

Technical Notification 0008

Benzodiazepine LC-MS/MS Screening

Column Comparison: Dr. Maisch HPLC GmbH
ReproSil Star Phenyl-Ether 3 µm, 50 mm x 2.0 mm
(P/N: star3.pet.s0502)

vs.

Phenomenex
Synergi Polar RP 4 µm, 50 mm x 2.1 mm
(P/N: 00B-4336-AN)

In this application 2 similar modified phenyl-phases (fully-porous silica modified with phenyl-ether & polar end-capping) were compared for the screening of different benzodiazepines with standards and different real blood samples.

ID No.: 0008

Analytes:

- 1) K7-Standard (Benzodiazepine Mix)
- 2) K7-Standard (Benzodiazepine Mix) – ZOOM 1
- 3) K7-Standard (Benzodiazepine Mix) – ZOOM 2
- 4) Blood Sample 19
- 5) Blood Sample 29
- 6) Blood Sample 34
- 7) Blood Sample 49

Eluent A: H₂O + 0.1% Formic Acid

Eluent B: ACN + 0.1% Formic Acid

Elution Type: Gradient

Gradient Method:

Time program for gradient (binary high-pressure pumps):

Time [min]	Flow Rate [µl/min]	%B	Comments
0.00	300	10	Start MS and pumps
1.00	300	10	Start gradient
7.50	300	90	N/A
8.50	300	90	N/A
8.60	300	10	N/A
12.00	300	10	End of program

Flow Rate: 300 ml/min

Temperature: 30 °C

Injection: 10 µl

MS Parameters: Sciex 3200 QTrap in MRM mode (MS/MS)

Ionization: Positive Electrospray Ionization

Spray Voltage: 4500 V

Temperature: 450 °C

Gas 1: 40

Gas 2: 60

Curtain Gas: 40

Collision Gas: 6

NOTE: MS/MS conditions tuned for each compound separately.

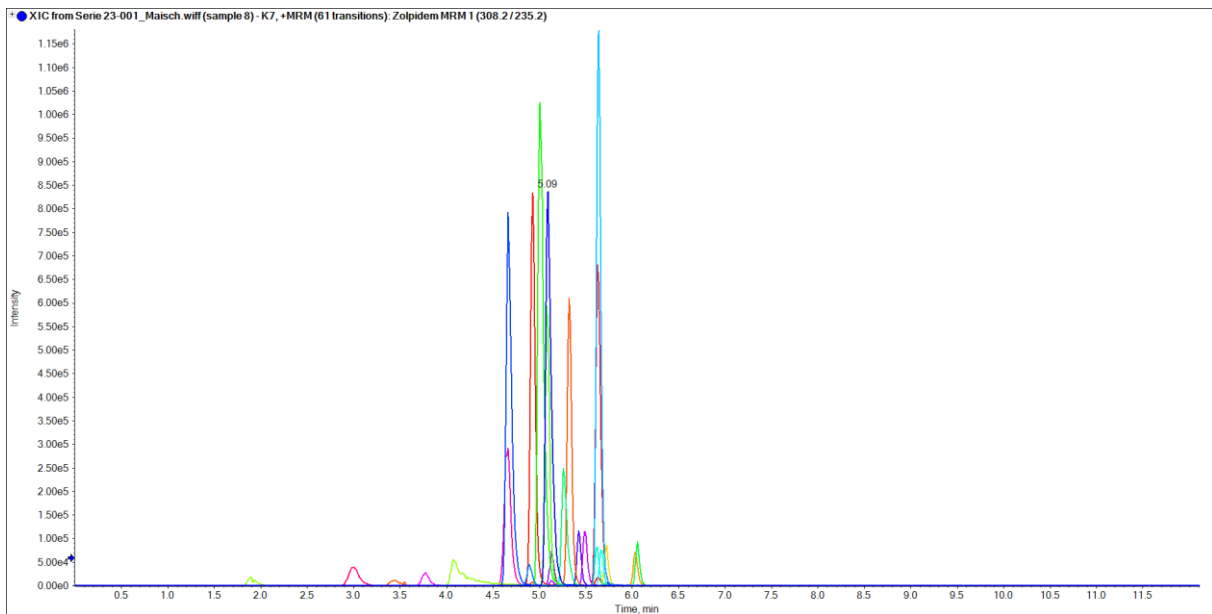
Separation of Benzodiazepines:

- | | |
|------------------------------------|--|
| • Clonazepam | Klonopin ¹⁾ , Ravotril ¹⁾ |
| • Norflunitrazepam | Desmethyflunitrazepam ²⁾ , N-Desmethyflunitrazepam ²⁾
Flunitrazepam metabolite |
| • Temazepam | Restoril ¹⁾ |
| • Zopiclone | Imovane ¹⁾ |
| • Nordiazepam | Nordazepam, Desoxydemoxepam,
Desmethyldiazepam ²⁾
Nordaz ¹⁾ , Stilny ¹⁾ , Madar ¹⁾ , Vegesan ¹⁾ , and Calmday ¹⁾ |
| • 7-NH ₂ -Flunitrazepam | Flunitrazepam metabolite |
| • Diazepam | Valium ¹⁾ |
| • Oxazepam | Alepam ¹⁾ |
| • Desalkylfluorazepam | Norflurazepam ²⁾ |
| • Aprazolam | Xanax ¹⁾ |
| • Flunitrazepam | Rohypnol ²⁾ |
| • Bromazepam | Lexotanil ¹⁾ |
| • Lorazepam | Ativan ¹⁾ |
| • Midazolam | Versed ¹⁾ |
| • Triazolam | Halcion ¹⁾ |
| • Flurazepam | Dalmane ¹⁾ Dalmadorm ¹⁾ |
| • Lormetazepam | Noctamid ¹⁾ |
| • Zaleplon | Sonata ¹⁾ |
| • Clobazam | Frisium ¹⁾ Onfi ¹⁾ |
| • Nitrazepam | Mogadon |
| • Alpha-hydroxymidazolam | Midazolam metabolite |

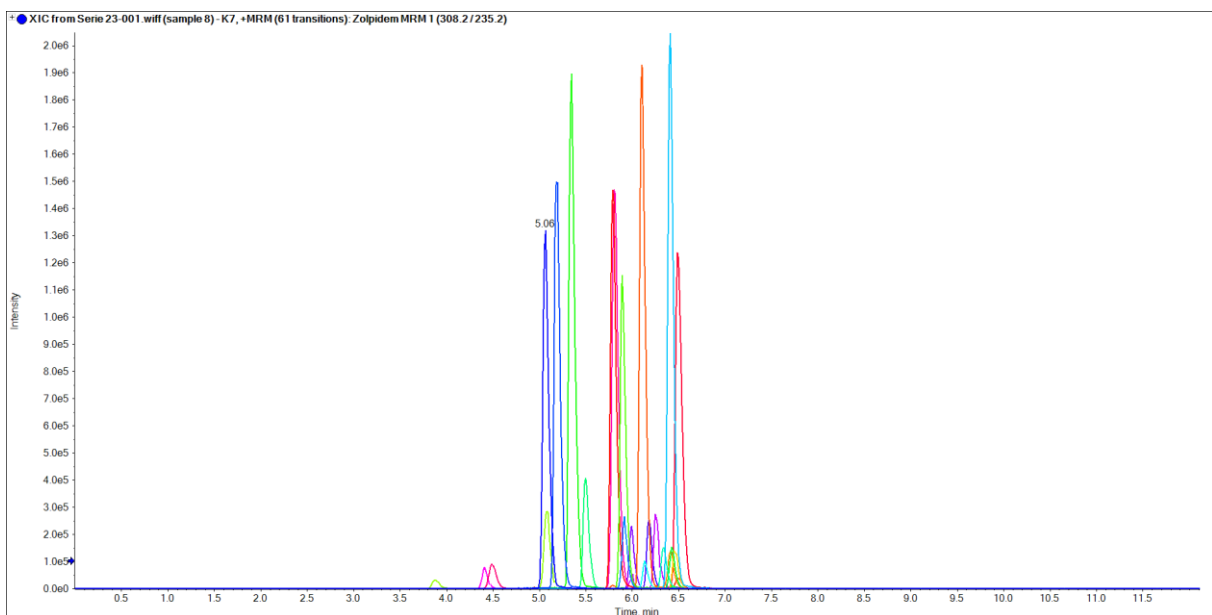
1) Brand Name 2) also known as

1) K7-Standard (Benzodiazepine Mix)

ReproSil Star Phenyl-Ether 3 µm, 50 mm x 2.0 mm - Dr. Maisch HPLC GmbH

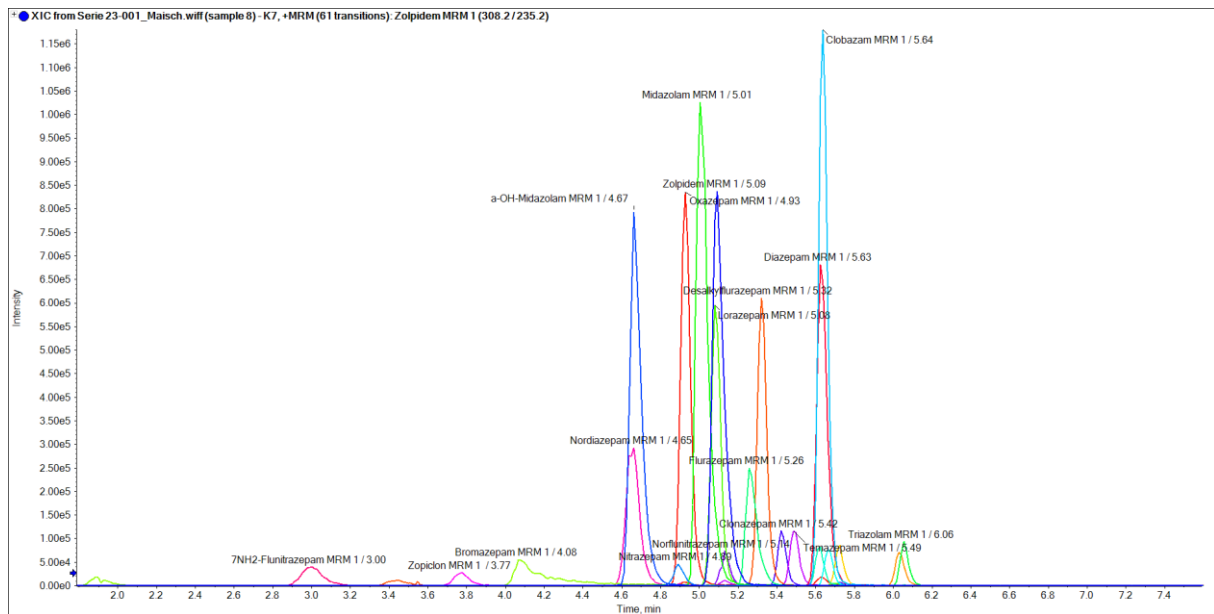


Synergi Polar RP 4 µm, 50 mm x 2.1 mm - Phenomenex

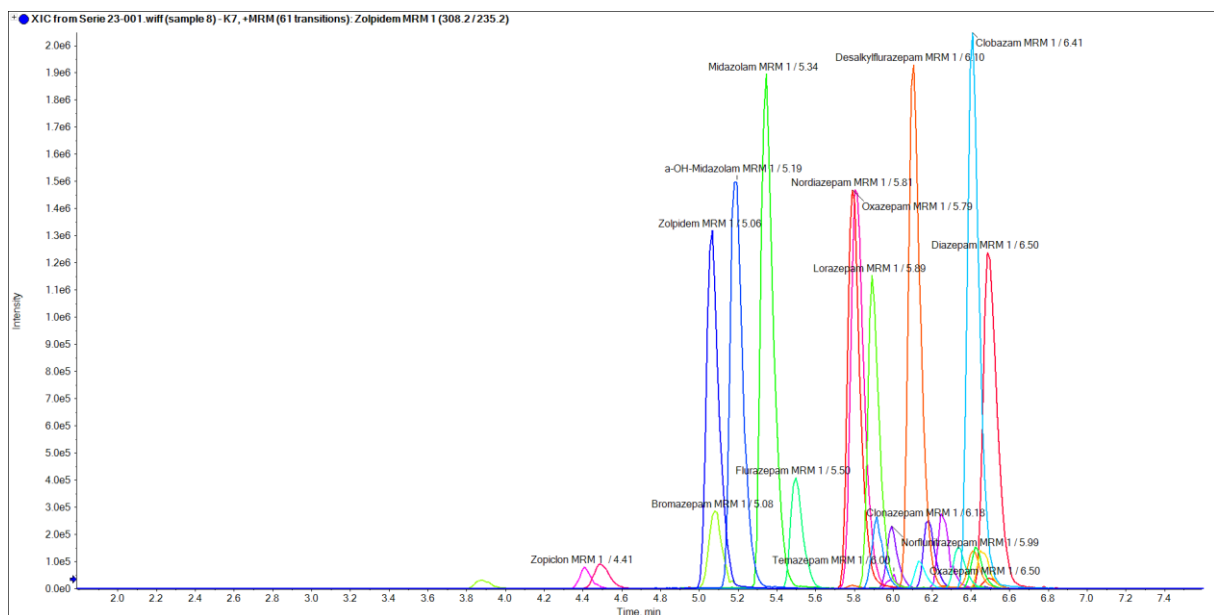


2) K7-Standard (Benzodiazepine Mix) – ZOOM 1

ReproSil Star Phenyl-Ether 3 µm, 50 mm x 2.0 mm - Dr. Maisch HPLC GmbH

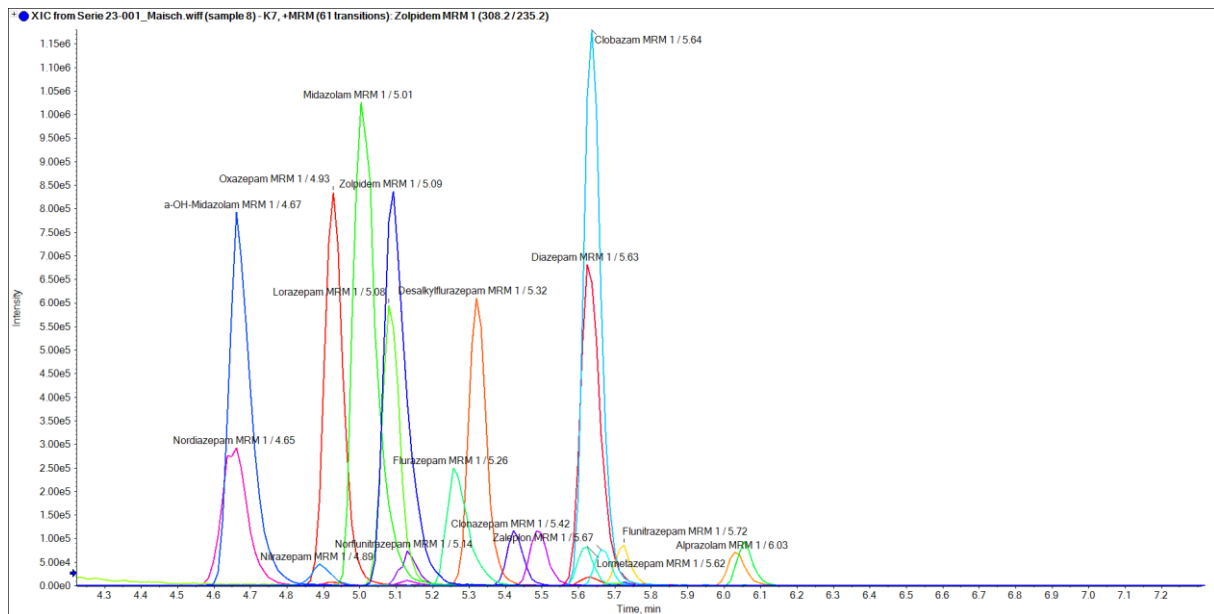


Synergi Polar RP 4 µm, 50 mm x 2.1 mm - Phenomenex

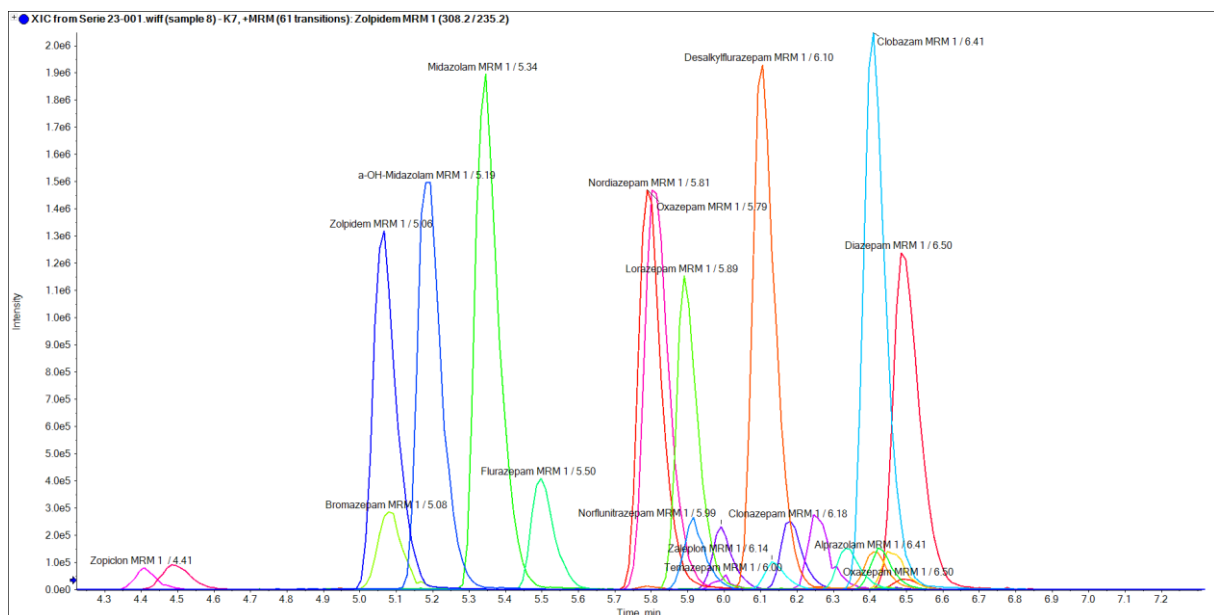


3) K7-Standard (Benzodiazepine Mix) – ZOOM 2

ReproSil Star Phenyl-Ether 3 µm, 50 mm x 2.0 mm - Dr. Maisch HPLC GmbH

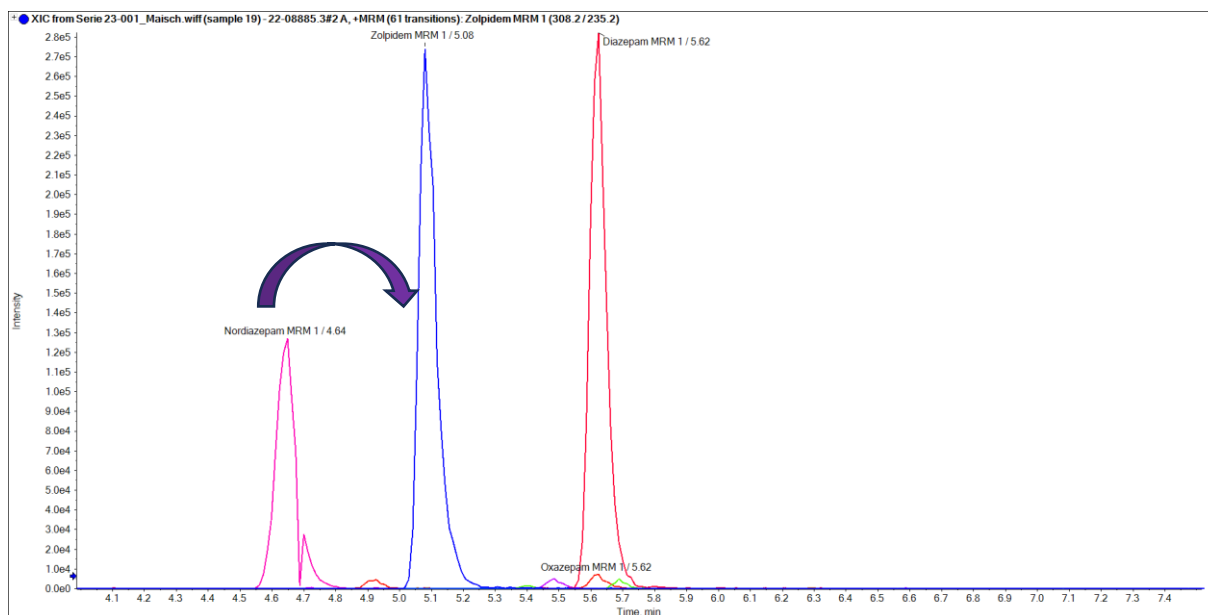


Synergi Polar RP 4 µm, 50 mm x 2.1 mm - Phenomenex

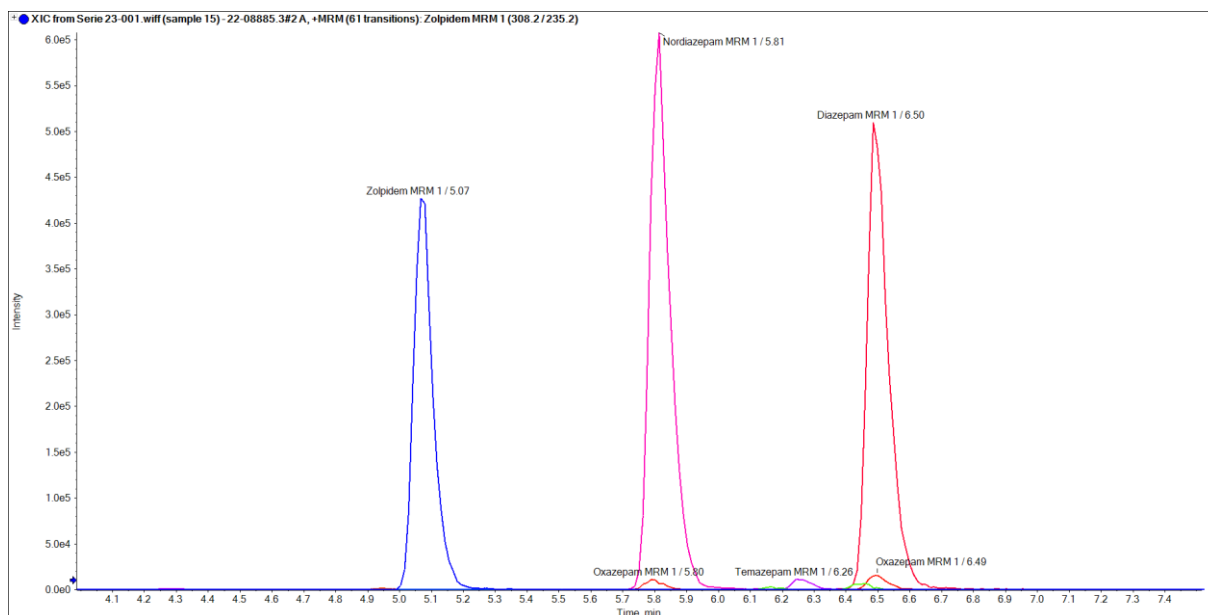


4) Blood Sample 19

ReproSil Star Phenyl-Ether 3 µm, 50 mm x 2.0 mm - Dr. Maisch HPLC GmbH

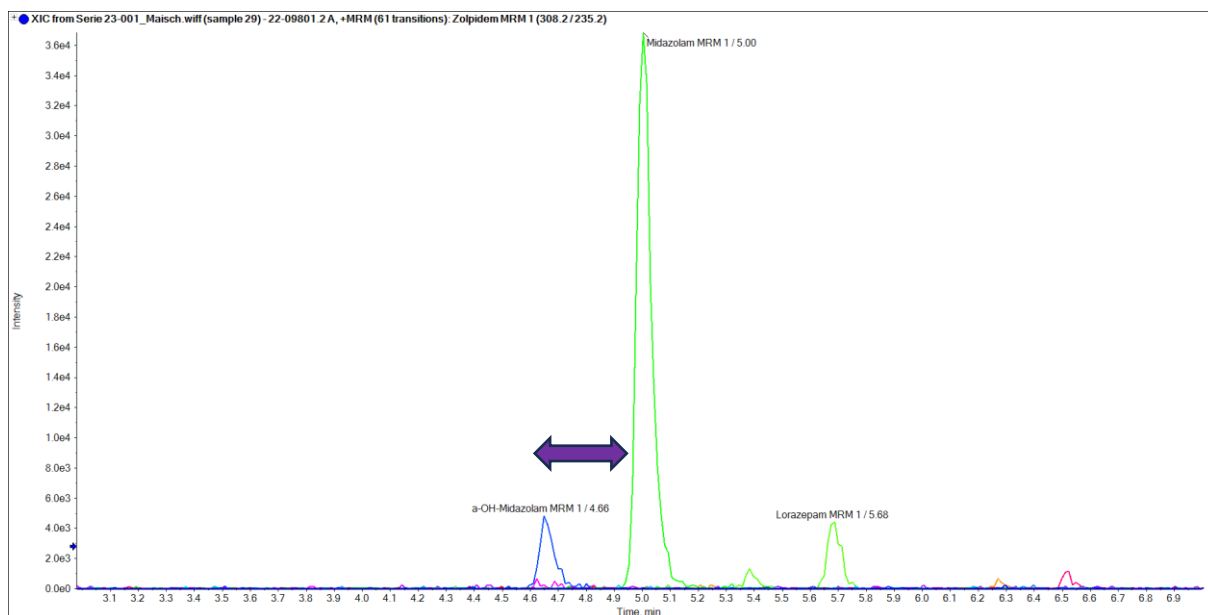


Synergi Polar RP 4 µm, 50 mm x 2.1 mm - Phenomenex

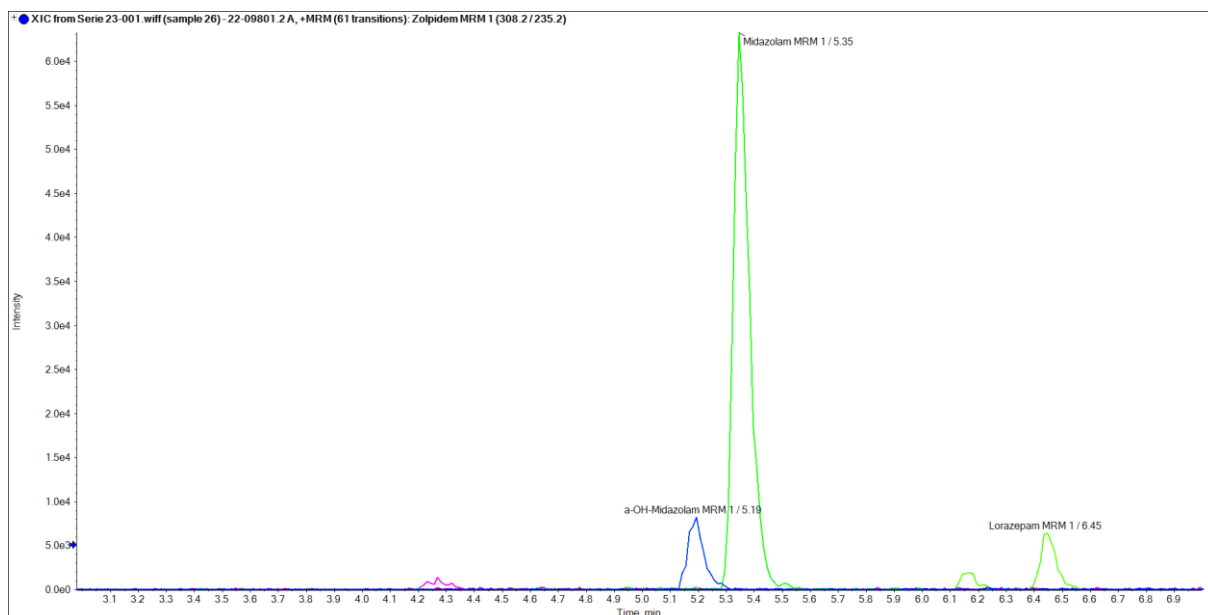


5) Blood Sample 29

ReproSil Star Phenyl-Ether 3 µm, 50 mm x 2.0 mm - Dr. Maisch HPLC GmbH

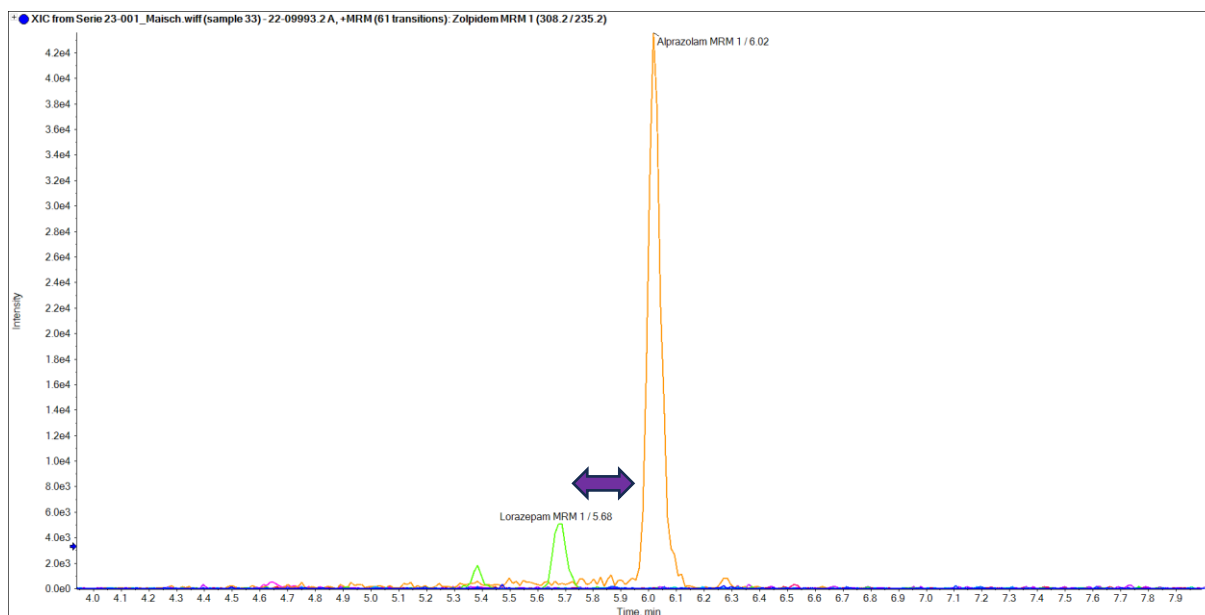


Synergi Polar RP 4 µm, 50 mm x 2.1 mm - Phenomenex

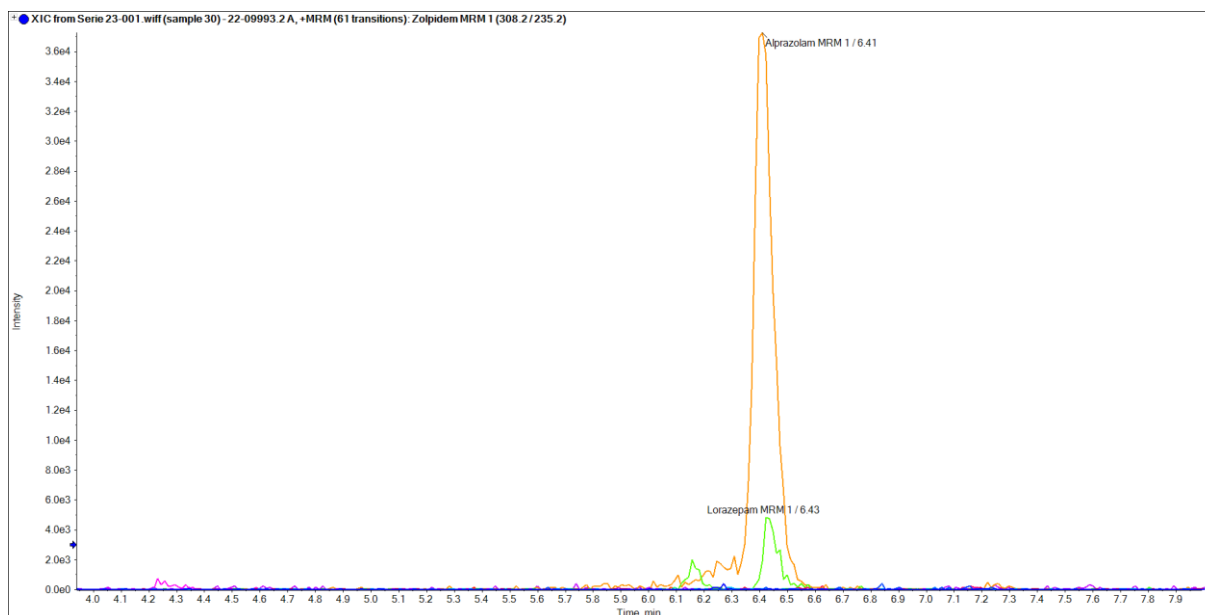


6) Blood Sample 34

ReproSil Star Phenyl-Ether 3 μ m, 50 mm x 2.0 mm - Dr. Maisch HPLC GmbH

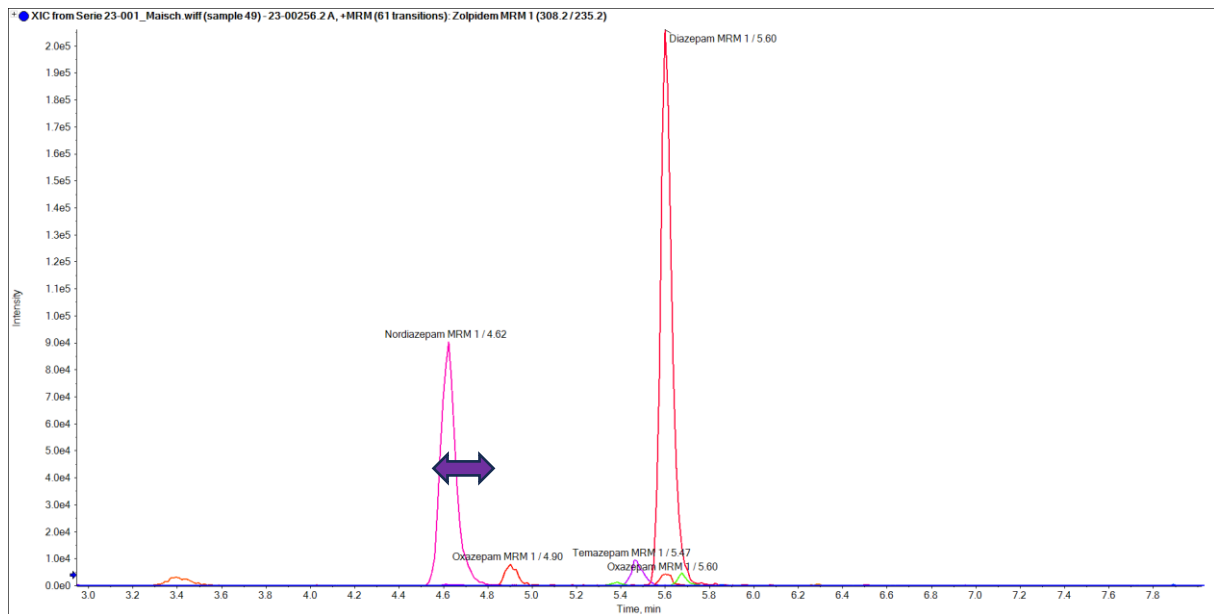


Synergi Polar RP 4 μ m, 50 mm x 2.1 mm - Phenomenex

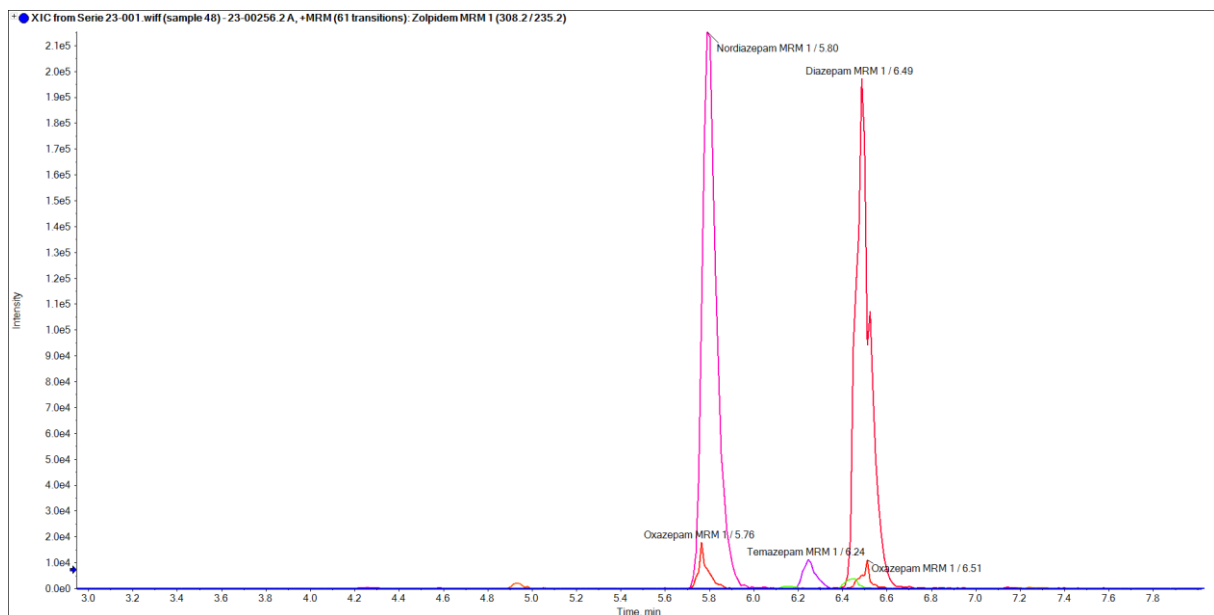


7) Blood Sample 49

ReproSil Star Phenyl-Ether 3 µm, 50 mm x 2.0 mm - Dr. Maisch HPLC GmbH



Synergi Polar RP 4 µm, 50 mm x 2.1 mm - Phenomenex



Data courtesy of Dr. Stefan König, Institut für Rechtsmedizin Bern (Switzerland), Forensische Toxikologie und Chemie.

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