IONIZATION OF PENEM β-LACTAM ANTIBIOTICS USING THERMOSPRAY (FILAMENT-ON) MASS SPECTROMETRY

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β-Lactams are widely used antibiotics and penems[1,2] which are a relatively new class of such antibiotics is of particular importance because of their broad spectrum and potency[3,4]. They have been found to be highly active against Gram-positive and Gram-negative organisms (excluding *Pseudomonas*).

In spite of the tremendous therapeutic importance and the amount of research activity on penems for over 10 years, there is very little literature data concerning MS analysis of these antibiotics. Like many other biological compounds, the penem β-lactams are non-volatile and thermolabile which are difficult to characterize by MS techniques such as electron impact (EI) and chemical ionization (CI). Ammonium chloride salt addition in EI[5] was used to observe some improvement in ion intensity in the MW region. Thermospray MS is one of the latest soft ionization techniques developed in recent

Fig. 1. Direct thermospray ionization (filament-off) (a) and methanol thermospray ionization (filament-on) (b) of Sch 36251 formate.

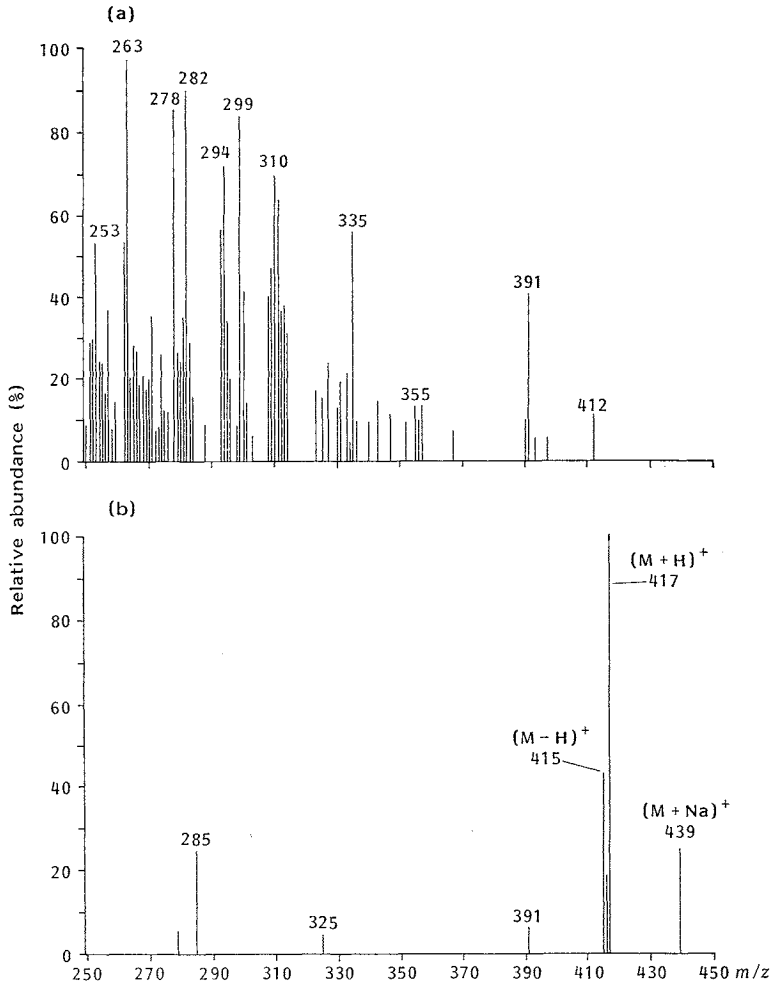
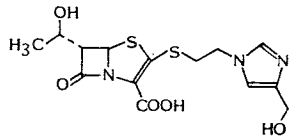
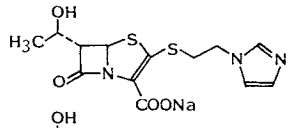
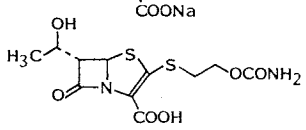
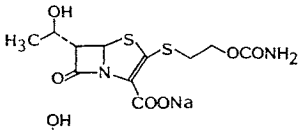
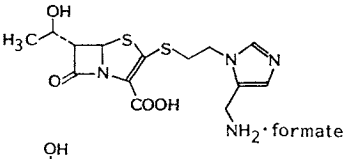
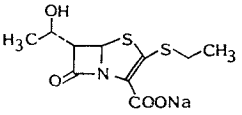


Table 1. Thermospray mass spectrometry of the six penems.

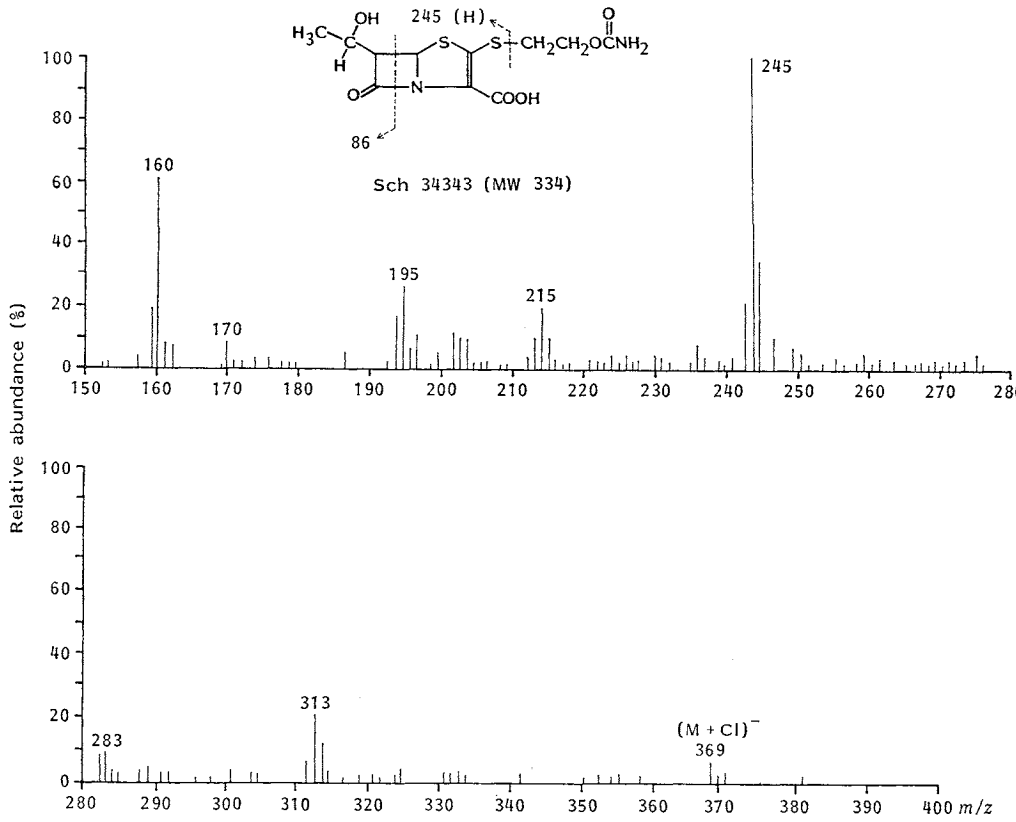
Compound	Structure	MW	Two most intense ions
Sch 35828		371	372 $(M+H)^+$, 394 $(M+Na)^+$
Sch 34390 Na salt		363	364 $(M+H)^+$, 386 $(M+Na)^+$
Sch 34343		334	352 $(M+NH_4)^+$, 350 $(M+NH_4-H_2)^+$
Sch 34343 Na salt		356	357 $(M+H)^+$, 379 $(M+Na)^+$
Sch 36251 formate		416	417 $(M+H)^+$, 439 $(M+Na)^+$
Sch 29482 Na salt		297	298 $(M+H)^+$[a], 320 $(M+Na)^+$

[a] Unlike the other penems, this was not the base peak and corresponded to an abundance of 80%; *m/z* 320, 20%.

years[6], and multiple ionization mechanisms are believed to exist in this method[7]. Thermal degradation studies of β-lactams using thermospray were recently reported[8], however, penem β-lactams are generally less stable than the cephalosporins and penicillins used in that study. We have utilized the thermospray technique (with filament-on) using 100% methanol to obtain MW information of these compounds[9]. We have reported the application of this method to steroids[10]. Direct thermospray ionization (without any external mode of ionization) failed to provide MW information for these penems because of the higher proportion of water in a water - methanol mixture (about 65% water in this case) required for such ionization. This necessitated using higher probe (210°C) and vapor temperatures for maximum sensitivity resulting in thermal degradation of the penems. However, with methanol thermospary (which requires an external ionization aid, such as a filament), this problem was greatly alleviated since much lower probe temperature (160°C) was required for obtaining maximum ion current of the molecular ion, $(M+H)^+$. Figs. 1 (a) and (b) display the MS from direct ionization and filament-on methanol thermospray for Sch 36251 formate.

An Extranuclear ELQ-400-1 quadrupole mass spectrometer with a thermospray ion source and interface from Vestec Corporation was used in this study. All samples were introduced into the mass spectrometer ionization source by direct flow injection technique with methanol flowing at 0.8 ml/minute, the solvent being pumped by a Hewlett Packard 1090 pump. Ammonium acetate (used as an electrolyte mostly to enhance ionization) was added to the sample solution (in methanol) instead of mixing with the mobile phase. Approximately 10~20 μg sample was required to obtain satisfactory MS for these compounds. Optimal instrument sensitivity was obtained by tuning on ion peaks *m/z* 65 (protonated dimer) and *m/z* 97 (pro-

Fig. 2. Negative ion methanol thermospray MS (discharge) of penem β-lactam using ammonium chloride.



tonated trimer) of methanol.

Table 1 lists the six penems used in this study and the two most intense ions observed in their respective spectra. We have included both free acids and sodium salts of the penems for this report. The base peak (100% abundance) in most cases was $(M+H)^+$ or $(M+Na)^+$ and fragments were minimal. The free acid of Sch 34343 provided less intense ions and ionization of this compound was relatively poor, in terms of total ion current. This was also the only penem amongst the five studied for which $(M+NH_4)^+$ and $(M+NH_4-H_2)^+$ ions were observed. The latter ion appears to be an ammoniated adduct of an oxidised form of Sch 34343, or it may be the protonated molecular ion of the species formed by the replacement of the NH_2 group in the amide functionality with OCH_3 by the reaction of methanol with the sample. This latter species would have a MW of *m/z* 349. The ammonium ion arises from the ammonium acetate used as the electrolyte in sample preparation.

Negative ion formation of penems was demonstrated, using Sch 34343 as an example, by the addition of ammonium chloride to the sample solution. The formation of $(M+Cl)^-$ adduct ion was very specific and easily recognizable from its chlorine isotope. To our knowledge this is the first report of an Cl^- attachment, by the deliberate addition of a salt, using the thermospray technique. The MS obtained with the filament and with the discharge were remarkably different in terms of fragmentation. Filament-on gave only $(M+Cl)^-$ ion whereas the discharge produced considerable fragments, Fig. 2. We have observed a similar enhancement of fragmentation with the discharge electrode for other classes of compounds[9] such as aminoglycosides, sugars and vitamin K_1.

These results demonstrate that methanol thermospray (filament-on ionization) is a very useful method for MS analysis of penem β-lactams, especially for MW information. However, integration of the information from both the filament and discharge techniques may

provide both MW and structural information. This was demonstrated, in the negative mode, for one of the penems used in this study.

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