

# Simplify and Speed Up Opiates Analysis

Using LC/MS/MS & an Allure® PFP Propyl HPLC Column

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- 7-minute analysis time, for increased sample throughput.
- Faster sample prep—no derivatization required.
- Separate compounds with similar mass spectra.

Opiates are one of the primary drug classes tested in clinical and forensic laboratories, and most confirmation methods use GC/MS. These methods require derivatization of the target compounds, which significantly lengthens sample preparation time. Here we present an alternative confirmation method, using LC/MS/MS, which can increase sample throughput by eliminating derivatization and shortening analysis time. This procedure also provides accurate confirmation and quantification of compounds that have similar mass spectra, by using an Allure® PFP Propyl column to chromatographically separate compounds that share product ions, allowing positive identification based on retention time.

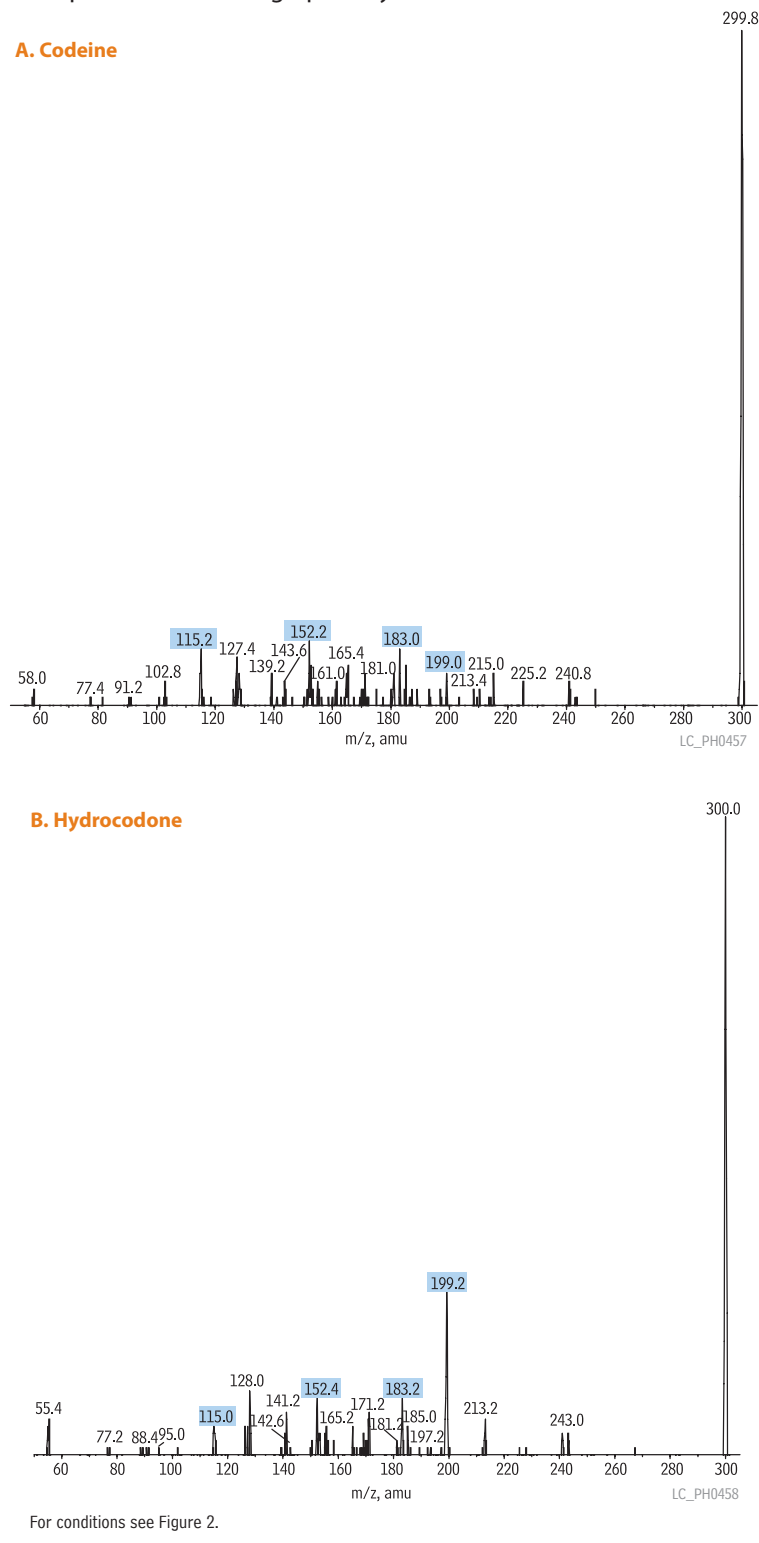
In developing this LC/MS/MS method for the analysis of opiates, our goals were to obtain baseline resolution of compounds having similar mass spectra while providing an analysis time of less than 10 minutes. To accomplish this, mass spectrometer conditions, column selection, mobile phase, and gradient profiling were evaluated and optimized. Several different stationary phases initially were evaluated including an aqueous C18, a biphenyl, a propyl cyano, and a pentafluorophenyl propyl stationary phase. Consistent column dimensions and base silica (50mm, 2.1mm ID, 5µm particle size, and 60Å pore size) were used for all phases; mobile phase conditions were optimized for each stationary phase. Mobile phases tested included: 0.1% formic acid in water, 0.1% formic acid in acetonitrile, and 0.1% formic acid in methanol in various combinations. A variety of gradient profiles also were evaluated.

**Table I** +MRM Transitions for Opiates.

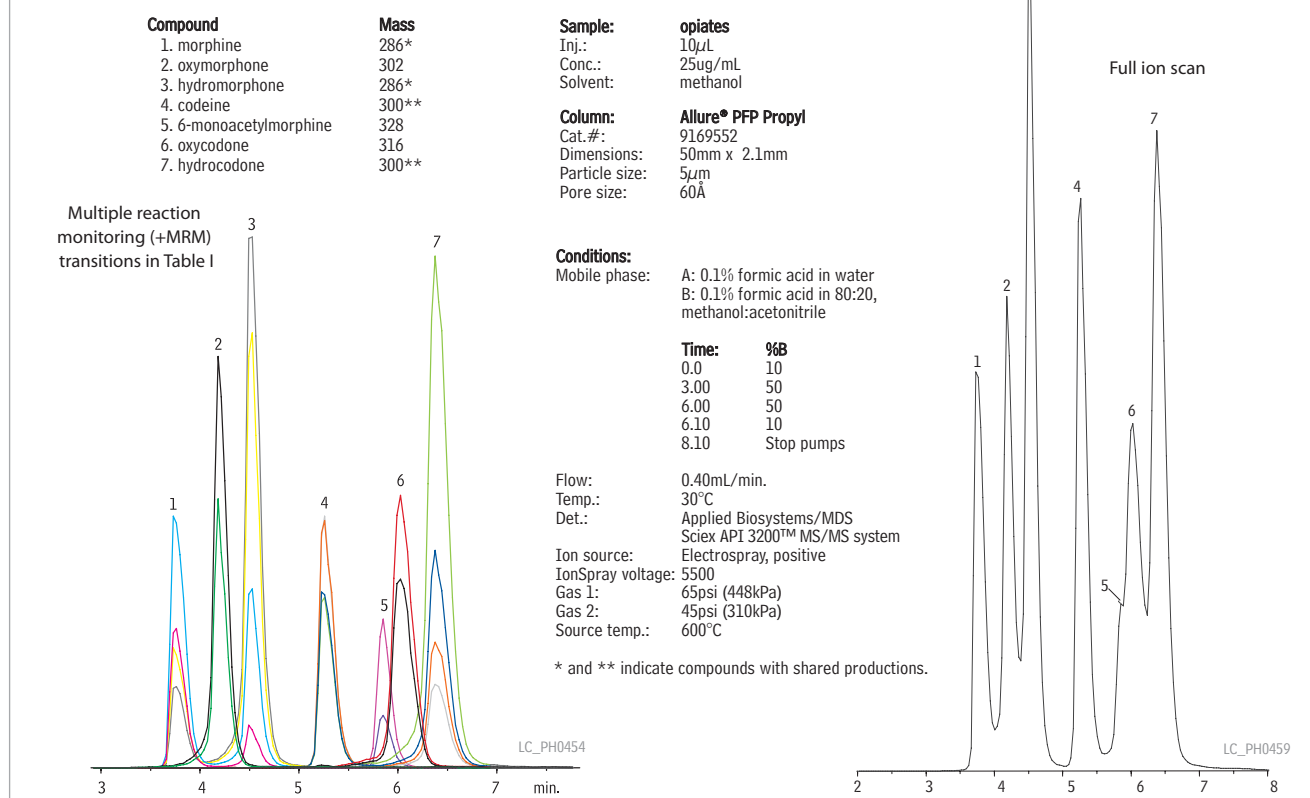
**Mass Spectrometer Experiments:**

| Compound             | Q1  | Q3  | Declustering Potential (V) | Collision Energy (V) |
|----------------------|-----|-----|----------------------------|----------------------|
| morphine             | 286 | 152 | 46                         | 79                   |
| morphine             | 286 | 165 | 46                         | 51                   |
| hydromorphone        | 286 | 185 | 46                         | 41                   |
| hydromorphone        | 286 | 157 | 46                         | 55                   |
| oxymorphone          | 302 | 227 | 36                         | 37                   |
| oxymorphone          | 302 | 198 | 36                         | 55                   |
| codeine              | 300 | 152 | 46                         | 85                   |
| codeine              | 300 | 115 | 46                         | 89                   |
| hydrocodone          | 300 | 199 | 46                         | 39                   |
| hydrocodone          | 300 | 128 | 46                         | 69                   |
| oxycodone            | 316 | 240 | 31                         | 39                   |
| oxycodone            | 316 | 256 | 31                         | 33                   |
| 6-monoacetylmorphine | 328 | 211 | 51                         | 55                   |
| 6-monoacetylmorphine | 328 | 193 | 51                         | 35                   |

**Figure 1** Codeine and hydrocodone share product ions and must be separated chromatographically.



**Figure 2** Fully resolve opiates with shared product ions on (morphine/hydromorphone and codeine/hydrocodone) an Allure® PFP Propyl column.



After mass spectrometry conditions were optimized for each compound, the resulting mass spectra were used to generate +MRM (multiple reaction monitoring) methods. Since MS/MS was used, we were able to target two +MRM transitions per compound to verify the identity of each compound. Table I shows the +MRM transitions and the mass spectrometer conditions. Standards contained morphine, hydromorphone, oxymorphone, codeine, hydrocodone, oxycodone, and 6-monoacetylmorphine (6-MAM) in methanol. The on-column concentration used for column evaluations was 250ng for all compounds.

Although two +MRM transitions were targeted for each compound, some compounds, such as codeine and hydrocodone, shared all monitored product ions (Figure 1). Since these compounds have similar mass spectra, two peaks appear in the extracted ion chromatograms. This made it necessary to separate codeine and hydrocodone chromatographically and identify compound peaks by retention time. Morphine and hydromorphone present the same challenge. Of the stationary phases tested, pentafluorophenyl propyl phase (Allure® PFP Propyl column) produced the best chromatographic separation and peak shape. Baseline resolution of opiates that shared the same product ions was achieved on an Allure® PFP Propyl column in a total analysis time of 7 minutes (Figure 2). Mobile phase gradient and composition had a significant effect on peak shape and resolution (data not shown) and optimized analytical conditions were used.

The Allure® PFP Propyl column, coupled with an LC/MS/MS, produced positive identification of opiates while reducing sample preparation time and keeping analysis time short. Use of the Allure® PFP Propyl column and the LC/MS/MS method shown here can increase sample throughput and is recommended for routine opiates analysis.

#### Acknowledgement

The authors wish to thank Applied Biosystems for supplying the Applied Biosystems/MDS Sciex API 3200™ MS/MS system used for this work.

### Allure® PFP Propyl Columns (USP L43) Excellent Columns for LC/MS and ELSD

#### Physical Characteristics:

particle size: 5µm, spherical  
 pore size: 60Å  
 carbon load: 17%

endcap: fully endcapped  
 pH range: 2.5 to 7.5  
 temperature limit: 80°C

| 5µm Column, 2.1mm                 | cat. #          |
|-----------------------------------|-----------------|
| 50mm                              | 9169552         |
| 50mm (with Trident Inlet Fitting) | 9169552-700     |
| Guard Cartridges                  | qty. cat. #     |
| 10 x 2.1mm                        | 3-pk. 916950212 |
| 10 x 4.0mm                        | 3-pk. 916950210 |
| 20 x 2.1mm                        | 2-pk. 916950222 |
| 20 x 4.0mm                        | 2-pk. 916950220 |

### Exempted Drug of Abuse Reference Materials: Opiates & Metabolites

Concentration is µg/mL. Volume is 1mL/ampul.

| Compound      | CAS#       | Solvent |       |       |
|---------------|------------|---------|-------|-------|
|               |            | Code    | Conc. | cat.# |
| codeine       | 76-57-3    | PTM     | 1,000 | 34000 |
| hydrocodone   | 34195-34-1 | PTM     | 1,000 | 34002 |
| hydromorphone | 71-68-1    | PTM     | 1,000 | 34063 |
| morphine      | 6211-15-0  | PTM     | 1,000 | 34006 |
| oxycodone     | 124-90-3   | PTM     | 1,000 | 34007 |
| oxymorphone   | 76-41-5    | PTM     | 1,000 | 34065 |

PTM=purge & trap grade methanol.

For a full product listing for these columns and reference materials, visit our website at [www.restek.com](http://www.restek.com).