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POSTER NOTE 65078

Troubleshooting LC-MS/MS Peak Shape and Recovery Problems for Polar Opiates

PROBLEM

While supporting multiple research laboratories developing reversed-phase LC-MS/MS methods to analyze diluted urine samples for polar opiates such as morphine, normorphine, hydromorphone and morphine glucuronides, we encountered several instances of peak fronting, peak splitting as well as poor recoveries as some of the analyte molecules eluted as “breakthrough” peaks far ahead of their expected retention times.

METHOD INFORMATION

All methods utilized

- 10 µL injections of calibrators (drug-free urine spiked with analytes and diluted 1:10 with water)
- Thermo Scientific™ Prelude™ SPLC system
- Thermo Scientific™ TSQ Endura™ mass spectrometer with heated electro-spray interface

Most methods utilized

- Columns (50 x 2.1 mm) packed with 2.6 um superficially-porous silica particles with a Phenomenex Kinetics Biphenyl bonded phase (Phenomenex Inc., Torrance, CA)
- Mobile phase gradients from water to methanol, both containing either 0.05% or 0.1% formic acid
- Autosampler Wash 1: water + 0.1% formic acid; Wash 2: 40% acetonitrile + 40% iso-propanol + 20% acetone

One method utilized

- Columns (50 x 2.1 mm) packed with 2.6 um superficially-porous silica particles with a pentafluorophenyl (PFP) bonded phase (Thermo Scientific™ Accucore™ PFP)
- Mobile phase gradient from water to methanol, both containing 0.1% formic acid
- Autosampler Wash 1: water + 2% acetonitrile + 0.1% formic acid; Wash 2: 40% acetonitrile + 40% iso-propanol + 20% acetone

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TROUBLESHOOTING STEPS

1. Changed starting gradient conditions from 5 or 10% methanol to 100% aqueous mobile phase. Figure 1 shows how the elution and shapes of polar opiates changed by this step.
2. Ensured complete column equilibration with 100% aqueous mobile phase. Figures 2 and 3 show column pressure traces typical of insufficient and sufficient equilibration.
3. Changed autosampler method by introducing an air gap between washes and samples.
4. Ensured that no residual Wash 2 remained in injection ports after washes. Excessive needle gaps during rinses with organic wash caused peak problems.

Figure 4 shows improved autosampler method with air gap and needle rinse parameters that further reduced peak problems.

RESULTS

Figure 1. Peak changes for breakthrough (Brkth) normorphine (NM), morphine (M), hydromorphone (HM), morphine-3-β-glucuronide (M-3β-Gluc) & morphine-3-β-glucuronide (M-6β-Gluc) eluted by 5% methanol vs 100% aqueous through PFP column.

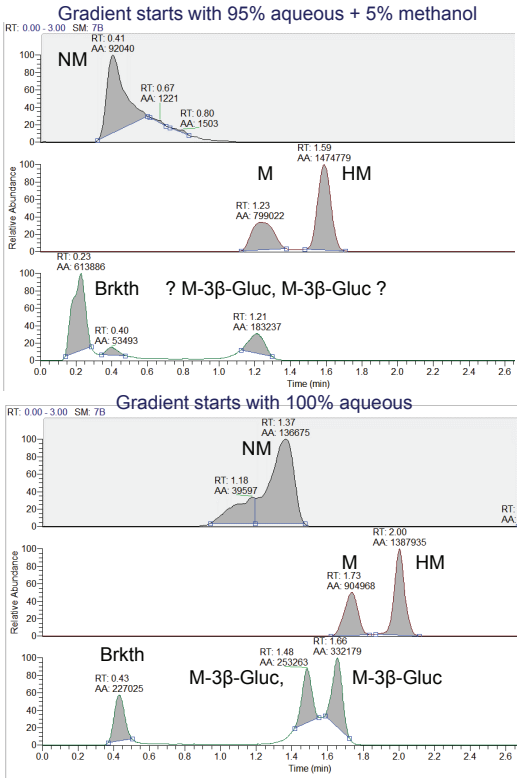


Figure 2. PFP column pressure trace showing insufficient equilibration.

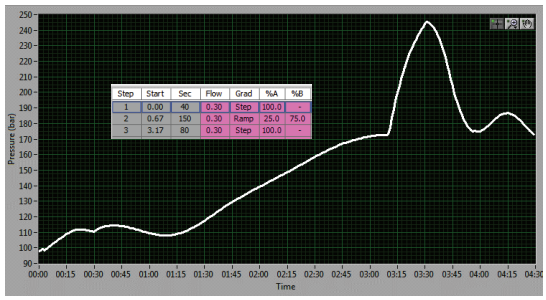


Figure 3. PFP column pressure trace showing sufficient equilibration.

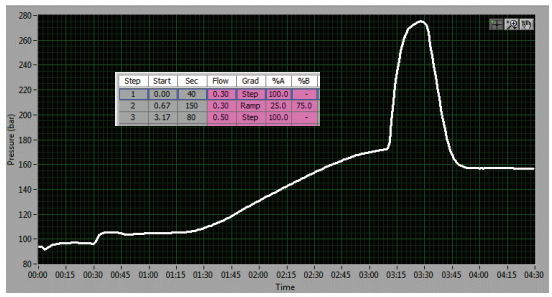
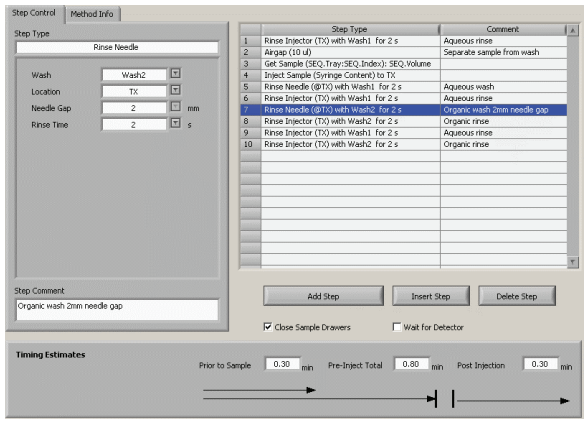


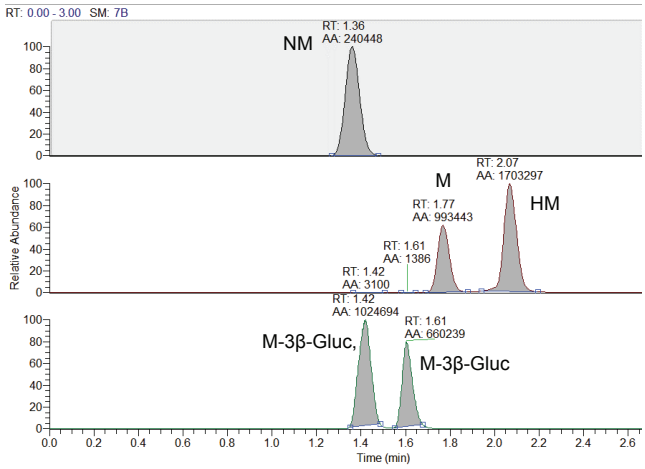
Figure 4. Autosampler method with air gap and organic needle wash parameters that remedied peak-shape problems.



OUTCOME

Our investigations revealed how the chromatographic behavior of polar opiates in both PFP and Biphenyl columns were very sensitive to small amounts of organic solvent in the mobile phase during injection as well as in the injected sample. Clinical research methods similar to that of Sartori et al (1) can be optimized to avoid peak anomalies and provide robust and rapid performance (Figure 5).

Figure 5. Reproducible peak shapes, retention and area counts for polar opiates from PFP column.



Similar results were experienced in other labs which used Biphenyl columns.

SUMMARY

Symmetrical peaks with reproducible retention times and recoveries for polar opiates were achieved when adequately equilibrating PFP and Biphenyl columns with 100% aqueous mobile phase and preventing introduction of organic solvent into the sample before and during the injection. Not only must the sample be free of organic solvent, it must not touch any residual organic solvent from the autosampler washes during injection.

REFERENCES

1. D. Sartori, T. Lewis, A. Breaud & W. Clarke, The development of a high-performance liquid chromatography-tandem mass spectrometric method for simultaneous quantification of morphine, morphine-3-β-glucuronide, morphine-6-β-glucuronide, hydromorphone and normorphine in serum. Clin. Biochem. 2015. 48:1283-1290.

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