RESTEK Pharmaceutical

Applicationsnote

Analysis of Narcotics and Narcotic / Acetaminophen Admixtures: What to do When Compendium Methods Don't Work

At some point in their careers, especially if performing raw materials or generic testing analyses for pharmaceuticals, analytical chemists are referred to compendium methodologies, most often to the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), or the British Pharmacopoeia (BP), but occasionally to other volumes. Often the methods described in these compendia provide less than the desired robustness in separation and reproducibility, or the results may be marginal—barely passing system suitability requirements. Sometimes a particular delivery system formulation absolutely will not work with a generic method, due to interference from other ingredients in the sample. Modifications must be made to the problem methodology, and the results compared statistically to the original. To improve analysis efficiency and reduce laboratory supply costs associated with revalidating and testing a method, it may be desirable to create and validate a single analytical method for a wide range of similar drug products.

Many narcotics are very similar in structure, often varying by only a single substitution. Morphine, codeine, hydrocodone, and oxycodone are quite similar, for example (Figure 1). Some of these closely related compounds—all but morphine, in fact—might be blended with other analgesics, such as acetaminophen (APAP). However, USP 25 describes more than 7 different methods to test these raw materials and admixtures, and some of these older raw material methods do not use HPLC as a primary test for purity.

One of the chromatographic applications in USP 25 is for the analysis of oxycodone raw material. After reading the mobile phase section, which lists sulfonic acids, triethylamine, water, phosphoric acid and methanol as the constituents, we saw potential problems with the method, including:

1) The use of methanol in this analysis can lead to high background absorption and loss of linear range, because the analytical wavelength is 206nm, and the UV cutoff point for methanol is 235nm. In extreme cases this also can reduce sensitivity, because the lamp is a finite energy source—the more energy the background absorbs, the less is available to the sample.

2) An ion-pairing agent (heptane sulfonic acid) is introduced into the mobile phase without a proper buffer to maintain pH at a known level. This situation can lead to widened peaks, tailing peaks, and retention time drift. The goal of ion pairing is to create a "neutral" species.

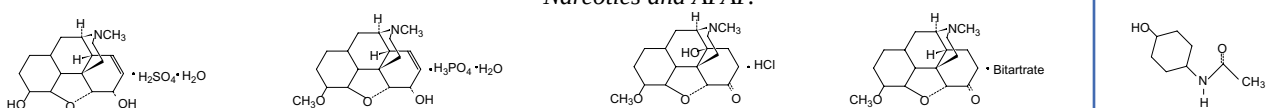
3) TEA modifier is included in the method. When basic compounds are analyzed on older-type HPLC columns, TEA often is added as competing base, to reduce the tailing caused by acidic silanol activity. If the analytical species are neutral, or have been "neutralized" by the addition of an ion-pairing agent, the addition of TEA should have no beneficial effect. Since TEA is a base, adding it to a mobile phase containing sulfonic acids will instantly cause an acid/base neutralization, producing a salt and water and reducing the effective concentration of the acidic ion-pairing agent. This reaction could lead to the formation of undesirable side products in the mobile phase that also will absorb in the low UV range, creating noisy baselines. Furthermore, TEA is volatile, and its composition might change over time if the mobile phase is sparged.

With these concerns in mind, we tested the USP method, using our Ultra and Pinnacle II™ C-8 columns (Application 1).

Initial analyses were performed according to USP 25. The Ultra C8 column gave the better performance at the specified detection wave-

Figure 1

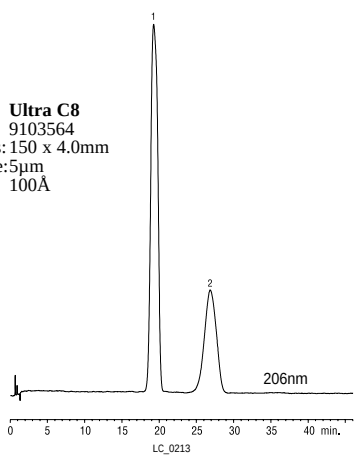
Narcotics and APAP.



Application 1

Oxycodone HCl raw material monograph performed per USP 25.

Column: **Ultra C8**
Catalog #: 9103564
Dimensions: 150 x 4.0mm
Particle size: 5µm
Pore size: 100Å

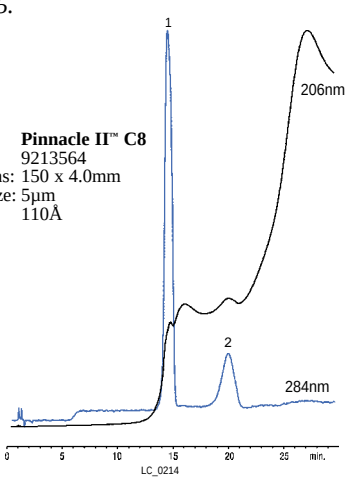


Peak List: **Conc.** (µg/mL)
1. codeine phosphate 13
2. oxycodone HCl 9

Sample:
Inj.: 10µL
Sample: resolution solution
Solvent: mobile phase

Conditions:
Mobile phase: A: 5mM hexane sulfonic acid with 2mL TEA and 3mL of phosphoric acid per liter. pH adjusted to 2.5
B: methanol
(90A:10B, v/v)
Flow: 1.5mL/min
Temp.: 40°C
Det.: UV @ 206 and 284nm

Column: **Pinnacle II™ C8**
Catalog #: 9213564
Dimensions: 150 x 4.0mm
Particle size: 5µm
Pore size: 110Å



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length, 206nm. The Pinnacle II™ C8 column performed well at 284nm, another wavelength commonly used for analyzing narcotics. At 206nm the Pinnacle II™ C8 column exhibited significant noise and took excessive time to equilibrate, due to the competing mobile phase constituents. A longer equilibration might have solved the problem. Both columns meet the criteria—with the exception of the wavelength change with the Pinnacle II™ column—for USP 25 system suitability.

Overall both columns behaved extremely well in performing the USP 25 oxycodone HCl raw material method. However many aspects of the method appear redundant and might actually be compromising the separation. In addition, some of the reagents, such as TEA, might not be necessary for modern columns. The fewer reagents a mobile phase contains, the smaller the control that should be needed to affect a robust and practical separation. After performing the USP 25 method as written, we made some tests to determine actual needs to achieve the system suitability requirements as specified.

The first step in simplifying a convoluted analysis is to apply the KISS principle (Keep it Simple, Scientist!). With peak shape, separation, and proper analytical technique in mind, we attempted to eliminate some of the perceived problems. We realized that by using 284 nm as the detection wavelength we might not see some impurities, but in real life the material should be tested against some known source for potency. Note that with the additional reagents removed, both columns provided good results at the 206nm wavelength.

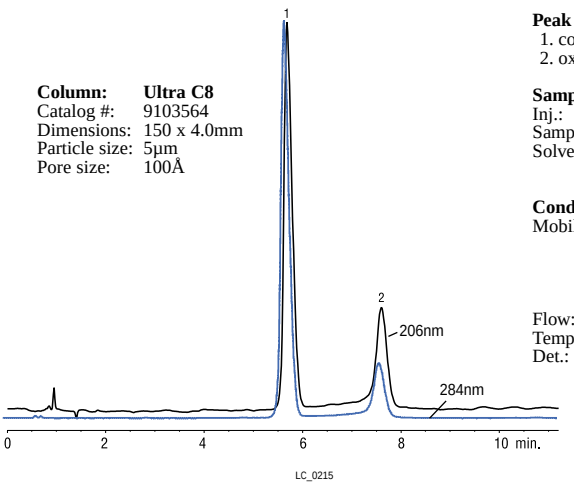
Next we removed the ion pairing agent and the TEA. We elected to keep a 20 mmolar phosphate buffer system to maintain a pH of 2.5. Then we reduced the temperature to 27°C. This reduced fluctuations in retention time caused by changes in air temperature (i.e., air conditioning), and prevented the increase in mass transfer and solubility in the mobile phase from masking other potential problems. The temperature change also helped promote column longevity; phosphate buffers tend to dissolve silica more readily at higher temperatures.

These changes led to a slight increase in tailing for all compounds on both columns, but the difference was acceptable, especially because the run time for the analysis was reduced by a factor of 3 and resolution was improved by 59% to 79%(Application 2). The system passed the system suitability requirements set forth in the USP monograph.

In the next experiment, we re-introduced the ion pair reagent hexane

Application 2

Ion pair agents and TEA removed—run time decreases 3x and resolution improves.

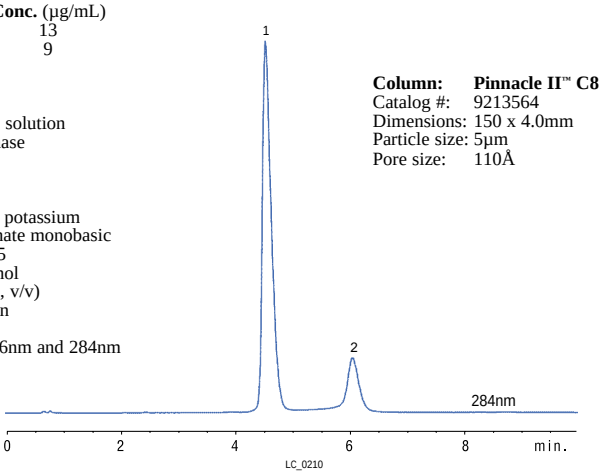


Column: **Ultra C8**
Catalog #: 9103564
Dimensions: 150 x 4.0mm
Particle size: 5µm
Pore size: 100Å

Peak List: **Conc.** (µg/mL)
1. codeine phosphate 13
2. oxycodone HCl 9

Sample:
Inj.: 10µL
Sample: resolution solution
Solvent: mobile phase

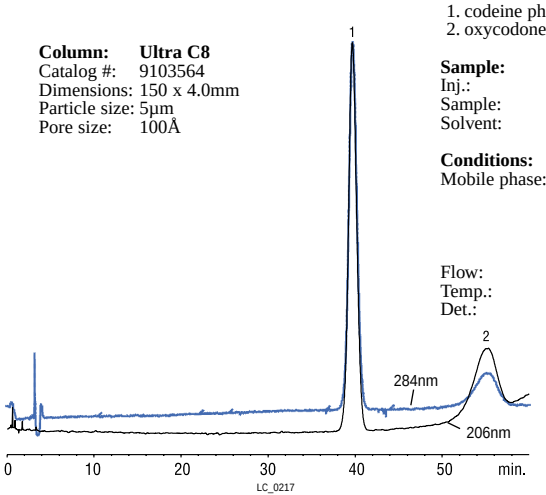
Conditions:
Mobile phase: A: 20mM potassium phosphate monobasic pH=2.5
B: methanol
(90A:10B, v/v)
Flow: 1.5mL/min
Temp.: 27°C
Det.: UV @ 206nm and 284nm



Column: **Pinnacle II™ C8**
Catalog #: 9213564
Dimensions: 150 x 4.0mm
Particle size: 5µm
Pore size: 110Å

Application 3

Hexane sulfonic acid plus a controlled buffer system doubles analysis time.

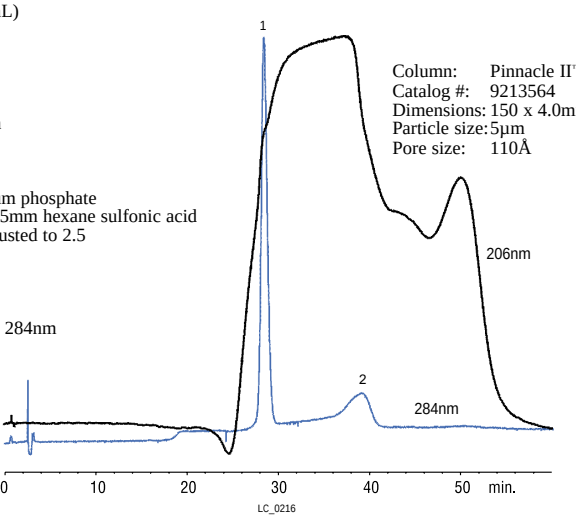


Column: **Ultra C8**
Catalog #: 9103564
Dimensions: 150 x 4.0mm
Particle size: 5µm
Pore size: 100Å

Peak List: **Conc.** (µg/mL)
1. codeine phosphate 13
2. oxycodone HCl 9

Sample:
Inj.: 10µL
Sample: resolution solution
Solvent: mobile phase

Conditions:
Mobile phase: A: 20mM potassium phosphate monobasic and 5mm hexane sulfonic acid in water pH adjusted to 2.5
B: methanol
(90A:10B, v/v)
Flow: 1.5mL/min
Temp.: 27°C
Det.: UV @ 206nm and 284nm



Column: Pinnacle II™ C8
Catalog #: 9213564
Dimensions: 150 x 4.0mm
Particle size: 5µm
Pore size: 110Å

sulfonic acid into the system under the control of the pH 2.5 phosphate buffer system. The run time doubled, demonstrating that TEA did affect the concentration of the ion-pairing agent. Reducing the concentration of ion pairing agent, or using a shorter chain length ion-pairing agent, might have been a better alternative to adding TEA. The system still passed the system suitability requirements listed by the USP, but the chromatogram was much noisier—and equilibration problems returned (Application 3).

In addition to oxycodone HCl raw material, we chromatographed hydrocodone bitartrate raw material, morphine sulfate, and an admixture of hydrocodone bitartrate and acetaminophen. All methods followed USP 25 requirements and all chromatograms passed system suitability requirements (Application 4).

After reviewing the monographs for admixtures containing structurally related narcotics and acetaminophen, we created a single separation for morphine sulfate, acetaminophen, codeine phosphate, oxycodone HCl, and hydrocodone bitartrate. The goal was to create an adequate separation while keeping the method as simple as possible. For this purpose we chose an Ultra C18 column and set detection to 235nm. All components, including a small unknown peak, were separated to baseline (Application 5).

Next, we increased the amount of buffer to 90% (a 5% increase), to determine the effect on the separation. The separation was very sensitive to the amount of buffer present; this simple 5% increase doubled the analysis time. Resolution doubled between most components, with the greatest change between acetaminophen and codeine. The unknown peak disappeared, however, and probably co-eluted with morphine (Application 6).

To further improve resolution for codeine and the other later eluting components, we adjusted the mobile phase ratio to 85:15, buffer:organic solvent, using a 90:10 mixture of acetonitrile and methanol as the organic solvent. Resolution improved, relative to the original mobile phase composition, analysis again was under 10 minutes, and the co-eluting unknown peak returned (Application 7).

In an effort to further improve peak shape, we heated the column to 35°C. The higher temperature reduced the analysis time by one minute, at the sacrifice of resolution between analytes, and with further distortion of the leading baseline for oxycodone and hydrocodone (Application 8). For this analysis, the conditions used to produce (Application 7) provided the most desirable results.

In summary, the goal of any method should be to achieve the most stable and robust separation possible. Too often methods are made more complicated than they need be, perhaps from lack of chromatographic experience or, possibly, to make analysis unnecessarily difficult. Even troubleshooting such methods adds to production costs. When preparing to follow a compendium method always

Application 4

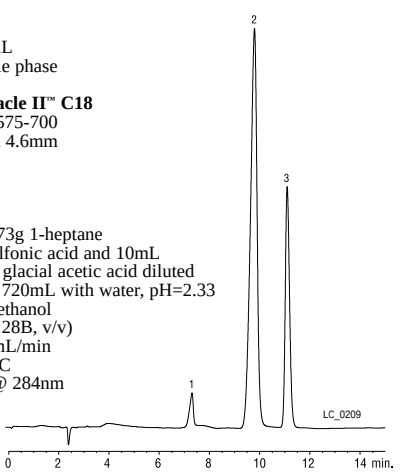
Analyses of morphine sulfate and a hydrocodone bitartrate / acetaminophen admixture according to USP 25

Peak List:	**Conc.** (mg/mL)	**Ret. Time** (min.)	**RRT**	**Tailing**	**Resolution**
1. unknown		7.1			
2. phenol	0.15	9.8	0.88		
3. morphine	0.24	11.0	1.00	1.17	3.2

Sample:
Inj.: 20.0µL
Solvent: mobile phase

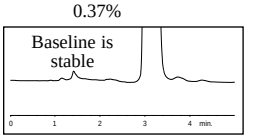
Column: **Pinnacle II™ C18**
Catalog #: 9214575-700
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 110Å

Conditions:
Mobile phase: A: 0.73g 1-heptane sulfonic acid and 10mL of glacial acetic acid diluted to 720mL with water, pH=2.33
B: methanol
(72A:28B, v/v)
Flow: 1.00mL/min
Temp.: 26.5°C
Det.: UV @ 284nm



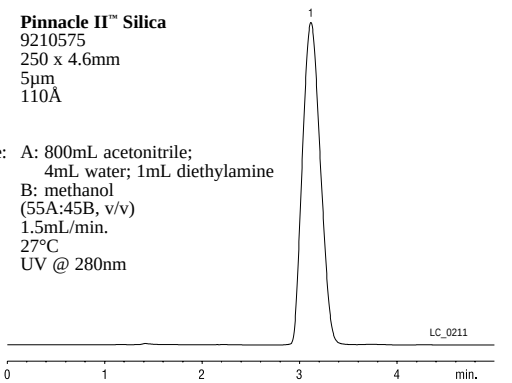
Peak List:	**Ret. Time** (min.)	**%Inj. RSD (n=5)**
1. hydrocodone bitartrate	3.1	0.37%

Sample:
Inj vol: 20µL
Conc.: 1 mg/mL
Sample: assay standard
Solvent: mobile phase



Column: **Pinnacle II™ Silica**
Catalog #: 9210575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 110Å

Conditions:
Mobile phase: A: 800mL acetonitrile; 4mL water; 1mL diethylamine
B: methanol
(55A:45B, v/v)
Flow: 1.5mL/min.
Temp.: 27°C
Det.: UV @ 280nm

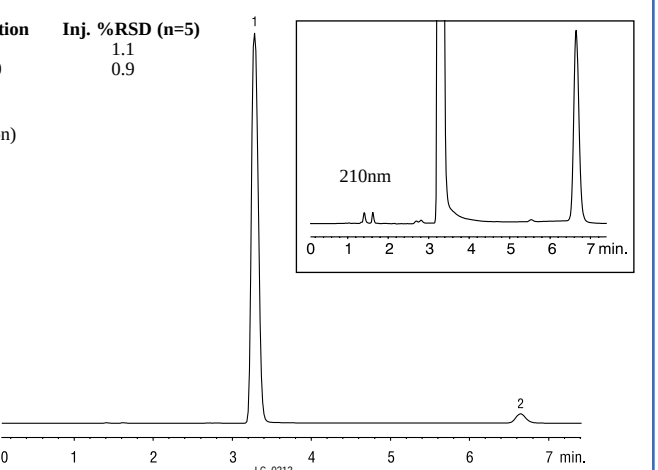


Peak List:	**Conc.** (mg/mL)	**Ret. Time** (min.)	**RRT**	**Tailing**	**Resolution**	**Inj. %RSD (n=5)**
1. acetaminophen	0.76	3.304	1.0	1.1	—	1.1
2. hydrocodone bitartrate	0.0076	6.664	2.0	1.1	17.0	0.9

Sample:
Inj.: 25µL
Sample: 500mg acetaminophen with 5mg hydrocodone bitartrate (standard solution)
Solvent: mobile phase

Column: **Ultra C18**
Catalog #: 9174575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: A: 6.8 g potassium monobasic with 0.2 mL of TEA per liter
B: acetonitrile
(85A:15B, v/v)
Flow: 1.5mL/min.
Temp.: 27°C
Det.: UV @ 210nm (hydrocodone bitartrate) and 295nm (acetaminophen). 210nm channel shown



attempt to determine the reason a reagent would be added to a mobile phase, but any change or modification should have an established scientific purpose. By creating more universal methods for analyses of structurally related compounds, it should be possible to reduce inventory costs for supplies, increase laboratory analysis efficiency, and reduce personnel training time.

Some analysts may have neither the time nor the experience to troubleshoot a problem methodology. If you encounter problems when analyzing your samples according to an established method, our experienced Technical Service chemists will be glad to help. Contact them at 800-356-1688, ext. 4 or 814-353-1300, ext. 4, or contact your Restek representative.

Application 5

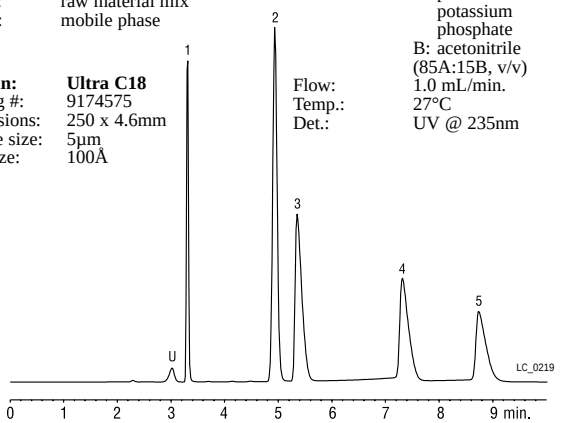
Separation of Narcotic Analgesics and Acetaminophen on Ultra C18.

Peak List:	Conc. (µg/mL)	Ret. Time (min.)	Tailing	Resolution
U. unknown	unknown	3.0	NA	NA
1. morphine sulfate	204	3.3	0.97	2.3
2. acetaminophen	92	5.0	1.1	14.9
3. codeine phosphate	216	5.3	1.8	2.1
4. oxycodone HCl	206	7.3	1.9	6.9
5. hydrocodone bitartrate	218	8.8	1.9	4.1

Sample:
Inj.: 10µL
Sample: raw material mix
Solvent: mobile phase

Column: **Ultra C18**
Catalog #: 9174575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile Phase: A: pH 2.8 10mm potassium phosphate
B: acetonitrile (85A:15B, v/v)
Flow: 1.0 mL/min.
Temp.: 27°C
Det.: UV @ 235nm



Application 6

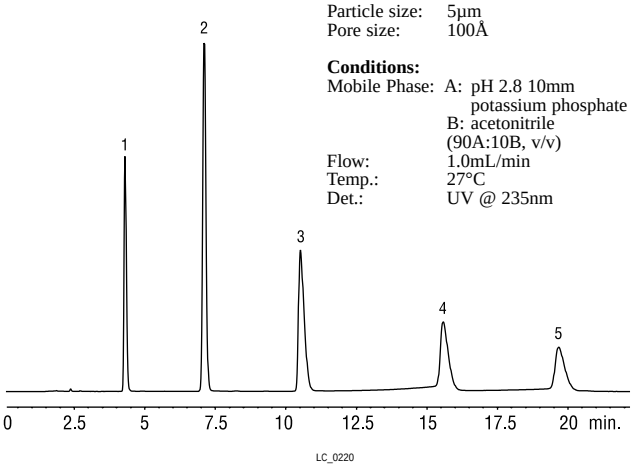
A small (5%) increase in the aqueous buffer doubles resolution and retention.

Peak List:	Conc. (µg/mL)	Ret. Time (min.)	Tailing	Resolution
1. morphine sulfate	204	3.3	1.2	NA
2. acetaminophen	92	5.0	1.1	15.0
3. codeine phosphate	216	5.3	1.7	11.3
4. oxycodone HCl	206	7.3	1.5	11.0
5. hydrocodone bitartrate	218	8.8	1.5	7.0

Sample:
Inj.: 4.0µL
Sample: raw material mix
Solvent: mobile phase

Column: **Ultra C18**
Catalog #: 9174575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile Phase: A: pH 2.8 10mm potassium phosphate
B: acetonitrile (90A:10B, v/v)
Flow: 1.0mL/min
Temp.: 27°C
Det.: UV @ 235nm



Application 7

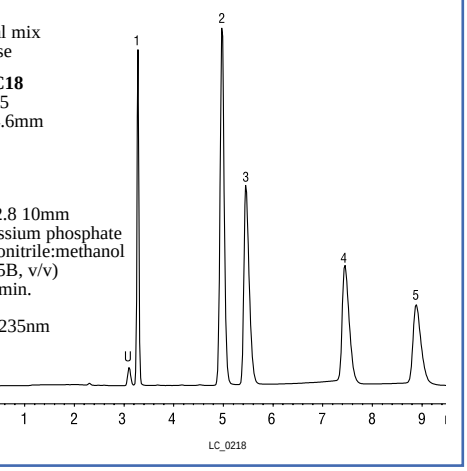
Further alteration of the organic phase improves resolution without increasing analysis time.

Peak List:	Conc. (µg/mL)	Ret. Time (min.)	Tailing	Resolution
U. unknown	unknown	3.1	NA	NA
1. morphine sulfate	204	3.3	1.0	1.8
2. acetaminophen	92	5.0	1.1	14.1
3. codeine phosphate	216	5.5	1.4	2.6
4. oxycodone HCl	206	7.5	1.4	8.5
5. hydrocodone bitartrate	218	8.9	1.4	5.0

Sample:
Inj.: 4.0µL
Sample: raw material mix
Solvent: mobile phase

Column: **Ultra C18**
Catalog #: 9174575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: A: pH 2.8 10mm potassium phosphate
B: acetonitrile:methanol (85A:15B, v/v)
Flow: 1.0mL/min.
Temp.: 27°C
Det.: UV @ 235nm



Application 8

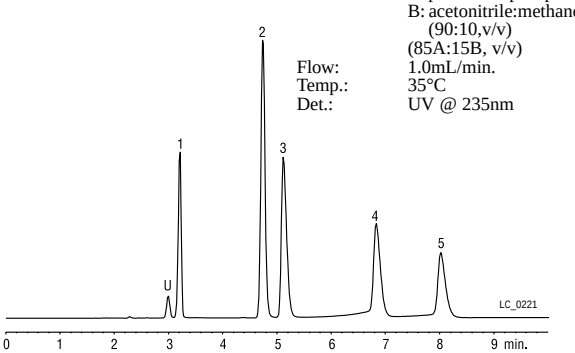
Increasing column temperature has detrimental effects on the chromatography of narcotic / acetaminophen admixtures.

Peak List:	Conc. (µg/mL)	Ret. Time (min.)	Tailing	Res.
U. unknown	unknown	3.0	NA	NA
1. morphine sulfate	204	3.2	0.90	1.9
2. acetaminophen	92	4.8	1.1	12.2
3. codeine phosphate	216	5.1	1.4	2.3
4. oxycodone HCl	206	6.9	1.4	8.2
5. hydrocodone bitartrate	218	8.0	1.4	4.8

Sample:
Inj.: 4.0µL
Sample: raw material mix
Solvent: mobile phase

Column: **Ultra C18**
Catalog #: 9174575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: A: pH 2.8 10mm potassium phosphate
B: acetonitrile:methanol, (90:10,v/v)
(85A:15B, v/v)
Flow: 1.0mL/min.
Temp.: 35°C
Det.: UV @ 235nm



Pinnacle II® C8 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9213531	9213532	9213533	9213535
50mm	9213551	9213552	9213553	9213555
100mm	9213511	9213512	9213513	9213515
150mm	9213561	9213562	9213563	9213565
200mm	9213521	9213522	9213523	9213525
250mm	9213571	9213572	9213573	9213575

Pinnacle II® C8 5µm Columns with Trident® Inlet Fitting

Length	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9213532-700	9213533-700	9213535-700
50mm	9213552-700	9213553-700	9213555-700
100mm	9213512-700	9213513-700	9213515-700
150mm	9213562-700	9213563-700	9213565-700
200mm	9213522-700	9213523-700	9213525-700
250mm	9213572-700	9213573-700	9213575-700

Pinnacle II® C18 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9214531	9214532	9214533	9214535
50mm	9214551	9214552	9214553	9214555
100mm	9214511	9214512	9214513	9214515
150mm	9214561	9214562	9214563	9214565
200mm	9214521	9214522	9214523	9214525
250mm	9214571	9214572	9214573	9214575

Pinnacle II® C18 5µm Columns with Trident® Inlet Fitting

Length	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9214532-700	9214533-700	9214535-700
50mm	9214552-700	9214553-700	9214555-700
100mm	9214512-700	9214513-700	9214515-700
150mm	9214562-700	9214563-700	9214565-700
200mm	9214522-700	9214523-700	9214525-700
250mm	9214572-700	9214573-700	9214575-700

Pinnacle II® Silica 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9210531	9210532	9210533	9210535
50mm	9210551	9210552	9210553	9210555
100mm	9210511	9210512	9210513	9210515
150mm	9210561	9210562	9210563	9210565
200mm	9210521	9210522	9210523	9210525
250mm	9210571	9210572	9210573	9210575

Pinnacle II® Silica 5µm Columns with Trident® Inlet Fitting

Length	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9210532-700	9210533-700	9210535-700
50mm	9210552-700	9210553-700	9210555-700
100mm	9210512-700	9210513-700	9210515-700
150mm	9210562-700	9210563-700	9210565-700
200mm	9210522-700	9210523-700	9210525-700
250mm	9210572-700	9210573-700	9210575-700

Ultra C8 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9103531	9103532	9103533	9103535
50mm	9103551	9103552	9103553	9103555
100mm	9103511	9103512	9103513	9103515
150mm	9103561	9103562	9103563	9103565
200mm	9103521	9103522	9103523	9103525
250mm	9103571	9103572	9103573	9103575

Ultra C18 5µm Columns

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9174531	9174532	9174533	9174535
50mm	9174551	9174552	9174553	9174555
100mm	9174511	9174512	9174513	9174515
150mm	9174561	9174562	9174563	9174565
200mm	9174521	9174522	9174523	9174525
250mm	9174571	9174572	9174573	9174575

Resprep® SPE Syringe Filters

- Solvent-resistant polypropylene housing.
- Better flow characteristics because of a glass fiber prefilter.
- Available in the most popular filter sizes and membrane porosities.
- Available in non-leaching nylon or PTFE.

Filter Diameter	Porosity	qty.	Nylon	PTFE
13mm	0.20µm	100-pk.	26066	26068
13mm	0.45µm	100-pk.	26067	26069
25mm	0.20µm	50-pk.	26070	26072
25mm	0.45µm	50-pk.	26071	26073
25mm	1.00µm	50-pk.	-	26074

Trident® Direct Guard Column System

Unlike "one size fits all" guard systems, the Trident™ Direct system gives you the power to select the right level of protection for your analysis. The system offers three levels of protection and guard cartridges in four dimensions, with a variety of bonded phases to match your analytical column. The economical, leak-free cartridge design provides an unprecedented combination of convenience, economy, and reliability. The foundation of the Trident™ Direct system is a reusable direct connect holder that easily attaches to any HPLC column using CPI- or Waters®-style end fittings.* The system is available in the following configurations to match different protection level needs: in-line filter, in-line filter with holder for 1cm guard cartridge, and in-line filter with holder for 2cm guard cartridge. The guard cartridges are available in 2.1 and 4.0mm ID and are interchangeable within the appropriate length holder.

*Replace tip when using Waters®-style fittings–see our catalog.

Trident® Direct provides three levels of protection

Trident™ Direct high-pressure filter
Protection against particulate matter.
cat.# 25082

Trident™ Direct 1cm guard cartridge holder with filter
Moderate protection against particulate matter and irreversibly adsorbed compounds.
cat.# 25084

Trident™ Direct 2cm guard cartridge holder with filter
Maximum protection against particulate matter and irreversibly adsorbed compounds.
cat.# 25086

RESTEK ISO 9001 REGISTERED