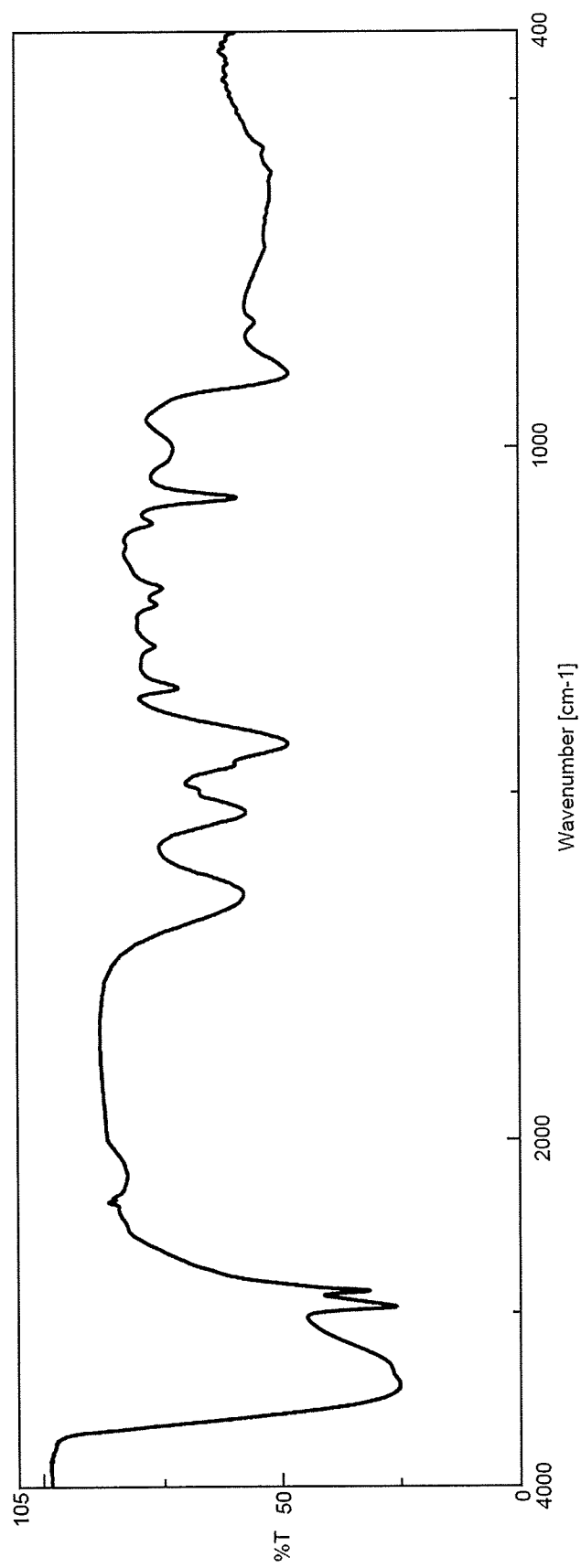


図 66 ピロリジン (水 200  $\mu$ L 添加 窓板閉)

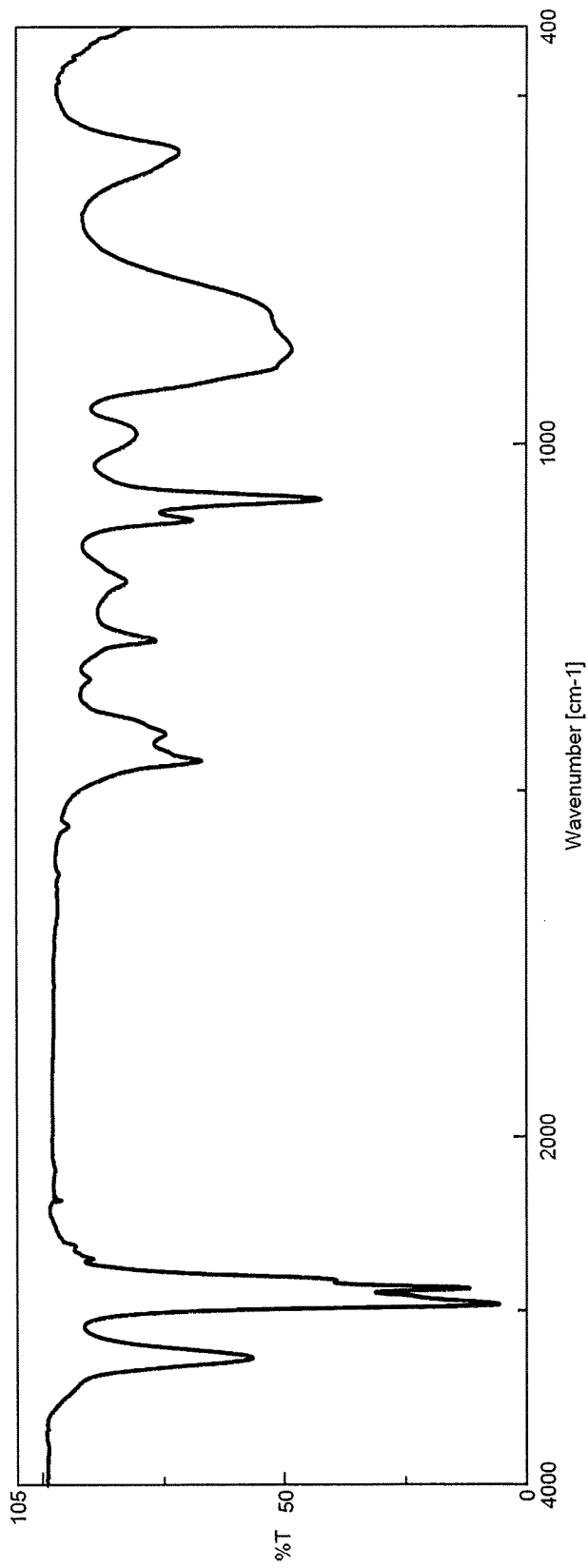


図67 ピロリジン  
(モレキュラーシーブ3Aで乾燥後, 素早く測定)



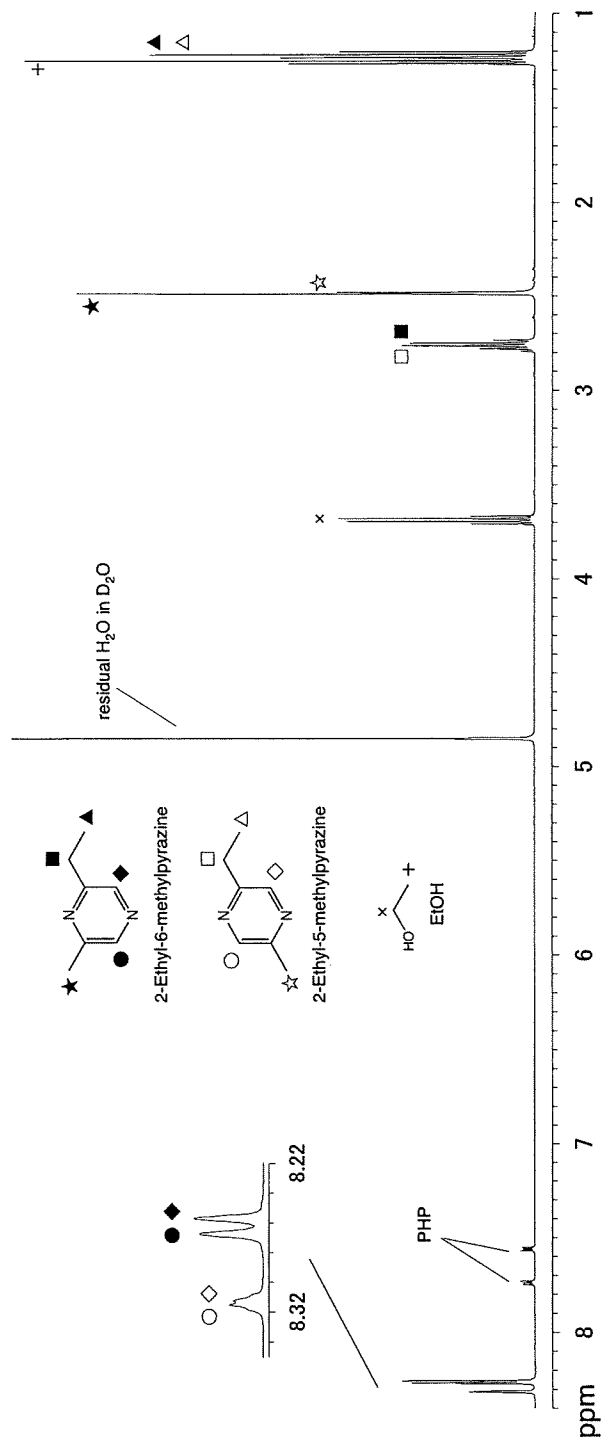


Fig. P1 Typical quantitative <sup>1</sup>H-NMR spectrum (D<sub>2</sub>O) of commercial 2-ethyl-6-methylpyrazine added EtOH. The assignments of signals were carried out using 2D-NMR such as COSY, HMQC and HMBC, and shown as symbols.

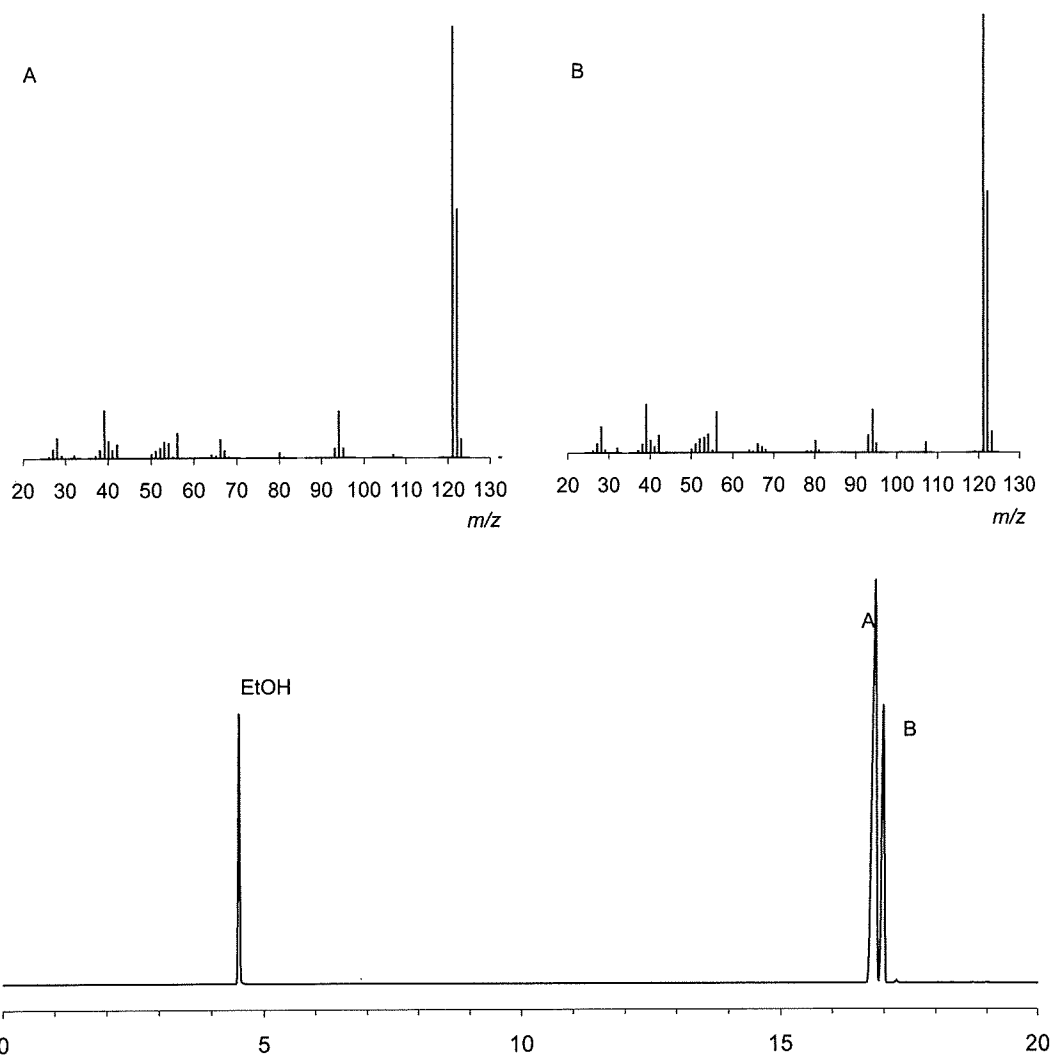


Fig. P2 Typical GC-FID profile of mixture of 2-ethyl-6-methylpyrazine and EtOH, together with the mass spectra of peak A and B  
The mass spectra of peak A and B were recorded on GC-MS.

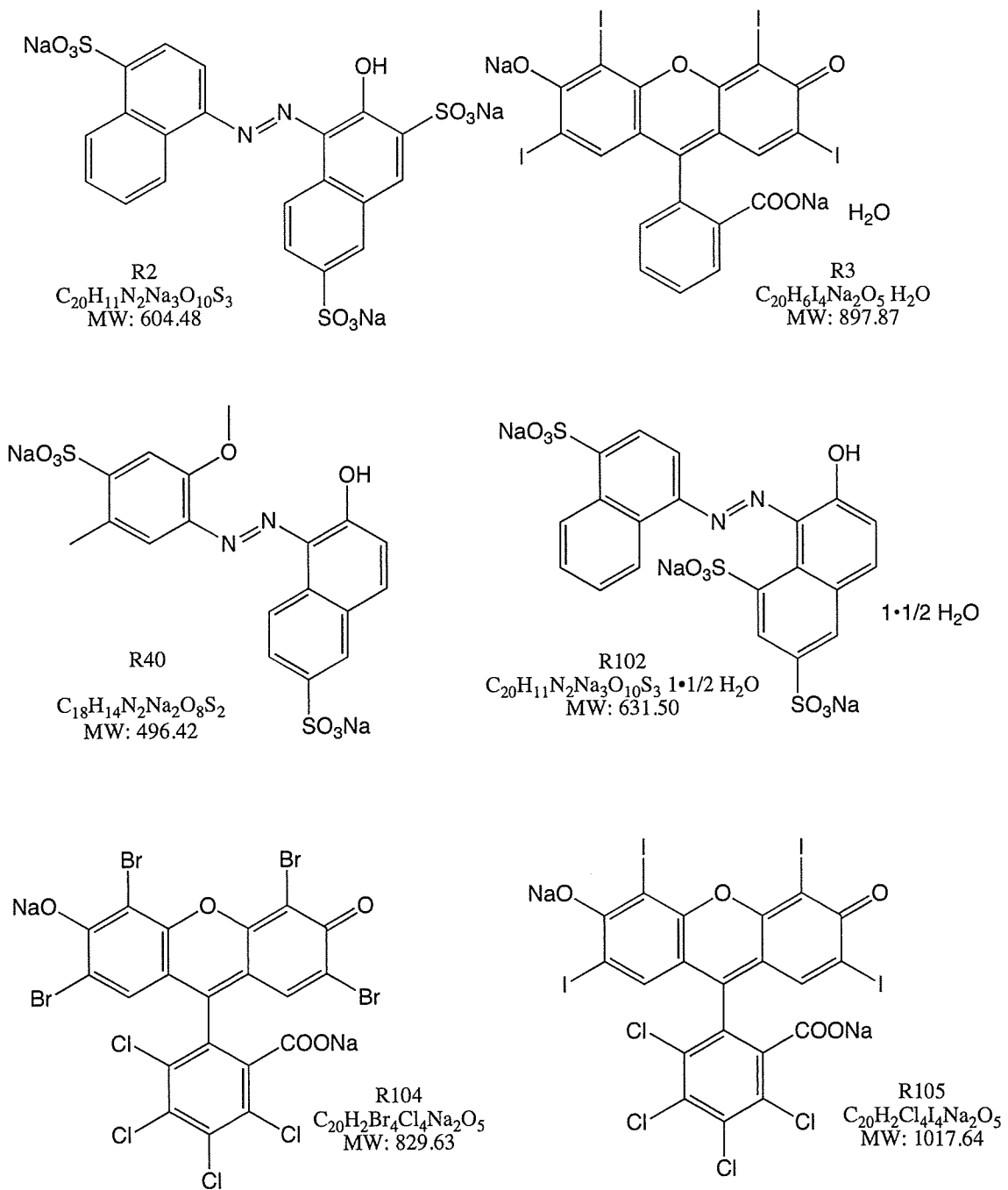
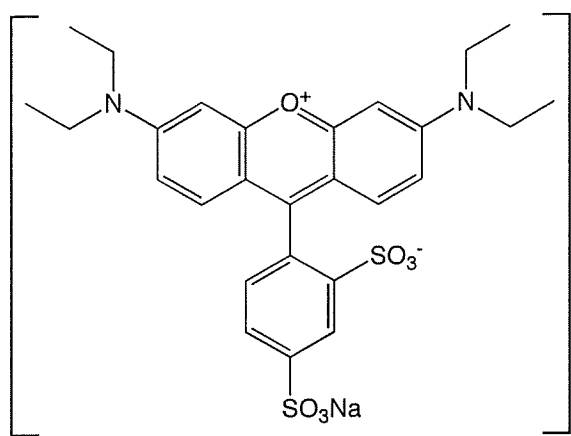
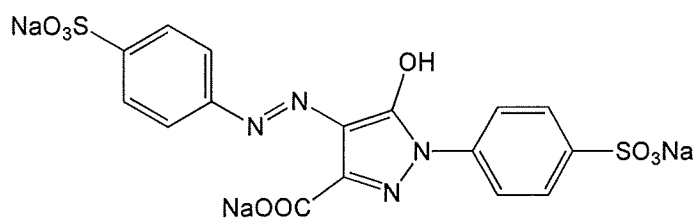


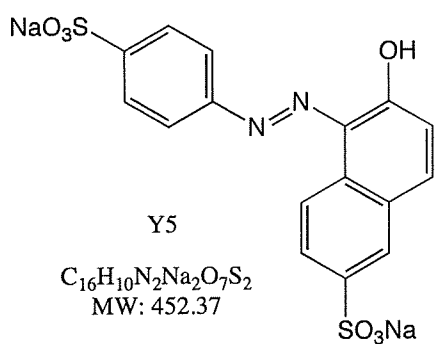
Fig. D1 Structures of tar dyes



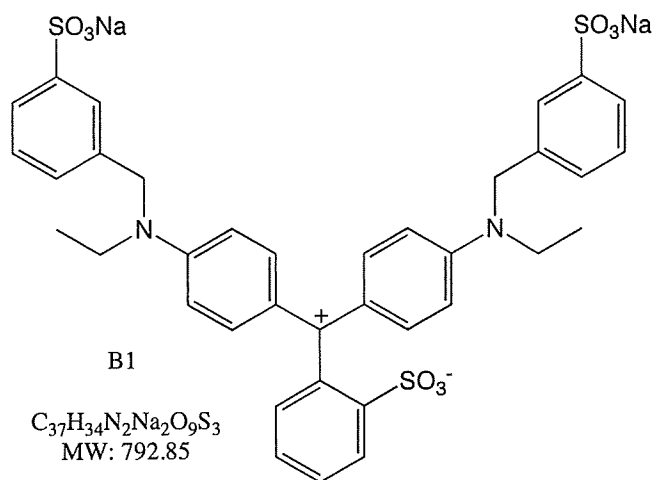
R106  
 $C_{27}H_{29}N_2NaO_7S_2$   
 MW: 580.65



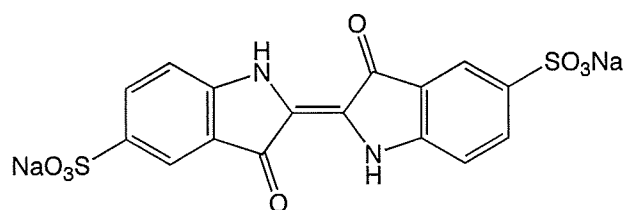
Y4  
 $C_{16}H_9N_4Na_3O_9S_2$   
 MW: 534.36



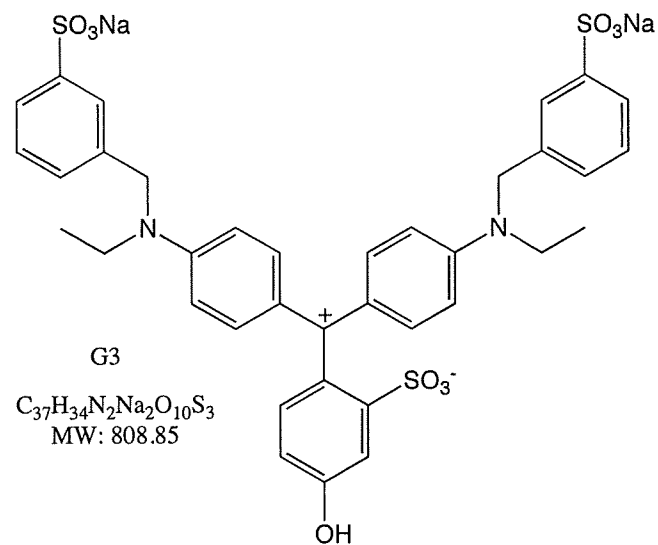
Y5  
 $C_{16}H_{10}N_2Na_2O_7S_2$   
 MW: 452.37



B1  
 $C_{37}H_{34}N_2Na_2O_9S_3$   
 MW: 792.85



B2  
 $C_{16}H_8N_2Na_2O_8S_2$   
 MW: 466.35



G3  
 $C_{37}H_{34}N_2Na_2O_{10}S_3$   
 MW: 808.85

Fig. D2 Structures of tar dyes

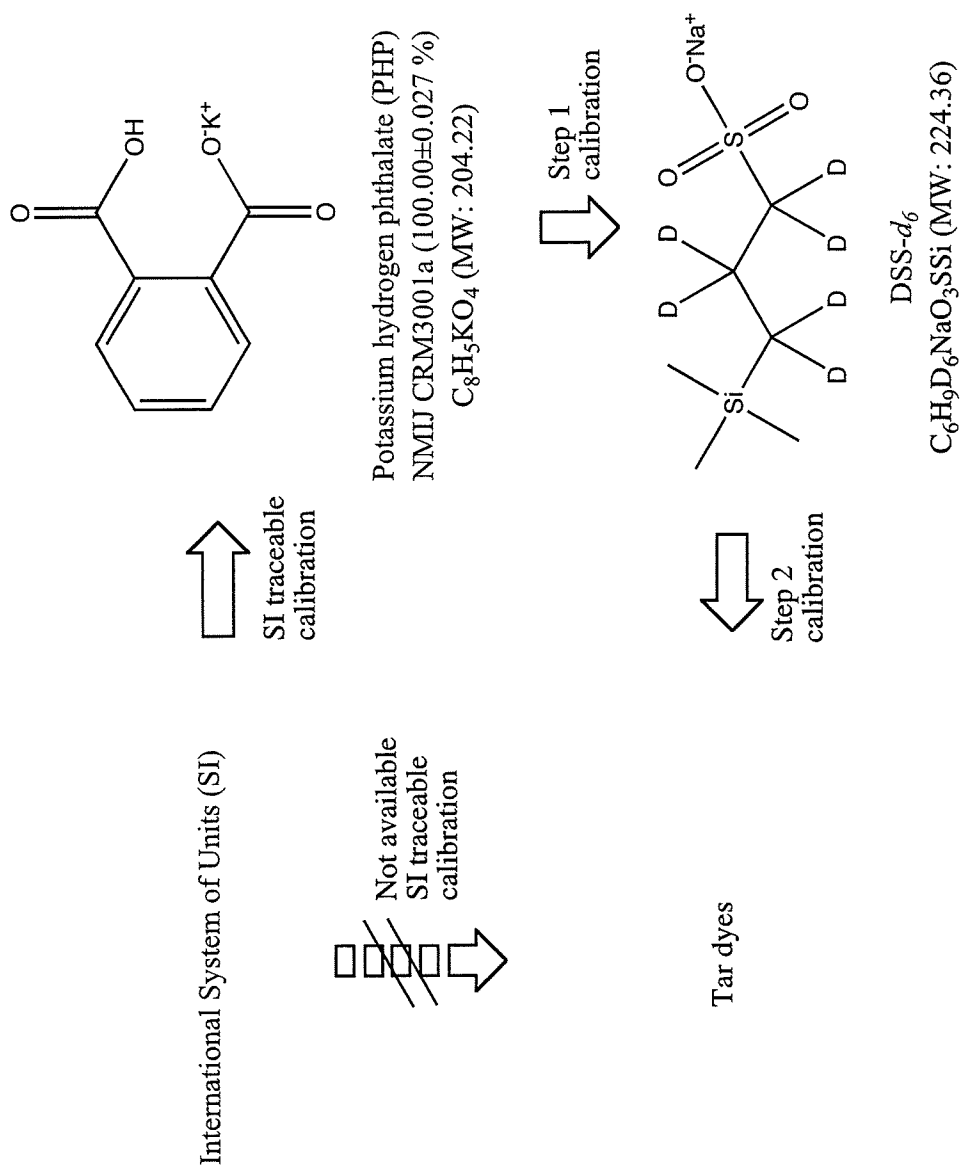


Fig. D3 Strategy of SI-traceable quantification based on qNMR.



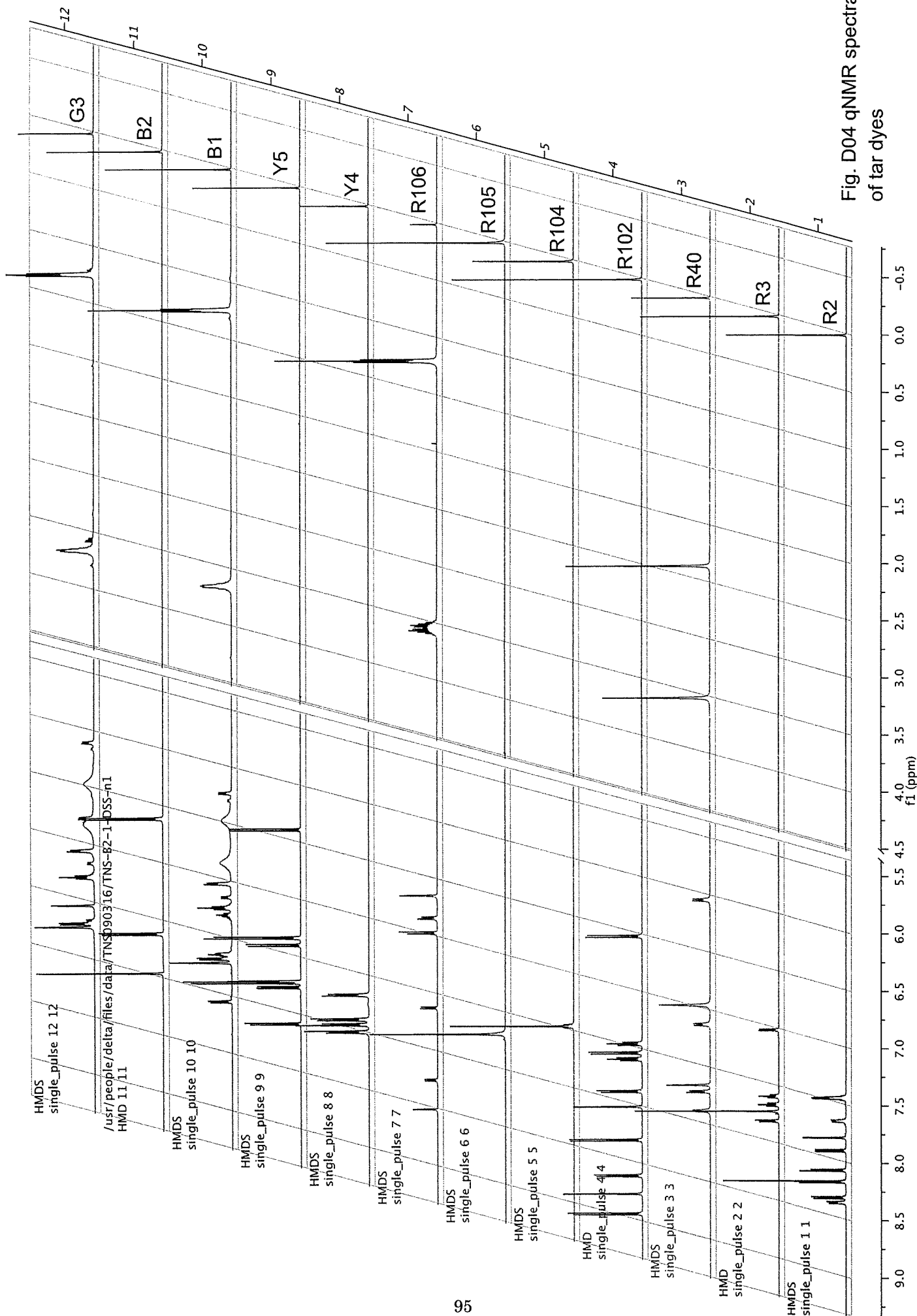


Fig. D04 qNMR spectra of far dyes

Table P1 Samples of pyrazine derivatives

Sample No.	Compound	Molecular weight	Manufacturer	Catalog No.	Grade <sup>a)</sup>	Remarks and specifications <sup>b)</sup>
P1	2-Ethyl-6-methylpyrazine	122.2	Pyrazine Specialties	726M	Food Additive	Liquid. Mixture of 2-ethyl-6-methylpyrazine and 2-ethyl-5-methylpyrazine
P2	2-Ethyl-5-methylpyrazine	122.2	Aldrich	W315400	Reagent	Liquid. Mixture of 2-ethyl-5-methylpyrazine and 2-ethyl-6-methylpyrazine, ≥98%, Kosher
P3	2-Ethyl-3-methylpyrazine	122.2	Acros Organics	25252	Reagent	Liquid. >98.5% (GC)
P4	Pyrazine (crystalline)	80.1	Sigma	P2145	Reagent	Crystals. Approx.99%
P5	2-Methylpyrazine	94.1	Aldrich	W330906	Reagent	Liquid. ≥99%, FCC, Kosher, FG
P6	2-Ethylpyrazine	108.1	Aldrich	W328103	Reagent	Liquid. ≥98%, Kosher, FCC
P7	2,3-Dimethylpyrazine	108.1	Aldrich	W327107	Reagent	Liquid. ≥95%, FCC, Kosher, FG
P8	2,5-Dimethylpyrazine	108.1	Aldrich	W327204	Reagent	Liquid. ≥98%, FCC, Kosher, FG
P9	2,6-Dimethylpyrazine	108.1	Aldrich	W327301	Reagent	Crystals. ≥98%, FCC, Kosher, FG
P10	2,3-Diethylpyrazine	136.2	Aldrich	W313602	Reagent	Liquid. ≥98%, Kosher
P11	2-Ethyl-3,(5 or 6)-dimethylpyrazine	136.2	Givaudan	4945003	Food Additive	Liquid. Mixture of 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine, 99% (combine isomers)
P12	2,3,5-Trimethylpyrazine	122.2	Aldrich	W324418	Reagent	Liquid. 2,3,5-trimethylpyrazine, ≥99%, FCC, Kosher, FG
P13	5,6,7,8-Tetrahydroquinoxaline	134.2	Aldrich	W332100	Reagent	Liquid. ≥97%, Kosher, FG
P14	6,7-Dihydro-5-methyl-5H-cyclopentapyrazine	134.2	Aldrich	W330604	Reagent	Liquid. ≥97%, Kosher, FG

a) Food Additive grade pyrazine derivatives were obtained through Japan Flavor and Fragrance Materials Association (JFFMA).

b) Remarks and specifications are reprinted from the specification sheets or label.

Table P2 Instrument and acquisition parameters

Spectrometer	JNM-ECA (500 MHz)
Probe	5 mm indirect detection probe
Probe temperature	25°C
Spectral width	- 2.5 - 12.5 ppm
Data points	64000
Flip angle	45°
Pulse delay	30 s ( $>5 \cdot T_1$ )
Scan times	8
Sample spin	15 Hz
Internal standard	Potassium hydrogen phthalate (PHP)

Table P3 Quantitative capability of qNMR and GC-FID

entry	Sample	Content (%) (n = 3)		
		qNMR	GC-FID	
1	2-Ethyl-6-methylpyrazine (sample No. P1)			
		2-Ethyl-5-methylpyrazine	28.7	29.4
		2-Ethyl-6-methylpyrazine	69.8	70.3
		Sum of isomers	98.5 (sd = 0.85)	99.7 (sd = 0.01)
2	2-Ethyl-6-methylpyrazine (sample No. P1) + EtOH (80:20 (weight))			
		2-Ethyl-5-methylpyrazine	23.1	25.1
		2-Ethyl-6-methylpyrazine	55.7	59.8
		Sum of isomers	78.8	84.9
		EtOH	19.2	14.8
		Total	98.0 (sd = 0.21)	99.8 (sd = 0.01)