

## Liquid Chromatography-Mass Spectrometry Analysis of High Molecular Weight Azines

Abdullah El-Alali

Department of Chemistry, Faculty of Science, Mu'tah University, Al-Karak, Jordan

---

### Abstract

(+)-Electrospray mass spectrometry ionization interface is a powerful tool in determining molecular weight of large, non-volatile or polar organic compounds. Mass analysis of three different azine derivatives 1-3, recently prepared in our lab., was conducted using LC<sub>DUO</sub> ion-trap quadrupole LC-MS equipped with (+)-electrospray ionization interface. Molecular ions observed were  $[M]^+$ ,  $[MH]^+$  as well as dimers, sodium and potassium adduct ions for the three compounds.

**Key words:** LC-MS, bicyclic azine, N-allyl pyrazole, tetraene azine.

---

### Introduction

Mass spectrometry measures gaseous ions (cations or anions) in a high vacuum environment. Different mass spectrometry methods are usually classified based on the way that gas phase ions are generated (i.e., ionization methods). Organic chemists may face difficulties in determining molecular weight of large, above 500 Daltons, low volatile or high polar compounds using electron impact (EI), chemical ionization (CI), GC-MS, or fast atom bombardment (FAB). Relatively new mass spectrometry ionization interfaces like MALDI (Matrix Assisted Laser Desorption Ionization), atmospheric-pressure chemical ionization (APCI) and atmospheric-pressure electrospray (ESI) are finding more useful application in solving such problems (Gaskell, 1997).

In ESI, the most popular "soft" ionization technique, a high voltage is applied to a liquid flow of sample containing solvent. The high potential disrupts the solvent into charged droplets, which will eventually result in gaseous ions during this process.

A number of solvents can be used for dissolving samples in ESI, including  $CH_3CN$ ,  $CH_3OH$ ,  $CHCl_3$ ,  $H_2O$ , and their mixtures. The routine solvent system used is 50/50  $CH_3CN/H_2O$ . About 0.1%  $HCOOH$  is normally added to the sample to help increase protonation and ion production. Different from most other mass spectrometry methods, ESI generates predominantly multiply charged ions due to multiple protonation. For large compounds, it is estimated that one charge will be deposited on the molecule for each 1000 Da. There are computer programs that can do the calculation and generate a transformed spectrum on the real mass scale. The mass range for this technique exceeds 100 kDa. It typically requires 10 - 50 pmol of samples. The mass accuracy is normally 0.01% of the compound molecular weight. Samples should be submitted in water or mixtures of water with acetonitrile and methanol (Gaskell, 1997)(Whitehouse *et al.*, 1965) (Fenn *et al.*, 1990), (Bruins, 1991).

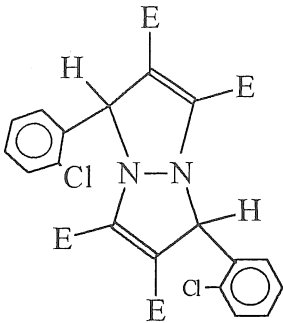
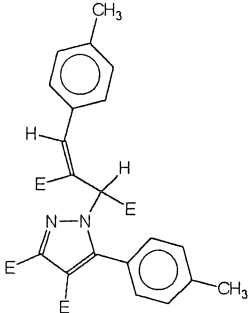
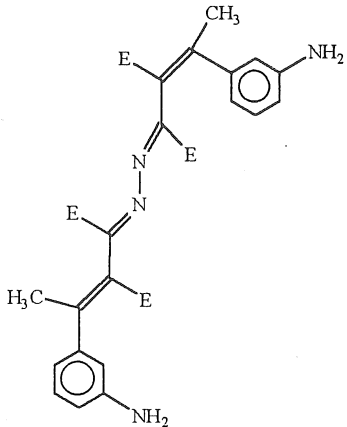
These relatively new ionization interfaces provide "soft" ionization in comparison with the widely used electron impact (EI) and chemical ionization (CI). Thus, fragmentation occurs in ESI to a much lesser extent than in EI or CI. Little is known about fragmentation mechanism in APESI (Gaskell, 1997).

In our research laboratory and recently, three different kinds of azines were prepared and characterized, ( El-Alali *et.al.*, 2002, 2003 ).

The reaction of symmetric aldazines or ketazines with dimethyl acetylenedicarboxylate gave in [3+2]-cycloaddition reaction, bicyclic azines, as final product, when electron-withdrawing groups were present on the aromatic moiety. The presence of electron-donating groups caused, in case of aldazines, a rearrangement into N-allyl pyrazoles, or a ring-opening into acyclic tetraene azines, when ketazines were used.

Three azine derivatives 1-3 ( El-Alali *et.al.*, 2002, 2003) ,were chosen for molecular weight determination by the new technique LC-MS, (Table 1)

Table 1 . The azine derivatives

Compound No.	Structure	Molecular formula and molecular weight (gmol <sup>-1</sup> )	Reference
1		C <sub>26</sub> H <sub>22</sub> N <sub>2</sub> O <sub>8</sub> Cl <sub>2</sub> 561.44	El-Alali and Al- Kamali
2		C <sub>28</sub> H <sub>30</sub> N <sub>2</sub> O <sub>8</sub> 522.52	El-Alali and Al-Kamali
3		C <sub>28</sub> H <sub>30</sub> N <sub>4</sub> O <sub>8</sub> 550.60	El-Alali and Al-Tarawneh

E = CO<sub>2</sub>CH<sub>3</sub>

In order to confirm their molecular weights, several unsuccessful attempts were conducted using classical mass spectrometric techniques like EI, CI, GC-MS or FAB. Probably, these compounds either fall apart quickly or non-volatile. Unlike other mass ionization techniques, ESI is a "soft" interface where most of the heat and voltage are deposited on the solvent but not on the compound (Gaskell, 1977), (Whitehouse *et al.*, 1965), (Fenn *et al.*, 1990), (Bruins, 1991).

## Experimental

Mass Analysis LC<sub>DUO</sub> ion-trap quadruple LC-MS, Finnigan, USA equipped with (+)-electrospray ionization interface was used in mass analysis. Solutions of 10 mg/mL in CH<sub>3</sub>CN:1% formic acid in water (50:50) for the three compounds were prepared. One at a time, each solution was loaded into a direct infusion syringe pump, Hamilton, USA which was coupled to the ionization interface using fused silica capillary tube with flow rate set at 0.2 mL/min. ESI ion source voltage was 30 V. Mass analysis was conducted using Xcalibur software, Finnigan, USA. Positive scanning ionization mode, full scan mode, from 150 to 550 amu was used. Minimum scan per peak was 10, probe voltage was 3 kV, and probe temperature was set at 200 °C.

## Results and Discussion

Due to quick fragmentation, non-volatility, large molecular weight or high polarity, molecular weight determination of certain organic compounds may become a challenge using mass spectroscopy. Traditional ionization interfaces like EI or CI use harsh conditions of high temperature and voltage (~70 eV) and thus, molecular ions of such compounds are commonly missing. In such cases it is recommended to switch to alternative "soft" ionization techniques like MALDI or APESI.

LC-MS analysis of compound **1** (Figure 1) which has two chlorine atoms revealed the following major ions peaks:  $m/z$  562.00 [MH]<sup>+</sup> (two <sup>35</sup>Cl isotopes),  $m/z$  563.07 [M]<sup>+</sup> (<sup>35</sup>Cl, <sup>37</sup>Cl),  $m/z$  564.07 [MH]<sup>+</sup> (<sup>35</sup>Cl, <sup>37</sup>Cl),  $m/z$  1145.60 [M-M-Na]<sup>+</sup>,  $m/z$  1160.93 [M-M-K]<sup>+</sup> (two <sup>35</sup>Cl isotopes) and  $m/z$  1162.60 [M-M-K]<sup>+</sup> (<sup>35</sup>Cl, <sup>37</sup>Cl). Compound **2** showed the following major ions:  $m/z$  522.20 [M]<sup>+</sup>,  $m/z$  1064.13 [M-M-2H-Na]<sup>+</sup> (Figure 2). Compound **3** displayed the following characteristic LC-MS ion peaks:  $m/z$  551 [MH]<sup>+</sup> (base peak) and  $m/z$  1100 **3** [M-M]<sup>+</sup> (Figure 3).

## Acknowledgements

The author would like to thank Dr. Feras Alali, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan for his kind technical help. Thanks are also due to ACDEMA, Amman – Jordan for their kind permission to use the LC-MS.

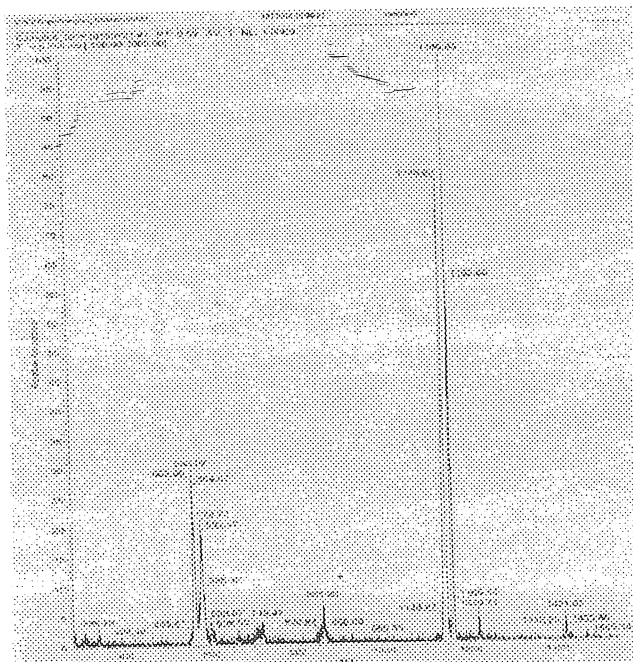


Fig. 1. LC-MS Spectrum of (Compound 1).

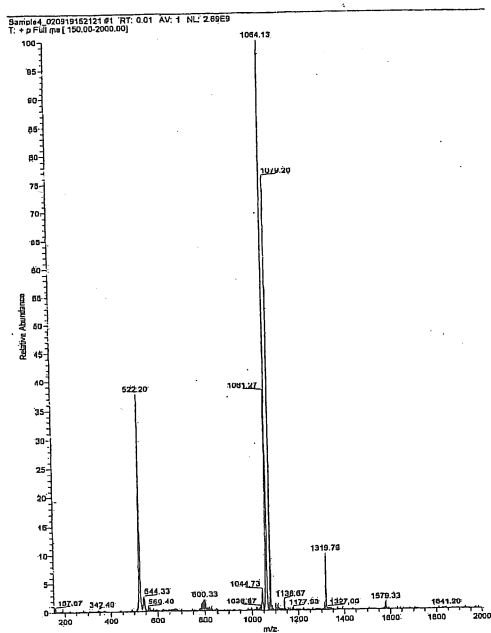


Fig. 2. LC-MS Spectrum of (Compound 2).

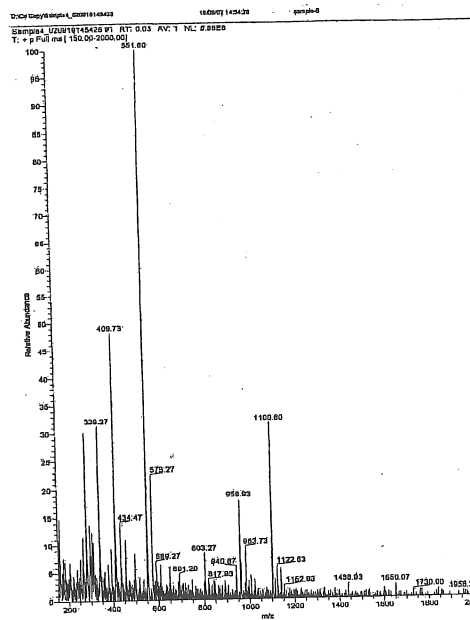


Fig. 3. LC-MS Spectrum of (Compound 3).

## References

- Bruins, A. B.( 1991); Mass Spectrometry with Ion Sources Operating at Atmospheric Pressure, *Mass Spectrom. Rev.* 10, 53-77 .
- El-Alali, A., and Al-Kamali A.S. (2002). Reactions of 1,3-dipolar aldazines and ketazines with the dipolarophile dimethyl acetylenedicarboxylate. *Can. J. Chem.* 80 : 1293-1301 .
- El-Alali, A. and Al-Tarawneh M. A. ( 2003). Some reactions of symmetrical aromatic ketazines with DMAD. *Mu'tah Lil-Buhuth wad-Dirasat* . 18 : 77-93.
- Fenn, J. B., Mann, M., Meng, Ch. K., Wong, Sh. F., Whitehouse, C. M. ( 1990). Electrospray Ionization – Principles and Practice. *Mass Spectrom. Rev.* 9: 37- 70.
- Gaskell, S. J. ( 1997). Electrospray: Principles and Practice. *J. Mass Spectrom.* 32: 677- 688.
- Whitehouse, C. M., Dreyer, R. N. , Yamashita, M., Fenn, J. B.( 1965 ). Electrospray Interface for Liquid Chromatographs and Mass Spectrometers. *Anal. Chem.* 57 : 675 – 679.

*Received: 15.0.,2003*

*Accepted:17.12.2003*