

# EPSRC National Mass Spectrometry Centre, Swansea NMSSC Application Note No 4

*The advantageous use of stepped in-source (cone) voltages in electrospray mass spectrometry for compound characterisation and the identification of mixtures.*

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## Introduction

The relative abundances of ions in electrospray are affected by the in-source voltages (*e.g.* cone voltage) applied. This phenomenon can be used to advantage in the characterisation of chemical compounds, the identification of unknown species and the recognition of mixtures, and is used routinely in our laboratory for all electrospray (ESI) analyses for these purposes.

The method should be generally useful on almost any ESI instrument, in either polarity, but in this illustration, for simplicity, we just describe it on the Waters ZQ4000 in positive ion mode.

## Experimental details

### INSTRUMENTATION

- Waters ZQ4000 quadrupole mass spectrometer (Micromass, Manchester, UK)
- Advion Nanomate-100 automated nanospray inlet (Advion BioSciences, Ithaca, NY) optimised for methanol and spraying for 2 minutes.

### MATERIALS AND REAGENTS

- Methanol (HPLC grade, Fisher Scientific)
- Ammonium acetate (BDH AnalaR) 50mM solution in HPLC grade water (Fisher Scientific)

### METHOD

#### MS Acquisition Parameters

Source voltage: 3kV

Positive ion mode: source temp.: 45°C, gas flow 40L/hr  
MCA acquisition for 2 minutes in total, acquiring for 30 seconds consecutively at each of the following cone voltages: 10V, 20V, 50V and 90V.

#### Inlet parameters

- Nanomate optimised on methanol and spraying 5µL for 2 minutes; gas pressure 0.1psi, voltage 1.55kV. (Flow injection or other inlet systems may also be used).

## Results and Discussion

### Underlying principles

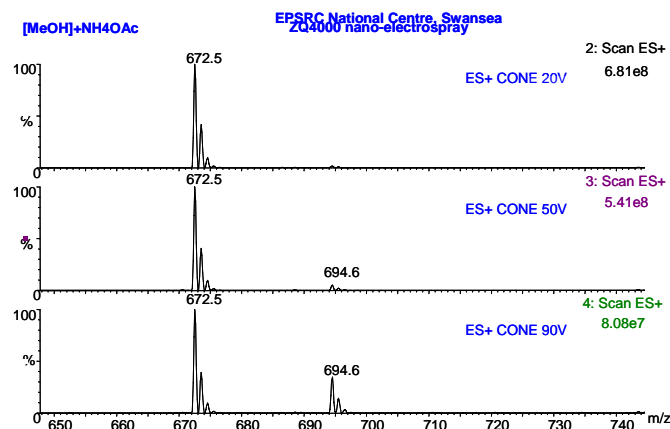
The reasons and mechanisms for these observations are beyond the scope of this note, but the following trends are generally observed when the cone voltages are altered in the manner suggested:

- Multiple charging is maximised at LOW cone voltage;
- Clustering and multimer formation is maximised at LOW cone voltage;
- Fragmentation is maximised at HIGH cone voltage;
- Higher molecular masses require HIGHER cone voltages to be observed;
- The ratio of  $[M+NH_4]^+$  /  $[M+Na]^+$  DECREASES as cone voltage INCREASES.

The combination of all these trends provides useful and meaningful information for the interpretation of ESI mass spectra as the following examples illustrate.

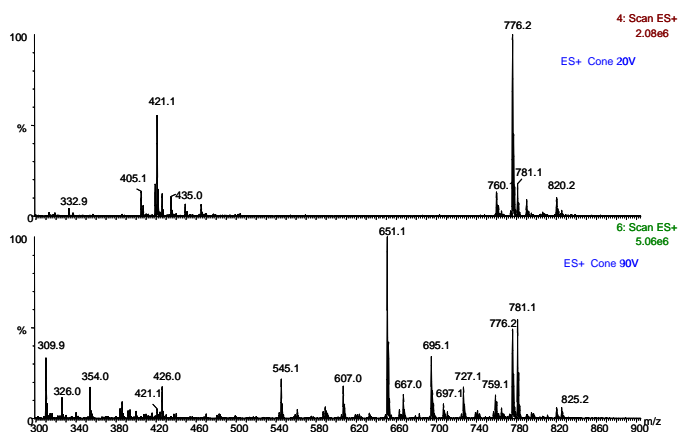
### EXAMPLES

#### Example 1: Determination of molecular mass



The relative abundances of the ions at m/z 672 and 694 suggest a molecular mass of 671, with the sodium adduct at m/z 694 predictably increasing in relative abundance with increasing cone voltage.

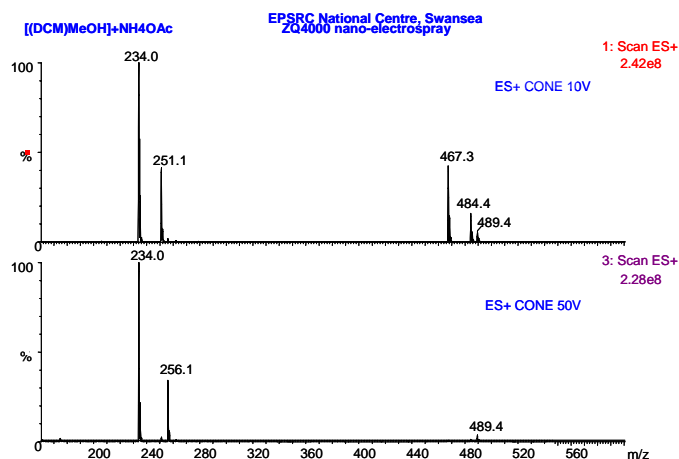
## Example 2 Identification of components in a mixture



In example 2 the sample is a mixture, and any single mass spectrum on its own would be inconclusive in determining the different molecular masses present. However the combination of the different ions promoted by the different cone voltages gives a very clear picture of the various molecular masses present, *viz.*: 403, 758, 802.

A key observation in drawing this conclusion is the relative abundances of the  $[M+Na]^+$  and  $[M+NH_4]^+$  ions of each molecule. Thus for  $M_r$  403, note the increase of  $m/z$  426 ( $[M+Na]^+$ ) relative to  $m/z$  421 ( $[M+NH_4]^+$ ) with increased cone voltage; for  $M_r$  758, the ions at  $m/z$  781 and 776 are key, and for  $M_r$  802, the relevant ions are  $m/z$  825 and 820.

## Example 3 Distinction of monomer from dimer



It is often important to answer the question whether a species is present in monomeric or dimeric form in a sample. As soft ionisation in mass spectrometry (MS) promotes dimer (and proton bound dimer) formation, MS may not be a reliable way to answer this question. However, by acquiring data at a range of cone voltages, the question can sometimes be answered even if both monomer and dimer are present.

In example 3, the question is whether the molecular mass is 233 or 466. If  $m/z$  467, 484 and 489 were molecular species, they should increase in intensity at higher cone voltage, whereas they can be seen to decrease in intensity as the voltage increases. This indicates that they are MS-induced adducts, and not molecular species. The molecular mass is therefore 233.

## Conclusion

The application of stepped in-source (cone) voltages can be very advantageous in characterisation of both "known" samples and the identification of "unknowns" or recognition of mixtures. This technique can also help to decrease the ambiguity of electrospray data that is acquired at a single source parameter.