Waters Alliance™ Systems for LC/MS ESI/APCI Applications

The Use of LC-API/MS with Photodiode Array Detection for the Determination of Impurities in Drug Synthesis

Highlights: The combination of mass spectrometry and photodiode array detection (PDA) provides a powerful tool for controlling quality in drug synthesis. The ability to scan a range of wavelengths with a PDA allows impurities to be detected without prior knowledge of absorbance. PDA information can be helpful in determining peak homogeneity and the UV spectrum can also give information about functional groups which may help determine the source and identity of the impurity. Data from atmospheric pressure ionization (API) mass spectrometry can provide both molecular weight information in the form of the protonated molecule ([M+H] +) and structural information by inducing " in-source" fragmentation which aids the structural elucidation of impurities. In addition, API-MS can detect the presence of impurities with no UV chromophore which would not be apparent from PDA data alone. The Platform LC is a dedicated API benchtop mass spectrometer controlled via MassLynx software. MassLynx software also simultaneously controls the Waters 2690 Sample and Solvent Management System for the acquisition of both MS and PDA data from a single injection. This note describes the application of an MS plus PDA system to the synthesis of a drug candidate.

Experimental:

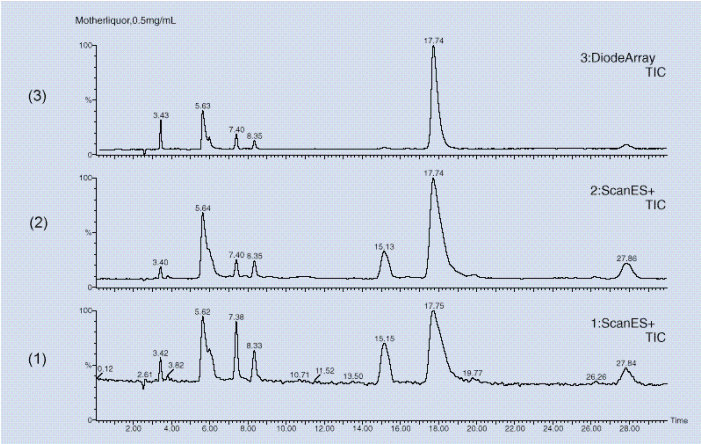
The analysis was performed using an HPLC system equipped with a photodiode array detector (PDA) coupled to a Platform-LC MS detector (Micromass UK Ltd., Wythenshawe, Manchester, UK) operating with an ESI source.

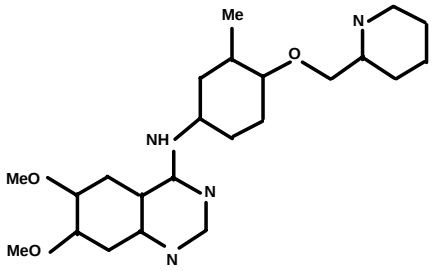
LC Conditions:

Column Hichrom RPB 250 x 4.6mm
Eluent 60% 0.1M aqueous ammonium acetate, 40% acetonitrile (isocratic)
Flow rate: 1.0mL/min
Injection volume: 20µL
The eluent was split after the column to allow 920µL/min to pass through the PDA and 80µL/min to enter the ESI source.
Photodiode Array Detection:
Scan range: 190-600nm

MS Detection:

Positive ion ESI; Source temp: 130°C
Scan function: 1
Mass range: 150-450 Da
Cone voltage 20V
Scan function 2
Mass range: 100-450amu
Cone voltage: 65V
Alternate scans at low and high cone voltages were acquired to generate both the molecular weight and some fragmentation.





Parent Compound

Figure 1: Chromatograms of 1)MS, low cone, 2)MS, high cone and 3)PDA

The acquired datafile contained three chromatograms (figure 1). The first chromatogram contained MS data acquired at a low cone voltage to give molecular weight information. The second contained MS data acquired a at high cone voltage in order to induce some fragmentation in the ESI source and yield structural information. The third chromatogram contained the PDA data. The parent compound eluted at 17.7 minutes with a number of impurities eluting both before and after the main peak.

Information about the impurity can be gained from the acquired mass spectra. Figure 2 shows the spectra acquired at cone voltages of 20V and 65V. At the lower voltage, there is no fragmentation and a [M+H] + at m/z 225 is observed with a distinctive isotope pattern indicating that chlorine is present. At a cone voltage of 65V, a number of fragments are produced.

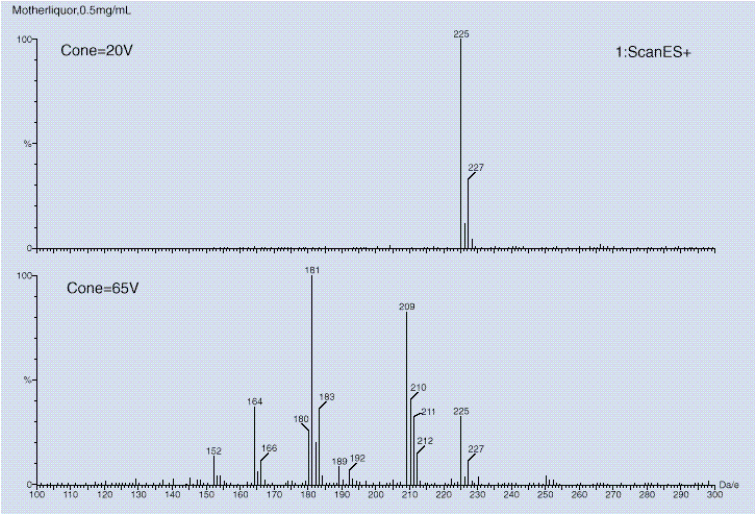
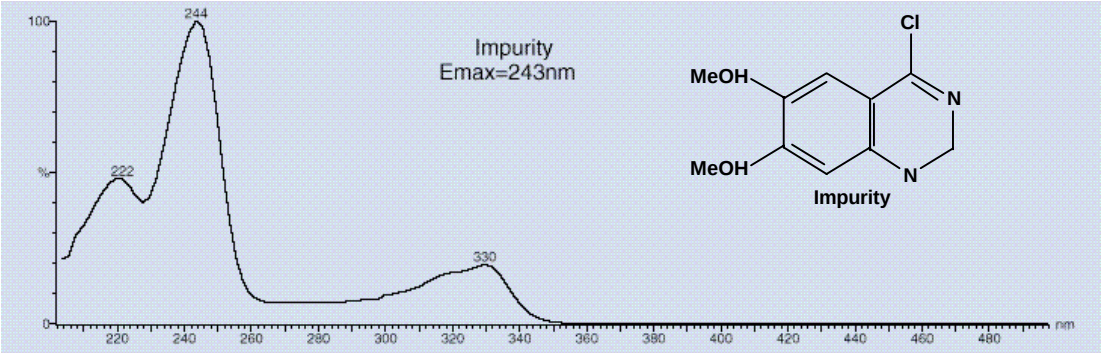


Figure 2 : Spectra of the impurity at cone voltages of 20V and 65V

The impurity at RT 8.35 min is known to be the compound shown here. It has a monoisotopic molecular weight of 224 which agrees with the [M+H] + at m/z 225 and has one chlorine atom giving the 3:1 ratio observed for the peaks at m/z 225 and 227. Figure 3 shows the UV spectrum of this impurity.

Figure 3



For quality control in the synthesis of drug candidates, the combination of PDA and MS detection offer many benefits. PDA data can be used to determine peak homogeneity and may show impurities which are not amenable to MS detection. ESI-MS allows the for the determination of analyte molecular weight and can be used to give some structural information through induced fragmentation to help with the identification of impurities. In addition, MS data may highlight impurities which do not contain a UV chromophore and are not apparent from PDA data. A single data file can contain a multitude of information from a single injection since the HPLC, the mass spectrometer and the facility to collect multifunction MS data at different cone voltages are all controlled from a single software package. Also, since all data and experimental records are acquired to a single source, data collection, storage and retrieval are simplified. This makes it easier to adhere to the regulations controlling scientific data in the pharmaceutical laboratory.

Acknowledgments: Steve Taylor 1 and Steve Preece 2
1 Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK.
2 Micromass UK Ltd., Floats Road, Wythenshawe, Manchester M23 9LZ, UK.