―原著―

LC/MSⁿ を用いた薬物スクリーニングシステムの構築
―液体クロマトグラフィー/質量分析における保持指標の適用―

志摩典明*1，中西啓子*1，片木宗弘*1，鎌田　徹*1，財津　桂*1，鎌田寛恵*1，
西岡　裕*1，三木昭宏*1，辰野道昭*1，土橋　均*2，鈴木廣一*2

大阪府警察本部科学捜査研究所*1，大阪医科大学法医学教室*2
〒541-0053　大阪市中央区本町 1-3-18*1
〒569-8686　大阪府高槻市大学町 2-7*2

Establishment of a Drug Screening System by LC/MSⁿ
―Applications of a Liquid Chromatography/Mass Spectrometry System based on Retention Indices―

Noriaki Shima*1, Keiko Nakanishi*1, Munehiro Katagi*1, Tooru Kamata*1, Kei Zaitsu*1, Hiroe Kamata*1, Hiroshi Nishioka*1, Akihiro Miki*1, Michiaki Tatsuno*1, Hitoshi Tsuchihashi*2 and Kouichi Suzuki*2

*Forensic Science Laboratory, Osaka Prefectural Police H.Q.*1*
1-3-18, Hommachi, Chuo-ku, Osaka 541-0053, Japan
*Department of Legal Medicine, Osaka Medical colleage*2*
2-7 Daigaku-machi, Takatsuki 569-8686, Japan

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As the first step to develop a liquid chromatography/multiple-stage mass spectrometry (LC/MSⁿ) analytical system which allows us to simultaneously screen relevant drugs and poisons in forensic specimens, an efficient and practical analytical method for correcting LC retention data has been produced utilizing a liquid chromatography/mass spectrometry (LC/MS). The method is based on retention indices (RI). LC/MS employed a C18 (ODS) semi-micro column with a methanol gradient elution program, and the RI values of target substances were calculated on the basis of the retention times of a substance and tri-n-alkylamines, which were chosen as reference markers (internal standards) and respectively assigned RI values. The present study has indicated that RI provides a very reproducible method for correction of retention data. This system has also been found to be a potentially important tool in simultaneously correcting LC retention data of target substances even when we modify the LC conditions such as the solvent-gradient and flow rate. This method will, thus, be successfully incorporated into routine work for forensic investigation.

Table 1 The lists of five internal standards (A) and 24 substances (B), and their parameters related to LC/MS analysis.

(A) Internal standard	Compound name	Molecular formula	Monitored ion	Ionization mode (Positive/Negative)	Assigned RI value
IS 1	Triethylamine	C6H15N	102.2	Positive	200
IS 2	Tri-n-propylamine	C9H21N	144.3	Positive	300
IS 3	Tri-n-butylamine	C12H27N	186.4	Positive	400
IS 4	Tri-n-pentylamine	C15H33N	228.4	Positive	500
IS 5	Tri-n-heptylamine	C21H45N	312.6	Positive	700
(B) Substance					
1	Ecgonine methylester	C10H17NO3	200.1	Positive	
2	Acetaminophen	C8H9NO2	152.1	Positive	
3	Salicylic acid	C7H6O3	137.0	Negative	
4	Salbutamol	C13H21NO3	240.1	Positive	
5	Methomyl	C5H10N2O2S	163.1	Positive	
6	Amphetamine	C9H13N	136.1	Positive	
7	Caffeine	C8H10N4O2	195.0	Positive	
8	Methamphetamine	C10H15N	150.1	Positive	
9	Pancuronium[a]	C35H60N2O4	286.4	Positive	
10	Main metabolite of Zolpidem[b]	C19H19N3O3	338.3	Positive	
11	5-methoxy-N,N-diisopropyltryptamine	C17H26N2O	275.2	Positive	
12	Phenobarbital	C12H12N2O3	231.0	Negative	
13	2,4-Dichlorophenoxyacetic acid	C8H6Cl2O3	219.1	Negative	
14	alfa-Hydroxytriazolam	C17H12Cl2N4O	359.1	Positive	
15	Triazolam	C17H12Cl2N4	343.2	Positive	
16	Zolpidem	C19H21N3O	308.2	Positive	
17	Risperidone	C23H27FN4O2	411.3	Positive	
18	Haloperidol	C21H23ClFNO2	376.3	Positive	
19	Sildenafil	C22H30N6O4S	475.3	Positive	
20	Vecuronium	C34H57N2O4	557.5	Positive	
21	Promethazine	C17H20N2S	285.0	Positive	
22	Levomepromazine	C19H24N2OS	329.1	Positive	
23	Chlorpromazine	C17H19ClN2S	319.2	Positive	
24	11-Carboxylic-Δ9-tetrahydrocannabinol	C21H28O4	343.3	Negative	

[a] Divalent cation
[b] 4-(3-Dimethylcarbamoylmethyl-6-methyl-imidazo [1,2-a] pyridin-2yl)-benzoic acid

で，薬毒物スクリーニングシステムの構築には不可欠である，RI による RT 補正法を確立することを目的とした．分離カラムには，一般に汎用される逆相系 ODS（Octadecyl Silyl）カラムを選択し，IS には，疎水性相互作用による保持特性が段階的に変化するように，側鎖の炭素数のみが異なる 5 種類の 3 級アミン，Tri-n-alkylamine を選定した（Table 1A）．これらの RT を基に試験対象薬物24種（Table 1B）の RI を算出し，その数値の再現性ならびに各種分析パラメータの変動による影響について検討した．また，分離を目的とした，あるいは分析時間短縮を目的とした条件（グラジエント条件，移動相流量，カラムサイズおよび充填剤粒子径）への変更時に，RI による RT 補正が効果的に機能するかについても併せて検討を行った．

材料および方法

1. 試薬類

1 mol/l 酢酸アンモニウム溶液および LC/MS 用メタノールは，和光純薬工業製を用いた．IS に

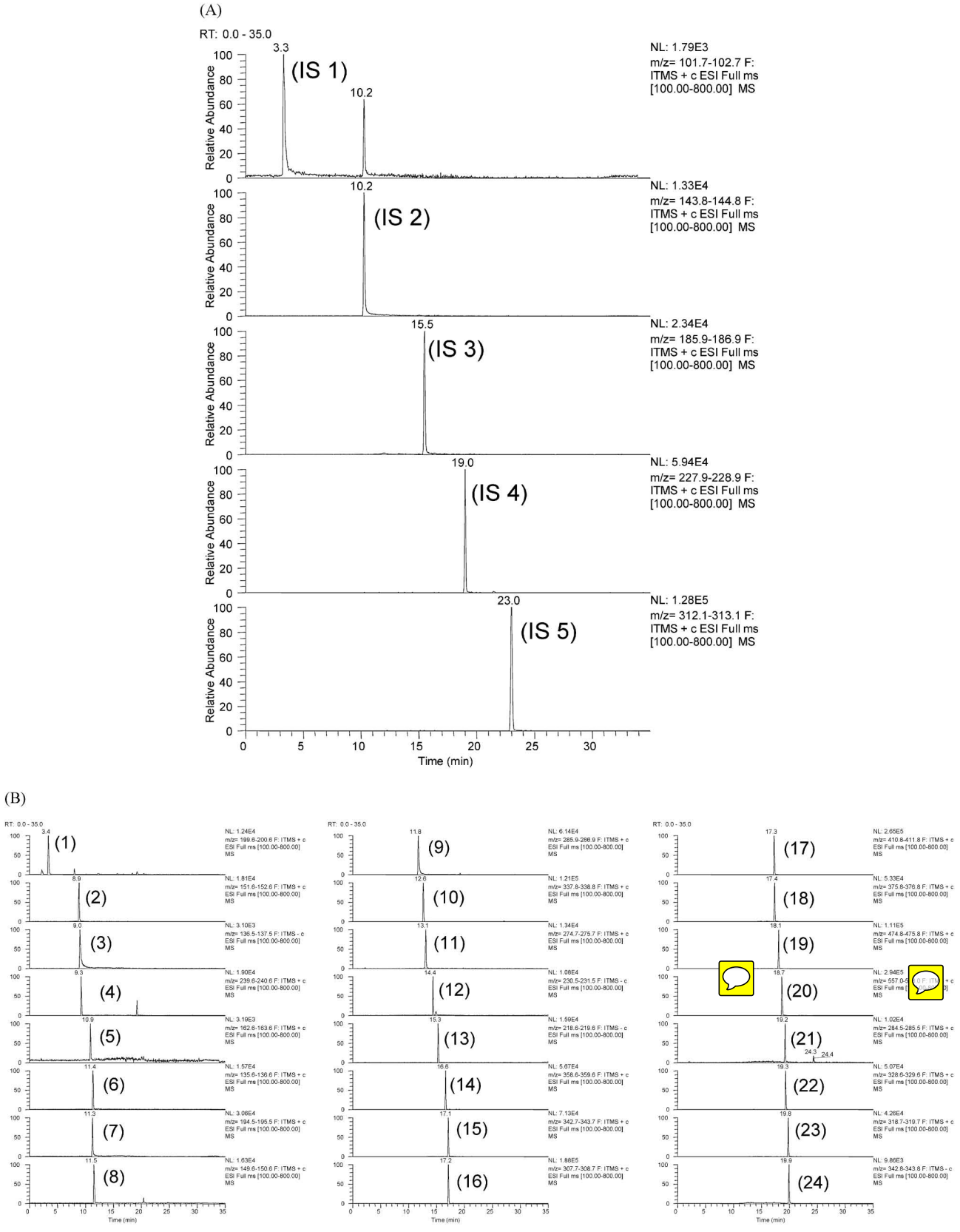

Fig. 1 Extracted mass chromatograms obtained from a standard solution spiked with (A) 5 tri-n-alkylamines (IS 1–IS 5) and (B) 24 substances