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

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.

For this study of benzodiazepine analysis by LC/MS, the influence of various cone voltage settings on compound fragmentation and sensitivity level was determined. The system was run automatically, with cone voltage levels varying from 25 volts to 55 volts. Results are shown in figure 1. However, one of these compounds doesn't fragment at 55 volts. It would have been necessary to go higher in voltage to get fragmentation, if needed. Although the chromatography was far from optimal, the unique selectivity of MS detection is such that optimization of the chromatographic separation was not imperative for this study.

Since analysts are often sample limited, a series of successive dilutions of the original sample was performed. External standard curves based on serial dilutions showed excellent correlation coefficients for all nine benzodiazepines studied. The dilutions ranged from 2048 to 8. Results are shown in figure 2.

The diluted material was also used to determine detection limits for each compound. TIC signals obtained for a 2µl injection of 2048 times diluted samples are shown in figure 3. Under the conditions used, the detection limits were typically observed to be less than 10 pg on column for most of the analytes.

Chromatographic Conditions:
HPLC: Waters 2690, 996
Column: Symmetry$_R$ 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

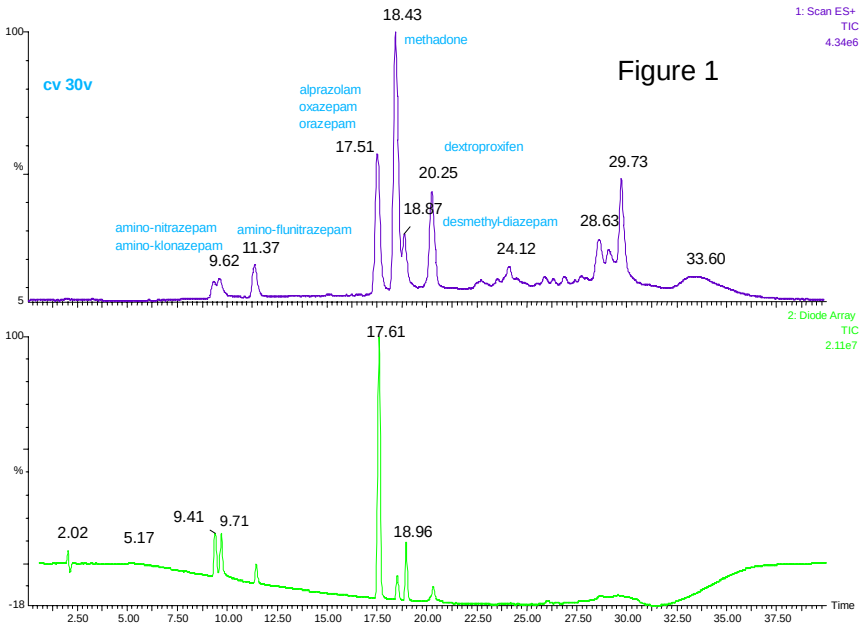

Figure 1 shows both the TIC (top) and the PDA chromatograms from a 2 µL injection of the benzodiazepine mixture with CID off. The MS scan range is 120-400 Da, and the PDA scan range is 200-350 nm.

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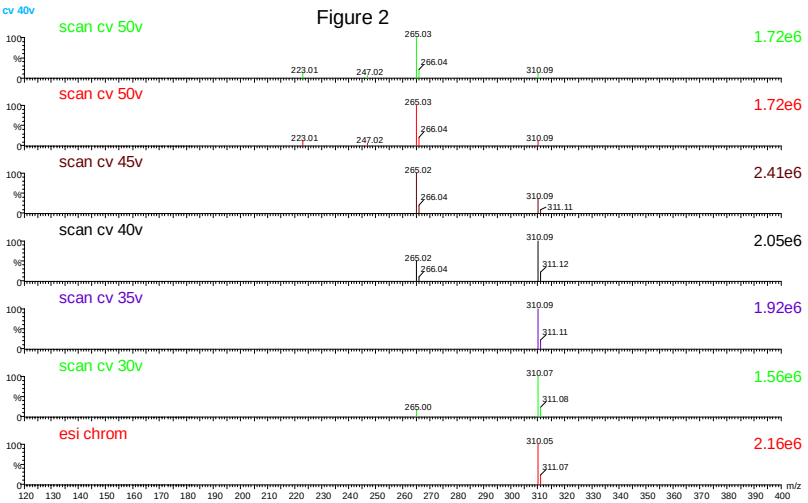
Figure 2 demonstrates the change in the fragmentation pattern when the Cone Voltage is varied from 25 to 55 volts. Note the increase in fragments and the decrease in signal of the protonated molecular ion as the cone voltage increases.



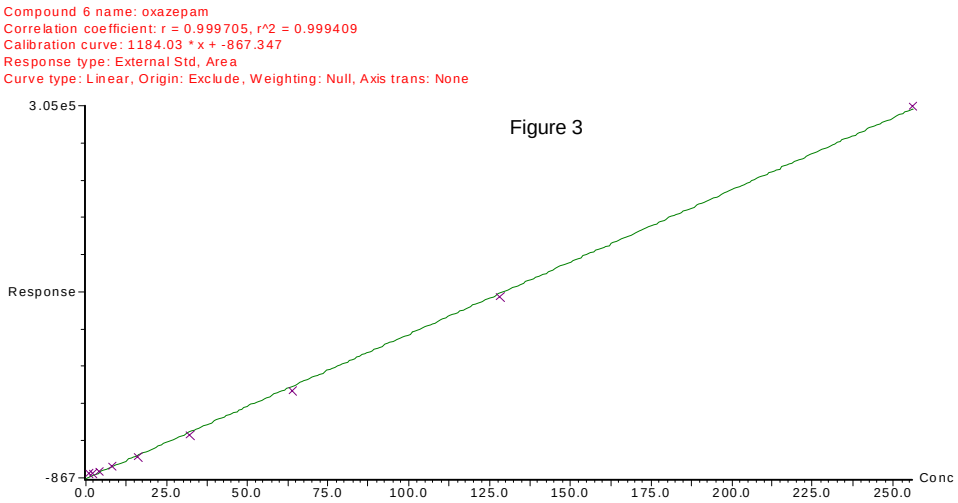


Figure 3 shows the external standard calibration curve based on serial dilution for methadone. Acquisition was in SIR mode at m/z 310. The injection volume was 2 µL of the 2048 times diluted sample. SIR was performed on nine simultaneous masses representative of significant masses for each of the nine benzodiazepines. Time programmed SIR was used for detection limit determination. The cone voltage was varied from 25 volts to 50 volts.

SIR of 9 channels:

	m/z	Cone Voltage
Nitrazepam :	252.0	30
Klonazepam :	270.9	45
Flunitrazepam:	284.0	45
Alprazolam :	286.0	50
Iorazepam :	286.9	25
Oxazepam :	309.0	25
Methadone :	310.0	35
Desmethyl-diazepam :	321.0	25
Dextroproxifen:	340.0	25

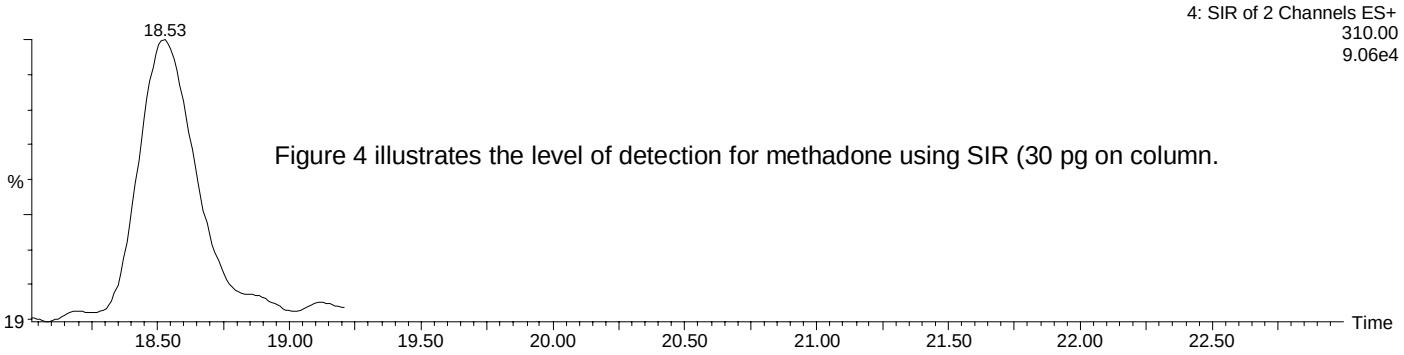


Figure 4 illustrates the level of detection for methadone using SIR (30 pg on column.

The Alliance™ LC/MS System Featuring the Platform LC Detector shows excellent feasibility for the analysis of benzodiazepines by ESI LC/MS. In a single run, the acquisition of nine different single ion chromatograms, each with optimized cone voltages (no time programming), is a unique and very useful feature of this system. SIR provides increased sensitivity and specificity for target compound analysis as well as linear calibration for compound quantification. Cone voltage switching within a single run allows the user to optimize fragmentation to yield important structural information.

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