Application Note

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A Simple Conversion of the USP Assay Method for Benzocaine Lozenges to the Agilent InfinityLab Poroshell 120 EC-C8 Column

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Abstract

The transfer of the USP Assay method for benzocaine lozenges is demonstrated using Agilent ZORBAX Eclipse Plus C8 and Agilent InfinityLab Poroshell 120 EC-C8 columns. The initial method uses a 5 µm 4.6 × 250 mm column and requires 15 minutes for the analysis. When InfinityLab Poroshell 120 EC-C8 columns (4.6 × 150 mm, 4.6 × 100 mm, and 4.6 × 75 mm, 2.7 µm) are used, analysis time is reduced from 40 to 70% of the original method time, without need for revalidation or changing the flow rate using the InfinityLab Poroshell 120 EC-C8 column. Pressure is monitored and considered as a factor in instrument transfer.

Introduction

Pharmaceutical companies routinely adopt U.S. Pharmacopeia (USP) compendial methods for testing raw materials and finished products. Successful implementation of the USP methods, and transferability between instruments are key steps to enhance throughput for routine analysis. Effective method transfer generates identical results for the same analysis, independent of the laboratory, instrument, and the resources for a specific method. By ensuring successful lab-to-lab method transferability, companies can replicate methods at additional sites or with partners such as contract research or manufacturing organizations (CROs and CMOs). Transferring an HPLC-based USP method to UPLC technology offers such organizations the additional opportunity to achieve productivity goals by reducing analysis time while ensuring reliable, high-quality chromatographic separations that are the basis for decisions about product quality. UHPLC technology offers QC and manufacturing facilities significant advantages in terms of increased throughput, improved quality, and reduced costs.

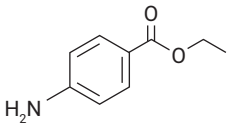
The costs associated with pharmaceutical testing can be reduced using adjustments to chromatography allowed under the general chapters in USP <621>. These costs are associated with chromatographic solvent and time. Of these two considerations, time is the most important.

In this application note, the current method for benzocaine lozenges published in the USP is adjusted within allowable limits to increase sample throughput using superficially porous particle columns.

The costs associated with pharmaceutical testing are considerable and many prudent lab managers are seeking ways to reduce costs by reducing solvent usage and improving productivity, while still using the LC instruments in their lab. Compendial methods from the USP are widely used in drug product and raw material testing. While efforts have been made to modernize these methods, they can be improved by taking advantage of newer technologies.

Benzocaine is the ethyl ester of *p*-aminobenzoic acid (PABA). It is a local anesthetic that is commonly used as a topical pain reliever or in cough drops. It is frequently found in many over the-counter products. The USP lozenge assay method uses a 5 µm C8 or L7 column.

The structure of benzocaine is shown in Figure 1. Its IUPAC name is ethyl 4-aminobenzoate.



Benzoic acid, 4-amino-, ethyl ester,
Ethyl *p*-aminobenzoate

Figure 1. Benzocaine structure.

InfinityLab Poroshell 120 columns are an LC column choice that can provide improved performance on a typical LC instrument. These columns have a 2.7 µm superficially porous particle that can provide faster analysis and higher resolution in shorter columns for testing more samples in less time on an existing instrument. The columns are available in many phases, including L1 (C18), L7 (C8), L11 (Phenyl), L10 (Cyano), as well as many others. The work in this application note used the L7 phase (InfinityLab Poroshell 120 EC-C8).

Experimental

An Agilent 1260 Infinity II LC was configured using 0.17 mm tubing throughout for this work. Table 1 shows corresponding details.

USP-grade monobasic potassium phosphate and phosphoric acid were purchased from Sigma-Aldrich. Acetonitrile was purchased from Honeywell (Burdick and Jackson HPLC-certified grade). Water was produced on site using a Millipore Milli-Q system (0.2 µm filtered, 18 MΩ). USP Benzocaine RS was purchased from the United States Pharmacopeia. A buffer was made per the USP method by preparing a 1.0 M monobasic potassium phosphate solution and adjusting the pH to 3.0 with phosphoric acid. The mobile phase consisted of mixing acetonitrile, water, and buffer (250 mL:500 mL:50 mL).

Table 1. Instrument configuration.

Agilent 1260 Infinity II LC	
Agilent 1260 Infinity II binary pump (G7117B)	
Agilent 1260 Infinity II multisampler (G7167A)	– Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (p/n 5182-0716) – Cap, screw, blue, PTFE/red silicone septa, 100/pk (p/n 5182-0717)
Agilent 1260 Infinity II multicolumn thermostat (MCT; G7116A)	– Standard flow heater G7116-60015 – Heater and column: Agilent InfinityLab Quick Connect assembly, 105 mm, 0.12 mm (p/n 5067-5961)
Agilent 1260 Infinity II diode array detector FS (G7117A)	– 10 mm 1 µL flow cell (G4212-60008) – 40 Hz
Agilent OpenLab CDS, version C.01.07	

Per the USP procedure, two diluents were prepared: designated Diluent A and Diluent B. Diluent A consists of 0.1 N hydrochloric acid and was prepared by diluting 8.3 mL of 37% HCl in 1 liter of water. Diluent B consists of a 1:1 solution of acetonitrile and water. Standard solution A is prepared by dissolving 0.01 mg/mL in Diluent A, and standard solution B is prepared by dissolving 0.01 mg/mL in Diluent B. These standard solutions are used for system suitability testing and calibration. The method conditions are summarized in Table 2.[1]

Columns used in this work:

- Agilent ZORBAX Eclipse Plus C8, 4.6 × 250, 5 µm (p/n 959990-906)
- Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 150 mm, 2.7 µm (p/n 693975-906)
- Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm (p/n 695975-906)
- Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 75 mm, 2.7 µm (p/n 697975-906)

Results and discussion

Table 3 describes the allowable adjustments within the USP method without the need for method validation. One example of an allowed change is the L/dp rule. The ratio of column length to particle size is kept constant within a range of −25 to +50%. By keeping the efficiency of the column nearly constant, a new method is not created. The intent is not to create a more efficient method, just a faster method. No changes can be made to the detection without revalidation. No changes are made to the mobile phase. While injection volume may be adjusted as far as is consistent with precision and detection limits, injection volumes are scaled geometrically. Precision is an important criterion in assay methods.

Table 2. Initial LC method conditions.

Parameter	Value
Column	L7, 4.6 × 250 mm, 5 µm (Agilent ZORBAX Eclipse Plus C8)
Buffer	1.0 M monobasic potassium phosphate titrated to pH 3.0 with phosphoric acid
Mobile Phase	Premix (acetonitrile:water:buffer, 200:750:50)
Flow Rate	1.5 mL/min
Run Time	15 min approximate
Temperature (Column)	25 °C
Injection Volume	20 µL (geometrically scaled for smaller columns)
Sample Concentration	0.01 mg/mL of USP Benzocaine RS in Diluent A or Diluent B
Detector	UV: 280 nm
System Suitability Requirements	Tailing factor: not more than 1.5% Relative standard deviation: not more than 2.0%

Table 3. Summary of allowable adjustments per USP General Chapter <621>.

Parameters for System Suitability	USP37-NF32S1	
	Isocratic	Gradient
Particle Size	L/dp: −25 to +50% or N: −25 to +50%	No changes allowed
Column Length		
Column Inner Diameter	Flexible, with constant linear velocity	No changes allowed
Flow Rate	Based on dp: $F_2 = F_1 \times [(dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)]$ Additional adjustments: ±50%, provided N decreases ≤20%	No changes allowed
Injection Volume	May be adjusted, as far as is consistent with precision and detection limits	May be adjusted, as far as is consistent with precision and detection limits
Column Temperature	±10 °C	±10 °C
Mobile Phase pH	±0.2 units	±0.2 units
Salt Concentration	Within ±10% if the permitted pH variation is met	Within ±10% if the permitted pH variation is met
Ratio of Components in Mobile Phase	Minor component (≤ 50%): ±30% relative, but cannot exceed ±10% absolute; may only adjust one minor component in ternary mixtures	No changes allowed *
Wavelength of UV-Visible Detector	No changes allowed	No changes allowed

* Not specified in <621>, assume no changes are allowed.

The initial column used in this method is a 250 mm (250,000 µm), 5 µm column. The L/dp for this column is calculated at 50,000. Table 4 shows a range of potential L/dp combinations that could be met using 5 and 3.5 µm totally porous particle (TPP) columns, as well as 4 and 2.7 µm superficially porous particle (SPP) columns. The L/dp calculation is applied identically for both TPP and SPP columns. Following the L/dp rule of –25 to +50%, and using a base of 50,000, the ratio is acceptable for adjustment without revalidation when the range is between 37,500 and 75,000. The extent to which savings can be found because of changes can be investigated by following the L/dp rule. By not changing the flow rate, this method can save time and solvent: Using the 4 µm 250 mm column, no time or solvent is saved. In contrast, using the 4 µm 150 mm, 3.5 µm 150 mm, or 2.7 µm 150 mm columns may save 40% time and solvent The 2.7 µm 100 mm column is slightly too short and too low to be used without revalidation under the L/dp rule. The possible scenarios are highlighted in Table 4. Based on these calculations, and the desire to improve throughput, the 4.6 × 150 mm, 4 and 2.7 µm SPP InfinityLab Poroshell 120 EC-C8 columns were chosen for evaluation. After proportionately reducing the injection volume, a faster analysis and chromatographic solvent savings, with analyte retention time decreased to 6.4 minutes or less was observed. The pressure using the InfinityLab Poroshell 2.7 µm 150 mm column is 421 bar, which works well with this instrument, but would not be possible on some older instruments. The 4 µm, 150 mm column, however, runs this method at 264 bar. These chromatograms are shown in Figure 2.

Table 4. Potential L/dp combinations.

L/dP (µm)	5	4	3.5	2.7
250	50,000	62,500	71,429	92,593
150	30,000	37,500	42,857	55,555
100	20,000	25,000	28,571	37,037
75	15,000	18,750	21,428	27,778
50	10,000	12,500	14,285	18,519

Following the L/dp rule of –25 to +50%, and using a base of 50,000, the ratio is acceptable when the range is between 37,500 and 75,000. Sections highlighted in orange are allowed adjustments within this rule.

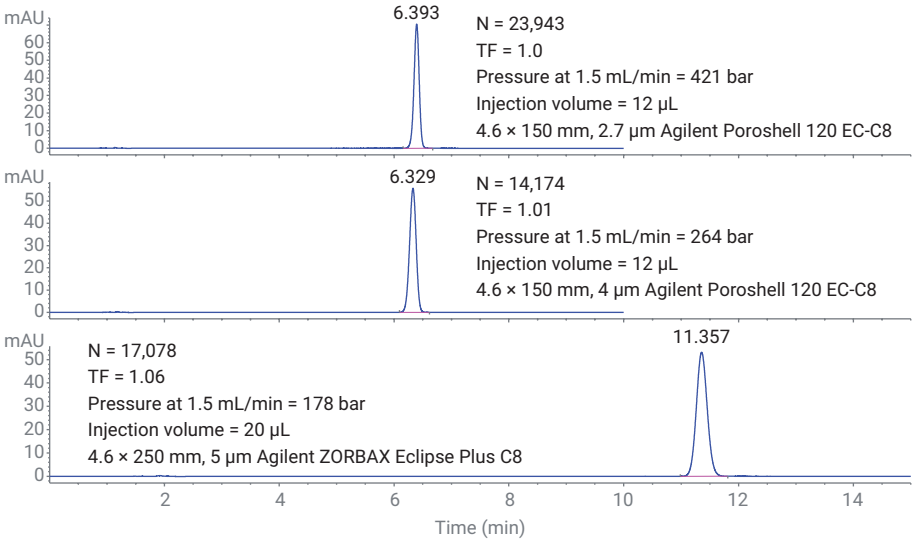


Figure 2. USP Benzocaine lozenge: Agilent ZORBAX Eclipse Plus C8 to Agilent InfinityLab Poroshell 120 EC-C8 using the L/dp rule.

It was noted that the 2.7 µm InfinityLab Poroshell 120 EC-C8 column generated approximately N = 24,000 or 160,000 N/meter. This calculation suggests that the method could meet the N –25 to +50% rule with a shorter 100 mm or 75 mm column. The base comparison for this is the original column (4.6 × 250 mm, 5 µm) with the analyte in the method. The range for the N rule would be between 12,808 and 25,617. Figure 3 shows the comparison chromatograms for the 2.7 µm, 75 and 100 mm columns. The 100 mm column was found to generate N = 18,325 at 315 bar, while the 75 mm column generates N = 14,065 at 263 bar. Both columns meet the N –25 to +50% rule. Additionally, even more time and chromatographic solvent are saved with the shorter column.[2]

The summary in Table 3 shows that allowable adjustments to particle size and column length can be made if either the L/dp ratio is within the limits of –25 to +50% or if the N –25 to +50% conditions are met.

Table 5 summarizes the experimental outcomes and demonstrates the time savings. As can be observed, the 4.6 × 75 mm column can easily run on the same instrument as the original column and save 70% of the time and chromatographic solvent, without the need to revalidate. This adjustment using the N –25 to +50% rule shows the true benefit of SPP columns.

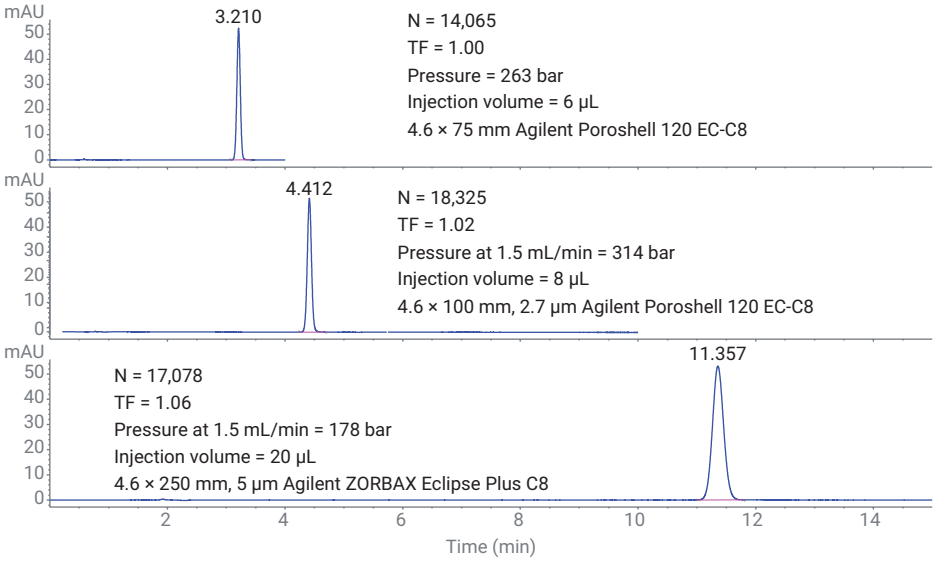


Figure 3. USP Benzocaine lozenge: Agilent ZORBAX Eclipse Plus C8 to Agilent InfinityLab Poroshell 120 EC-C8 4.6 × 75 or 100 mm 2.7 µm using the N rule.

System suitability requirements are the acceptance criteria for adjustments. In the case of the benzocaine lozenge assay, this method was able to reduce the analysis time from 15 minutes on the original method to 4.5 minutes in a method on an InfinityLab Poroshell 120 EC-C8, 4.6 × 75 mm, 2.7 µm column. A 70% decrease in solvent consumption is gained in addition to the efficiency changes. Such a decrease is typical of methods adapted to 75 mm InfinityLab Poroshell 120 2.7 µm columns. System suitability requirements using the benzocaine lozenge method are carried out separately using 0.01 mg/mL solutions made in Diluent A (0.1 N hydrochloric acid), and Diluent B (Acetonitrile and Water (1:1)). The requirements of Tailing Factor NMT (not more than) 1.5, RSD not more than 2.0% are met, and summarized in Table 6. System suitability data when RSD is equal to 2 or less are typically generated using five chromatograms. If a run time of 15 minutes is used, over 75 minutes would pass before a single real sample could be injected. Using the faster 75 mm method with a 2.7 µm SPP column, only 27 minutes would be required.

Table 5. Summary of USP adjustment outcomes to benzocaine assay on Agilent InfinityLab Poroshell 120 EC-C8 columns.

Column	Column Length (L, mm)	Particle Size (dp, µm)	L/dp Ratio	Allowable L/dp Range (–25% to +50%)	N Benzocaine Standard	Allowable N Range (–25 to +50%)	Percentage of Time Saved	Pressure (Bar)
ZORBAX Eclipse Plus C8	250	5	50,000	37,500 to 75,000	17,078	12,808 to 25,617		176
Poroshell 120 EC-C8	150	4	37,500	Meets specification	23,943	No need to check	40%	264
Poroshell 120 EC-C8	150	2.7	55,555	Meets specification	14,174	No need to check	40%	421
Poroshell 120 EC-C8	100	2.7	37,037	Does not meet specification	18,325	Meets specification (+7%)	60%	314
Poroshell 120 EC-C8	75	2.7	27,778	Does not meet specification	14,128	Meets specification (–17%)	70%	287

Conclusion

Laboratories performing compendial analyses with fully porous 5 µm columns can benefit from the increased speed and solvent savings that superficially porous 2.7 µm Agilent InfinityLab Poroshell 120 EC-C8 columns can provide, without needing to replace instrumentation. Perhaps more importantly, the method described allows the laboratory manager flexibility to assign work to any instrument in the laboratory. Faster analysis times, leading to higher throughput, can lead to a more productive laboratory. By applying permitted adjustments to these shorter columns, additional validation is not required. In this case, superficially porous columns can achieve faster results than 5 µm columns, resulting in a more productive laboratory while easily meeting system suitability requirements.

References

1. USP Benzocaine Lozenge Method, *United States Pharmacopeia* **2020**, *42(4)*, Rockville, MD.
2. USP General Chapter 621, USP 37-NF32, First supplement.

Table 6. Results of the system suitability test and analysis time summary.

	System Suitability Requirements	Agilent Poroshell 120 EC-C8 4.6 × 75 mm, 2.7 µm 1.5 mL/min Std A		Agilent Poroshell 120 EC-C8 4.6 × 75 mm, 2.7 µm 1.5 mL/min Std B	
USP Tailing Factor	Not more than 2.0%	1.0		1.0	
Relative Standard Deviation	Not more than 2.0%	Area	0.3%	Area	0.3%
		Retention time	0.3%	Retention time	0.2%

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