

# Application

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## Quantitative Analysis of Gabapentin from Serum by Reversed Phase HPLC Using Discovery C18 Columns

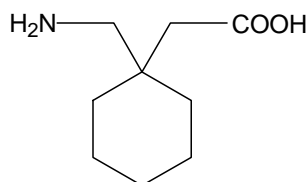
The feasibility of quantitative analysis of gabapentin extracted from serum was studied by reversed phase HPLC on Discovery C18 columns. The results showed very good resolution, excellent column stability, and reproducibility for columns from different batches.

### Key Words:

- gabapentin
- reversed phase HPLC
- Discovery C18 column

Gabapentin (1-[aminomethyl] cyclohexaneacetic acid, Figure A) is an analogue of  $\gamma$ -aminobutyric acid (GABA), a substance approved by the US Food and Drug Administration (FDA) for add-on therapy in treating seizures thought to be associated with interference in GABA-ergic pathways or provoked by excitatory amino acids (1-3). Gabapentin circulates essentially unbound to serum proteins.

Figure A. Gabapentin



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Most patients dosed within the recommended range have serum concentrations between 2 and 12  $\mu\text{g}/\text{mL}$ . Therefore, monitoring serum concentration is useful to determine whether a patient is above the therapeutic threshold. Also, monitoring may assist in assessing compliance and suspected toxicity (1-3).

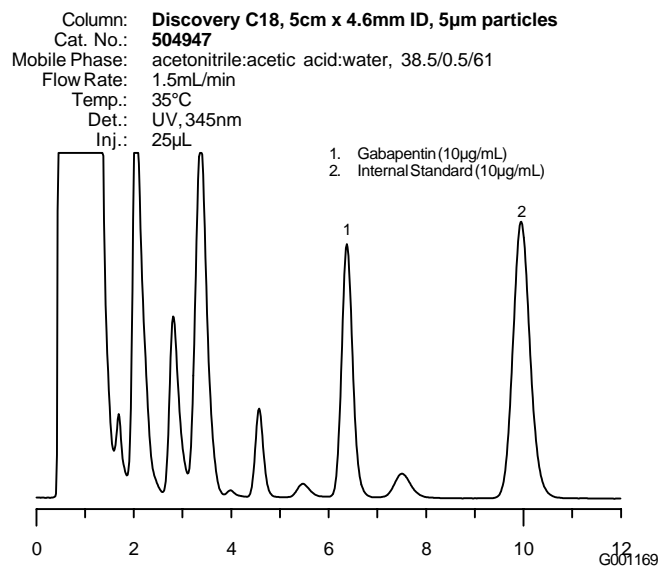
We developed a method to quantitate gabapentin in serum using Discovery<sup>®</sup> C18 reversed phase HPLC columns (5cm x 4.6mm ID, 5 $\mu\text{m}$  particles). Proteins are precipitated from serum with acetonitrile. Following centrifugation, the supernatant is spiked with an internal standard. After derivatization with picrylsulfonic acid (1-3), gabapentin and the internal standard were separated using a Discovery C18 column. To test the robustness of this method, we looked at four different bond lots from three different silica batches of the Discovery C18 phase. A comparison of the retention times among the four columns reveals that the coefficient of variation was less than 1.8% for each compound (Table 1), indicating a robust method on the columns. Method ruggedness was to be tested in an independent laboratory.

Table 1. Retention Time Variation Among Columns from Different Batches

| Column Batch No.       | Retention Time (min) |           |
|------------------------|----------------------|-----------|
|                        | Gabapentin           | Int. Std. |
| Column 1 (3142, PS73)  | 6.48                 | 10.121    |
| Column 2 (3167, PS77)  | 6.662                | 10.387    |
| Column 3 (3172, PS77)  | 6.386                | 9.967     |
| Column 4 (3306, PS104) | 6.564                | 10.198    |
| Average                | 6.523                | 10.168    |
| STD                    | 0.11                 | 0.174     |
| CV (%)                 | 1.80                 | 1.71      |

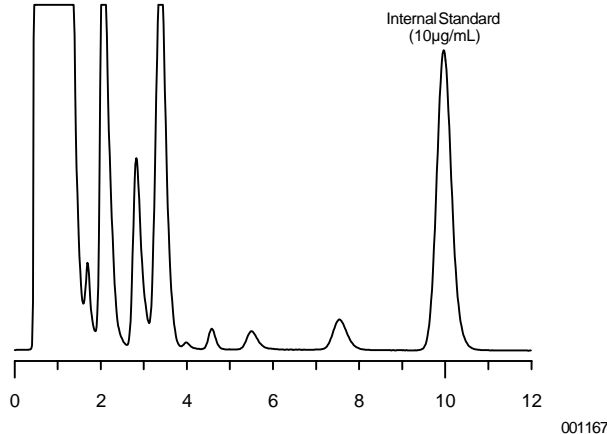
A separation of a serum sample containing 10  $\mu\text{g}/\text{mL}$  gabapentin (Figure B) resulted in excellent resolution and peak shape. Note that interfering peaks from the matrix and derivatizing reagent were resolved completely from gabapentin and the internal standard. We found that a mobile phase consisting of 38.5% acetonitrile / 0.5% acetic acid / 61% deionized water yielded the best separation. This analysis was completed in less than eleven minutes. Figures C and D show the internal standard peak and the derivatizing agent peak, respectively.

Figure B. Gabapentin Extracted from Bovine Serum



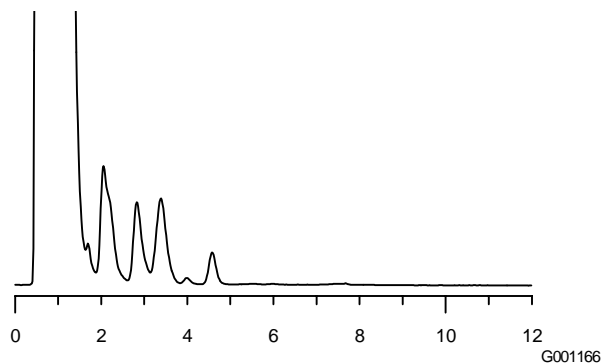
### Figure C. Gabapentin Internal Standard Only in Bovine Serum

Conditions: see Figure B.



### Figure D. Derivatizing Agent (Picrylsulfonic Acid) in Bovine Serum

Conditions: see Figure B.



A Discovery C18 column is an excellent choice in the quantitative analysis of gabapentin in serum. This column provides excellent resolution and retention time reproducibility.

### Ordering Information:

| Description  | Cat. No.      |
|--|---------------|
| <b>Discovery C18 Column</b>                              |               |
| 5cm x 4.6mm ID, 5µm particles                            | <b>504947</b> |
| Discovery is a registered trademark of Sigma-Aldrich Co. |               |

#### References

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  - Upton, N. *Mechanisms of Action of New Antiepileptic Drugs: Rational Design and Serendipitous Findings*. *Trends. Pharmacol. Sci.* **15**: 456-463 (1994).
- References not available from Supelco.

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