ApplicationNOTE

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THE ANALYSIS OF ORGANOMETALLIC COMPOUNDS USING ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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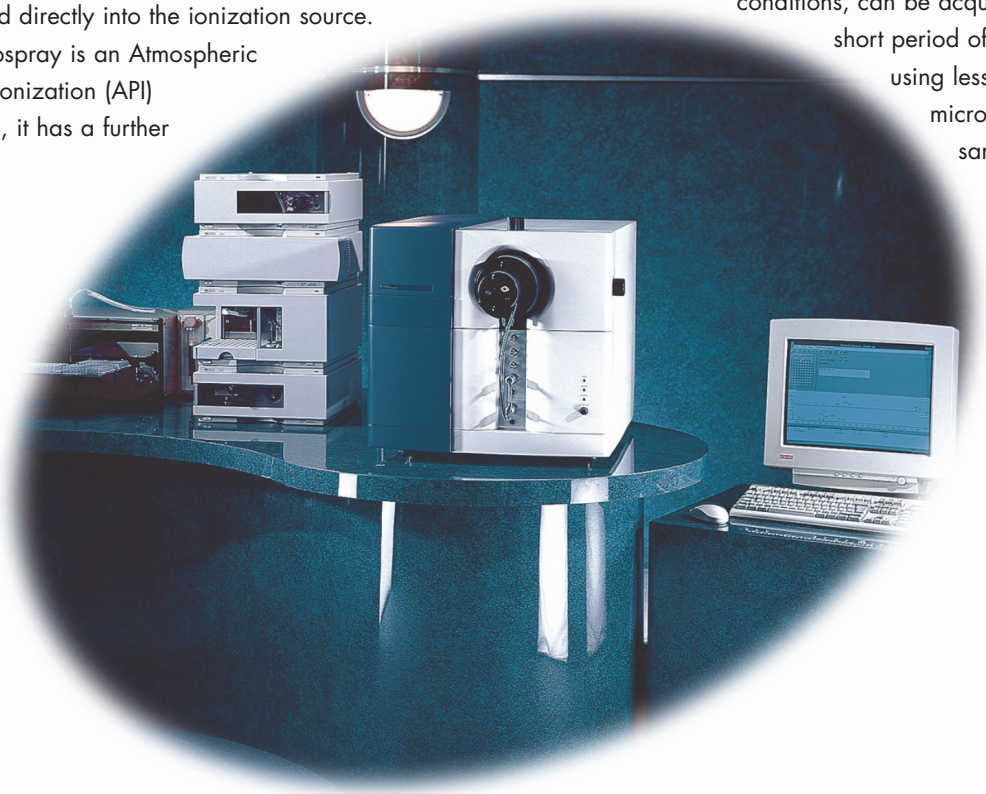
Introduction

The analysis of organometallic compounds by mass spectrometry has traditionally been carried out using Fast Atom Bombardment (FAB) as the mode of ionization. The advent of electrospray ionization offers an alternative method of analysis with a number of inherent advantages. This Application Note demonstrates the analysis of synthetic compounds using electrospray ionization on a Micromass® Platform LC (Waters Corporation, Altrincham, Cheshire, UK) benchtop mass spectrometer.

Unlike FAB ionization, electrospray requires no matrix to aid the ionization process which means that the background ions relating to chemical noise are much lower in intensity. All that is required for electrospray analysis is a suitable solvent for the compound, and then a solution at the required concentration can be introduced directly into the ionization source. As electrospray is an Atmospheric Pressure Ionization (API) technique, it has a further advantage over FAB in that there is no need to insert the sample via a vacuum lock. Electrospray is well known for use with solvents used in reverse phase chromatography, such as water, methanol and acetonitrile, but it is also possible to use a wider range of solvents, such as dichloromethane and toluene, which may be more suitable for organometallic compounds which are either insoluble in other solvents, or require careful choice of solvent to prevent ligand exchange reactions.

Once in solution, the sample can be introduced either by loop injection into a carrier solvent flowing to the electrospray source or by direct introduction via an infusion pump. Conventional electrospray can be operated at flows as low as 5 µL/min, such that a 100 µL aliquot of a sample solution can be infused for 20 minutes allowing easy optimization and data acquisition. Several spectra, using different conditions, can be acquired in a short period of time using less than a microgram of sample.



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As electrospray is a soft ionization technique which tends to keep analytes intact and provide molecular weight information, it can be used to confirm the molecular weight of synthetic organometallic compounds.

However, electrospray has an added advantage of allowing fragmentation to be produced when required. By increasing the source cone voltage it may be possible to produce controlled and reproducible fragmentation, giving useful information for the elucidation or confirmation of the structure of the sample.

Experimental

For each sample, a suitable solvent was chosen and a solution was prepared in the concentration range 25-50 ng/µL. The solution was then introduced into the electrospray source at a flow rate of 5 µL/min using a Harvard Apparatus™ 22 syringe pump. The electrospray source conditions were optimized for the generation of the molecular related ion and data were acquired by accumulating several continuum spectra over a suitable mass range. Subsequent spectra were acquired at higher cone voltages to generate in-source fragmentation.

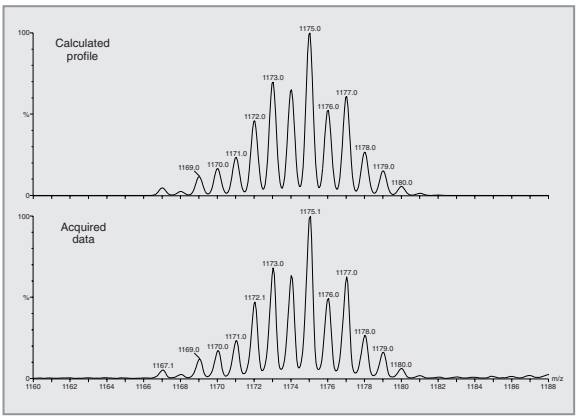


Figure 1. The cost of drug development

Results

Sample 1

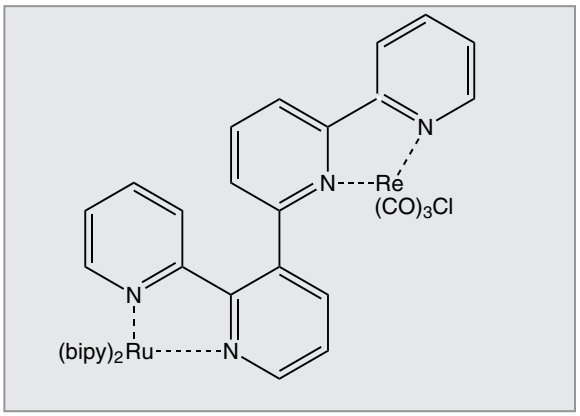
Formula: $C_{43}H_{30}N_8RuReClO_3.2PF_6$

Average molecular weight = 1319.4

Solvent: acetonitrile

Concentration: 25 ng/µL

(note : bipy = 2,2′-bipyridine, $C_{10}H_8N_2$)



Molecular Weight Information: Sample 1 contains ruthenium, rhenium and chlorine atoms so the expected isotope pattern is complex. When using electrospray ionization in positive ion mode, with a cone voltage of 30V, the compound loses a PF6- counter ion to give a singly charged cation with an average mass to charge ratio (*m/z*) of 1174.5.

Continuum spectra for the singly charged molecular ions and the isotope model (Figure 1) show that the isotope profile for the acquired data closely matches that predicted by the isotope model.

Isotope Models: As organometallic compounds often contain metals with complex isotope patterns, the expected isotope pattern of the compound can be used to help confirm its structure. The MassLynx™ software, which controls the Platform LC™ mass spectrometer, has an isotope pattern calculator which can model the expected isotope distribution. By entering the expected chemical formula of the compound, a model of the isotopes can be produced in either continuum or centroid format which can then be compared to the data acquired by electrospray ionization to confirm the presence of the expected atoms.

Resolution of Doubly Charged Ions: The sample also loses the second PF6- counter ion to give intense doubly charged ions with an average mass of 1029.5 and thus an average mass to charge (*m/z*) ratio of 515.7. Due to having two charges, the resulting spectrum shows isotopes which are separated by only *m/z* 0.5. The Platform-LC is easily able to resolve doubly charged isotopes as shown in Figure 2.

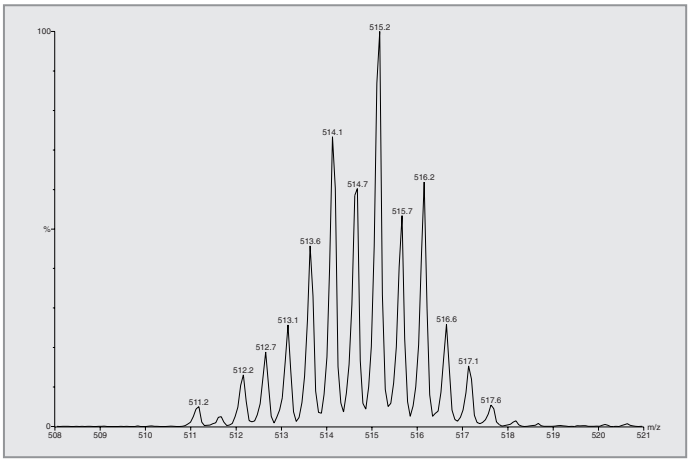


Figure 2. Doubly charged ion produced by the loss of two counter ions from Sample 1

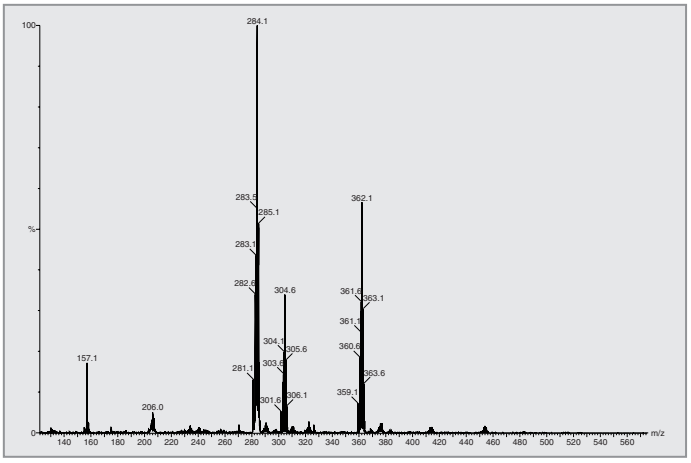


Figure 3. Induced fragmentation of Sample 1

Gaining Structural Information: As stated previously, it is possible to induce some fragmentation in the source in order to gain some structural information about the sample. By increasing the sample cone voltage in the source to a value of 55V, the doubly charged ion was completely dissociated to give a number of fragment ions as shown in Figure 3. The peaks centered around *m/z* 362 are due to a doubly charged fragment (charge state determined by the isotope separation) produced by the loss of the two PF6- counter ions and rhenium with its associated ligands, $[M - 2(PF_6^-) - Re(CO)_3Cl]^{2+}$. A further loss of a 2,2′-bipyridine ligand from the ruthenium centre gives the intense group of doubly charged ions centered around *m/z* 284 and loss of the second (bipy) ligand gives a group of ions around *m/z* 206. The ions around *m/z* 304.6 are again due to a doubly charged species which has a mass of 609. The odd mass indicates that the fragment contains an odd number of nitrogen atoms and is likely to be due to the cleavage of the compound backbone to lose one nitrogen. There is also a singly charged peak at *m/z* 157 which can be assigned as protonated 2,2′-bipyridine.

Molecular Weight Information: Sample 2 gives a molecular ion at *m/z* 738 (Figure 4) which matches the expected isotope pattern generated by the software. The base peak of the spectrum at *m/z* 659 is due to the facile loss of bromine from the complex which occurs even at a low cone voltage of 18V. Indeed, this fragmentation occurs so easily that the intact molecular ion for this complex has not previously been observed using FAB.

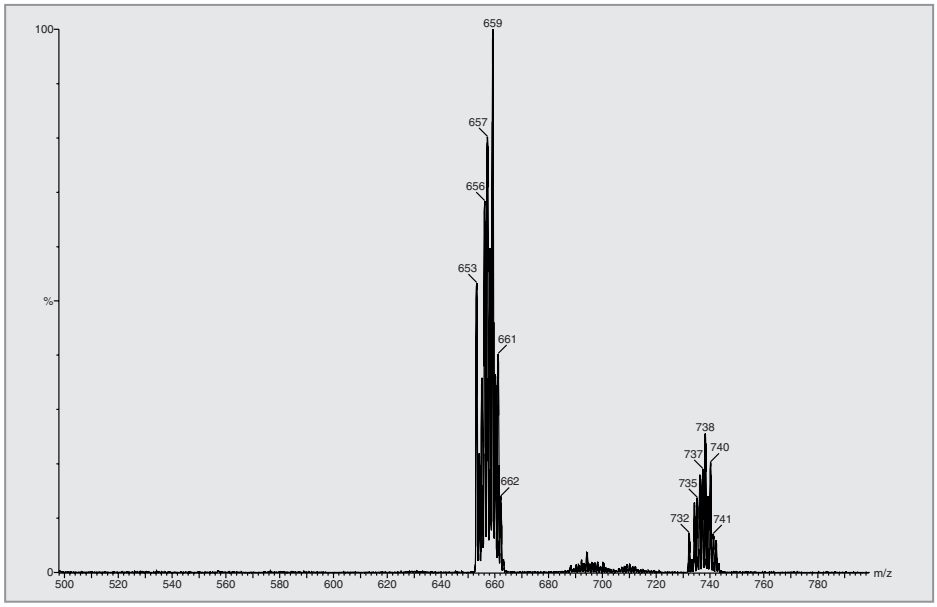
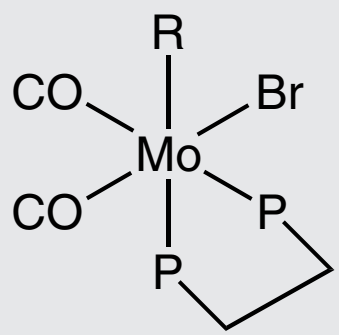


Figure 4. Spectrum for Sample 2 at a cone voltage of 18V



R = 1-3-η : 5-6-C_8H_{11}
P – P = dppe = $Ph_2PCH_2CH_2PPh_2$

Sample 2
Formula: $C_{36}H_{35}O_2BrMoP_2$
Average molecular weight = 737.5
Solvent: dichloromethane
Concentration: approx 50 ng/μL

Summary

The data presented show that electrospray is a suitable technique for the analysis of organometallic compounds. Like FAB, electrospray can be used to confirm the molecular weight of a compound, but it has the added advantage of controlled and reproducible fragmentation which can confirm an expected structure or can help with the elucidation of an unknown. The data were acquired on a benchtop mass spectrometer equipped with an electrospray ionization source which offers an advantage in cost while maintaining the required performance. The high performance allows the facility to resolve multiply charged ions to help with interpretation of the data.

Acknowledgments

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