Impurity Profiling with Capillary Electrophoresis/Ion Trap Mass Spectrometry

Application Note

Christian Sauber and Gordon Ross
Agilent Technologies

Introduction

Drugs, drug impurities, and metabolites are routinely monitored using HPLC with various detectors. However, capillary electrophoresis (CE) offers greater resolution and a different selectivity when compared to reversed-phase (RP) HPLC. For example, Phase II metabolites are often too polar to give sufficient retention with RP HPLC, but are easily separated by CE. Therefore, CE has become established as a powerful tool for the separation of charged drugs, impurities, and metabolites in crude biological matrices. When impurity standards are not available, some method is needed to characterize the impurities on line. Ion trap mass spectrometers are capable of acquiring multiple-stage MS^n data and therefore have the ability to generate characteristic impurity "fingerprints," comprised of a precursor ion mass plus corresponding product ion masses. We evaluated the use of CE with an ion trap detector for the determination of impurities; making use of the automated, data-dependent MS/MS capabilities of the ion trap detector.

Experimental

All experiments were performed using an Agilent 1100 Series LC/MSD Trap ion trap mass spectrometer coupled to an Agilent 1100 Series CE system. The CE/MS coupling was achieved using a triple-tube nebulizer held at ground potential. The sheath liquid was delivered using an Agilent 1100 Series quaternary pump fitted with a 1:100 splitter.

The mass spectrometer was operated with the electrospray (ESI) source in the positive ion mode. Clenbuterol and isoproterenol were used as model compounds because of their similar chemical structures and properties.

Four samples were prepared with concentrations of clenbuterol of 100% to 0.1% relative to the main compound, isoproterenol. The concentration of isoproterenol was held at 0.6 mg/ml. The two drugs were separated using a CE separation buffer of 20 mM ammonium formate, pH 3.2. The ion trap was operated in full scan MS/MS mode to obtain MS and data-dependent MS/MS spectra during the run.

ANALYSIS METHOD

CE/MS/MS

CE Conditions

Buffer:	20 mM ammonium formate, pH 3.2
Capillary:	80 cm (20 cm to UV) x 50 µm i.d.
Detection:	200 nm
Injection:	5 s @ 50 mbar
Temperature:	25°C
Voltage:	25 kV
Sheath liquid:	1% formic acid in 50:50 methanol:water
Sheath flow:	6 µl/min

MS Conditions

Ionization mode:	Positive ESI
Drying gas flow:	10.0 l/min
Nebulizer:	10 psig
Drying gas temperature:	150°C
Skim 1:	32.1 V
Skim 2:	6.0 V
Capillary exit offset:	72.1 V
Averages:	6
ICC:	On
Maximum accumulation time:	200 ms
Target:	30000
Scan range:	50 – 500 *m/z*
Auto-MS/MS:	On
Isolation width:	4.0 *m/z*
Fragmentation amplitude:	1 V
No. of parents:	2

Results and Discussion

Figure 1 shows the MS/MS spectra of clenbuterol and isoproterenol acquired during their infusion in manual MS/MS mode. No optimization of the collision energy is required, since it is ramped during the fragmentation process. This SmartFrag feature provides highly reproducible MS/MS spectra in either manual-MS/MS or data-dependent MS/MS modes. The main fragment for both compounds is generated by the loss of water (18 u).

In automated, data-dependent MS/MS mode, the ion trap mass spectrometer has the ability to generate MS/MS spectra from the most abundant *m/z* peaks of the previous full scan MS spectrum. Therefore MS and MS/MS spectra of the main compound and its impurities can be generated simultaneously during the analysis.

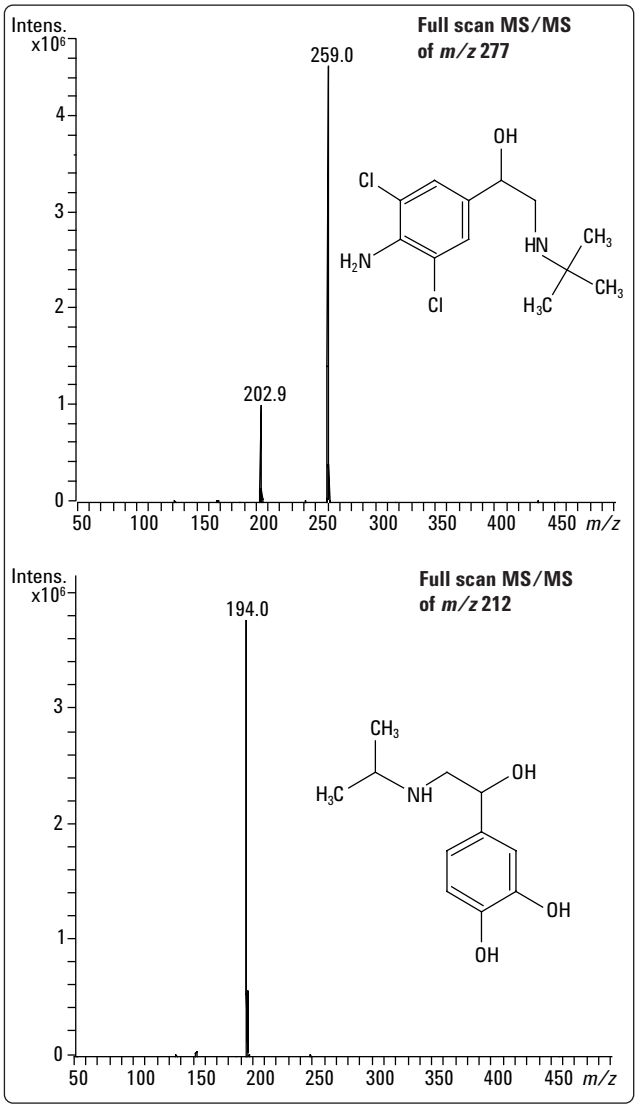

Figure 1. MS/MS spectra of A) clenbuterol and B) isoproterenol acquired during infusion of standard solutions of each compound at 100 ng/ml in methanol/water.

Having MS and MS/MS spectra from data-dependent MS mode allows the construction of a neutral-loss electropherogram. For example, the loss of glucuronic acid from Phase II metabolites can be monitored without knowing the masses of the precursor or product ions.

Figure 2 shows the extracted ion electropherograms of isoproterenol and clenbuterol at *m/z* 212 and *m/z* 277 respectively and the corresponding MS/MS spectra from the apex of each peak.

Typically, impurity levels are monitored down to 0.1% of the main compound. In this experiment, the concentration of clenbuterol was varied between 100% and 0.1% relative to the main compound, isoproterenol. The dynamic range of the ion trap is sufficient for a detection down to the 0.1% level (Figure 3).

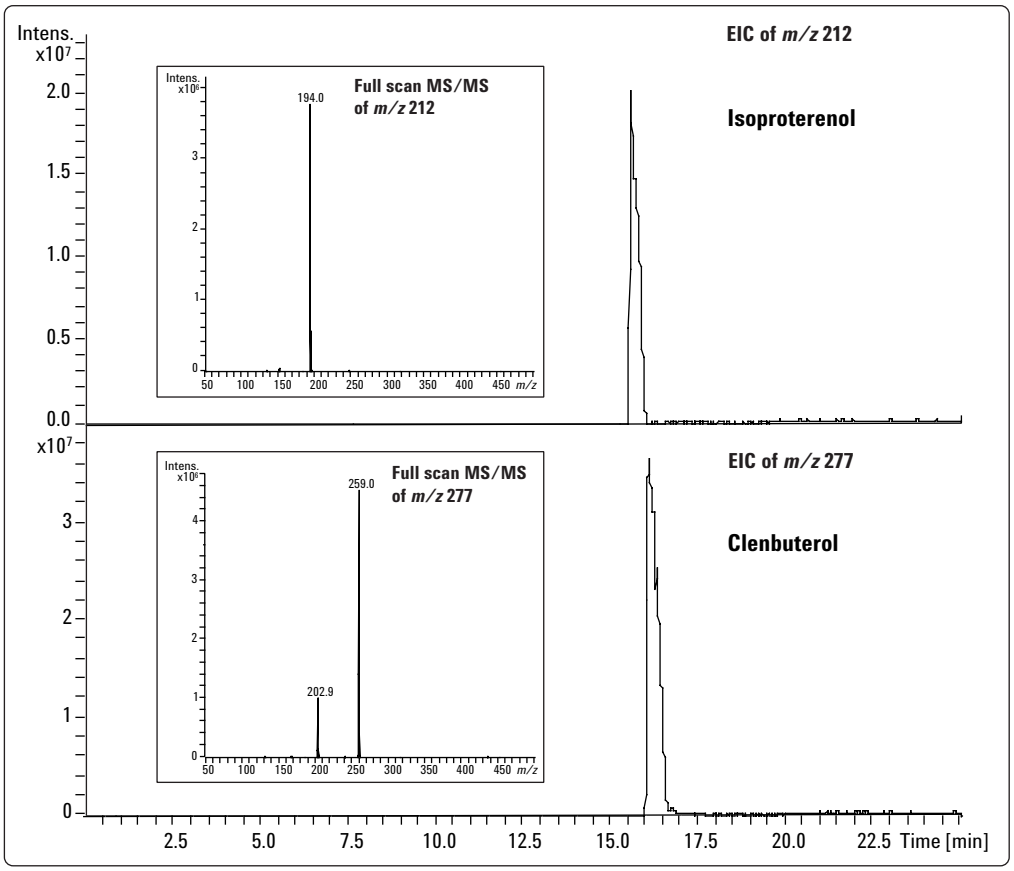

Figure 2. Extracted ion electropherograms of isoproterenol and clenbuterol at concentrations of 0.6 mg/ml with associated full scan MS/MS spectra (inset)

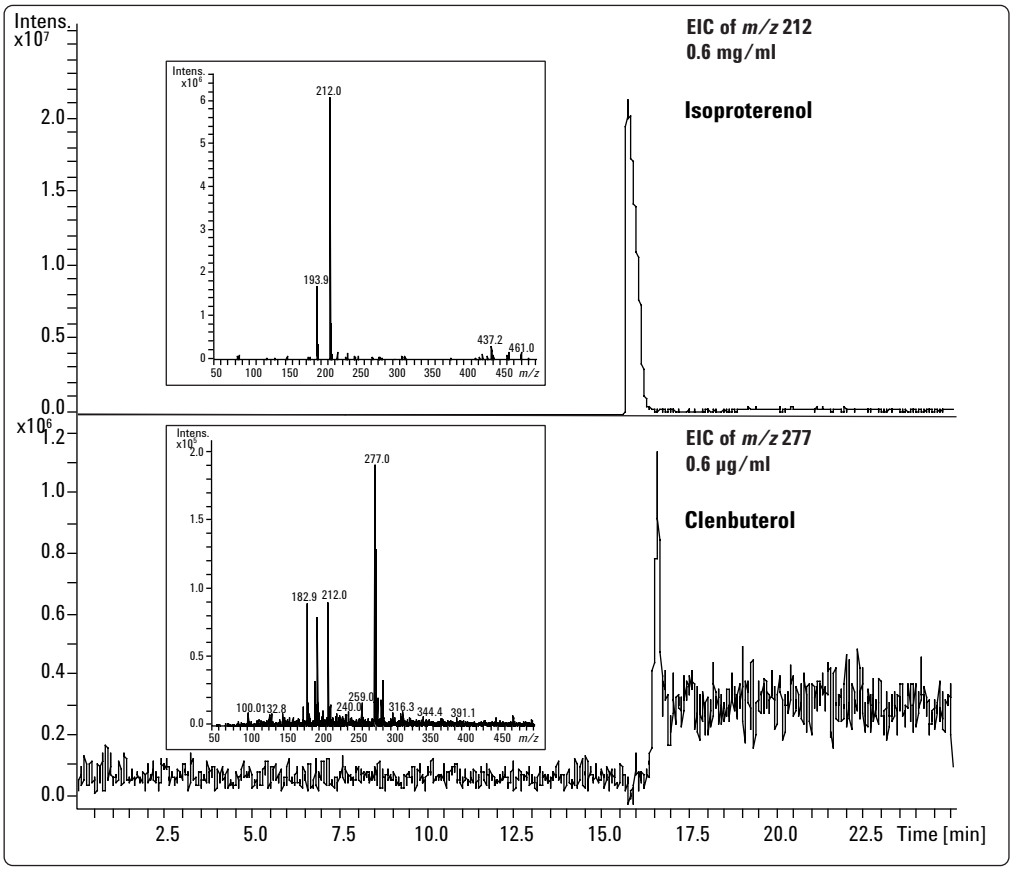

Figure 3. Extracted ion electropherograms of isoproterenol and clenbuterol at concentrations of 0.6 mg/ml and 0.6 µg/ml respectively with associated full scan MS spectra

Conclusions

Capillary electrophoresis with ion trap mass spectrometery is a powerful tool for monitoring impurities in drugs. CE is an orthogonal technique to LC, providing a different selectivity and higher resolution. The ion trap mass spectrometer allows the automatic, data-dependent acquisition of MS and MS/MS spectra without the need to select specific *m/z* values prior to the analysis. Moreover, it offers excellent full scan sensitivity. The dynamic range allows the detection and confirmation of impurities at least at the 0.1% level (w/w) of the main compound.

Authors

Christian Sauber and ***Gordon Ross*** are applications chemists at Agilent Technologies in Waldbronn, Germany

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