 Establishment of a Drug Screening System by LC/MS^n
—Applications of a Liquid Chromatography/Mass Spectrometry System based on Retention Indices—

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As the first step to develop a liquid chromatography/multiple-stage mass spectrometry (LC/MS^n) 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

| Substances | Compound name | Molecular formula | Monitored ion | Ionization mode (Positive/Negative) | Assigned RI value |
--- | --- | --- | --- | --- | --- |
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<sup>a)</sup> | C35H60N2O4 | 286.4 | Positive | |
10 | Main metabolite of Zolpidem<sup>b)</sup> | 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 | C21H23ClFN02 | 376.3 | Positive | |
19 | Sildenafl | C22H30N6O4S | 475.3 | Positive | |
20 | Vecuronium<sup>a)</sup> | 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-9-tetrahydrocan-nabinol | C21H28O4 | 343.3 | Negative | |

<sup>a</sup> Divalent cation  
<sup>b</sup> 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 酰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.