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# USP Method Modernization for Lidocaine Formulations Using XBridge Columns and Different LC Systems

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## Abstract

In this application note, USP methods from different lidocaine formulations were modernized by switching from older Atlantis dC$_{18}$ and XTerra C$_{18}$ Columns to contemporary XBridge BEH C$_{18}$ Columns. Improved method speed and resolution originated from both the change in particle and the change in particle size.

### Benefits

- Improved resolution of the USP methods by transferring to a newer column chemistry (XBridge BEH)
- Increased sample throughput by decreasing the analysis time
- Demonstrated use of Waters columns on LC instruments from different manufacturers


## Introduction

The analysis of pharmaceutical semisolid dosage forms, such as jellies, ointments, and creams, can be difficult due to excipients used in their formulation. In contrast, oral and injectable solutions are simpler and therefore easier to analyze. Lidocaine, an anesthetic used to treat pain from certain procedures, comes in both semisolid and solution forms. In this application note, we discuss the modernization of the USP liquid chromatography (LC) methods, using XBridge Columns, for both types of lidocaine formulations. In this context, modernization is chromatographic analysis speed improvement through the use of smaller particles, shorter columns, and higher flow rates, without sacrificing performance. Herein we also demonstrate equivalent outcomes across various LC instruments from different manufacturers.

## Experimental

Sample preparation

### Semisolid formulations

*Lidocaine hydrochloride jelly and lidocaine ointment*

Diluent: 0.1% aq phosphoric acid/acetonitrile (50:50, v/v)

System suitability solution (USP assay method): 0.1 mg/mL of lidocaine RS (Reference Standard), *1*, and 0.04 mg/mL of ropivacaine related compound A RS, *2*, in diluent.

Sample solution for lidocaine hydrochloride jelly (USP assay method): Generic lidocaine hydrochloride jelly (2%, w/v) was used in this study. The sample solution was prepared as per the lidocaine hydrochloride jelly USP method. Specifically, 0.3 mL of jelly was transferred to a 50 mL volumetric flask containing about 25 mL of diluent. The solution was sonicated for 5 minutes. Further diluent was added to the volume mark to obtain a lidocaine hydrochloride concentration of 0.12 mg/mL.

Sample solution for lidocaine ointment (USP assay method): Generic lidocaine ointment (5%, w/w) was used in this study. The sample solution was prepared as per the lidocaine ointment USP method. Specifically, 100 mg of ointment was weighed into a 50 mL volumetric flask. Diluent (25 mL) was added and the mixture was sonicated for 10 minutes. Further diluent was added to the volume mark to obtain a lidocaine concentration of 0.1 mg/mL.

## Solution formulation

*Lidocaine hydrochloride oral topical solution*

Buffer: aq monobasic potassium phosphate buffer, 4.85 g/L, adjusted to pH 8.00 with 10 N sodium hydroxide.

Diluent: buffer/acetonitrile (70:30, v/v).

System Suitability Solution (USP assay method): 0.0043 mg/mL of lidocaine RS, *1*, 0.00065 mg/mL of ropivacaine related compound A RS, *2*, and 0.005 mg/mL of lidocaine related compound H, *3*, in diluent.

Sample solution for lidocaine hydrochloride oral topical solution (USP assay method): Generic lidocaine hydrochloride oral topical solution (2%) was used in this study and was prepared as per the USP lidocaine hydrochloride oral topical solution method. Specifically, 2.0 mL of topical solution was added to a 20 mL vial containing 6 mL of mobile phase and mixed well to obtain a concentration of 5 mg/mL of lidocaine hydrochloride. A 1.0 mL aliquot of this solution was transferred to another 20 mL vial containing 4 mL of mobile phase to get a concentration of 1 mg/mL of lidocaine hydrochloride.

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| Instruments: | Alliance e2695 Quaternary HPLC with 2489 UV/Visible Detector |

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| | Shimadzu Nexera-I Quaternary LC 2040C 3D |
| | Agilent 1100 Binary LC with Agilent 1100 DAD Detector |
| | Agilent 1260 Infinity Quaternary LC with Agilent 1260 DAD Detector |
| Data management: | Empower 3 CDS Software |

Method conditions

## Semisolid formulations

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| Columns: | Atlantis $dC_{18}$, 5 µm, 4.6 x 150 mm (p/n: 186001344) (USP Method, $L/d_p$=30,000) |
| | XBridge BEH $C_{18}$, 5 µm, 4.6 x 150 mm (p/n: 186003116) (Modernized Method, $L/d_p$ = 30,000) |
| | XBridge BEH $C_{18}$, 3.5 µm, 4.6 x 150 mm (p/n: 186003034) (Modernized Method, $L/d_p$ = 42,800) |
| | XBridge BEH $C_{18}$ *XP*, 2.5 µm, 4.6 x 75 mm (p/n: 186006038) (Modernized Method, $L/d_p$ = 30,000) |
| Mobile phase A: | 0.1 % aq phosphoric acid |
| Mobile phase B: | Acetonitrile |
| Composition profile: | 10% B to 90% B in 10 min (Atlantis $dC_{18}$, 5 µm, 4.6 x 150 mm) |

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| | 10% B to 90% B in 10 min (XBridge BEH $C_{18}$, 5 µm, 4.6 x 150 mm) |
| | 10% B to 90% B in 7 min (XBridge BEH $C_{18}$, 3.5 µm, 4.6 x 150 mm) |
| | 10% B to 90% B in 2.5 min (XBridge BEH $C_{18}$ *XP*, 2.5 µm, 4.6 x 75 mm) |
| Flow rate: | 0.8 mL/min (Atlantis $dC_{18}$, 5 µm, 4.6 x 150 mm) |
| | 0.8 mL/min (XBridge BEH $C_{18}$, 5 µm, 4.6 x 150 mm) |
| | 1.14 mL/min (XBridge BEH $C_{18}$, 3.5 µm, 4.6 x 150 mm) |
| | 1.6 mL/min (XBridge BEH $C_{18}$ *XP*, 2.5 µm, 4.6 x 75 mm) |
| Column temp.: | 25 °C |
| Detection (UV): | 210 nm |
| Injection volume: | 5 µL (Atlantis $dC_{18}$, 5 µm, 4.6 x 150 mm) |
| | 5 µL (XBridge BEH $C_{18}$, 5 µm, 4.6 x 150 mm) |
| | 5 µL (XBridge BEH $C_{18}$, 3.5 µm, 4.6 x 150 mm) |
| | 2.5 µL (XBridge BEH $C_{18}$ *XP*, 2.5 µm, 4.6 x 75 mm) |

## Solution formulation

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| Columns: | XTerra Shield RP18, 3.5 µm, 4.6 x 150 mm (p/n: 186000536) (USP Method, $L/d_p$ = 42,800) |
| | XBridge BEH Shield RP18, 3.5 µm, 4.6 x 150 mm (p/n: 186003045) (Modernized Method, $L/d_p$ = 42,800) |
| | XBridge BEH Shield RP18 *XP*, 2.5 µm, 4.6 x 100 mm (p/n: 186006063) (Modernized Method, $L/d_p$ = 40,000) |
| Mobile phase A: | Buffer |
| Mobile phase B: | Acetonitrile |
| Composition profile: | 30% B (XTerra RP18, 3.5 µm, 4.6 x 150 mm) |
| | 30% B (XBridge BEH Shield RP18, 3.5 µm, 4.6 x 150 mm) |
| | 30% B (XBridge BEH Shield RP18 *XP*, 2.5 µm, 4.6 x 100 mm) |
| Flow rate: | 1.0 mL/min (XTerra RP18, 3.5 µm, 4.6 x 150 mm) |
| | 1.0 mL/min (XBridge BEH Shield RP18, 3.5 µm, 4.6 x 150 mm) |
| | 1.4 mL/min (XBridge BEH Shield RP18 *XP*, 2.5 µm, 4.6 x 100 mm) |
| Column temp.: | 45 °C |
| Detection (UV): | 230 nm |

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| Injection volume: | 20 µL (XTerra RP18, 3.5 µm, 4.6 x 150 mm) |
| | 20 µL (XBridge BEH Shield RP18, 3.5 µm, 4.6 x 150 mm) |
| | 13.3 µL (XBridge BEH Shield RP18 *XP*, 2.5 µm, 4.6 x 100 mm) |

## Results and Discussion

Lidocaine solution[1] and semisolid formulations,[2,3] sample solutions were analyzed using XTerra $C_{18}$, 3.5 µm, 4.6 x 150 mm (p/n: 186000536) and Atlantis d$C_{18}$, 5 µm, 4.6 x 150 mm (p/n: 186001344) compendial columns, respectively, with USP methods as specified in their respective monographs. The Alliance and Agilent 1100 HPLCs plus the Agilent 1260 and Shimadzu Nexera-i UHPLCs were used. As shown in Figure 1 and Figure 2, similar chromatograms were observed across all the instruments, with slight variation in the retention times which may be due to system volume differences.

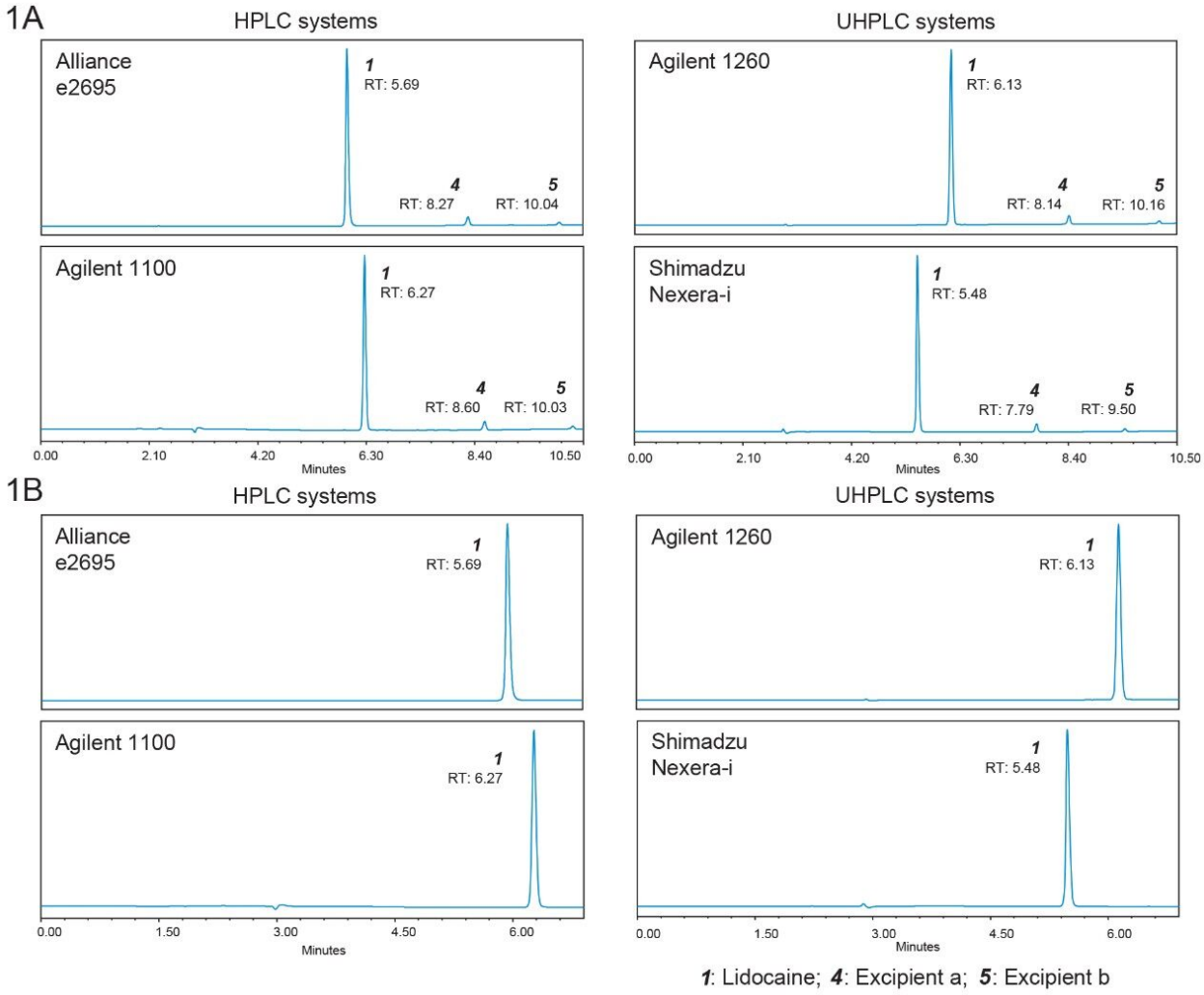


*Figure 1. Chromatograms obtained on different LC instruments (HPLC and UHPLC) from the sample solution of (A) lidocaine hydrochloride jelly and (B) lidocaine ointment using the USP method and compendial column.*

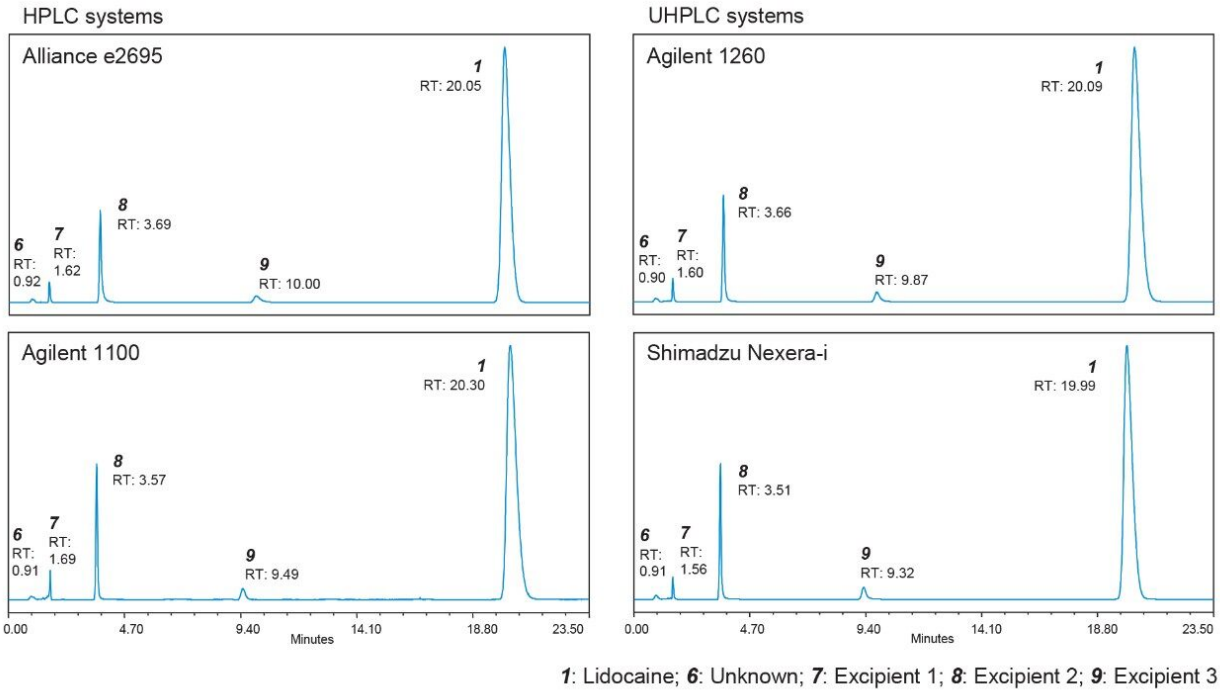


*Figure 2. Chromatograms obtained on different LC instruments (HPLC and UHPLC) from the sample solution of oral topical lidocaine hydrochloride using the USP method and compendial column.*

Even though semisolids are considered to be more difficult formulations to work with, no challenges were encountered during the analysis of these formulations. Hence, method modernization was carried out on an Alliance HPLC System using the system suitability solution. The modernized methods were then demonstrated across instruments from different vendors as discussed below.

We can modernize[4] a USP LC method by scaling to smaller particle sizes. At a given column diameter, the speed of an LC method can be expressed as the delivery of the required number of mobile phase column volumes,[5] ***# CVs***, per time interval, ***t***, shown in eq 1. Thus method speed increases with flow rate, *F*, and decreases with column length (via the lower calculated column void volume, $V_0$).

*LC method speed* = (#*CVs*)/t = $F/V_0$ - eq. 1

For isocratic USP methods, the USP General Chapter <621> specifies the allowed method modernization changes in particle size, flow rate, and column geometry.[6] Eq 2 gives the relationship between the flow rates $F_1$ and $F_2$, the column diameters $dc_1$ and $dc_2$, and the particle sizes $dp_1$ and $dp_2$ for the original and modernized conditions, respectively.

$F_2 = F_1 \cdot [(dc_2)^2 \cdot dp_1/(dc_1)^2 \cdot dp_2]$ - eq. 2

Decreasing the particle size alone allows a faster flow rate per USP guidelines, assuming the pressure ceiling of the LC instrument is not reached. A smaller particle size also permits use of a shorter column as long as the original ratio of column length, *L*, to particle size, $d_p$, is maintained in the modernized method. This "equivalent $L/d_p$" USP guideline[7] provides an additional method speed increase as the particle size decreases since smaller particles can give equivalent efficiency using higher flow rates and shorter columns.

## Semisolid formulations: method modernization

The USP method for lidocaine ointment and lidocaine hydrochloride jelly specifies an older column, Atlantis $dC_{18}$, using a gradient method. USP General Chapter <621> guidelines do not allow changes to a gradient chromatographic method without a revalidation. However, if a faster analysis is desired, modernization can be performed and then the resulting method subjected to the USP validation,[8,9] process.

We began the method modernization by switching from the Atlantis $dC_{18}$, 5 µm, 4.6 x 150 mm (p/n: 186001344) compendial column to the more recent L1 ($C_{18}$), XBridge BEH $C_{18}$, 5 µm, 4.6 x 150 mm (p/n: 186003116) Column. This is typically done to gain access to more robust column particles such as the BEH hybrid technology. Figure 3a and Figure 3b shows the chromatograms observed for these columns with the original USP method. The XBridge Column gives an 11% shorter analysis time (due to reduced analyte retention) and improved resolution between the two closely eluting peaks from lidocaine, *1*, and ropivacaine related compound A, *2* (due to increased selectivity of the BEH particle). The method was then scaled to a smaller particle size, by switching to an XBridge BEH $C_{18}$, 3.5 µm, 4.6 x 150 mm (p/n: 186003034) Column. There is a change of only the particle size so the method speed increases due to a scaled flow rate adjustment alone, per eq 2. Specifically, the flow rate rises from 0.8 mL/min to 1.14 mL/min, affording a 38% analysis time reduction from the compendial conditions, Figure 3c. The USP method was further modernized to an XBridge BEH $C_{18}$ *XP*, 2.5 µm, 4.6 x 75 mm (p/n: 186006038) Column. This is an "equivalent $L/d_p$" type of modernization which produces a larger increase in method speed. The analysis time drops by ca. 73% from the original method without compromising the resolution, as shown in Figure 3d.

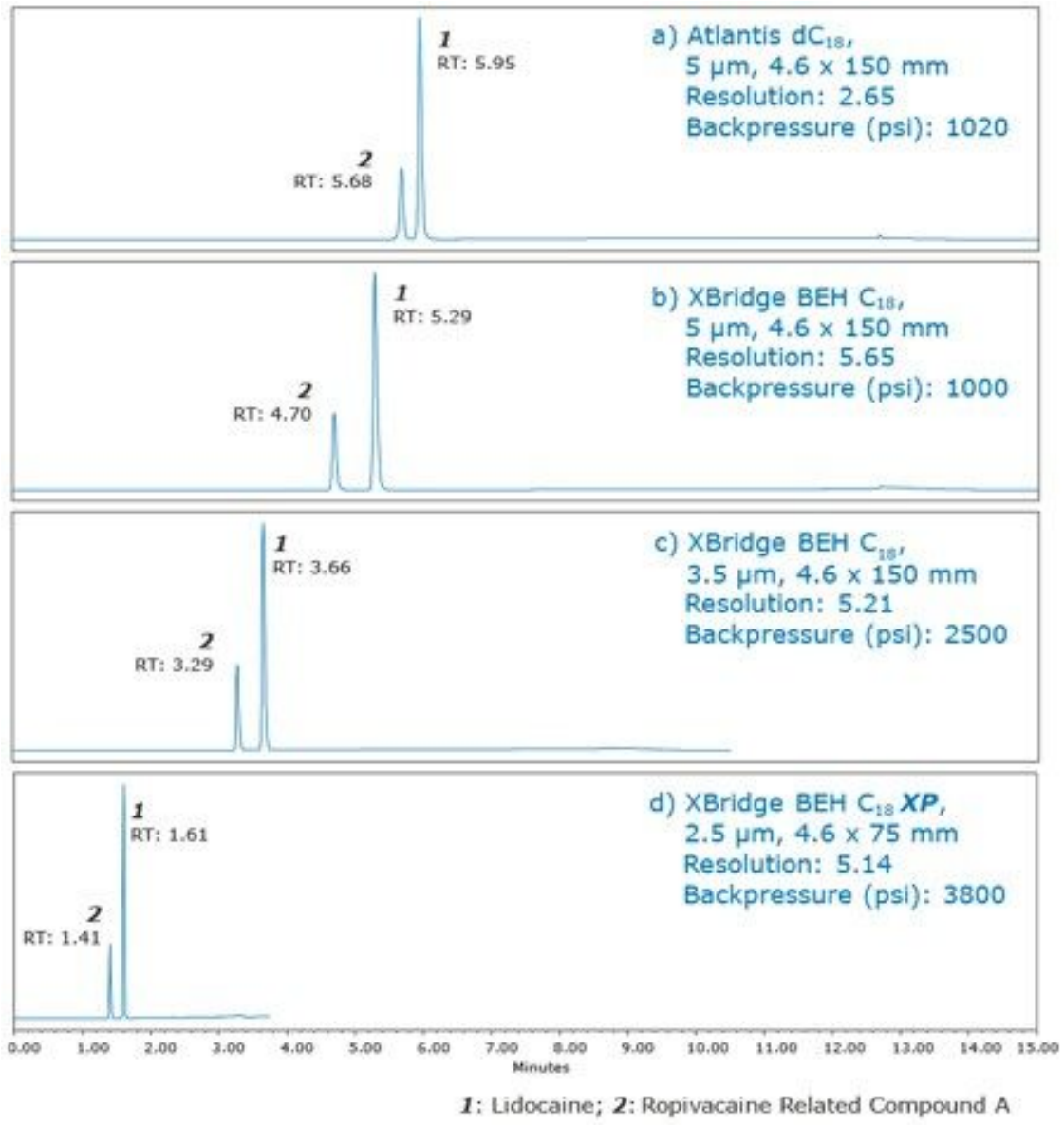


*Figure 3. Chromatograms obtained on the Alliance HPLC for the system suitability solution of the semisolid formulations using different columns.*

## Semisolid formulations: using different LC instruments

The fastest modernized method, using the XBridge BEH $C_{18}$ *XP*, 2.5 µm, 4.6 x 75 mm (p/n: 186006038) Column, was run on Agilent 1100, Agilent 1260, and Shimadzu Nexera-i instruments with the semisolid formulations system suitability solution. The results are shown in Figure 4. The Alliance HPLC and Agilent 1260 instruments gave the narrowest peaks whereas the Agilent 1100 and Shimadzu Nexera-i instruments gave wider peaks. The Agilent 1260 and particularly the Shimadzu Nexera-i provided higher backpressures

for the same column and method, consistent with these being UHPLC class instruments.

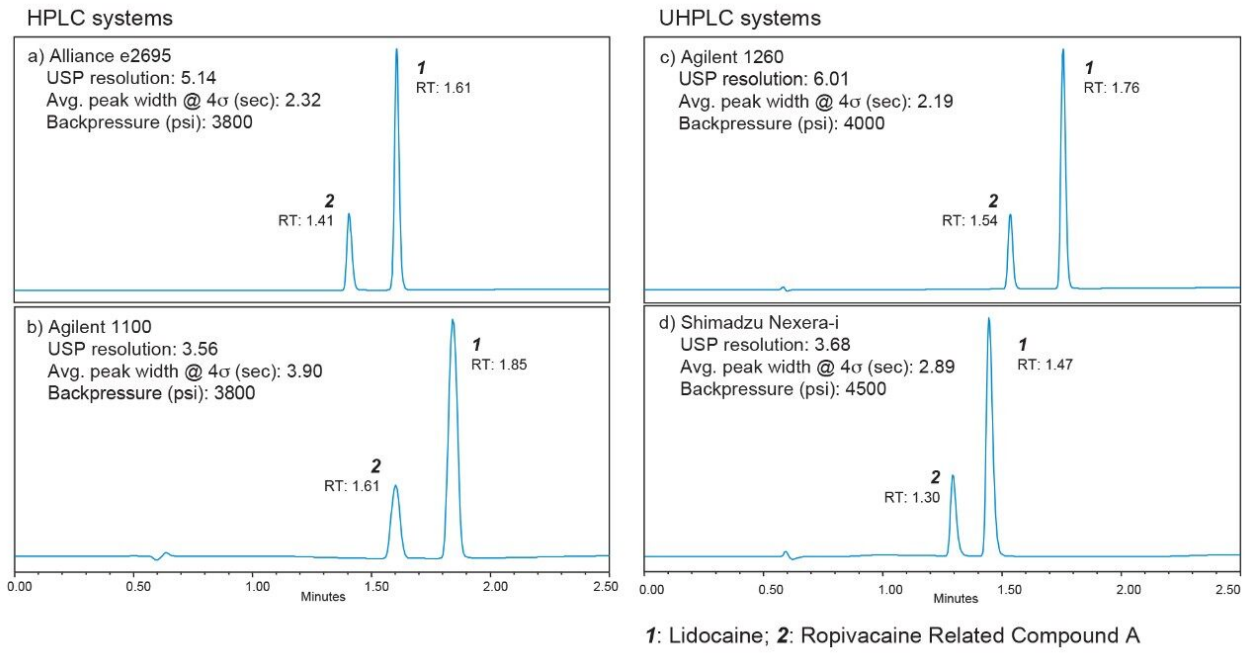


*Figure 4. Chromatograms obtained on different LC instruments (HPLC and UHPLC) for the system suitability solution of the semisolid formulations using the XBridge BEH $C_{18}$ XP, 2.5 µm, 4.6 x 75 mm Column.*

## Solution formulation: method modernization

The USP method for the lidocaine solution formulation also recommends an older column, XTerra RP18, 3.5 µm, 4.6 x 150 mm (p/n: 186000536). Although XTerra Columns have more sturdy particles compared to predecessors, we also modernized by transferring this USP method to an even better column particle, BEH. This change resulted in improved resolution between ropivacaine related compound A, *2*, and lidocaine related compound H, *3*, as depicted in Figure 5a and Figure 5b. An "equivalent $L/d_p$" modernization of the compendial method requires use of a 2.5 µm, 4.6 x 100 mm column. This modification provided the chromatogram in Figure 5c with a 50% decrease in the analysis time.

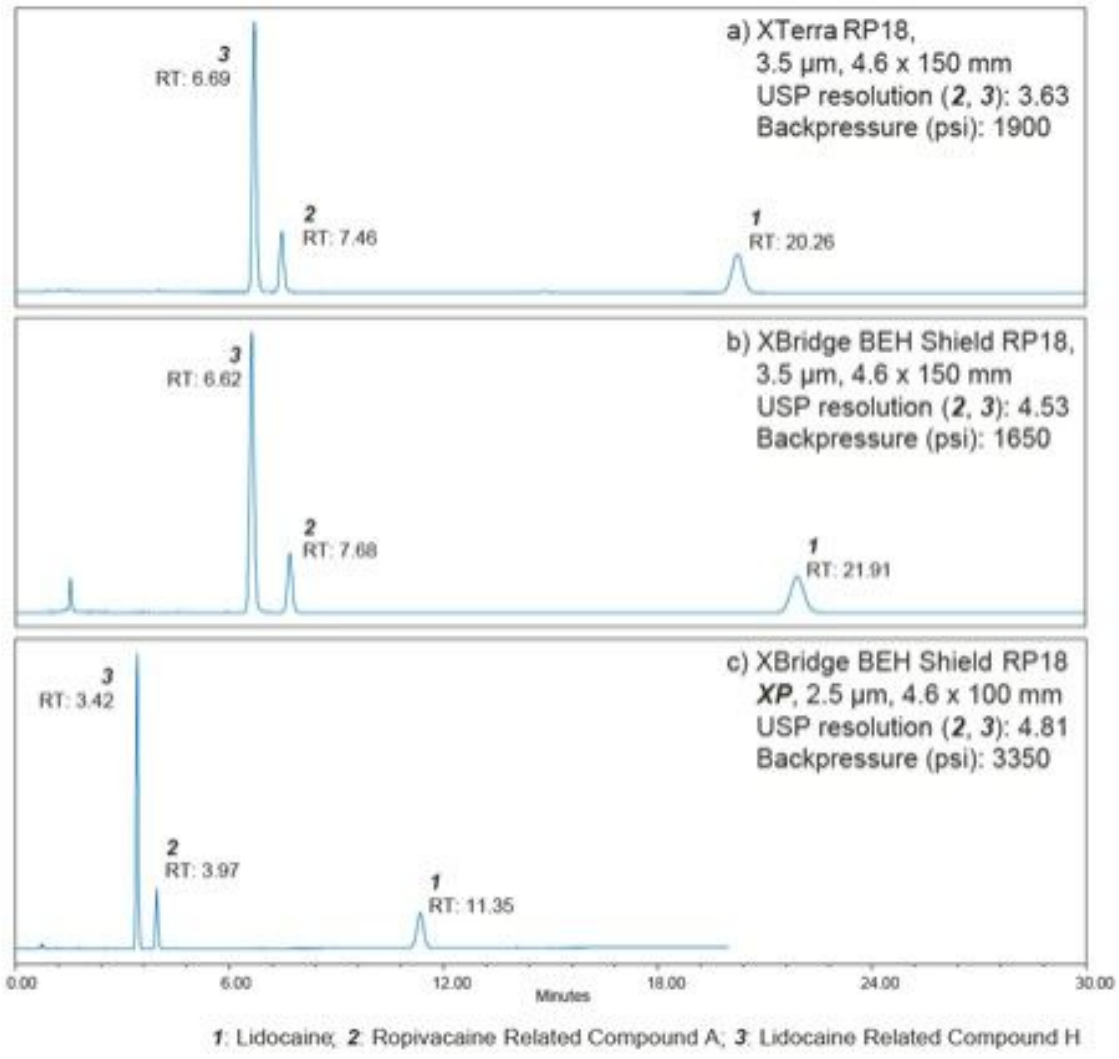


*Figure 5. Chromatograms obtained on the Alliance HPLC for the system suitability solution of the solution formulation using different columns.*

## Solution formulation: using different LC instruments

The system suitability solution was run with the modernized scaled method using the XBridge BEH Shield RP18 *XP*, 2.5 µm, 4.6 x 100 mm (p/n: 186006063) Column on different LC instruments. Similar results were obtained across these instruments, as shown in Figure 6.

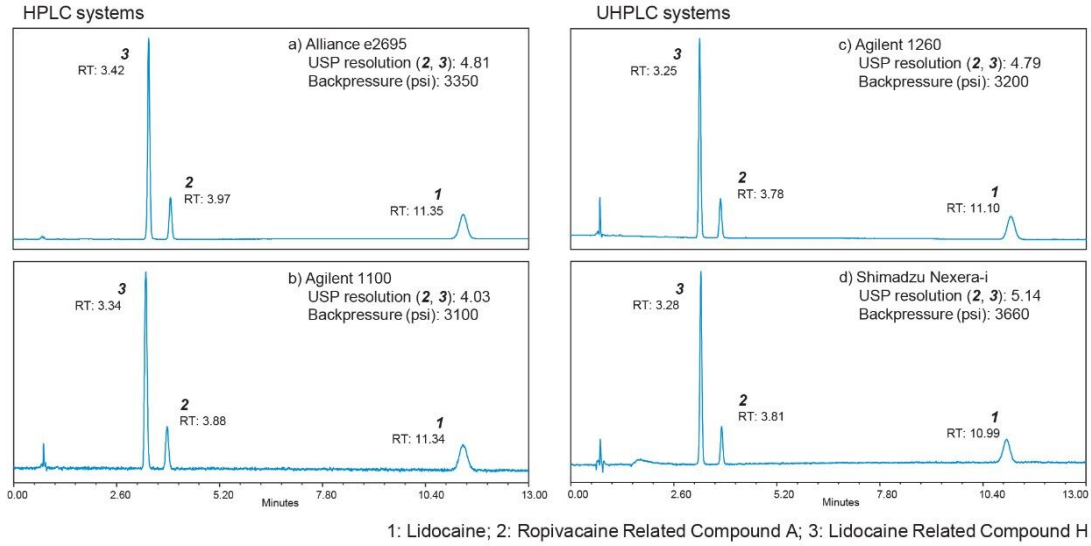


*Figure 6. Chromatograms obtained on different LC instruments (HPLC and UHPLC) for the system suitability solution of the solution formulations using the XBridge BEH Shield RP18 XP, 2.5 µm, 4.6 x 100 mm Column.*

## Conclusion

In this application note, USP methods from different lidocaine formulations were modernized by switching from older Atlantis $dC_{18}$ and XTerra $C_{18}$ Columns to contemporary XBridge BEH $C_{18}$ Columns. Improved method speed and resolution originated from both the change in particle and the change in particle size. Running the modernized methods on four LC instruments from three different vendors afforded comparable excellent separations regardless of the system used. This demonstrates the ability to achieve quicker analytical answers and higher sample throughput with such modern robust columns when moving from 5 µm to 3.5 µm to 2.5 µm particles on various manufacturers' LC instruments.

## References

1. USP40 – NF35 S1 Monograph: Lidocaine Hydrochloride Oral Topical Solution.

2. USP40 – NF35 S1 Monograph: Lidocaine Hydrochloride Jelly.

3. USP40 – NF35 S1 Monograph: Lidocaine Ointment.

4. Chromatographic methods were scaled using the Waters Column Calculator, version 2.0 (http://www.waters.com/waters/support.htm?lid=134891632&type=DWNL). The duration of a chromatographic method can be set via appropriate truncation of the composition profile after the last peak. We therefore use the retention time of the last peak in different chromatograms to express changes in method speed and analysis time during method modernization efforts.

5. The packed column volume, CV (in µL), also called the column void volume, $V_0$, is calculated using the following relationship: $CV = V_0 = \varepsilon_t L\pi(D/2)^2$. The terms L and D are the column length and diameter (in mm), respectively. The term εt is the total column porosity which is the fraction of the column taken up by the mobile phase in the space between particles and in the internal pores of the particles. A nominal value of total column porosity is generally applied in such calculations. In well packed columns, $\varepsilon_t = 0.66$ has been used for fully porous particles and $\varepsilon_t = 0.49$ has been used for solid-core particles. For example, see the Waters Columns Calculator.

6. USP40 – NF35 S1 General Chapter <621> Chromatography.

7. Swann, T.; Nguyen, J. M. USP Method Modernization Using "Equivalent $L/d_p$ " and "Equivalent N" Allowed Changes with CORTECS $C_8$ and CORTECS UPLC $C_8$ Columns. (2016). Waters Application Note (p/n: 720005666EN).

8. USP40 – NF35 S1 General Notices: 6. Testing Practices and Procedures, Section 6.30 Alternative and Harmonized Methods and Procedures.

9. USP40 – NF35 S1 General Chapter <1225> Validation of Compendial Procedures

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