

The Analysis of Benzodiazepines by LC/MS Using Positive Ion Electrospray

Highlights: A feasibility study of benzodiazepine analysis by benchtop, single quadrupole LC/MS was performed using the Waters Alliance™ LC/MS System Featuring the Micromass Platform LC Detector. A mixture of nine benzodiazepines was analyzed by positive ion Electrospray Ionization (ESI). Cone Voltage Fragmentation, or "In-Source" Collision Induced Dissociation (CID), was utilized in order to generate some compound structural information. Single Ion Recording (SIR), the ability to collect the signal from selected ions only, was used to check linearity for quantification and to determine detection limits.

Instrument: [Finnigan LCQ Duo LC/MS/MS with surveyor HPLC](#)

Chromatographic Conditions:

HPLC: Waters 2690, 996

Column: Symmetry C18, 150 mm x 2 mm

Column temperature: 50 deg C

Mobile Phase:

A: 10 mM Ammonium acetate

B: MeOH

Flow rate: 0.25 mL/min

Gradient: initial: A: 80 B: 20

15 mn: A: 30 B: 70

25 mn: A: 10 B: 90

30 mn: A: 80 B: 20

40 mn: A: 80 B: 20

Injection volume and dilution: variable

MS Conditions:

Detector: Micromass Platform LC

Interface: ESI +

Capillary Potential: 3.5 kV

Cone voltage: See further

Scan from 120 to 400 Da in 1 second
or

SIR at specific masses

Source Temperature: 100 °C

Nitrogen flow rate: 215 L/h

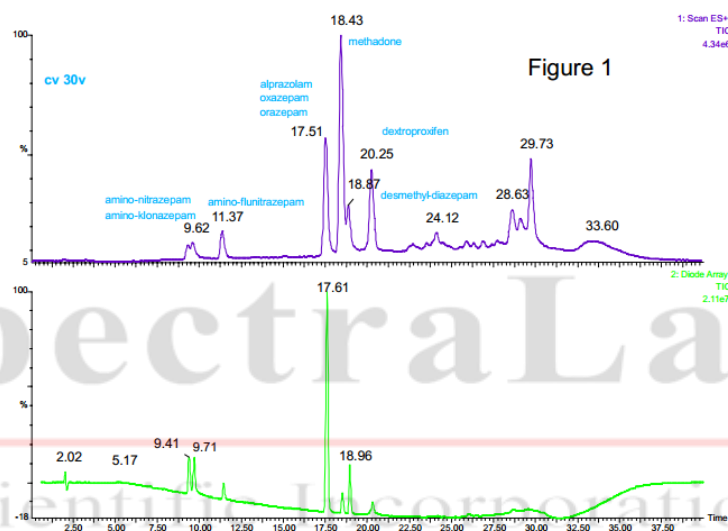
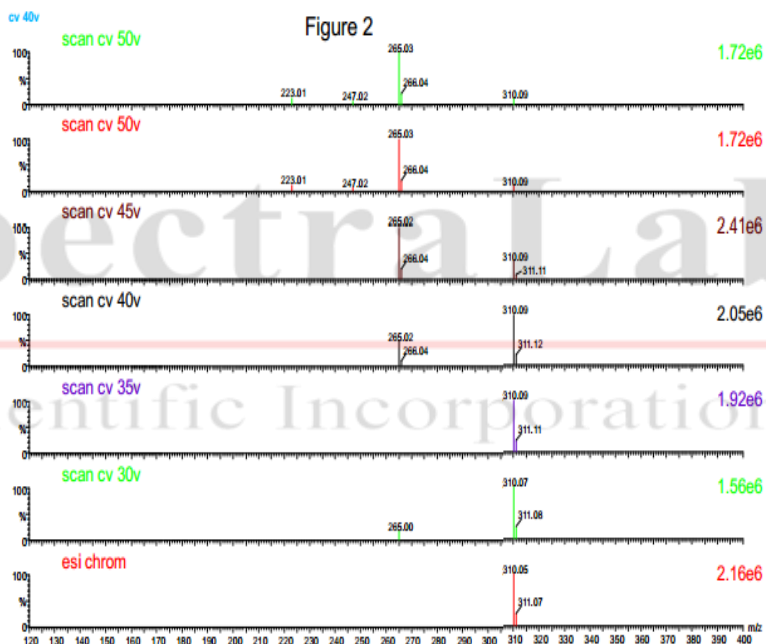


Figure 1 shows both the TIC (top) and the PDA chromatograms from a 2 μ L injection of the benzodiazepine mixture with CID off. The MS scan range is 120-400 Da, and the PDA scan range is 200-350 nm.

Figure 2 demonstrates the change in the fragmentation pattern when the Cone Voltage is varied from 25 to 55 volts. Note the increase in fragments and the decrease in signal of the protonated molecular ion as the cone voltage increases.



Reference: <http://www.waters.com/webassets/cms/library/docs/amd10.pdf>