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Applikationsberichtsammlung

Improving Peak Shape for Combination Drug Products Using Charged Surface Hybrid Particle Technology

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Abstract

Fixed dose combination (FDC) drug products are a wide range of available over-the-counter and prescription drugs. The most common FDCs are cold and cough medicine which contain as many as four active pharmaceutical ingredients (API). These drug products present several challenges during development and analysis, including the different concentrations of the APIs in the formulated drug, leading to one analytical peak being overloaded or with high signal, while the lower dose molecules are barely detectable. This necessitates the need for multiple LC methods to accurately analyze all the components without having issues of peak overloading. If the overloading of the higher dose analyte can be mitigated, and the peak shape improved, a single method for detecting all APIs can be developed, saving time and money. Improvements in peak shape for high concentration analytes can be done in a variety of ways.

In this application note, a single analytical method was developed for the APIs in the prescription drug, Contrave, a combination of naltrexone, and bupropion in different doses. Contrave is an FDC to manage chronic obesity, which was approved for use in the United States and other countries starting in 2014. Two different approaches to improve the peak shape of bupropion, the high concentration API, are shown, including the use of alternate mobile phase additives and different stationary phases.

Benefits

- Development of a single analytical method for the FDC drug product, Contrave
- Improved peak shape for high dose bupropion using Charged Surface Hybrid (CSH™) Particles
- Good linearity is achieved for both APIs using the developed method

Introduction

A variety of pharmaceutical products are available over the counter as fixed dose combination (FDC) products. Examples of FDCs include cough syrup, certain allergy medication, and multivitamins. FDCs are created to deliver multiple active ingredients to the consumer to combine benefits for a given purpose. In the case of cough syrups, one ingredient may act as a decongestant, while another reduces fever, and yet another acts as an expectorant. From a consumer standpoint, FDCs are beneficial in that only one capsule or dose of the product is needed. From an analytical science perspective, however, FDCs present real challenges in analysis. In FDCs, it is not uncommon for one or more of the APIs to be in very high concentrations compared to the others present. This is caused by the relative "strength" of each API and the dose required of each to achieve the desired medicinal effect. Having different concentrations of analytes in an analysis can present challenges in quantitation, as attempting to analyze the lower concentration peak can lead to overloading of the higher dosage API. Meanwhile, if the higher dose API is analyzed under certain conditions, the lower dosage APIs may be undetectable via LC-UV analyses.

One product which suffers from this issue is the drug, Contrave, which combines a drug used to manage alcohol and opioid use, naltrexone, with an atypical antidepressant, bupropion. Both drugs are available separately, however, when combined they have been found to curb cravings; and when combined with diet and exercise they have been shown to help with weight loss.[1–3] The combination of naltrexone and bupropion in a extended-release formulation was approved in 2014 in the United States, with subsequent approvals by the European Union and Canada in 2015 and 2018, respectively. The dosage of Contrave is listed as 8 mg naltrexone and 90 mg bupropion per extended-release tablet. The difference in concentrations between the two APIs is 82 mg, which is 10x the concentration of naltrexone, potentially leading to quite the discrepancy in LC-UV signal for the two analytes. This formulation can present some real challenges in analysis, especially considering that both compounds have basic moieties. This application note will examine an assay of a neat standard containing the

same ratio of naltrexone/bupropion as is present in the extended-release tablets to simulate dilution of a tablet for analysis. Improvements to peak shape for bupropion will be examined, and a final method including linearity testing will be shown. A single method was able to be developed for this formulation with good linearity and acceptable peak shape for both APIs present using an ACQUITY™ Premier CSH Phenyl-Hexyl Column on an ACQUITY UPLC™ H-Class Plus System.

Experimental

Sample Description

Two stock solutions at 1 mg/mL for both naltrexone and bupropion in water. Stock solutions were then combined to create a final working sample containing 80 µg/mL naltrexone and 900 µg/mL bupropion using water as the sample diluent. This created a 11.25:1 ratio of bupropion:naltrexone which matches the concentration ratio of the extended-release tablets.

LC Conditions

LC system:	ACQUITY UPLC H-Class Plus System with Quaternary Solvent Manager (QSM) with optional solvent select valve, Sample Manager Flow Through Needle (SM-FTN), Column Manager, Two Column Manager Auxs, and QDa™ mass detector
Detection:	UV at 254 nm
Columns:	ACQUITY Premier BEH™ C_{18}, 2.1 x 50 mm, 1.7 µm (p/n: 186009452) ACQUITY Premier CSH Phenyl-Hexyl, 2.1 x 50 mm, 1.7 µm (p/n: 186009474)

	ACQUITY Premier CSH C_{18}, 2.1 x 50 mm, 1.7 µm (p/n: 186009460) CORTECS™ UPLC C_{18}+, 2.1 x 50 mm, 1.7 µm (p/n: 186007114)
Column temperature:	30 °C
Sample temperature:	10 °C
Injection volume:	1.0 µL
Flow rate:	0.50 mL/min
Mobile phase A:	Milli-Q Water
Mobile phase B:	Acetonitrile
Mobile phase C:	Methanol
Mobile phase D1:	2% Formic acid in water
Screening gradient conditions:	Constant 5% Dx maintained throughout gradient. Linear Gradient of 5–95% B/C in 6.86 minutes, hold for 1.14 minutes. Return to 5% B/C and hold for 2.3 minutes. Total run time: 10.30 minutes. Optimized gradient conditions described in figure captions

Data Management

Chromatography software:	Empower™ 3 Feature Release 4

Results and Discussion

When analyzing fixed dose combination (FDC) drugs, it is common for one active pharmaceutical ingredient (API) to be in much higher concentration than another. This is caused by the required doses being different for the APIs to achieve the desired medicinal effect. For analytical scientists attempting to quantify both compounds in a single run, this difference in concentration can lead to either over-saturation of the higher dose or an inability to detect the lower dose. In the case of the weight loss drug, Contrave, two APIs are present, shown in Figure 1. Bupropion is present at 90 mg, while naltrexone is present at only 8 mg. This is a concentration ratio of 11.25:1 for bupropion:naltrexone. Compounding the differences in concentration, both APIs have basic functional groups, which can present analytical challenges in the form of peak tailing due to secondary interaction between the basic functional group and the base particle. This is especially true at low pH using low ionic strength mobile phases like formic acid.

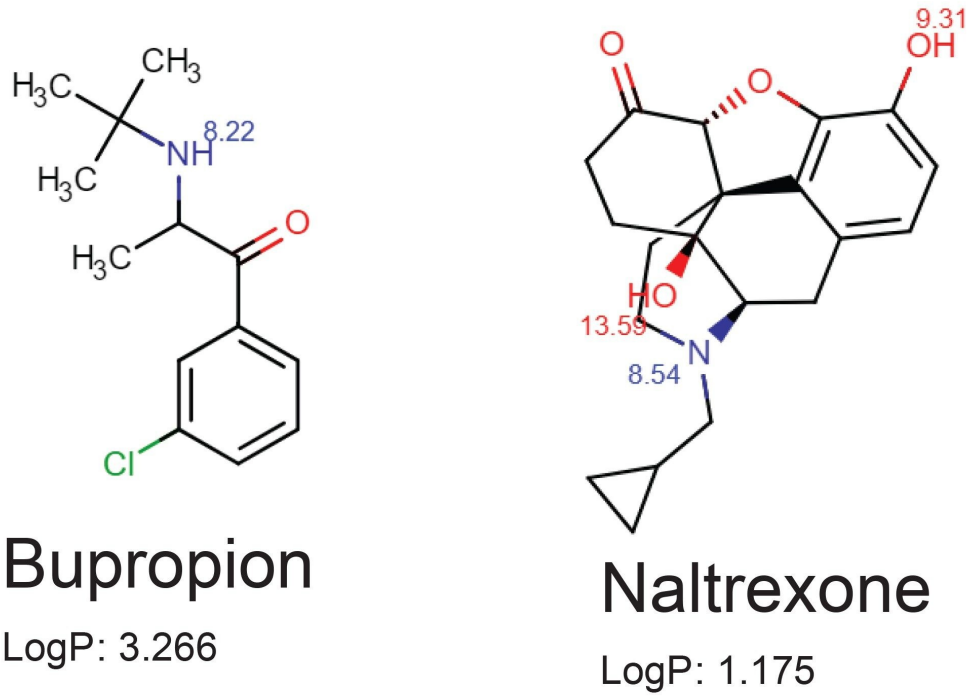

Figure 1. Chemical structures, pKa values, and LogP values for bupropion and naltrexone, the active ingredients in Contrave weight loss drugs.

When analyzing basic compounds at low pH using silica based stationary phases, the charged basic functional groups can interact with residual silanols on the surface of the particle, leading to peak shape distortions. These distortions are augmented at higher concentrations as the peak becomes mass overloaded on the column worsening the peak shape.[4–6] Figure 2 shows a chromatogram of bupropion and naltrexone analyzed at low pH using an ACQUITY Premier BEH C_{18} Column using formic acid-modified mobile phases.

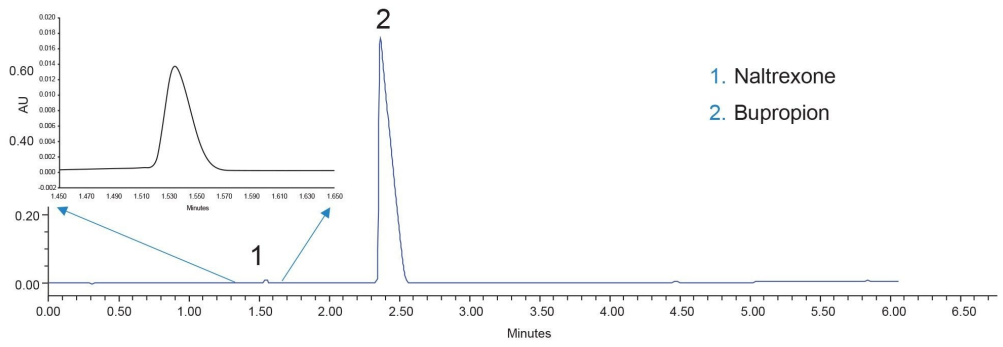

Figure 2. Analysis of naltrexone (1) and bupropion (2) using an ACQUITY Premier BEH C $_{18}$ Column using formic acid-modified mobile phases at the concentrations listed in the method section.

The bupropion peak (2) shows poor peak shape with a “shark fin” indicative of not only mass overloading, but also secondary interaction between the basic functional group of the API and the silanols of the base particle. The peak shape of naltrexone (1) is also showing slight peak asymmetry which would be caused by the secondary interactions between the analyte and base particle, as it is not being overloaded. In either case, while good separation is achieved between these analytes, having better peak shape for both will improve integration quality and therefore quantitation results.

One method for addressing the peak shape concern is to reduce the on-column load. This would reduce the amount of mass loading happening and should improve peak shape. However, for FDCs, reducing the mass load will have an impact on the lower dosage API as well, potentially leading to an inability to detect the peak. As such, for FDCs, reducing mass load is not always possible. Another route to examine is the use of high pH mobile phases using a hybrid particle column like the ACQUITY Premier BEH C_{18} Column, Figure 3.

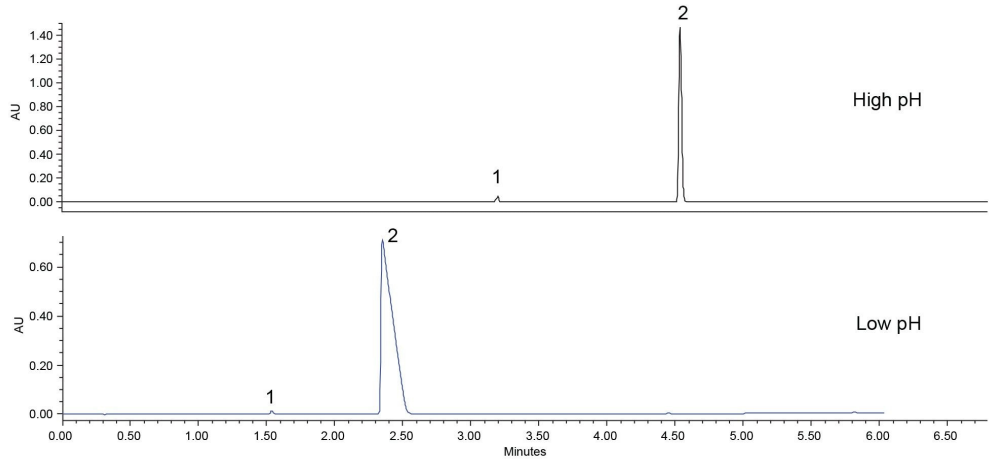

Figure 3. Analysis of naltrexone (1) and bupropion (2) using an ACQUITY Premier BEH C $_{18}$ Column at high pH and low pH.

At high pH, the basic functionality of the analytes is neutral, eliminating the possibility of secondary interactions with the base particle and improving loadability onto the stationary phase. In this example, the bupropion peak at high pH shows much more symmetrical results than at low pH. While changing pH may alleviate the issue, it may not always be appropriate depending on the method being used, or the stability of the compounds.

Another way to improve the peak shape of bupropion is to use a charged base particle, which is specifically designed to reduce secondary interactions between analyte and silanols by employing a slight surface charge. This causes the charged basic analytes to be repelled from the base particle leading to sharper and more symmetrical peaks.[7–8] The CORTECS C_{18}+ Column and the entire CSH Column line all employ a charged base particle in the design, which address the concern of poor peak shape for basic analytes at low pH. The CORTECS C_{18}+ Column is a solid-core silica particle which boasts higher column efficiency compared to fully-porous particles.[9–11] The particle is created with a slight positive charge during the manufacturing process and is then bonded with a full-coverage C_{18} ligand. The CSH family of columns, including the CSH C_{18} Column, CSH Phenyl-Hexyl Column, and CSH PFP Column are all based on hybrid particle technology, providing not only improved peak shape for bases at low pH, but also high pH stability for method development.[7–8, 12–14] Figure 4 shows the analysis of naltrexone and bupropion on four different columns.

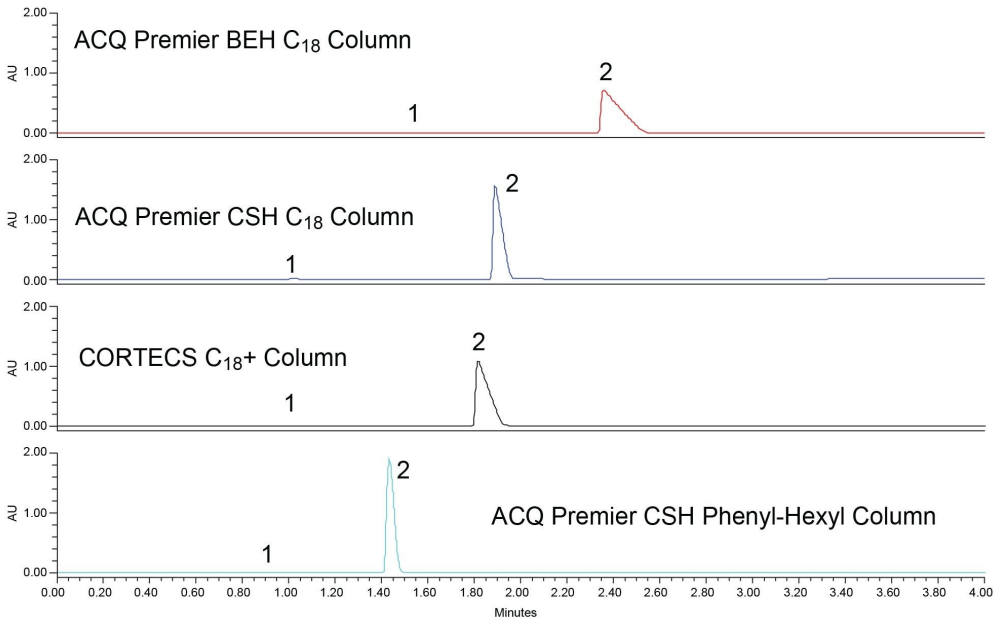

Figure 4. Analysis of naltrexone (1) and bupropion (2) on four different analytical columns. ACQUITY (ACQ) Premier BEH C_{18} Column, ACQ Premier CSH C_{18} Column, and ACQ Premier CSH Phenyl-Hexyl all use MaxPeak™ Premier Hardware. Y-axis linked between chromatograms to show difference in peak heights.

The worst peak shape is seen on the ACQUITY Premier BEH C_{18} Column, which was expected given the initial results. Both CSH particle columns show good improvement of peak shape for bupropion with the CSH Phenyl-Hexyl Column having even better symmetry compared to the CSH C_{18} Column. The CORTECS C_{18}+ Column improved the peak shape slightly compared to the BEH C_{18} Column, however, it is still not symmetrical. The poor peak shape on the CORTECS C_{18}+ Column could be caused by the decreased loading capacity of the solid-core particle compared to fully-porous phases.[15] The differences in peak shape obtained on the CSH C_{18} Column and CSH Phenyl-Hexyl Column can be explained by the ligands bonded to the surface. The Phenyl-Hexyl ligand allows more interaction between the base particle, improving peak shape even further by repelling the analyte through the column. The final method conditions for naltrexone and bupropion were developed on the ACQUITY Premier CSH Phenyl-Hexyl Column as that showed the best overall peak shape at low pH, which is preferred to high pH for this analysis.

After method optimization, which included reducing cycle time and speeding up analysis, linearity for the two

compounds was tested over as wide a dynamic range as possible. The dynamic range was determined by serial dilution of the neat standard shown in the experimental section. A calibration curve was created with the low-level standards selected to ensure naltrexone could be detected and the high-level standards being selected to prevent overload of the bupropion peak in the detector. Figure 5 shows the overlay of the calibration curve standards for naltrexone and bupropion.

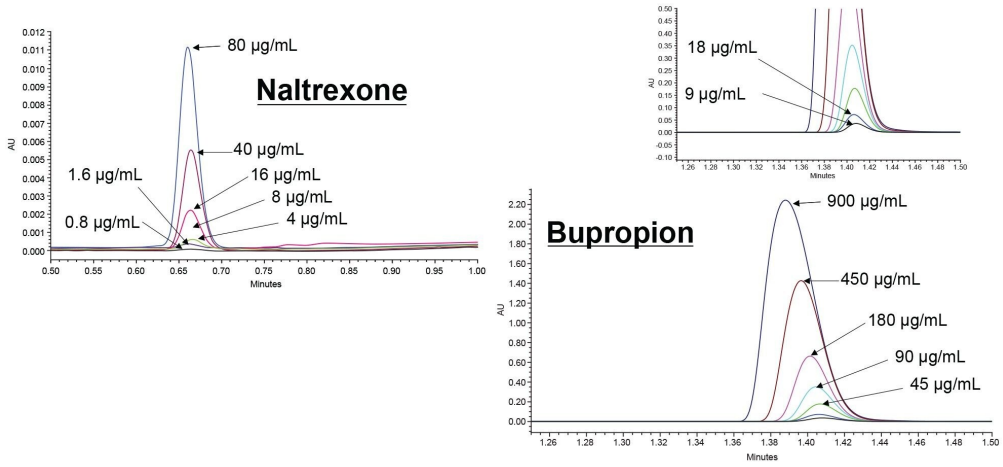

Figure 5. Chromatograms of calibration curve standards for naltrexone and bupropion. Inset is of low-level standards of bupropion.

Naltrexone was able to be detected and quantitated over a range of 4–80 µg/mL, with the 1.6 µg/mL and 0.8 µg/mL being under the typical detection limit of signal to noise ratio greater than three. Bupropion was able to be calibrated over a range of 9–900 µg/mL as the high-level standard showed no signs of detector saturation. Figure 6 shows the plotted calibration curves, including R^2 values for the two APIs over the dynamic ranges indicated.

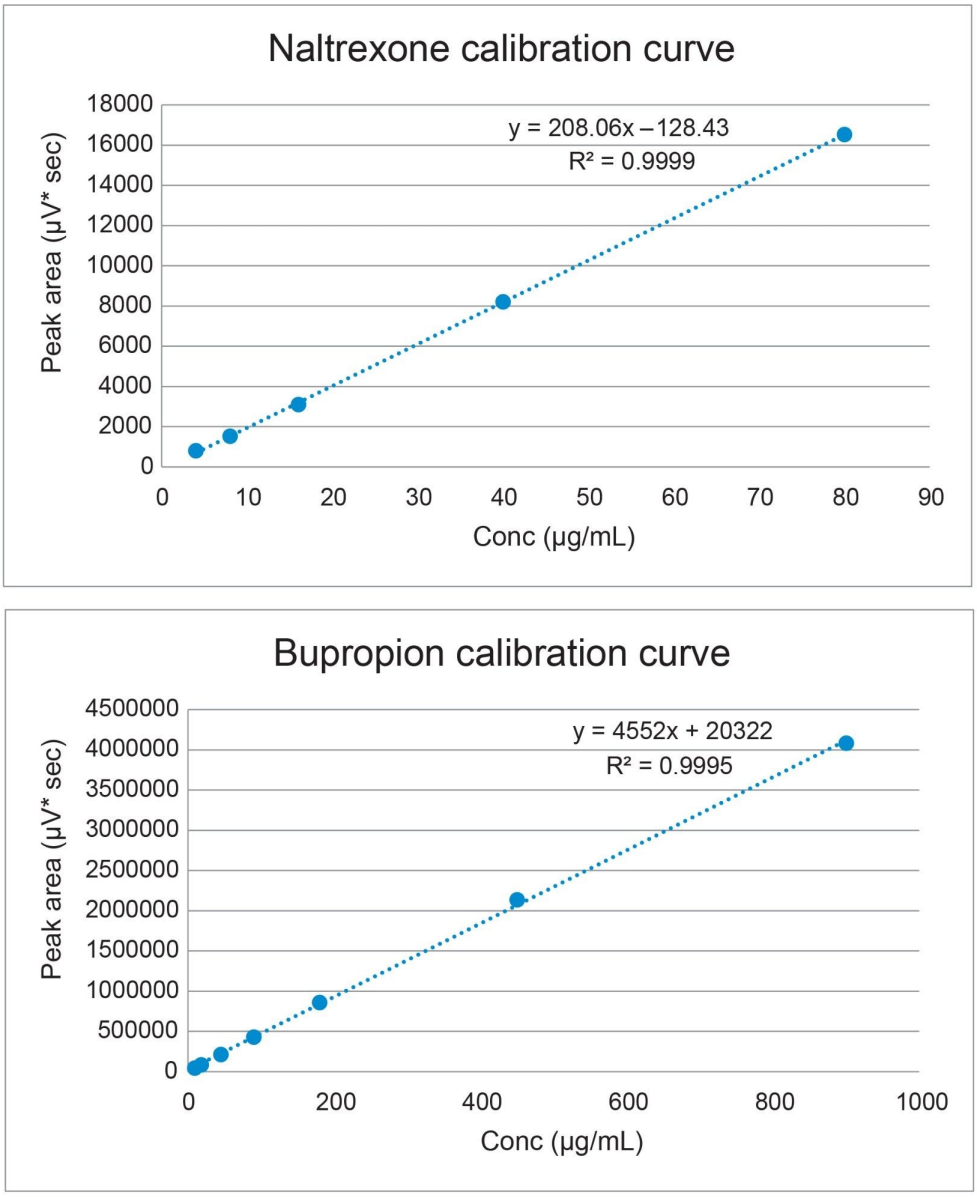

Figure 6. Analyte calibration curves.

Good linearity as defined as R^2 values being greater than 0.999 was achieved for both analytes using the final method conditions. This method, using the ACQUITY Premier CSH Phenyl-Hexyl Column to improve peak shape of bupropion, can now be used for batch release testing, or other workflows. Without the use of the ACQUITY Premier CSH Phenyl-Hexyl Column, poor peak shape for the high dose basic analyte, bupropion, may have

necessitated the use of specialized mobile phases, or worse yet, two separate analytical conditions to analyze the sample. By taking advantage of the charged base particle to improve the peak shape of bases at low pH, a single method could be created to analyze both compounds in a single run.

Conclusion

Fixed dose combination (FDC) drugs are common drug products available in a variety of formats, including tablets and syrups. These products prove to be challenging in analytical workflows due to the differences in active pharmaceutical ingredient (API) concentrations, as often one API will be overloaded, while others are barely detected. Overloaded peaks present their own challenges as they are harder to reproducibly integrate and quantitate. Improving the peak shape of overloaded peaks can be achieved in a variety of ways, including the use of high pH mobile phases, or changing the stationary phase being used. This can be especially true for basic analytes, like those found in the FDC drug, Contrave, which contains both naltrexone and bupropion. For this drug product, the use of a charged base particle with a phenyl-hexyl ligand was able to provide the best peak shape without the need for going to high pH mobile phases. The ACQUITY Premier CSH Phenyl-Hexyl Column provided the best peak shape for bupropion compared to three other columns tested. This column was then used to test linearity of the method for both naltrexone and bupropion. Good linearity was achieved indicating the method could now be used for other workflows such as batch release testing.

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