Application Note 20689

# Determination of Zolmitriptan in Serum by SPE-LC-MS/MS

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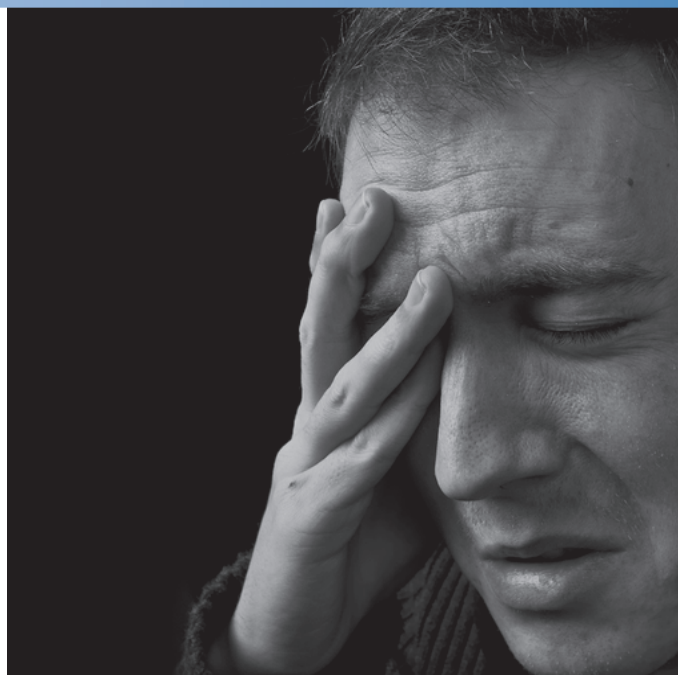
## Key Words
Zolmitriptan, diphenhydramine, SOLA, SOLA CX, Hypersil GOLD

## Abstract
A liquid chromatography-tandem mass spectrometry method for the analysis of zolmitriptan in serum has been developed. A limit of quantitation of 1 ng/mL is readily achieved. When using Thermo Scientific™ SOLA™ CX solid phase extraction cartridges, sample preparation is fast and efficient, giving a recovery of 88.2% with excellent reproducibility. The Thermo Scientific™ Hypersil GOLD™ 1.9 µm UHPLC column was used to give a fast run time of 2 minutes. The dynamic range was linear between 1 and 100 ng/mL with a $r^2$ of 0.996 and accuracies of +/- 15% for standards and quality control samples.



## Introduction
Zolmitriptan is used in the treatment of migraines and headaches. It is a triptan that acts as a selective serotonin receptor agonist. The method described in this application note allows the testing of serum for the presence of zolmitriptan.

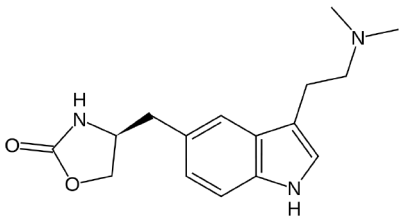



Figure 1: Structure of zolmitriptan

SOLA is a revolutionary solid phase extraction (SPE) device. This first-in-class SPE product range introduces next generation, innovative technological advancements, giving unparalleled performance characteristics compared to conventional SPE, phospholipid removal, and protein precipitation products.

These include:

- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Reduced sample and solvent requirements
- Increased sensitivity

SOLA has a significant advantage for the analyst when processing compounds in complex matrices particularly in high-throughput bioanalytical and clinical research laboratories where reduced failure rate, higher analysis speed, and lower sample/solvent requirements are critical. The increased performance offered by SOLA SPE gives higher confidence in analytical results and lowers cost without compromising ease of use or requiring complex method development.

Hypersil GOLD 1.9 µm UHPLC columns, based on highly pure silica, provide very symmetrical peaks, even when analyzing compounds that give notoriously poor peak shape on traditional silica-based chemistries. Hypersil GOLD media provides a stationary phase with C18 selectivity and a predictable elution order.

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The excellent peak shape offered by Hypersil GOLD 1.9 µm UHPLC columns contributes to high resolution, increased peak capacity, greater sensitivity especially for trace compound analysis, and the highest confidence in the accuracy and quality of results. The 1.9 µm particles give higher efficiency than 3 µm or 5 µm particles, and this efficiency is delivered over a greater range of linear velocities. This makes it possible to operate at high flow rates without losing performance. Obtaining symmetrical peak shapes is critical to ensuring that optimum resolution and sensitivity are achieved for basic pharmaceutical compounds. Hypersil GOLD columns use proprietary bonding technology to make certain that symmetrical peaks are obtained, producing data with the highest confidence in the accuracy and quality of results.

Using SOLA CX solid phase extraction cartridges and Hypersil GOLD UHPLC columns for the extraction of zolmitriptan, a fast 2-minute run time was achieved.

## Experimental Details

| Consumables | Part Number |
|---|---|
| Fisher Scientific™ LC/MS grade methanol | M/4062/17 |
| Fisher Scientific LC/MS grade water | W/0112/17 |
| Fisher Scientific LC/MS grade acetonitrile | 51101 |
| Fisher Scientific Analytical Reagent grade formic acid 98/100% | F/1900/PB08 |
| Fisher Scientific HPLC grade ammonia solution | A/3295/PB05 |
| Zolmitriptan, LGC standards | |
| Diphenhydramine, Sigma-Aldrich® | |
| Serum | |

| Sample Handling Equipment | Part Number |
|---|---|
| Thermo Scientific™ FinnPipette™ (100-1000 µL) | 642090 |
| Thermo Scientific FinnPipette (10-100 µL) | 4642070 |
| Thermo Scientific FinnPipette (1-10 µL) | 4642040 |

| SPE Hardware | Part Number |
|---|---|
| Thermo Scientific™ UltraVap™ | CLS-229070 |
| Thermo Scientific™ HyperSep™ glass block manifold | 60104-232 |
| Centrifuge | |

| SPE Consumables | Part Number |
|---|---|
| SOLA CX, 10 mg/1 mL | 60109-002 |

| Column | Part Number |
|---|---|
| Hypersil GOLD 1.9 µm, 50 × 2.1 mm | 25002-052130 |

| Vials and Closures | Part Number |
|---|---|
| Thermo Scientific™ Chromacol™ 22 mL clear storage vial combi pack, PTFE lined cap | 22-SV-CP |
| Thermo Scientific Convenience kit, 8-425 standard opening clear screw vial, black cap, white Silicone/ red PTFE seal | 60180-600 |

| Sample Preparation | |
|---|---|
| Compound: | Zolmitriptan, diphenhydramine (IS) |
| Matrix: | Serum |
| Sample pretreatment: | Pipette 180 µL of serum into a centrifuge tube. Add 10 µL of standard spiking solution, 10 µL of internal standard spiking solution, and 200 µL of 0.1% formic acid in water. Mix well and centrifuge for 10 minutes at 14,000 rpm. |

| | |
|---|---|
| SPE cartridge type: | SOLA CX 10 mg/1 mL |
| Conditioning stage: | Apply 500 µL of methanol followed by 500 µL of 2.5% formic acid in water onto the SPE cartridge. |
| Application stage: | Load all of the supernatant onto the SPE cartridge. |
| Washing stage: | Apply 500 µL of 2.5% formic acid in water followed by 500 µL of 0.1% formic acid in methanol onto the SPE cartridge. |
| Elution stage: | Apply 2 × 250 mL 5% ammonia in methanol sequentially onto the SPE cartridge. |
| Additional stage: | Evaporate eluent to dryness under nitrogen at room temperature. Reconstitute in 200 µL of water. Gently vortex mix and sonicate for 5 minutes. |

| **Solutions Preparation** | |
|---|---|
| Zolmitriptan stock: | Weigh 1 mg and dissolve in 1 mL of methanol. |
| Diphenhydramine stock: | Weigh 1 mg and dissolve in 1 mL of methanol. |

| **Separation Conditions** | | **Part Number** |
|---|---|---|
| Instrumentation: | Thermo Scientific™ Dionex™ UltiMate™ 3000 | |
| Column: | Hypersil GOLD 1.9 µm, 50 × 2.1 mm | 25002-052130 |
| Mobile phase A: | 0.1% formic acid in water | |
| Mobile phase B: | 0.1% formic acid in acetonitrile | |
| Gradient: | 5%-95% B in 1 min, hold for 30 s, return to 5%, and equilibrate for 1 min | |
| Flow rate: | 1 mL/min | |
| Run time: | 2.5 min | |
| Column temperature: | 60 °C | |
| Injection details: | 10 µL full loop | |
| Injection wash solvent: | Acetonitrile / water (50:50 v/v) | |

| **MS/MS Conditions** | |
|---|---|
| Instrumentation: | Thermo Scientific™ TSQ Vantage™ MS |
| MS/MS run time: | 2.5 min |
| Ionization conditions: | APCI Positive |
| Discharge current: | 4 µA |
| Vaporizer temperature: | 350 °C |
| Sheath gas pressure: | 60 arb |
| Ion sweep gas pressure: | 0 arb |
| Auxiliary gas pressure: | 40 arb |
| Capillary temperature: | 270 °C |
| Ions monitored: | Table 1 |

| Name | Precursor (*m/z*) | Product (*m/z*) | Collision Energy (eV) | S-lens (V) |
|---|---|---|---|---|
| **Zolmitriptan** | 288.2 | 182.2 | 22 | 74 |
| **Diphenhydramine (IS)** | 256.2 | 167.2 | 11 | 49 |

Table 1: Ions monitored

| | |
|---|---|
| Scan time: | 0.02 s |
| Q1 peak width: | 0.70 FWHM |
| Q3 peak width: | 0.70 FWHM |
| Q2 gas pressure: | 1.5 mTorr |
| Chrom filter: | 5 arb |

**Data Processing**

| Software: | Thermo Scientific™ LCQuan™ 2.6 |
|---|---|

## Results

### Chromatography

The Hypersil GOLD 1.9 µm UHPLC column gave excellent peak shape. The chromatography of the QCM at 15 ng/mL is shown in Figure 2.

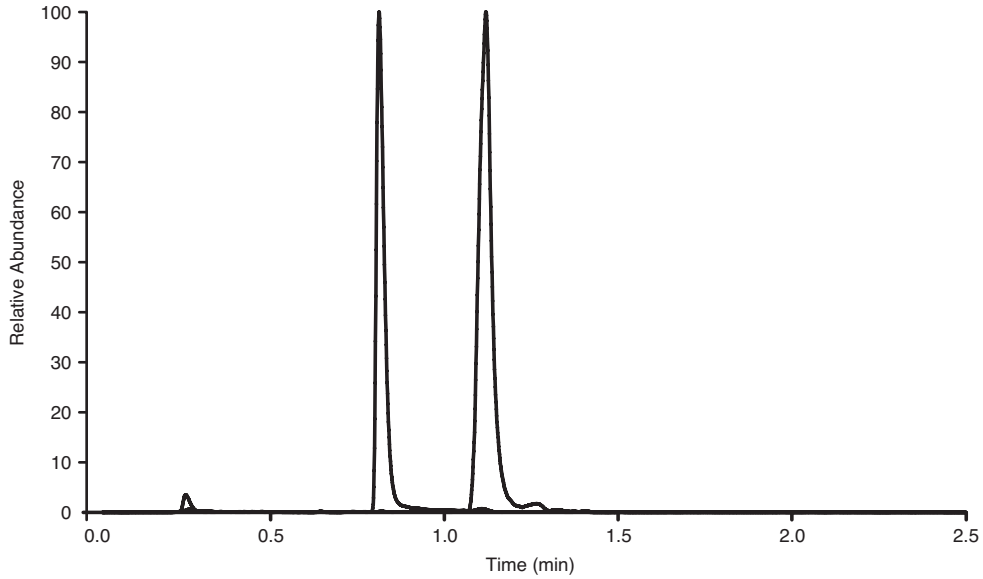


Figure 2: Representative chromatogram of (1) zolmitriptan and (2) diphenhydramine SRM, extracted from serum at 100 ng/mL

### Linearity

Extracted zolmitriptan standards from serum gave a linear calibration curve over the dynamic range of 1 to 100 ng/mL with an $r^2$ of 0.9955 (Figure 3 and Table 2).

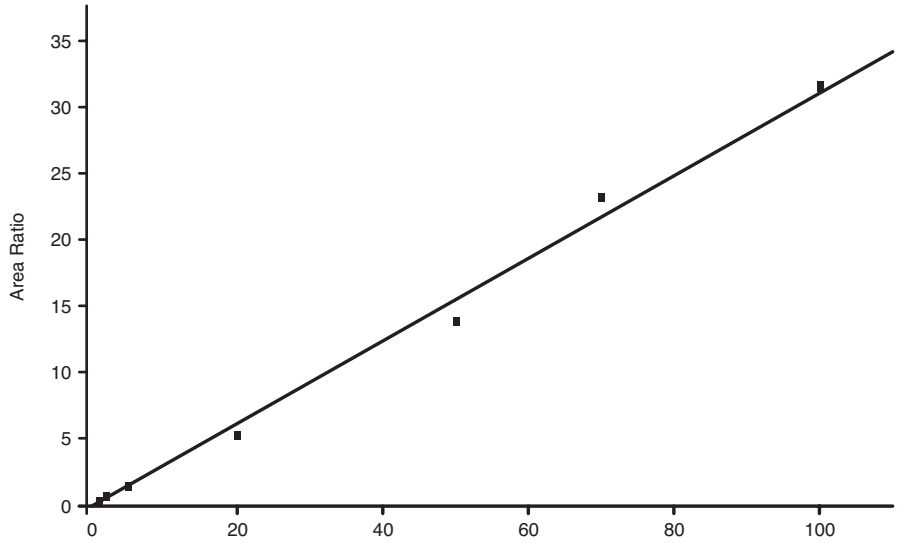


Figure 3: Zolmitriptan linearity over the dynamic range 1 to 100 ng/mL

| Standard | Specified Concentration (ng/mL) | Calculated Concentration (ng/ml) | %Diff |
|---|---|---|---|
| **S1 (average of 2 replicates)** | 1 | 1.03 | 4 |
| **S2** | 2 | 2.25 | 13 |
| **S3** | 5 | 4.75 | -5 |
| **S4** | 20 | 17.1 | -14 |
| **S5** | 50 | 44.6 | -11 |
| **S6** | 70 | 74.8 | 7 |
| **S7 (average of 2 replicates)** | 100 | 101 | 1 |

Table 2: Accuracy data for seven extracted zolmitriptan standards over the linear range 1 to 100 ng/mL

### Accuracy and Precision

QC samples were run in replicates of six at concentrations of 3.5, 15, and 60 ng/mL. The precision of the QC level was <7.5% CV in all cases (Table 3).

| Standard | Concentration (ng/mL) | Average Calculated Concentration (n=6) | %CV |
|---|---|---|---|
| **QCL** | 3.5 | 3.90 | 3.4 |
| **QCM** | 15 | 16.7 | 3.5 |
| **QCH** | 60 | 66 | 7.4 |

Table 3: Average precision data for six replicate QCs at three levels for zolmitriptan

### Recovery

Overspikes were run in triplicate at concentrations of 3.5, 15, and 60 ng/mL and used to calculate the percentage recovery level for zolmitriptan of 88.2% (Table 4).

| | Standard | Response | % Recovery | Average % Recovery |
|---|---|---|---|---|
| **Zolmitrptan** | **Average QCL response** | 18880.2 | 91.7 | **88.2** |
| | **Average QCL overspike response** | 20588.8 | | |
| | **Average QCM response** | 86579.5 | 91.4 | |
| | **Average QCM overspike response** | 94769.2 | | |
| | **Average QCH response** | 342945.6 | 81.5 | |
| | **Average QCH overspike response** | 420674.4 | | |
| **Diphenhydramine** | **Average QC response** | 16923.7 | **79.1** | |
| | **Average QC overspike response** | 21382.9 | | |

Table 4: Recovery data for zolmitriptan and diphenhydramine

## Conclusion

- The use of SOLA CX SPE and a Hypersil GOLD 1.9 µm UHPLC column allows for a simple extraction and quantification of zolmitriptan from serum.
- The method on SOLA CX SPE was extremely reproducible.
- A LOQ of 1 ng/mL for zolmitriptan in serum was achieved.
- Extraction recovery was high (>88%).
- The method showed excellent precision with %RSD (n=6) <7.5%.



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