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

The Analysis of Clenbuterol by Positive Ion Electrospray Using the Waters Alliance LC/MS System Featuring the Platform LC Detector

Highlights: Clenbuterol, a anti-asthmatic compound used primarily in veterinary medicine, was examined by positive ion electrospray LC/MS. In addition to determining the feasibility of analysis, the effects of cone voltage fragmentation, quantification, linearity and reproducibility for this compound were also investigated. Limits of detection for this analysis were also determined in both full scan acquisition mode and Selected Ion Recording (SIR) mode.

Chromatographic Conditions:

HPLC: Waters Alliance™ (2690 Separations Module plus the Waters 2487 UV Detector)
Column: Symmetry® C_{18} 2X50mm
Mobile Phase:
A: H_2O (0.1%HCOOH)
B: CH_3CN
Flow rate: 250 uL/min
Injection Volume: 20 uL
UV Wavelength: 230 nm

MS analytical conditions:

Detector: Micromass Platform LC
Capillary Potential : 3.3 kV
Cone voltage: 30V and 50V
Source Temperature: 135 °C
Nitrogen flow rate: 220 l/h

Since electrospray ionization mass spectrometry (ESI) primarily yields molecular weight (MW) information, the technique of "In-Source" Collision Induced Dissociation (CID) is commonly used to produce limited fragmentation information in addition to MW information on a benchtop Atmospheric Pressure Ionization (API) LC/MS system. The resulting fragmentation obtained when utilizing "In-Source" CID, or alternately, Cone Voltage Fragmentation, can be manipulated to yield varying degrees of fragmentation. This is accomplished by increasing or decreasing the voltage applied to the sampling cone located beyond the source region of the mass detector. Figure 1 shows the resulting CID spectrum for Clenbuterol at low cone voltage (30 volts), while figure 2 illustrates more extensive fragmentation as the voltage is increased to 50 volts.

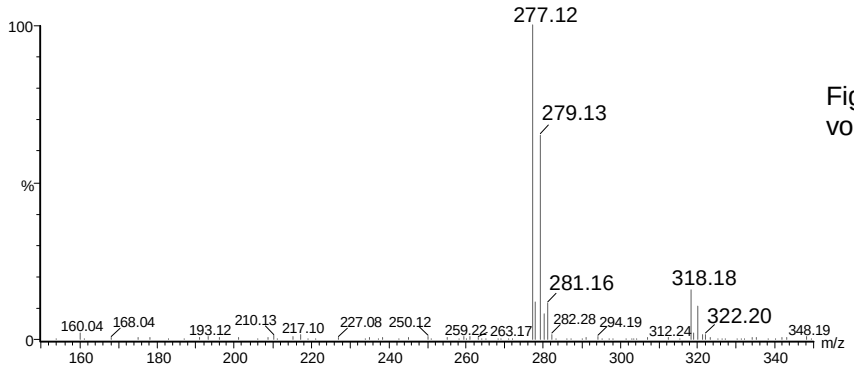


Figure 1: ESI Spectrum of Clenbuterol: Cone voltage @ 30 volts

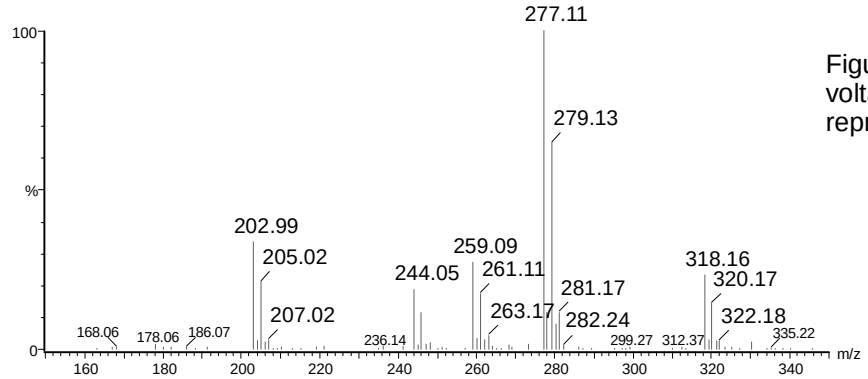


Figure 2: ESI spectrum of Clenbuterol: Cone voltage @ 50 volts The ion at m/z 318 represents acetonitrile adduct formation.

Compound 1 name: Clenbuterol 277
Correlation coefficient: r = 0.999692, r^2 = 0.999383
Calibration curve: 3942.29 * x + -1971.89
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None

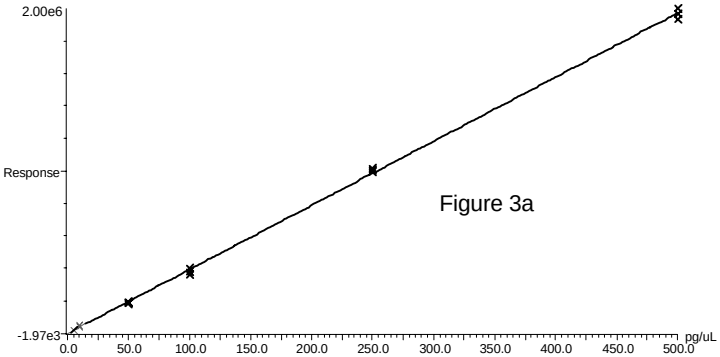


Figure 3a

Compound 2 name: Clenbuterol 203
Correlation coefficient: r = 0.999701, r^2 = 0.999401
Calibration curve: 1034.24 * x + -2545.60
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None

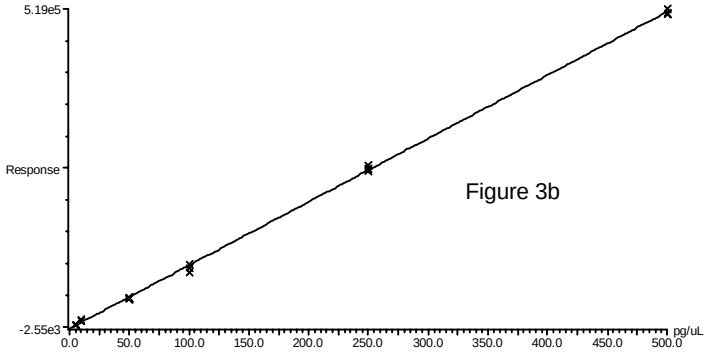


Figure 3b

Figure 3a and 3b are both examples of linear calibration curves for Clenbuterol. Two representative ions (m/z 277 and m/z 203) were selected for quantification and data was acquired in SIR mode. Each calibration curve was generated using six points corresponding to a concentration range of 5 to 500 pg/uL. There are triplicate injections for each concentration. Curve 3a was generated using the signal from the m/z 277 ion which represents [M+H]+. Curve 3b was generated using m/z 203 which is [[M -H2O - tButyl] +H]+. Note the linear relationship and appropriate correlation coefficient for each curve.

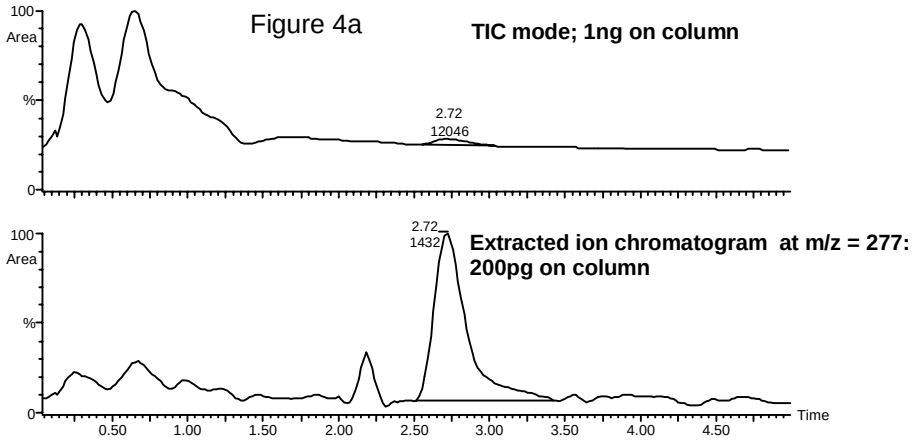
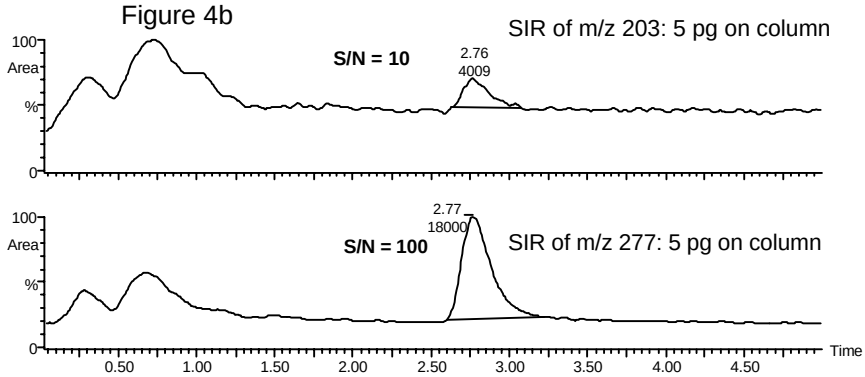


Figure 4a and 4b illustrate the detection limits (DL) achieved for Clenbuterol by positive ion ESI on the Platform LC detector. 4a compares the DL using full scan acquisition vs. an extracted ion chromatogram for m/z 277. 4b demonstrates the increased sensitivity obtained by utilizing SIR mode at m/z 203 and m/z 277.

The list below summarizes the DL results (in quantity injected on column) for the scan mode vs. the SIR mode of acquisition.

TIC Mode: *Scan:* 1ng DL *m/z= 277:* <200 pg DL
SIR Mode: *m/z= 277:* < 10pg DL *m/z= 203:* 100pg DL



The Waters Alliance LC/MS System Featuring the Platform LC Detector affords the chromatographer the ability to couple mass spectrometric detection with HPLC analysis for the ultimate in compound detection and confirmation capabilities. The current example demonstrates the use of such techniques as "In-Source" CID and SIR acquisition for increased speed of analysis as well as better confidence in analytical results.