行政院國家科學委員會專題研究計畫 期中進度報告

1 溶劑及液相與固相微萃取結合電灑質譜及氣相層析化學游離串聯質譜於環境藥物生化的應用 2 自身離子分子反應(1/3)

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中文摘要:

本計劃主要進行溶劑微萃取法、液相微萃取法與固相微萃取法結合電灑質譜及氣相層析質譜於環境,藥物的應用及自身離子/分子反應。具體的研究內容主要包括下列：(1)液相微萃取結合氣相層析儀/串聯質譜在負離子化學游離法模式下區分氯酚之同分異構物。(2)利用溶劑微萃取法結合氣相層析儀/化學游離法/串聯質譜鑑別及定量茴香醛結構異構物之混合物。(3)二甲苯在內部游離式離子阱質譜儀中自身離子/分子反應的反應機構的研究,利用離子阱質譜儀的碰撞活化解離與 isolation 的技術，另外再加上理論計算的輔助，探討這些特殊離子生成的反應機構。(4)離子阱串聯質譜儀偵測二甲苯及同分異構物鑑別。(5)溶劑微萃取法連結電灑質譜/串聯質譜於農藥鑑別及定量的應用。(6)利用固相微萃取法結合氣相層析質譜儀檢測市售藥物及血清中維他命 E 之混合物。(7)內部游離式離子阱質譜儀中碰撞活化解離(CAD)的自身離子/分子反應的研究。

關鍵詞：液相微萃取法，固相微萃取法，溶劑微萃取法，氣相層析儀/離子阱質譜儀，化學游離法，電灑質譜，串聯質譜,離子阱質譜儀，自身離子/分子反應、碰撞活化解離

本研究計劃的成果:

(1) **利用固相微萃取法結合氣相層析質譜儀檢測市售藥物及血清中維他命 E 之混合物**

α-tocopherol、β-tocopherol 以及 γ-tocopherol 為市售維他命 E 中常見的三種形式，因為它具有抗氧化的作用，可以減緩細胞的老化，甚至是預防癌化的效用。近來在在社會保健意識逐漸提昇下各類維他命的需求量有日日增加的趨勢，但由於維他命 E 為脂溶性之大分子且揮發性不佳，這大大增加在 GC/MS 上分析的困難度，因此，如何建立一套快速而準確之分析方法於維他命 E 的檢測上是值得研究的。本實驗主要是探討利用固相微萃取法結合氣相層析質譜儀進行維他命 E 之分析與偵測。以固相微萃取纖維萃取水中的維他命 E 之混合物，再經由衍生化後分析偵測。同時將探討固相微萃取纖維萃種類、萃取時間、磁石攪拌速度、萃

取溫度及鹽類添加濃度、衍生化條件調控等因素對於萃取效率的影響，企圖找出最佳的萃取條件。最後再將此方法應用於市售健康食品及真實血清樣品之分析。

(2) **A Novel Liquid-Phase Microextraction Method: a Dual Gauge Microsyringe with a Hollow Fiber Membrane**

A novel liquid-phase microextraction method (LPME) by combining a dual gauge microsyringe with a hollow fiber membrane (LPME/DGM-HF) for the extraction and determination of organochlorine pesticides (OCPs) in aqueous solution then followed detection by Gas Chromatography/Ion Trap Mass Spectrometry (GC/ITMS) has been demonstrated. The advantages of this technique include simplicity, easy of use, fast and little solvent consumption. Influence of parameters including extraction time, solvent selection, salt concentration and sample stirring rate have been investigated in order to optimize the extraction efficiency for this method. The viability of this methodology is evaluated by measuring the linearity and detection limit. The detection linearity for the OCPs has been obtained over a range of concentrations between 1 to 500 μg/l ($r^2 > 0.930$), with detection limit of 0.1 μg/l for each of OCPs.

(3) **Combination of Liquid-Phase Hollow Fibre Membrane Microextraction with Gas Chromatography/Negative Chemical Ionization Mass Spectrometry for the Determination of Dichlorophenol Isomers in Environmental Sample**

A method for the determination of trace amount of dichlorophenol isomers in environmental samples using the combination of liquid-phase hollow fibre microextraction (LPME-HF) with gas chromatography–negative chemical ionization mass spectrometry (GC/NCI MS) has been demonstrated. The method has been optimized with respect to several parameters including the effects of NCI reagent pressure, the hollow fibre length, extraction time, stirring rate, sample PH and salt concentraction for the analysis of dichlorophenol isomers in environmental samples. The correlation coefficients for the calibration curves (ranging from 0.970 to 0.994, < 8.5% RSD) and the average recovery rates (ranging from 93% to 97%, n = 3 for each dichlorophenol) indicate that the methodology is feasible for the determination of trace amounts of dichlorophenol isomers in environmental samples. Method detection limits (MDL) have been found to be in the range of 5 – 20 ng/ml.

(4) **Single-Drop Microextraction and Gas Chromatography-Mass Spectrometric Determination of Anisaldehyde Isomers in Human Urine and Blood Serum**

The application of single drop microextraction (SDME) followed by gas chromatography/chemical ionization mass spectrometry (GC/CI-MS) was investigated for the determination of anisaldehyde isomers in human urine and blood serum. The effects of extraction solvent, sample agitation rate, salt addition, sampling time and temperature on the extraction efficiency were examined and optimized. Analytical parameters such as linearity, reproducibility, detection limit and relative recovery were evaluated under the optimized experimental conditions. Good reproducibilities of replicate extractions (n = 5) were obtained, with relative standard deviation (RSD) values below 6%. The limits of detection (LOD) using an extraction time of 5 minutes were found to be in the range of 2 to 5 ng/ml under the selective ion-monitoring (SIM) mode of GC/MS. 82-98% recoveries were achieved after 5 minutes extraction.

(5) **Differentiation of xylene isomers by SIMR**

This study presents the Self-Ion/Molecule Reactions (SIMR) spectra of the three xylene isomers, and proposes an approach to differentiating them based on observed differences in relative abundances of ions formed by SIMR in an internal source ion trap instrument. It also demonstrates the applicability of SIMR for isomer discrimination, which is better than electronic ionization (EI) and dimethyl ether chemical ionization (DME CI) in the ion trap mass spectrometer (ITMS) since no CI reagent, metal ions or internal standards were required to perform SIMR. The merits of the new method for distinguishing the isomers include simple, rapid and economic. So far, the methyne addition products ($[M+13]^+$ ions) have been observed for several nitrogenated compounds including aza-crown ethers, aniline and dopamine from SIMR in the ITMS. While the xylene isomers are the first three compounds that can produce the methyne addition ions in SIMR for non-nitrogenated compounds.

(6) Self - Ion / Molecule Reaction to produce the protonated molecules during collisionally activated dissociation in an ion trap

This study describes the formation of protonated molecules ($[M+H]^+$) and adduct ions owing to Self - Ion / Molecule Reaction (SIMR) during CAD of methyne addition ions ($[M+CH]^+$) produced from chemical ionization (CI) or SIMR in both external and internal source ion trap mass spectrometer (ITMS). The CAD results for the methyne addition ions of dopamine produced from both SIMR and dimethyl ether (DME) CI undertaken in the external and internal source ITMS were compared in order to prove the occurrence of SIMR during CAD processes. Compared with the external source ITMS, the internal source ITMS are much more easily to undergo this type of reaction due to large population of neutral analytes present in the trap.

(7) **Probing the unusual product ions observed in Self-Ion/Molecule Reactions of xylene isomers by deuterium labeling, isolation experiments and collisionally activated dissociation in an ion trap mass spectrometer**

This study investigates the mechanisms of addition of the methyne ions produced in the Self-Ion/Molecule Reactions (SIMR) of o, m and p-xylene isomers by deuterium labeling and isolation experiments in an ion trap mass spectrometer (ITMS). Identical SIMR products including $[M-3H]^+$, $[M-H]^+$, $M^{+\cdot}$, $[M+H]^+$, $[M+CH]^+$, $[M+CH+C]^+$, $[2M-3H]^+$ and adduct ion of fragments ($[M+F]^+$, where F represents fragment ions) have been produced by all the xylene isomers. The isolation experiments have been applied to trace the source and the formation of SIMR product ions. The main sources for formation of the methyne ions for these three isomers have been attributed to the benzyl cations (m/z 91). Highly unusual products including $[M+25]^+$, $[M+37]^+$, $[M+47]^+$, $[M+49]^+$, $[M+50]^+$, $[M+62]^+$ and $[M+131]^+$have been observed in the SIMR or after isolation experiments. The assignments of these unusual ions have been confirmed by deuterium labeling experiments. The collisionally activated dissociation technique (CAD) has also been applied to elucidate the structures of the SIMR products.

計劃成果自評:

In this project, we already published several SCI journals so far. In addition, we also demonstrated part of our results in the posters in some conferences within this year. All the information in regards to publications and poster presentations is provided in the references.

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Observation of Self - Ion / Molecule Reaction during collisionally activated dissociation in an ion trap mass spectrometer

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Abstract

This study describes the formation of protonated molecules ($[M+H]^+$) and adduct ions owing to Self - Ion / Molecule Reaction (SIMR) during CAD of methyne addition ions ($[M+CH]^+$) produced from chemical ionization (CI) or SIMR in both external and internal source ion trap mass spectrometer (ITMS). The CAD results for the methyne addition ions of dopamine produced from both SIMR and dimethyl ether (DME) CI undertaken in the external and internal source ITMS were compared in order to prove the occurrence of SIMR during CAD processes. Compared with the external source ITMS, the internal source ITMS are much more easily to undergo this type of reaction due to large population of neutral analytes present in the trap.

Introduction

Tandem mass spectrometry is a rapid and powerful analytical technique due to its capability for structural elucidation of compounds. The ion trap mass spectrometer (ITMS) possesses excellent capability for tandem mass analysis and low detection limits for collisionally activated dissociation (CAD) [1,2]. Unusual adduct ions have been observed during the CAD process in an ITMS, corresponding to addition of water or oxygen, using when chemical ionization (CI) or electrospray ionization [2-5]. In this study, the observation of Self - Ion / Molecule Reaction (SIMR) during CAD, when examining CI and SIMR in an ITMS, is

reported. SIMR lead to the formation of the methyne addition reactions in an ITMS has been investigated previously [6-9]. Brodbelt et al have examined methyne addition reactions by dimethyl ether (DME) CI for many compounds using an ITMS [10-26]. The purpose of the study is to describe the phenomenon when CAD is performed on the methyne adduct ions produced from both SIMR or CI, using both external and internal source ITMS instruments, the SIMR can occur and lead to the formation of the protonated molecules or adduct ions.

Experimental

Dopamine was purchased from Sigma (St Louis, MO, USA). DME, aniline, p-xylene and all aza-crown ethers were obtained from Aldrich (Milwaukee, WI, USA). o- and m-Xylene were purchased from Fluka (Buchs, Switzerland). Toluene was purchase from J. T. Backer (Phillipsburg, NJ, USA). Methane was purchased from the San-Fu Chemical Company (Hsin-Chu, Taiwan). n-Hexane was purchased from Tedia (Fairfield, OH, USA). All compounds were used as received without further purification. Figure 1 shows the structures of compounds investigated. All experiments were performed using both external and internal source ITMS (Finnigan MAT GCQ and Varian Saturn 2000 GC/MS, respectively) under SIMR or CI ionization methods. DME and methane were applied as CI reagent gases. Both ITMS instruments were operated in the mass selective instability mode. For experiments performed in the external source ITMS (Finnigan MAT GCQ, San Jose, CA, USA), the temperature of

the ion source was maintained at 200 ^{o}C. The ion injection time was 25 msec. CAD experiments were performed using a supplementary tickle voltage to the endcaps of the ITMS at q_z = 0.225. The collisional activation time was 15 msec. Signal width for selection of the precursor ions was from 0.1-1 Da. The CAD voltage for fragmentation of the parent ions was from 0.7-1.0 V. The analyte was introduced to the ion source of GCQ *via* a temperature controlled direct insertion probe (DIP) or a Gas Chromatograph (GC). Solutions of analyte were prepared in methanol or n-hexane at concentrations of 1 x 10^{-1} to 1 x 10^{-5}g/ml. For DIP experiments, 1 µL of the solutions were subjected to an evaporation step by a heater to eliminate the solvent. The probe temperature was increased from 200 to 350 ^{o}C at 100 ^{o}C/min.

For experiments undertaken in the internal source ITMS (Saturn 2000, Varian, Walnut Creek, CA, USA), selective ejection chemical ionization (SECI) was used to perform both CI and SIMR experiments. The ionization time was controlled by the automatic reaction control (ARC) [27]. The temperatures of trap, transfer line and manifold were 120, 240 and 80 ^{o}C, respectively. QISMS (Varian) research version software was used to implement the RF voltage sequences. The ITMS was connected by a heated transfer line (240 ^{o}C) to a Varian 3800 model GC equipped with split/splitless injection and programmable on-column injector. CAD experiments were performed by applying a resonant excitation voltage at q_z = 0.45. Signal width for selection of the parent ions was 1.0 Da. The collision voltage for fragmentation of the parent ions was from 0.6-0.7 V. Full scan data acquisition was achieved for the range m/z

40-600 at 1 sec/scan. The emission current was 9.4 x 10^{-6}A and the multiplier voltage was 1500 V. All compounds were introduced to the ion trap by GC with a 1079 injection port for 1 μg / μL injection. A 30m DB5-MS capillary column with an internal diameter of 0.25mm and a film thickness of 1 μm was used. The carrier gas was helium. The flow rate was 1ml/min. The ionization times were varied from 0.3ms to 2ms.

Results and Discussion

Figures 2 and 3 display the CAD spectra of $[M+13]^+$ ions (m/z 166, assigned as $[M+CH]^+$) of dopamine produced by both SIMR [8] and DME CI [4] in an internal source ITMS, respectively. Notably, the formation of the base peak at m/z 154 in Figures 2 and 3 seems unusual due to the apparent loss of 12 Da from m/z 166. This process is attributed to SIMR during CAD processes of m/z 166. The m/z 154 is assigned as the protonated molecule ($[M+H]^+$), produced from the following reactions. The CAD of methyne adduct ions at m/z 166 produces fragment ions in the low-mass region, and these low - mass fragment ions then act as CI reagent ions and undergo secondary ion/molecule reactions with the neutral dopamine molecules to generate the protonated molecules. In order to confirm this assumption, we compared the CAD results for $[M+13]^+$ ions for DME CI of dopamine in an internal source ITMS with that of an external source ITMS. Although the CAD of $[M+13]^+$ ions of dopamine produced from DME CI in an external source ITMS has been reported previously [4], it is

shown again in Figure 4 to facilitate the present comparison. In Figure 4, the concentration of dopamine is $1x10^{-1}$ g/mL for 1 µL loading on the DIP. Although the concentration is two orders of magnitude higher than that used to obtain Figures 2 and 3, no protonated molecules ($[M+H]^+$) at m/z 154 were observed in the external source ITMS. Besides, the base peak switches to m/z 149 via elimination of one molecule of NH_3. This is because no SIMR occurred during CAD in the configuration with the external source ITMS, since the ionization is separated from the ion storage and analysis. Only ions are allowed to enter the ion trap mass analyzer, and neutral molecules are removed by the vacuum pumps. Thus, formation of the protonated molecules was not observed in Figure 4. In contrast, the internal source ITMS allows all CI reagent molecules and neutral dopamine to ionize and react inside the ion trap. The large population of neutral dopamine molecules inside the ion trap mass analyzer can react with the fragment ions produced from CAD. Thus, SIMR can easily occur. This type of reaction would only be observed when using a DIP with thermal desorption of analytes or during GC applications because the neutral analyte must be present in the trap during the CAD period, thus allowing fragment ions to react with the neutral analytes to form the SIMR products including protonated analyte molecules or the adduct ions $(M+F)^+$ where F represents the EI fragment ions. Besides, one may also expect this type of reaction to be only common for basic analytes, such as the nitrogen-containing compound. In order to confirm this assumption, we examine many other compounds in order to show the range of occurrence for

this type of reactions. The results are shown in Table 1. It lists CAD results of $[M+CH]^+$ ions formed by SIMR or CI in both internal (Saturn 2000) and external (GCQ) source ITMS for 1 μL loading on the GC or DIP. From the results shown in Table 1, three phenomena were observed. First, this type of reactions is routinely occurs for less basic compounds such as toluene and xylene isomers (the proton affinities for toluene and m-xylene are 784 and 811 KJ/mol, respectively [28]. Second, it can also occur in an ITMS with an external source for more basic compounds such as aza-crown ethers. Third, reduce the concentration of analyte can effectively reduce the observation of the SIMR during the CAD processes in an ITMS.

Conclusion

This study presents the novel observation of SIMR during CAD in an ITMS. The proponated molecules ($[M+H]^+$) or adduct ions can be routinely observed during CAD in both internal and external source ITMS. The internal source ITMS are extremely easy to observe this type of reaction due to large population of the neutral molecules present in the trap region. The best way to avoid this type of reactions and to obtain the successful CAD results is to reduce the sample concentration.

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Captions

Figure 1. Structures of the compounds examined.

Figure 2. CAD spectrum of $[M+CH]^+$ ions (m/z 166) of dopamine formed by self-ion/molecule reactions in an internal source ion trap mass spectrometer at a concentration

corresponding to $1x10^{-3}$ g/mL for 1 µL loading on the GC.

Figure 3. CAD spectrum of $[M+CH]^+$ ions (m/z 166) of dopamine formed by chemical ionization with dimethyl ether ions in an internal source ion trap mass spectrometer at a concentration corresponding to $1x10^{-3}$ g/mL for 1 µL loading on the GC.

Figure 4. CAD spectrum of $[M+CH]^+$ ions (m/z 166) of dopamine formed by chemical ionization with dimethyl ether ions in an external source ion trap mass spectrometer at a concentration corresponding to $1x10^{-1}$ g/mL for 1 µL loading on the DIP.

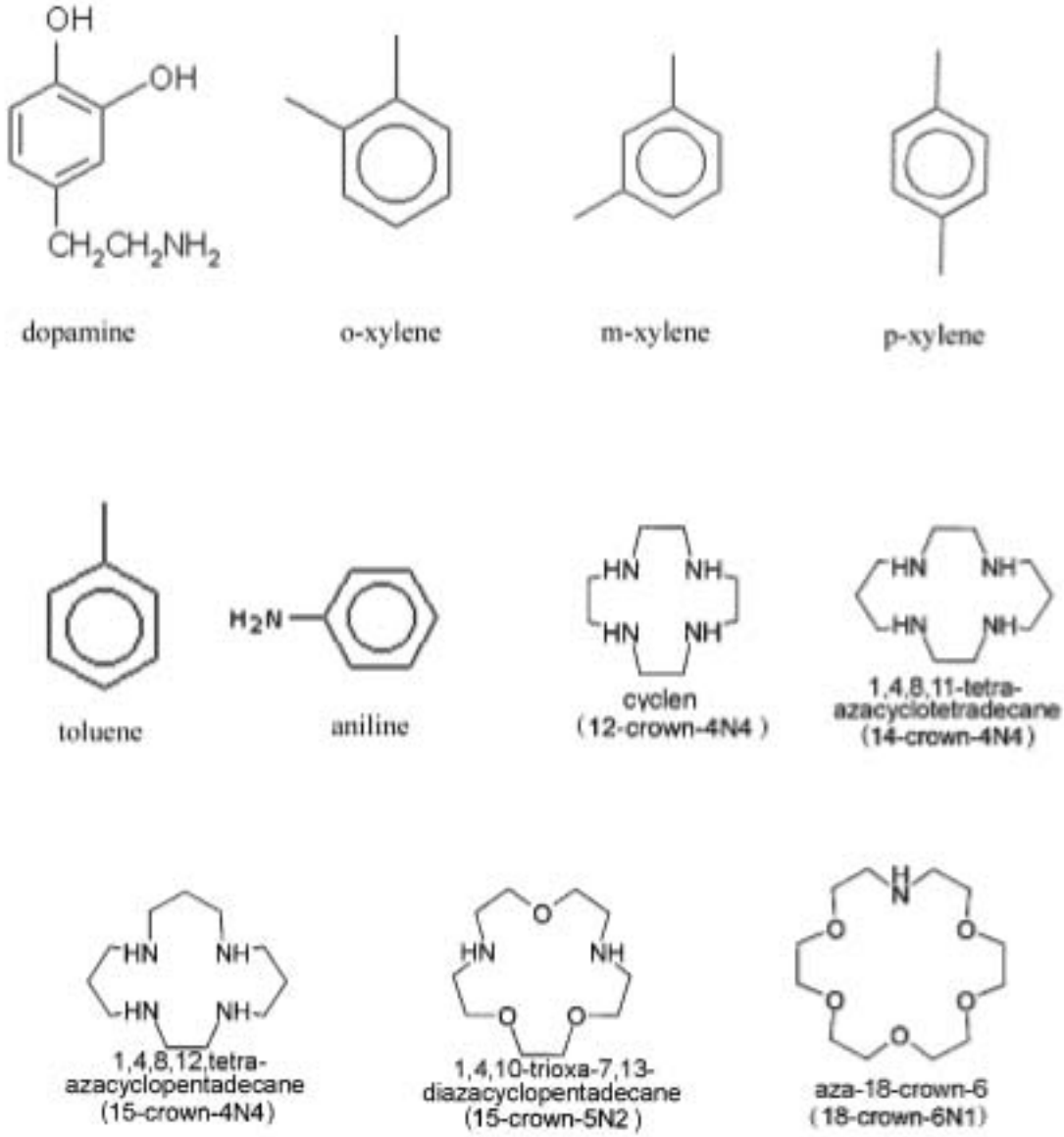
OH
OH
$CH_2CH_2NH_2$
dopamine
o-xylene
m-xylene
p-xylene
toluene
H_2N
aniline
HN
NH
HN
NH
cyclen
(12-crown-4N4)
1,4,8,11-tetra-
azacyclotetradecane
(14-crown-4N4)
1,4,8,12,tetra-
azacyclopentadecane
(15-crown-4N4)
O
1,4,10-trioxa-7,13-
diazacyclopentadecane
(15-crown-5N2)
H
N
aza-18-crown-6
(18-crown-6N1)

Figure 1

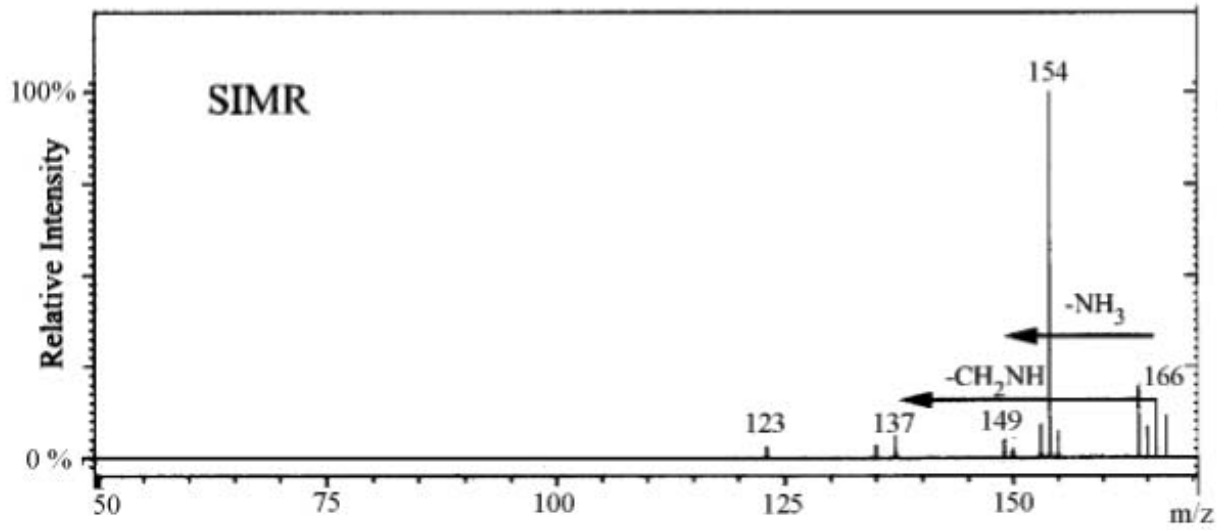
SIMR
100%
0 %
Relative Intensity
154
-NH3
-CH2NH
166
123
137
149
50
75
100
125
150
m/z

Figure 2

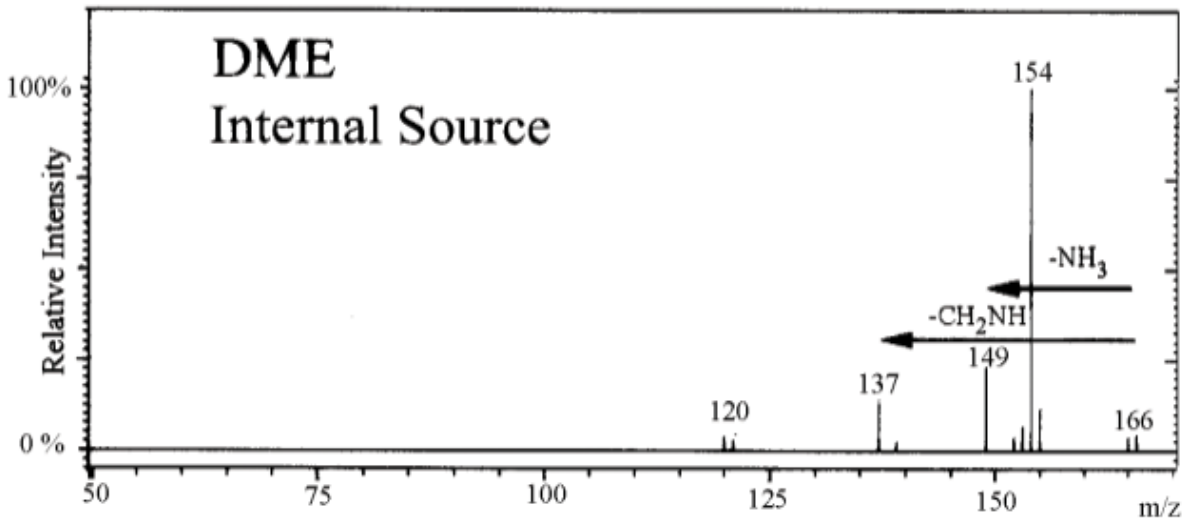
DME
Internal Source
100%
0 %
Relative Intensity
154
-NH3
-CH2NH
149
137
120
166
50
75
100
125
150
m/z

Figure 3

Table 1. CAD results of $[M+CH]^+$ ions formed by self-ion/molecule reactions or chemical ionization in both internal source and external source ion trap mass spectrometers.

Compound	Precursors $[M+13]^+$ (m/z)	Concentration	Product ions of CAD of $[M+13]^+$	Ionization Method	Instrument
Toluene (92)	105	0.01 mg mL^{-1}	78(63%) 79(100%) 91(56%) 103(48%)	CH_4-CI	GCQ
	105	0.1 mg mL^{-1}	78(43%) 79(100%) 91(35%) 103(47%)	CH_4-CI	GCQ
	105	0.1 mg mL^{-1}	93(100%),$[M+H]^+$ 103(9%) 117(34%) 169(10%) 195(13%)	SIMR	Saturn 2000
	105	1 mg mL^{-1}	79(100%) 91(27%) 93(73%) 178(18%) 119(9%) 195(23%)	CH_4-CI	GCQ
o-Xylene (106)	119	0.01 mg mL^{-1}	79(7%) 91(100%) 103(6%) 107(<1%),$[M+H]^+$ 115(15%) 117(436)	CH_4-CI	GCQ
	119	1 mg mL^{-1}	91(72%) 105(33%) 107(28%),$[M+H]^+$ 115(100%) 197(17%) 209(17%) 221(59%)	SIMR	Saturn 2000

m-Xylene (106)	119	0.01mg mL^{-1}	79(9%) 91(100%) 103(6%) 107(<1%),$[M+H]^+$ 115(13%) 117(41%)	CH_4-CI	GCQ
	119	1mg mL^{-1}	79(9%) 91(28%) 107(51%),$[M+H]^+$ 117(100%) 131(12%) 197(45%) 209(22%) 221(86%)	SIMR	Saturn 2000
p-Xylene (106)	119	0.01 mg mL^{-1}	79(8%) 91(100%) 103(6%) 107(<1%),$[M+H]^+$ 115(15%) 117(35%)	CH_4-CI	GCQ
	119	1 mg mL^{-1}	79(4%) 91(12%) 107(30%),$[M+H]^+$ 105(100%) 117(75%) 131(13%) 197(11%) 221(11%)	SIMR	Saturn 2000
Aniline (93)	106	1 mg mL^{-1}	94(100%),$[M+H]^+$ 93(84%) 79(2%)	SIMR	Saturn 2000
12-Crown-4N4 (172)	185	1 mg mL^{-1}	183(18%) 173(100%),$[M+H]^+$ 111(11%) 101(15%)	SIMR	Saturn 2000
14-Crown-4N4 (200)	213	1 mg mL^{-1}	201(43%),$[M+H]^+$ 156(100%)	SIMR	Saturn 2000
15-Crown-4N4 (214)	227	1 mg mL^{-1}	215(38%),[M+H] 210(100%) 184(44%) 153(23%) 143(32%) 141(34%) 139(32%) 129(41%) 127(29%)	DME-CI	GCQ

	227	1 mg mL^{-1}	216(10%) 215(100%),$[M+H]^+$	SIMR	Saturn 2000
15-Crown-5N2 (218)	231	1 mg mL^{-1}	219(100%),$[M+H]^+$ 203(20%) 186(26%) 174(30%) 158(27%) 114(53%)	DME-CI	GCQ
	231	1 mg mL^{-1}	220(12%) 219(100%),$[M+H]^+$	SIMR	Saturn 2000
	231	1 mg mL^{-1}	219(100%),$[M+H]^+$ 186(6%) 158(5%)	SIMR	GCQ
18-Crown-6N1 (263)	276	1 mg mL^{-1}	264(100%),$[M+H]^+$ 258(9%) 214(20%) 126(17%) 113(7%)	DME-CI	GCQ
	276	1 mg mL^{-1}	264(100%),$[M+H]^+$ 232(3%) 214(8%) 190(5%) 170(5%)	SIMR	GCQ

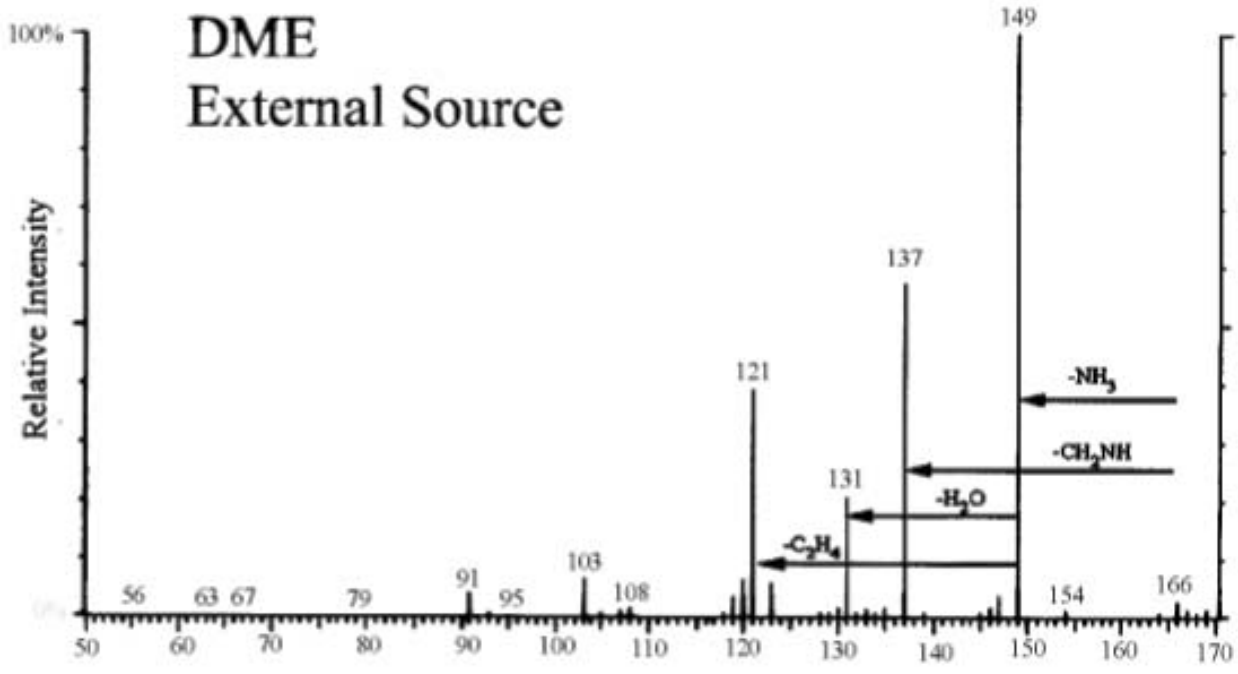

Figure 4