**LC-MS/MS Method for Quantitative Analysis of Gabapentin and Pregabalin in serum or plasma**

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Background

Gabapentin and pregabalin are analogs of the inhibitory neurotransmitter gamma-amino butyric acid (GABA). Both analogs can be prescribed as full or partial anticonvulsants for some types of seizures as well as for postherpetic neuralgia and Restless Legs Syndrome (gabapentin) or for pain from diabetes, shingles, fibromyalgia, or spinal cord injury (pregabalin). Gabapentin and pregabalin are commonly tested to monitor therapeutic efficacy and patient compliance.

Objective

Our objective was to develop a rapid, sensitive, specific, and robust LC-MS/MS method to replace a less sensitive LC-MS/MS assay for Gabapentin quantification, while adding quantitation of an additional GABA analog pregabalin and achieving chromatographic separation.

Methods

We developed a 3.5-minute LC-MS/MS method used for the quantification gabapentin and pregabalin in serum. Sample preparation procedure was as follows: 20 µL of patient sample, calibrator, or control was combined with 380 µL of precipitating solution containing internal standards (gabapentin-D10 and pregabalin-D6), and the mixture was vortexed and centrifuged. A portion of the resulting supernatant (20 µL) was further diluted 1:20 with mobile phase A. The diluted supernatant (10 µL) was injected onto Phenomenex Kinetex 2.6u Biphenyl 100A HPLC column (2.1 mm x 50 mm), separated using an Agilent 1200 HPLC system, and detected using Triple Quad ™ 4000 (AB SCIEX) with electrospray ionization in positive ion mode. Baseline chromatographic separation of both analytes was achieved.

## Results

The method was linear from 0.0 to 50.0 µg/mL for both gabapentin and pregabalin. Intra-assay imprecision (CV) ranged from 1.1 to 4.1% and 1.0 to 4.4% for gabapentin and pregabalin, respectively. Inter-assay imprecision (CV) ranged from 3.6 to 7.6% and 9.3 to 10.7% for gabapentin and pregabalin, respectively. Method comparison of gabapentin was assessed by an in-house UPLC-MS/MS gabapentin assay with 95% confidence intervals yielded: (gabapentin LC-MS/MS) = 1.01 ± 0.06(gabapentin UPLC-MS/MS) - 0.15 ± 0.81 µg/mL, Sy/x = 1.84, r = 0.977 (n=50). Comparing the pregabalin method with an external reference laboratory using LC-MS/MS methodology yielded the following results with 95% confidence intervals: (pregabalin LC-MS/MS) = 0.99 ± 0.03(comparator pregabalin assay) - 0.16 ± 0.20 µg/mL, Sy/x = 0.55, r = 0.993 (n=62).

## Conclusion

This assay demonstrates good linearity, reproducibility, and is comparable to an in-house method for gabapentin as well as a national reference laboratory for pregabalin. We have developed a rapid, sensitive, specific, and robust LC-MS/MS method for quantification of gabapentin and pregabalin in serum.