# Quantitation and Confirmation of Gabapentin

## Table of Contents

# Quantitation and Confirmation of Gabapentin

## 1 Introduction

Gabapentin is a drug used for its anticonvulsant and analgesic properties.Structurally, gabapentin resembles GABA (γ-amino butyric acid) but does not interact or interfere with $GABA_A$ or $GABA_B$ neurotransmitter sites within the body; furthermore, gabapentin crosses the blood-brain barrier unlike GABA. Gabapentin is not protein bound to any significant extent and is eliminated as the parent drug with a half-life of approximately five to seven hours.

## 2 Scope

| | |
|---|---|
| Analyses | ☒ Screening ☒ Confirmation ☒ Quantitation |
| Matrices | Blood |
| Analytes | Gabapentin |
| Personnel | This document applies to authorized personnel who perform the described tasks, singly or in combination. |

## 3 Principle

Specimens are mixed with a deuterated analog internal standard. The specimens are extracted using SPE and eluted from the cartridges using a solvent mixture. The eluent is then taken to dryness and reconstituted in a methanol-water mixture and analyzed by liquid chromatography – electrospray – mass spectrometry (LC-ESI-MS).

## 4 Specimen Criteria

This procedure is validated for whole blood. Typically, 2 x 0.5 mL samples are analyzed; however, samples suspected to be above the procedure's linear range may be diluted before extraction.

## 5 Equipment

### 5.1 Equipment

A. Centrifuge
B. Evaporator w/ Nitrogen
C. Routine laboratory supplies, including disposable pipettes, wooden sticks, test tube racks, graduated cylinders, etc.
D. Volumetric flasks (5 mL)
E. Vortex mixer

#### *5.1.1 LC Column*

Xterra C-18 MS, 3.0 x 150 mm, 3.5 µm dp; or equivalent

## 5.2 Consumables

A. 12 x 75 mm culture tubes with polypropylene snap-tops
B. 16 x 100 mm screw-top tubes with caps
C. Clean Screen DAU® SPE cartridges (regular flow) - 200 milligrams

## 5.3 Instruments

A. Thermo LTQ Orbitrap XL
B. Shimadzu HPLC

## 5.4 Software

| Component | Software | Version |
|---|---|---|
| **Operating System** | Microsoft Windows | 7 Pro SP 1 / XP Professional |
| **Mass Spectrometer** | Foundation | 1.0.2 or higher |
| | Xcalibur | 2.1.0 SP1 / 2.0.7 |
| | LTQ Tune Plus | 2.5.5 |
| | Shimadzu LC Controller | 5.4 / 6.5 |

## 5.5 Chemicals/Reagents

Storage/stability determined by manufacturer unless otherwise noted.

### *5.5.1 Purchased*

| Chemical or Reagent | Minimum Grade or Purity |
|---|---|
| Acetonitrile | Optima |
| Ammonium Hydroxide (concentrated) | Certified ACS |
| Formic Acid (concentrated) | Optima |
| Hydrochloric Acid (concentrated) | Certified ACS |
| Isopropanol | HPLC |
| Methanol | Optima |
| Methylene Chloride | Optima |
| Sodium Phosphate (monobasic monohydrate) | Reagent |
| Sodium Phosphate (dibasic heptahydrate) | Reagent |
| Water | Deionized (DI) and Optima |

### *5.5.2 Prepared*

| |
|---|
| Mobile Phase 2 (0.1% Formic Acid in Acetonitrile): |
| To a 500 mL graduated cylinder, add 500 mL acetonitrile and 0.5 mL formic acid, mix well; store at room temperature in glass. Solution is stable for one month. |
| Mobile Phase 1 (0.1% Formic Acid in Water) |
| To a 500 mL graduated cylinder, add 500 mL water (Optima grade) and 0.5 mL formic acid, mix well; store at room temperature in glass. Solution is stable for two weeks. |

| 0.1 M Sodium Phosphate Buffer (pH 6.0): |
|---|
| To a 500 mL volumetric flask, add 400 mL DI water, 6.1 g sodium phosphate monobasic monohydrate, and 1.6 g sodium phosphate dibasic heptahydrate. Mix well to dissolve, verify that the pH is between 5.8 and 6.1; fill to the mark with DI water. Store refrigerated in glass. Solution is stable for two months. |
| 0.1 M Hydrochloric Acid (0.1 M HCl): |
| To a 100 mL graduated cylinder, add 80 mL DI water and 0.8 mL concentrated hydrochloric acid. Bring to 96 mL with deionized water and mix well; store at room temperature in glass. Solution is stable for six months. |
| Methanol:Water (10:90 v:v): |
| To a 50 mL graduated cylinder, add 5 mL methanol and 45 mL water (Optima grade), mix well; store at room temperature in glass. Solution is stable for one year. |
| Elution Solvent [Methylene Chloride/Isopropanol/Ammonium Hydroxide (78:20:2)]: |
| To a 100 mL graduated cylinder, add 20 mL of isopropanol and 2 mL of concentrated ammonium hydroxide and mix well. Then add 78 mL of methylene chloride and mix well. Solution is to be made and used on the same day. |

## 5.6 Standards/Controls

Storage/stability determined by manufacturer unless otherwise noted.

### *5.6.1 Purchased*

| $d_{10}$-Gabapentin Stock Standard (100 µg/mL): |
|---|
| Purchased from Cerilliant International or equivalent manufacturer. Storage conditions and stability determined by manufacturer. |
| Gabapentin Stock Standard (1.0 mg/mL): |
| Purchased from Cerilliant International and Lipomed or equivalent manufacturers. Storage conditions and stability determined by manufacturer. |

### *5.6.2 Prepared*

#### *5.6.2.1 Control*

| Low Control Working Solution (100 µg/mL): |
|---|
| Add 0.5 mL of the 1.0 mg/mL Standard Stock Solution in a 5 mL volumetric flask and bring to the mark with Methanol. Store in glass at or below 0°C; stable for at least six months. |

#### 5.6.2.2 *Control Scheme*

| Exam Type | Level | Matrix (mL) | Solution | Spike (mL) | Final Conc. (µg/mL) | Replicates |
|---|---|---|---|---|---|---|
| **Qualitative** | Low | 0.5 | Low Control W/S | 0.075 | 15 | 1 |
| **Quantitative** | Low | 0.5 | Low Control W/S | 0.075 | 15 | 2 |
| **Quantitative** | High | 0.5 | High Control W/S | 0.040 | 80 | 2 |

#### 5.6.2.3 *Calibration*

| Low Calibration Working Solution (100 µg/mL): |
|---|
| Add 0.5 mL of the 1.0 mg/mL Standard Stock Solution in a 5-mL volumetric flask and bring to the mark with Methanol. Store in glass at or below 0°C; stable for at least six months. |

#### 5.6.2.4 *Calibration Scheme*

| Calibrator Level (µg/mL) | High Cal WS (1 mg/mL) Volume (µL) | Low Cal WS (100 µg/mL) Volume (µL) | Matrix (mL) |
|---|---|---|---|
| **5** | - | 25 | 0.5 |
| **10** | - | 50 | 0.5 |
| **20** | - | 100 | 0.5 |
| **30** | - | 150 | 0.5 |
| **46** | 23 | - | 0.5 |
| **60** | 30 | - | 0.5 |
| **80** | 40 | - | 0.5 |
| **100** | 50 | - | 0.5 |

#### 5.6.2.5 *LC/MS Performance Standard*

| Column Performance Mix |
|---|
| Dilute 0.010 mL of the Internal Standard Working Solution with 0.090 mL of Methanol:Water (10:90 v:v). Prepare fresh. |

#### 5.6.2.6 *Matrix*

| Negative Control Blood: |
|---|
| Purchased from Diagnostics Products Corporation, UTAK Laboratories, Inc., Cliniqa, or obtained in-house from a drug-free donor. Store refrigerated or frozen. Stability determined by manufacturer. A Negative Control Blood sample will be extracted and analyzed with every blood assay. |

## 6 PROCEDURE

| | Step | Note | Reference/Lot |
|---|---|---|---|
| | **A. Samples/Calibrators/Controls** | | |
| | 1. To labeled 16 x 100 mm screw-top tubes add: | | |
| ☐ | i. 0.5 mL of biological fluid | | |
| | **B. Controls** | | |
| ☐ | 1. Negative Control Source | | |
| | 2. Prepare Positive Control(s) | | |
| ☐ | i. Low Control Working Solution | | |
| ☐ | ii. High Control Working Solution | | |
| | iii. Control Scheme | | |
| | **C. Calibrators** | | |
| | 1. Prepare Calibrators | | |
| ☐ | i. Low Calibrator Working Solution | | |
| ☐ | ii. High Calibrator Working Solution | | |
| | iii. Calibration Scheme | | |
| | **D. Internal Standard** | | |
| ☐ | 1. Add 30 µL of Internal Standard Working Solution | | |
| ☐ | 2. Bring all samples to approximately 5 mL with deionized water | | |
| ☐ | 3. Vortex | | |
| | **E. Buffer** | | |
| ☐ | 1. Add 2 mL of 100mM phosphate buffer | | |
| ☐ | 2. Vortex and allow to stand for 5 minutes | | |
| ☐ | 3. Check pH: 6 ± 0.5 | | |
| ☐ | 4. Centrifuge for 10 minutes at 3500 rpm | | |
| | **F. Extract (SPE)** | | |
| | 1. Condition cartridges (1 mL/min) | | |
| ☐ | i. Add 3 mL methanol | | |
| ☐ | ii. Add 3 mL deionized water | | |
| ☐ | iii. Add 1 mL 100mM phosphate buffer | | |
| ☐ | 2. Load samples (1 mL/min) | | |

| | | | |
|---|---|---|---|
| | 3. Wash cartridges (1 mL/min) | | |
| ☐ | i. Add 2 mL of deionized water | | |
| ☐ | ii. Add 2 mL 100mM HCl | | |
| ☐ | iii. Add 3 mL of methanol | | |
| ☐ | 4. Dry cartridge under full vacuum for 1.5 minutes | | |
| | 5. Elute (1 mL/min) | | |
| ☐ | i. Add 3 mL Elution Solvent | | |
| ☐ | ii. Collect eluent in 12 x 75 mm tubes | | |
| | **G. Concentrate** | | |
| ☐ | 1. Evaporate the eluent to dryness under nitrogen at 40°C | | |
| | **H. Reconstitute** | | |
| ☐ | 1. Add 200 µL of methanol:water (10:90)<br>2. Vortex | | |
| ☐ | 3. Add 100 µL of extract to ALS vial and cap. | | |
| ☐ | **I. Instrumental Analysis**<br>1. LC/MS: analyze 10 µL<br>i. Analyze LC/MS Performance Standard prior to batch analysis | | |
| | ii. Mobile Phase 1 (aqueous) | | |
| | iii. Mobile Phase 2 (organic) | | |
| | iv. LC Column | | |

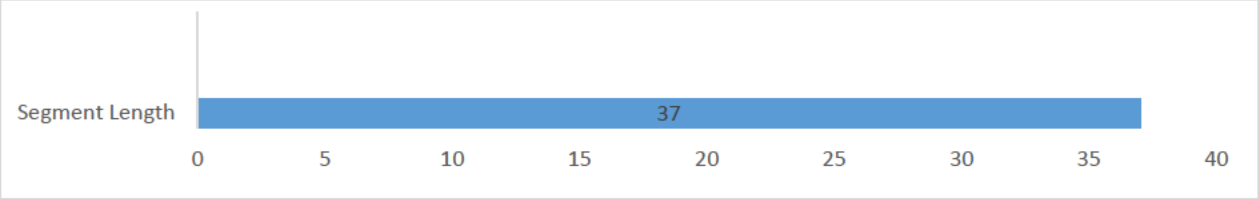
## 7 ANALYTICAL PARAMETERS

### 7.1 Shimadzu HPLC Gradient/Conditions

| Time (min) | Mobile Phase % | | Flow Rate | | |
|---|---|---|---|---|---|
| | 1-Aqueous | 2-Organic | (mL/min) | Column Heater (°C) | 30 |
| 0 | 90 | 10 | 0.3 | Autosampler (°C) | 15 |
| 5 | 90 | 10 | 0.3 | Run Time (min) | 37 |
| 20 | 10 | 90 | 0.3 | | |
| 30 | 10 | 90 | 0.3 | | |
| 31 | 90 | 10 | 0.3 | | |
| 37 | 90 | 10 | 0.3 | | |

### 7.2 LTQ-XL Orbitrap

A. Source Mode: ESI (+)




| Event | Mode | Range (m/z) | Analyzer | Resolution |
|---|---|---|---|---|
| 1 | Full Scan | 85-400 | FTMS | 15000 |

# 8 DATA ANALYSIS

## 8.1 Decision Criteria

### *8.1.1 Batch Acceptance Criteria*

No gabapentin should be detected in the Negative Control.

Gabapentin should be present in the Positive Control. Each Quantitative Positive Control will quantitate within ±20% of the target value. See TOX-101 for more information.

### *8.1.2 Sample Acceptance Criteria*

#### *8.1.2.1 Chromatography*

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. Ion peaks are typically extracted at ±0.005 m/z. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

##### 8.1.2.1.1 Retention Time

The retention time of the peak should be within ±5% of the retention time (relative or absolute, as appropriate) obtained from injection of an extracted Positive Control or extracted calibrator.

##### 8.1.2.1.2 Signal-to-Noise

To justify the existence of a peak, its signal-to-noise ratio should exceed 3. Note: nonsensical signal to noise values may result from high resolution mass spectral data. Further, the baseline signal for the peak of interest should be at least ten-fold greater than that for any observed peak at similar retention time in a Negative Control or solvent blank injected just prior to the sample.

#### *8.1.2.2 Mass Spectrometry*

The M+1 for gabapentin in each sample should be 172.133 ±0.005 m/z.

The M+1 for d10-gabapentin in each sample should be 182.196 ±0.005 m/z.

## 8.2 Calculations

Quantitation is performed by constructing a multi-point calibration curve based on the ratio of the area for the M+1 peak for the analyte to the internal standard. Ion traces are drawn at a 0.005 m/z mass tolerance. See TOX-101 for acceptable practices in calculating quantitative results.

### *8.2.1 Calibration Model*

| | |
|---|---|
| **Model** | Linear |
| **Weighting** | 1/x |

## 9 REPORTING

### 9.1 Measurement Uncertainty

Reference CHEM-100, TOX-100 and TOX-101 for guidance.

## 10 CORRECTIVE MEASURES

Refer to TOX-101 for guidance.

## 11 PERFORMANCE CHARACTERISTICS

### 11.1 LOD

1 µg/mL

### 11.2 LOQ

5 µg/mL

### 11.3 Linearity

5 – 100 µg/mL

### 11.4 Bias/Precision

| | 6 µg/mL | 40 µg/mL | 80 µg/mL |
|---|---|---|---|
| Bias (n=15) | -13.66 | 1.38 | -2.96 |
| Repeatability (n=15) | 4.82 | 5.75 | 6.00 |
| Intermediate Precision (n=15) | 7.53 | 8.98 | 8.27 |

### 11.5 Carryover

For extracted negative control samples analyzed immediately following extracted 100 µg/ml calibrator samples, no carryover was observed.

## 12 LIMITATIONS

### 12.1 Interferences

None observed.

### 12.2 Processed Sample Stability

The calculated difference between day 0 and day 1, 3, and 7 did not exceed ±10%.

## 13 SAFETY

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

## 14 REVISION HISTORY

| Revision | Issued | Changes |
|---|---|---|
| 01 | 02/11/2022 | Document reformat.<br>Minor updates to language and phrasing throughout. |