RESTEK Pure Chromatography

# Strategies for Mitigating the Effects of High Gabapentin Concentrations in Urine Specimens

Haley Berkland, MS
Restek Corporation, Bellefonte, PA

## Introduction

Gabapentin is an anti-convulsant drug prescribed for the treatment of neuropathic pain and seizures, as well as for many off label uses. Gabapentin is prescribed in high doses relative to other therapeutic drugs and is eliminated in urine predominantly in its unchanged form, which often results this compound being extremely concentrated in urine samples. Heltsley et al (2011) reported the mean concentration of gabapentin to be 430.9 µg/mL in patient urine specimens.[1]


When analyzed by LC-MS/MS, high concentrations of gabapentin can present significant analytical implications, particularly for the compound amphetamine. Interference between gabapentin and amphetamine has been well documented and can result in signal suppression, poor peak shape, and shifting retention times.[2] Saturation of the mass spectrometer and column overload are also a concern. In this work, we explored several strategies to mitigate the effects of high gabapentin concentrations in urine samples.

## Materials and Methods

Urine samples spiked with various concentrations of gabapentin and amphetamine were analyzed using an LC-MS/MS method (Method 1) developed for the analysis of 60 drugs of abuse in urine. Samples were fortified with varying amounts of gabapentin and amphetamine in urine, as described in Table I. Data was examined to determine how analyte performance was impacted by high levels of gabapentin when analyzed by Method 1. Based on these results, a new method (Method 2) was developed to mitigate the analytical challenges presented by samples with high gabapentin concentrations.

**Table I.** Analyte concentrations of Samples 1, 2, and 3 in urine.

| Sample | Gabapentin Concentration (µg/mL) | Amphetamine Concentration (µg/mL) |
|---|---|---|
| **1** | 0.1 | 0.1 |
| **2** | 250 | 0.1 |
| **3** | 500 | - |

**Table II.** Method 1 instrument conditions.

| | | | |
|---|---|---|---|
| **Column** | Raptor Biphenyl 50 x 2.1 mm, 2.7 µm | | |
| **Guard** | Raptor Biphenyl EXP Guard Cartridge, 5 x 2.1 mm, 2.7 µm | | |
| **Mobile Phase A** | 0.1% formic acid, water | | |
| **Mobile Phase B** | 0.1% formic acid, methanol | | |
| **Column Temperature** | 45°C | | |
| **Diluent** | 90:10 MPA:MPB (v/v) | | |
| **Injection Volume** | 5 µL | | |
| **Flow Rate** | 0.6 mL/min | | |
| **Gradient** | Time (min) | %A | %B |
| | 0.00 | 90 | 10 |
| | 6.00 | 25 | 75 |
| | 7.00 | 0 | 100 |
| | 8.00 | 0 | 100 |
| | 8.01 | 90 | 10 |
| | 9.00 | 90 | 10 |

**Table III.** Method 2 instrument conditions.

| | | | |
|---|---|---|---|
| **Column** | Raptor Biphenyl 100 x 2.1 mm, 2.7 µm | | |
| **Guard** | Raptor Biphenyl EXP Guard Cartridge, 5 x 2.1 mm, 2.7 µm | | |
| **Mobile Phase A** | 10 mM ammonium formate, water | | |
| **Mobile Phase B** | 0.1% formic acid, 90:10 methanol:2-propanol (v/v) | | |
| **Column Temperature** | 45°C | | |
| **Diluent** | 90:10 MPA:MPB (v/v) | | |
| **Injection Volume** | 2 µL | | |
| **Flow Rate** | 0.5 mL/min | | |
| **Gradient** | Time (min) | %A | %B |
| | 0.00 | 90 | 10 |
| | 7.00 | 25 | 75 |
| | 9.00 | 0 | 100 |
| | 10.00 | 0 | 100 |
| | 10.01 | 90 | 10 |
| | 11.00 | 90 | 10 |

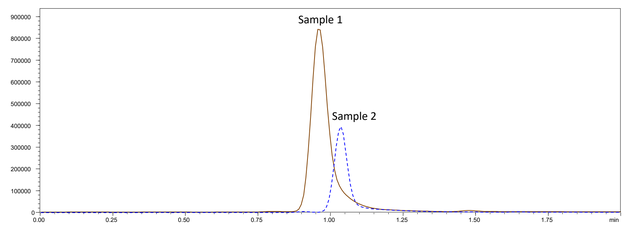
## Results

### Method 1

*Amphetamine*

Sample 1 (0.1 µg/mL of gabapentin/amphetamine) and Sample 2 (250 µg/mL of gabapentin/0.1 µg/mL of amphetamine) were analyzed using Method 1. When compared, the peak height and area for amphetamine are significantly lower in Sample 2 than in Sample 1, indicating that under these conditions, the signal of amphetamine is suppressed by the high concentration of gabapentin.

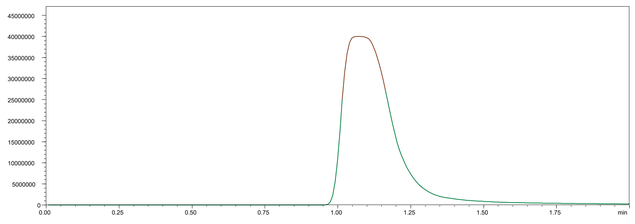
**Figure 1.** Amphetamine in Sample 1 and Sample 2, analyzed using Method 1.




*Gabapentin*

Sample 3 (500 µg/mL of gabapentin) was analyzed using Method 1. The high concentration of gabapentin results in a wide peak that is tailing significantly. The apex of the peak is beginning to flatten, indicating that the detector is being overloaded.

**Figure 2.** Gabapentin in Sample 3, analyzed using Method 1.

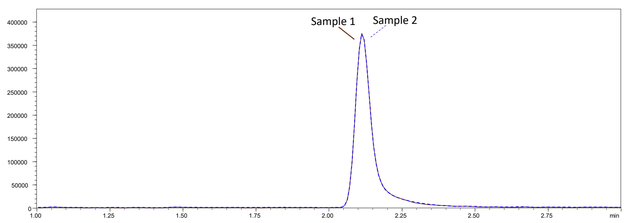



### Method 2

*Amphetamine*

Sample 1 (0.1 µg/mL of gabapentin/amphetamine) and Sample 2 (250 µg/mL of gabapentin/0.1 µg/mL of amphetamine) were analyzed using Method 2. When compared, the peak height and area for amphetamine are consistent in Sample 1 and Sample 2, despite the high concentration of gabapentin in Sample 2. This indicates that under these conditions, gabapentin is sufficiently separated from amphetamine so that its signal is not being suppressed.

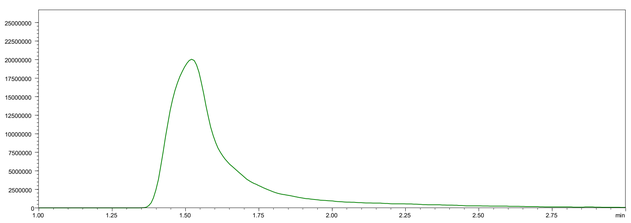
**Figure 3.** Amphetamine in Sample 1 and Sample 2, analyzed using Method 2.




*Gabapentin*

Sample 3 (500 µg/mL of gabapentin) was analyzed using Method 2. Under these method conditions, the peak shape of gabapentin has improved compared to those used in Method 1 (Figure 4). The peak is not as wide, and the tailing has significantly improved. The collision energy was deoptimized for gabapentin to mitigate detector saturation.

**Figure 4.** Gabapentin in Sample 3, analyzed using Method 2.

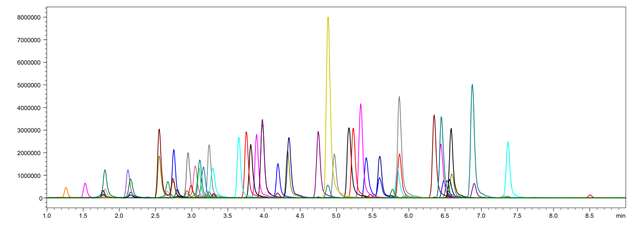



Disclosure: I have (or a member of my immediate family has) a financial relationship with a company as defined in the AACC policy on potential bias or conflict of interest.

## Results

The redeveloped method included 60 total compounds, for a comprehensive analysis of drugs of abuse in urine. In addition to resolving the interference between gabapentin and amphetamine, all isobars in the analyte list had a resolution of 1.5 or better. Isobaric compounds are highlighted in the table below.

**Figure 5.** 500 ng/mL all analytes in urine, analyzed using Method 2.




| Analyte | RT (min) | Analyte | RT (min) | Analyte | RT (min) |
|---|---|---|---|---|---|
| Pregabalin | 1.26 | Desmethylvenlafaxine | 3.30 | Desmethyldoxepin | 5.60 |
| Gabapentin | 1.53 | N-Desmethyltapentadol | 3.65 | Haloperidol | 5.77 |
| Morphine | 1.78 | Norfentanyl | 3.75 | Dextromethorphan | 5.84 |
| Oxymorphone | 1.80 | Benzoylecgonine | 3.82 | PCP | 5.86 |
| Amphetamine | 2.12 | Hydroxybupropion | 3.89 | Fentanyl | 5.87 |
| Hydromorphone | 2.15 | Tramadol | 3.97 | Norfluoxetine | 5.93 |
| Methamphetamine | 2.55 | Meprobamate | 4.19 | EDDP | 6.35 |
| Noroxycodone | 2.66 | Norketamine | 4.20 | Trazodone | 6.45 |
| Phentermine | 2.74 | Normeperidine | 4.32 | Cyclobenzaprine | 6.46 |
| O-Desmethyltramadol | 2.75 | Zolpidem carboxylic acid | 4.34 | Nortriptyline | 6.48 |
| Norhydrocodone | 2.80 | Venlafaxine | 4.75 | Lorazepam | 6.52 |
| Codeine | 2.92 | 7-aminoclonazepam | 4.88 | Buprenorphine | 6.55 |
| MDMA | 2.95 | 4'-hydroxy nitazene | 4.89 | Amitriptyline | 6.56 |
| 6-Acetylmorphine | 2.96 | Norbuprenorphine | 4.92 | Sufentanil | 6.58 |
| Oxycodone | 3.05 | 7-Hydroxyquetiapine | 4.96 | Oxazepam | 6.60 |
| Ritalinic acid | 3.12 | 9-Hydroxyrisperidone | 5.17 | Methadone | 6.88 |
| Naloxone | 3.13 | LSD | 5.23 | α-Hydroxyalprazolam | 6.90 |
| Naltrexone | 3.15 | Acetyl fentanyl | 5.34 | Dehydro aripiprazole | 7.31 |
| 6-β-Naltrexol | 3.24 | Mirtazapine | 5.41 | Temazepam | 7.36 |
| Hydrocodone | 3.29 | Citalopram | 5.46 | Δ9-THC-COOH | 8.50 |

## Discussion

Several strategies were employed to improve the performance of gabapentin at high concentrations and to mitigate interference with amphetamine.

Column Length—Method 1 used a 50 mm column, while Method 2 used a 100 mm column. Longer column lengths provide more resolving power over shorter lengths, which allowed for more chromatographic space between gabapentin and amphetamine.

Mobile Phase Additives—Method 1 used formic acid as a mobile phase additive, while Method 2 used ammonium formate. Switching from formic acid to ammonium formate affected the elution order of early eluting compounds, allowing gabapentin to elute before amphetamine. This mitigated much of the suppression of amphetamine by high concentrations of gabapentin.

Injection Volume--To improve the performance of gabapentin, the injection volume was decreased from 5 µL in Method 1 to 2 µL in Method 2. This allowed for improved peak shape for gabapentin, even at high concentrations.

Carryover—Significant carryover was observed from gabapentin. 2-propanol was added to Mobile Phase B in Method 2 to help mitigate carryover by washing contaminants off the analytical column more efficiently.

## References

1. Heltsley, Rebecca & Depriest, Anne & Black, David & Robert, Tim & Caplan, Yale & Cone, Edward. (2011). Urine Drug Testing of Chronic Pain Patients. IV. Prevalence of Gabapentin and Pregabalin. Journal of analytical toxicology. 35. 357-9. 10.1093/anatox/35.6.357.
2. Ana Celia Muñoz-Muñoz, Teresa Pekol, Dana Schubring, Robin Hyland, Charlene Johnson, Lawrence Andrade, Characterization of an Amphetamine Interference from Gabapentin in an LC–HRMS Method, Journal of Analytical Toxicology, Volume 44, Issue 1, January 2020, Pages 36–40, https://doi.org/10.1093/jat/bkz046