

The example data presented here represents typical expected performance and is provided for the purposes of illustration and user guidance only. All Waters analytical instruments are installed and tested in accordance with Waters Standard Performance Tests. In presenting these data no agreement is implied by Waters to perform any additional non-standard performance tests during the installation.

## XEVO TQ-XS UPLC/MRM

### System Sensitivity – Positive Ion ESI

#### 10fg Midazolam on Column

This document presents example data obtained using an ACQUITY UPLC / Xevo TQ-XS system when operating in the ESI positive MRM mode. The test is based on monitoring the UPLC MRM response for replicate injections of a solution of Midazolam to demonstrate the sensitivity and reproducibility of this system configuration. Please see the relevant document for a full listing of the Xevo TQ-XS performance specifications.

#### EXPERIMENTAL CONDITIONS

##### Sample

Test sample: 10 fg/μL Midazolam (in 100% acetonitrile)  
Blank: 100% acetonitrile

##### LC Conditions

Instrument: ACQUITY I-Class  
Column: ACQUITY BEH, 100mm x 2.1mm, 1.7μm  
Injection volume: 1 μL  
Flow rate: 0.8 mL/min  
Mobile phase A: water + 2mM ammonium formate  
Mobile phase B: methanol  
Sample Manager Wash: methanol  
Purge syringe: 90% water, 10% methanol  
Seal Wash: 90% water, 10% methanol  
Column Temp: 70°C  
Sample Temp: 4°C

##### UPLC Gradient

| Time (min) | %A | %B | Curve |
|------------|----|----|-------|
| 0.0        | 15 | 85 | -     |
| 0.7        | 15 | 85 | 6     |
| 1.5        | 1  | 99 | 6     |
| 2.0        | 1  | 99 | 6     |
| 2.1        | 15 | 85 | 6     |

##### MS Conditions

Instrument: Xevo TQ-XS  
Ionization mode: ESI positive  
MS acquisition: MRM = m/z 326 – m/z 291, dwell = 200ms

Resolution: Peak width at half-height of precursor and product ions <1 Da  
MRM spike rejection: Enabled

## Software

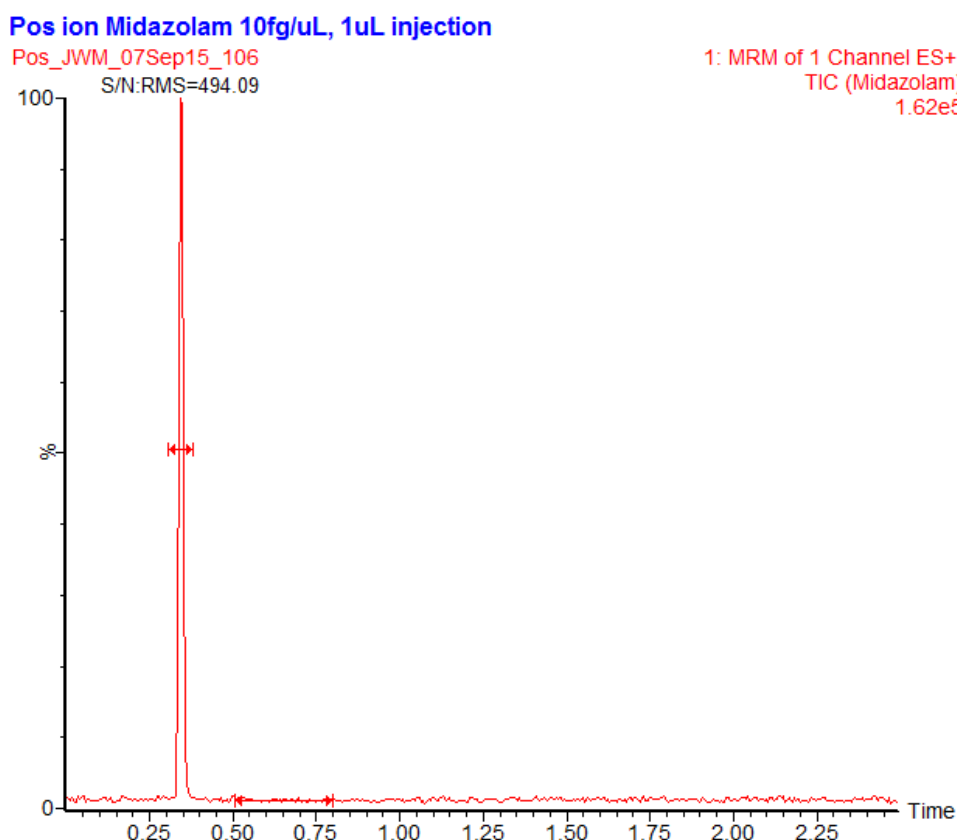
MassLynx: V4.1, SCN905

## RESULTS

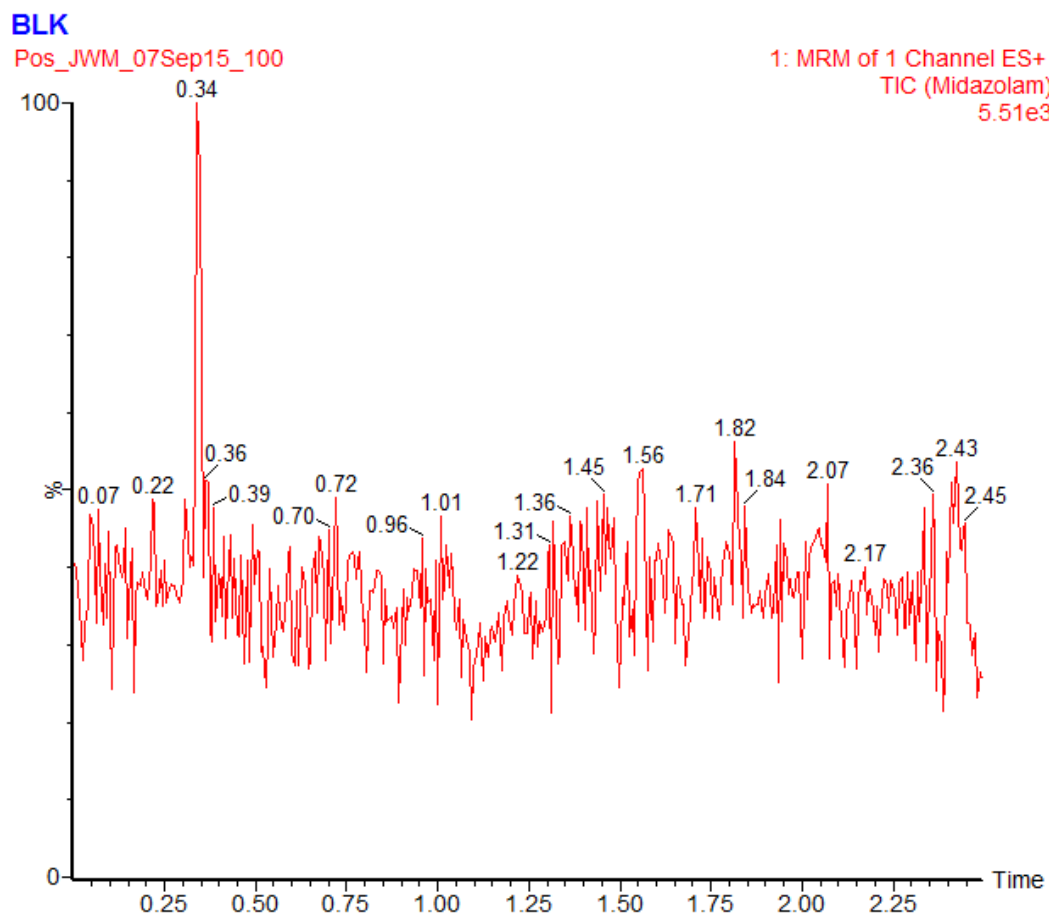
Figure 1 shows a typical UPLC MRM chromatogram obtained using the Midazolam standard solution (10fg on column). The example shows that the Midazolam was detected with a signal to noise ratio that was greater than 350:1 (Signal is the height of the chromatographic peak of interest and noise is the RMS of a continuous section of the mass chromatogram between 0.5 and 0.8 minutes). The signal to noise shown is based on raw (unsmoothed) data.

Figure 2 shows a typical UPLC MRM chromatogram obtained using the solvent blank (sample diluent). The blank response was less than 10% of that obtained for the Midazolam standard.

Ten replicate injections of the 10fg/μL Midazolam standard solution were made. The areas under the peak of interest were measured as illustrated in figure 3. The data presented in figure 4 shows that the relative standard deviation in chromatographic peak area reproducibility was less than 5%.

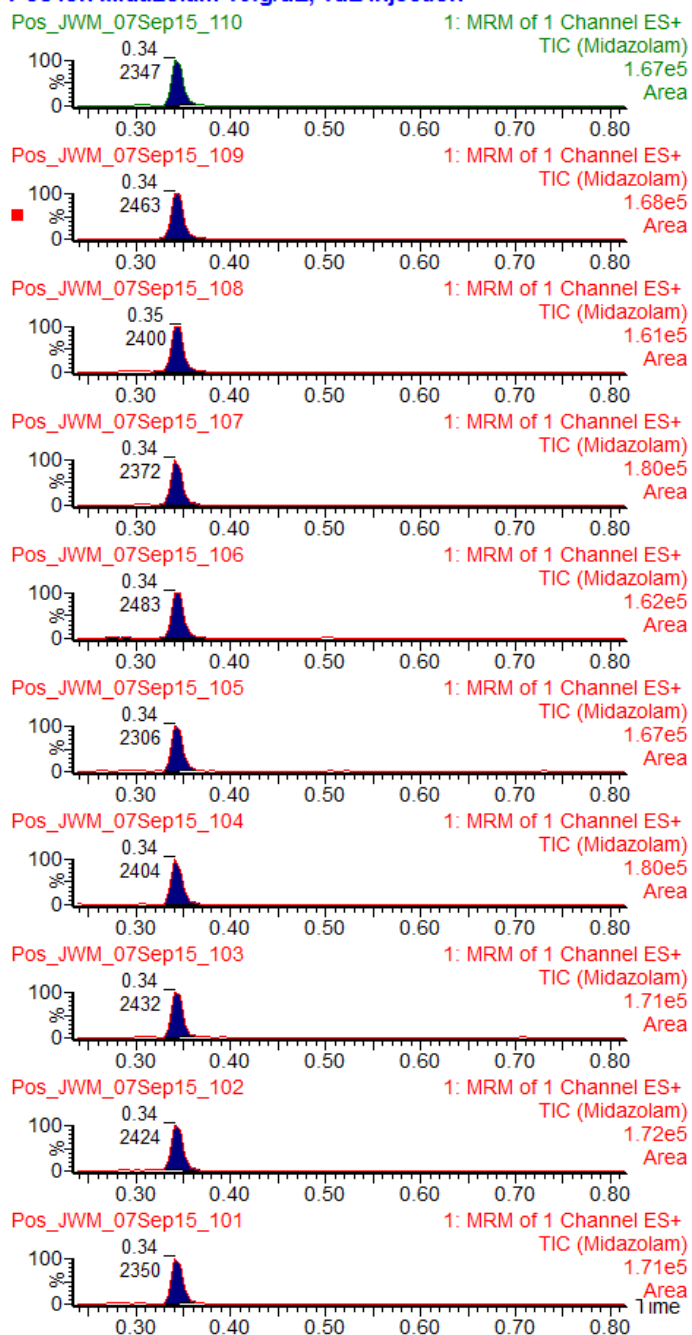


**Figure 1.**  
10fg/μL Midazolam (10fg on column)  
Signal:Noise > 350:1



**Figure 2.**  
Blank (sample diluent)  
Contribution = <10% (3.4% actual)

## Pos ion Midazolam 10fg/uL, 1uL injection



**Figure 3.**  
UPLC MRM peak areas for replicate injections.  
RSD of peak area < 5%

| Injection    | Peak areas  |
|--------------|-------------|
| Blank        | n/a         |
| Blank        | n/a         |
| Blank        | n/a         |
| injection 1  | 2350        |
| injection 2  | 2424        |
| injection 3  | 2432        |
| injection 4  | 2404        |
| injection 5  | 2306        |
| injection 6  | 2483        |
| injection 7  | 2372        |
| injection 8  | 2400        |
| injection 9  | 2463        |
| injection 10 | 2347        |
| Average      | <b>2398</b> |
| Std dev      | 55.1        |
| % RSD        | <b>2.3</b>  |

**Figure 4.**  
 UPLC MRM Peak area reproducibility  
 RSD of peak area <5% (2.3% actual)



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