The characterisation of synthetic and natural-product pharmaceuticals by electrospray ionisation-mass spectrometry (ESI-MS) and liquid chromatography (LC)-ESI-MS

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This article considers the application of topical analytical techniques – electrospray ionisation-mass spectrometry (ESI-MS) and liquid chromatography (LC)-ESI-MS – to the characterisation of selected small molecular-mass synthetic and natural-product pharmaceuticals, as well as bioactive peptides. We chose the synthetic drugs according to selected structural classes in which they give ESI signals primarily as $[M + H]^+$ ions. The structural classes chosen to illustrate the application of ESI-MS and LC-ESI-MS to such drug characterisation are drugs with amine-containing side chains, drugs with *N*-containing saturated ring structures, and 1,4-benzodiazepines and other heterocyclic hypnotics. We then discuss potential natural-product pharmaceuticals, such as selected quinolines and nicotines. We next apply the technique to the characterisation of molecules of unknown structure present in plant extracts or fractions that possess antibacterial activity. Finally, we discuss the isolation and the characterisation of bioactive peptides from frog-skin secretions utilising an arsenal of MS tools as well as Edman degradation sequencing and cDNA cloning.

Keywords: Bioactive peptide; Drug characterisation by LC-ESI-MS; Drugs of small molecular mass; Electrospray ionisation-mass spectrometry; ESI-MS; Fragmentation pattern; LC-ESI-MS; Liquid chromatography-electrospray ionisation-mass spectrometry; Natural-product pharmaceutical

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1. Introduction

Electrospray ionisation-mass spectrometry (ESI-MS) was introduced by Yamashita and Fenn [1] in 1984 with drugs and their related molecules being subjected to increasing investigation by this soft-ionisation technique. The Royal Swedish Academy of Sciences awarded The Nobel Prize in Chemistry for 2002 partly to John B. Fenn for his pioneering work in ESI-MS.

Detailed structural information on drugs can be obtained by resort to techniques such as cone-voltage fragmentation with a single MS instrument, collision-induced dissociation (CID) with triple-quadrupole MS instruments and MS^n techniques using quadrupole ion-trap instrumentation.

ESI is now the most important ionisation technique for the on-line coupling of liquid-phase separation techniques, such as liquid chromatography (LC) with MS. The Web of Knowledge database reveals 29, 35, 55, 72 and 87 publications in the years 2001, 2002, 2003, 2004 and 2005, respectively, on the LC-ESI-MS of drug molecules. Perusal of these database publications shows how LC-ESI-MS is being used for the analysis of a wide variety of drug molecules in matrices such as body

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fluids, foods, natural waters, and pharmaceutical formulations.

This article considers the application of the analytical techniques ESI-MS and LC-ESI-MS to the characterisation of selected small molecular mass synthetic and natural-product pharmaceuticals, as well as bioactive peptides. The synthetic drugs are chosen according to selected structural classes in which they give ESI signals primarily as $[M + H]^+$ ions.

The structural classes chosen to illustrate the application of ESI-MS and LC-ESI-MS to such drug characterisation are drugs with amine-containing side chains, drugs with *N*-containing saturated ring structures and 1,4-benzodiazepines/other heterocyclic hypnotics.

We then discuss potential natural-product pharmaceuticals, such as selected quinolines and nicotines.

The article continues with the application of the technique to the characterisation of molecules of unknown structure present in plant extracts/fractions that possess antibacterial activity.

Finally, we discuss the isolation and the characterisation of bioactive peptides from frog-skin secretions utilising an arsenal of MS tools as well as Edman degradation sequencing and cDNA cloning.

2. Drugs with amine-containing side chains

Joyce et al. [2] investigated the characterisation of selected drugs with amine-containing side chains using ESI-MS^n. From this study, certain rules were formulated with respect to the ESI-MS^n behaviour of drugs with amine-containing side chains. For example, the synthetic opiate agonist methadone has the following structure:

$H_3C–CH_2–CO–C(C_6H_5)_2–CH_2–CH(CH_3)–N(CH_3)_2$

It therefore has a carbon chain ending in a tertiary nitrogen atom with at least two methylene or substituted methylene groups separating this nitrogen atom from the other end of the carbon chain. Such a molecule, according to [2], will first lose the end nitrogen atom as the corresponding secondary amine, $HN(CH_3)_2$, followed by loss of the corresponding alkene, $CH_2{=}CHCH_3$, formed from these two methylene/substituted-methylene groups. Such structural information from ESI-MS^n, obtained sequentially for this and other drugs with amine-containing side chains and supported by electrospray ionisation-quadrupole time of flight-mass spectrometric (ESI-QToF-MS^2) elemental analyses, contrasts with that from the hard ionisation technique, electron impact-mass spectrometry (EI-MS), which, for methadone, gives no molecular ion at *m/z* 309, a single base peak at *m/z* 72 and several small signals of less than 5% relative abundance. If there is only one methylene group adjoining the end nitrogen atom, as is the case with lignocaine, then the end of this carbon chain becomes the detectable charged species, as in $CH_2{=}N^+(C_2H_5)_2$, and no neutral amine and alkene is formed [2].

These MS^n experiments, supported by ESI-QToF-MS^2 data, therefore show certain characteristic fragmentations with respect to the amine-containing side chains. The data therefore provide useful information on the structure of these compounds with amine-containing side chains and can be used in the characterisation of such drugs and their structurally-related metabolites. The ESI-MS^n data of such compounds can be held in a database and neutral mass losses/low molecular mass ions cross-referenced with such data obtained from analytes of unknown structure, which can then be of value in their structural characterisation with respect to those molecules with amine-containing side chains. A table giving such mass losses/signals at low *m/z* values in the range *m/z* 15–176 for drugs studied in our laboratory was presented in a review [3], which will be of value in the characterisation of unknown metabolites and natural-product pharmaceuticals isolated from plants, for example. Table 1 is a sample table for the range *m/z* 17–42 taken from [3] and featuring some of the molecules discussed in this article.

The ESI-MS^n and LC-ESI-MS^2 of selected antidepressant drugs, some with amine-containing side chains (i.e. citalopram, fluoxetine, sertraline and venlafaxine) has recently been investigated in our University of Ulster Coleraine (UUC) laboratory [4]. Following elucidation of the fragmentation mechanisms using ion-trap (IT)-MS, supported by ESI-QToF-MS^2 measurements, these molecules can be unambiguously identified and determined in mixtures at low-ng/ml concentrations by the application of LC-ESI-MS^2, as illustrated in Fig. 1.

Saliva is increasingly being used for drug testing to monitor illicit and licit drug use. Saliva is a natural ultrafiltrate of plasma with molecules transported across epithelial membranes. Highly protein-bound drugs are unlikely to cross the cellular membranes, so saliva testing offers the possibility of direct comparison of unbound, pharmacologically-active drug concentrations with the observed effects. Improved technology, such as LC-ESI-MS, makes it possible to make many diagnoses from saliva analysis that are currently made from blood analysis. Collection of saliva is safe and patient-friendly and requires no qualified personnel, and that could make it an important diagnostic matrix of the future. Four of the drugs with amine-containing side chains studied [2] (i.e. amphetamine, clenbuterol, flurazepam and methadone) have been simultaneously identified and determined in saliva samples at low-ng/ml concentrations by the application of LC-ESI-MS [2].