Simplify and Speed Up Opiates Analysis

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

By Kristi Sellers, Innovations Chemist

- 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.

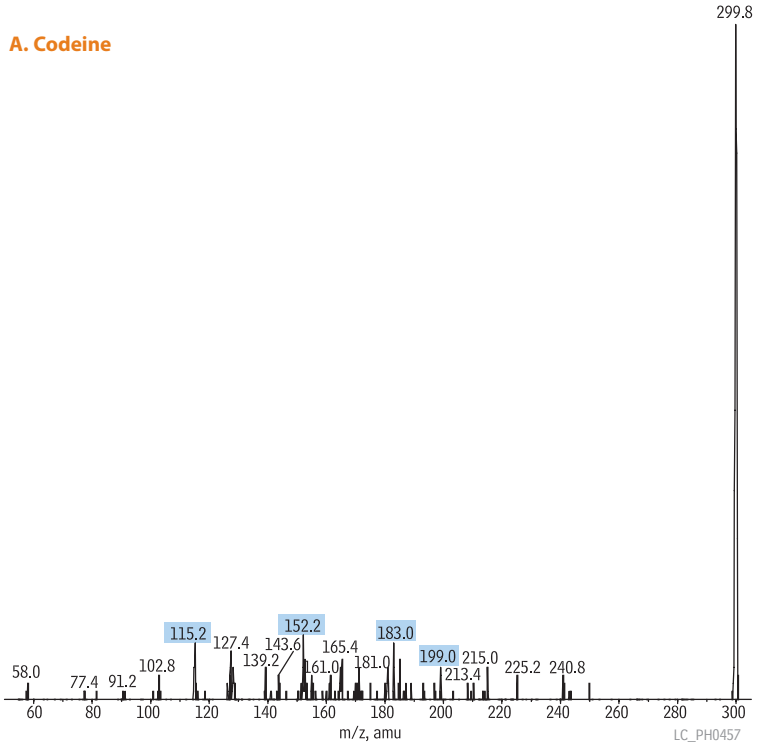
In developing this LC/MS/MS method for the analysis of opiates, our goals were to obtain baseline resolution of compounds having similar mass spectra while providing an analysis time of less than 10 minutes. To accomplish this, mass spectrometer conditions, column selection, mobile phase, and gradient profiling were evaluated and optimized. Several different stationary phases initially were evaluated including an aqueous C18, a biphenyl, a propyl cyano, and a pentaflurophenyl 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.

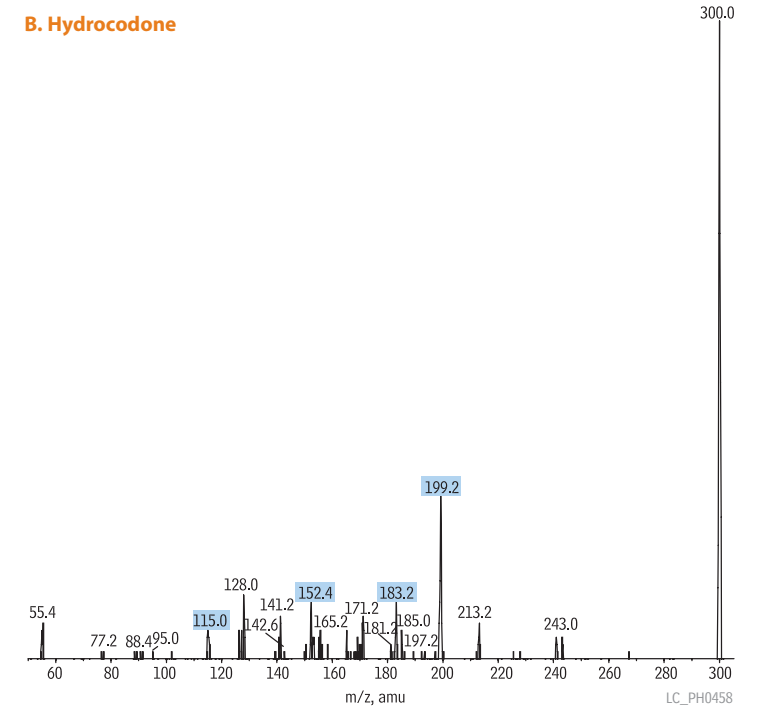
Table I +MRM Transitions for Opiates.

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

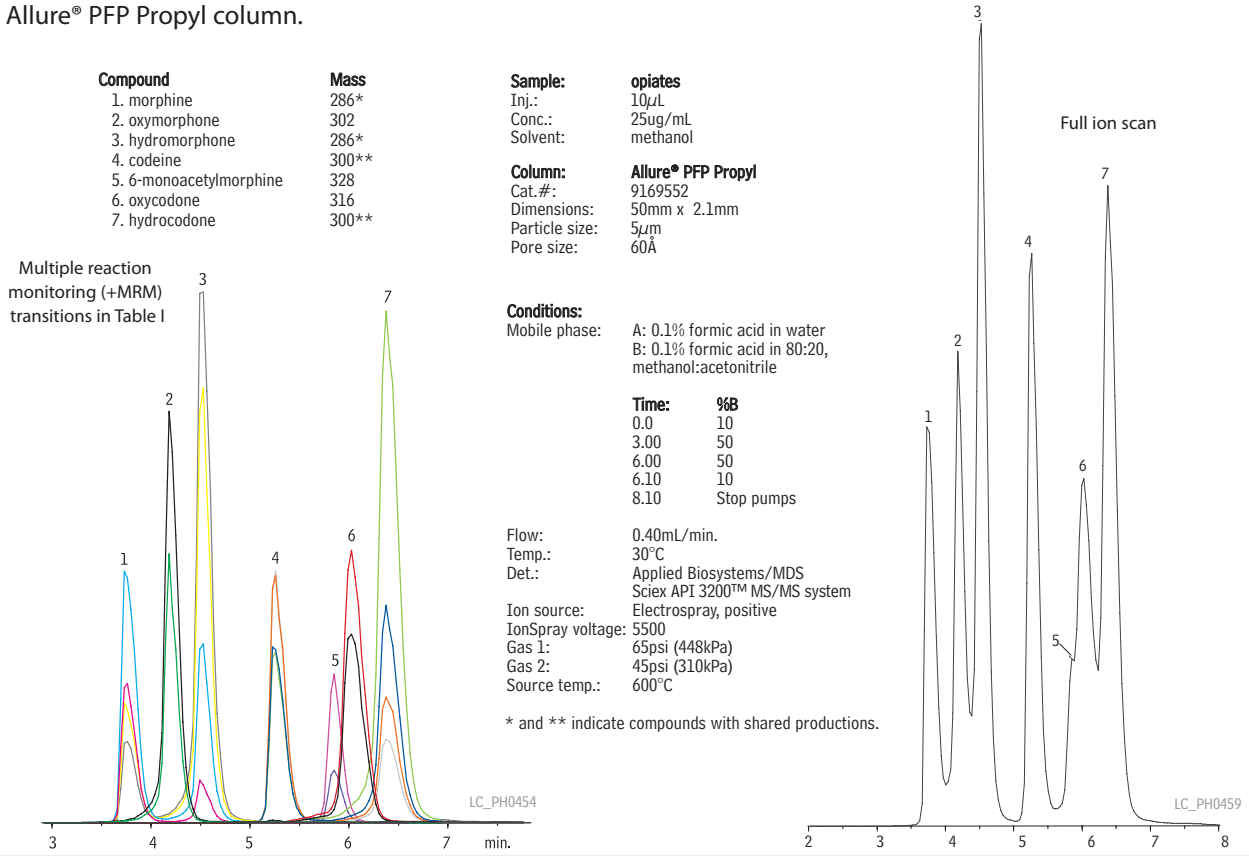
Figure 1 Codeine and hydrocodone share product ions and must be separated chromatographically.





For conditions see Figure 2.

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**.