Application Note

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A Simple Conversion of the USP Method for Diphenhydramine HCl Impurities to the Agilent InfinityLab Poroshell 120 EC-C8 Column

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Abstract

The transfer of the USP Impurities method for diphenhydramine hydrochloride 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 40 minutes for the analysis. When InfinityLab Poroshell 120 EC-C8 columns (4.6 × 100 mm, 2.7 µm) are used, analysis time is reduced from 40 to 33% of the original method time, without need for revalidation using the InfinityLab Poroshell 120 EC-C8 column. Pressure is monitored and considered a factor in instrument transfer. This transfer is consistent with allowed adjustments under USP37-NF32S1 (official August 1, 2014), and USP Stage 4 Harmonization, to be official December 1, 2022.

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, or 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 diphenhydramine HCl published in the USP is adjusted within allowable limits to increase sample throughput using superficially porous particle columns. This work is consistent with allowed adjustments under USP37-NF32S1 (official Aug 1, 2014), and USP Stage 4 Harmonization, to be official December 1, 2022.

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.

Diphenhydramine is found in pharmacies throughout the world. It is found in many over-the-counter products.

Diphenhydramine was discovered in 1943, and in 1946, it became the first prescription antihistamine approved by the U.S. FDA. The USP Diphenhydramine HCl impurity method uses a 5 µm C8 or L7 column. The structure of diphenhydramine HCl is shown in Figure 1. Its IUPAC name is 2-(diphenylmethoxy)-N,N-dimethylethanamine hydrochloride. Structures of other compounds listed in this method are also found in Figure 1.

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 uses the L7 phase (InfinityLab Poroshell 120 EC-C8).

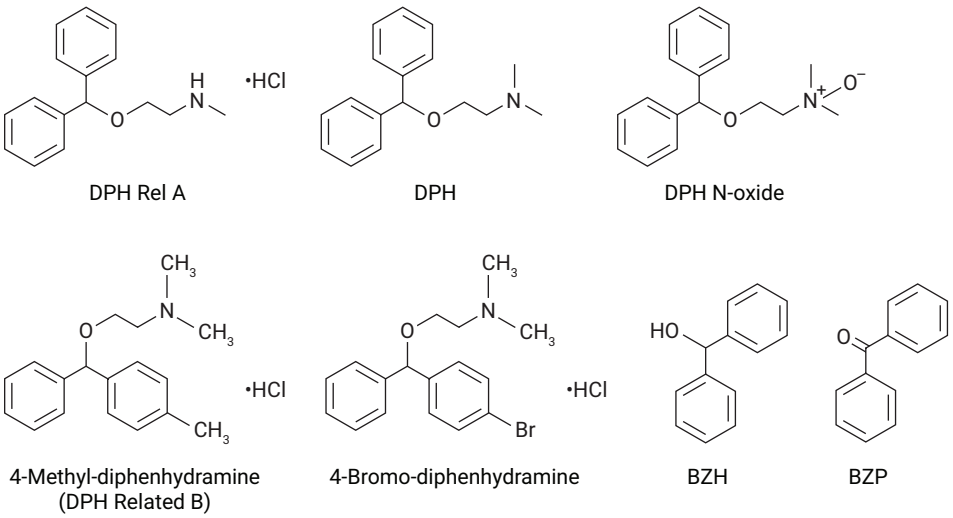

Figure 1. Diphenhydramine-related compound structures (diphenhydramine N-oxide hydrochloride is not on the USP list but is frequently found).

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 onsite using a Millipore Milli-Q system (0.2 µm filtered, 18 MΩ). USP Diphenhydramine RS, USP Diphenhydramine Related A RS, and USP Diphenhydramine Related B RS were purchased from the United States Pharmacopeia. 4-Bromodiphenhydramine, diphenylmethanol, benzophenone, and diphenhydramine N-oxide were purchased from Sigma-Aldrich. The premixed mobile phase consisted of buffer and acetonitrile. Samples were prepared in mobile phase. The mobile phase consisted of mixing acetonitrile and buffer (350:650 mL). The method conditions are summarized in Table 2.

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 × 100 mm, 2.7 µm (p/n 695975-906)

Table 1. Instrument configuration.

1260 Infinity II LC System	
Agilent 1260 Binary Pump (G7117B)	
Agilent 1260 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 (5182-0717)
Agilent G7116A Multicolumn Thermostat (MCT)	– Standard flow heater G7116-60015 – Heater and column: InfinityLab Quick Connect assembly, 105 mm, 0.12 mm (p/n 5067-5961)
Agilent 1260 Diode Array Detector (G7117A)	– G4212-60008 10 mm 1 L flow cell – 80 Hz
Agilent OpenLAB CDS, version C.2.6	

Table 2. Initial LC method conditions.

Parameter	Value
Column	L7, Agilent ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm (p/n 959990-906)
Mobile Phase	Premix (buffer: 5.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0. Mobile phase: acetonitrile and buffer (35:65)
Flow Rate	1.2 mL/min
Run Time	Not less than seven times retention of diphenhydramine
Temperature (Column)	25 °C (not in method, but under USP recommendations, 25 is used unless otherwise stated)
Injection Volume	10 µL (geometrically scaled for smaller columns)
Sample Concentration	0.7 mg/mL of USP diphenhydramine hydrochloride in mobile phase
Detector	UV 220 nm (40 Hz)
System Suitability Requirements	Rs: NLT 2.0 between diphenhydramine related compound A and diphenhydramine using system suitability solution consisting of 0.1 mg/mL each of USP Diphenhydramine Related Compound A RS, benzhydrol, and USP Diphenhydramine Hydrochloride RS in mobile phase

Results and discussion

Previously, under allowable adjustment guidelines, all columns and particle size adjustments could follow the L/dP rule. In that adjustment, 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.

For transfer of methods from totally porous to totally porous under Guidance, under December 1, 2022, Harmonization, the particle size and length of the column may be modified, provided that the ratio of the column length (L) to the particle size (dp) remains constant or in the range between −25 to +50% of the prescribed L/dp ratio.

For the application of particle-size adjustment from totally porous to superficially porous particles, other combinations of L and dp can be used, provided that the plate number (N) is within −25 to +50%, relative to the prescribed column. These changes are acceptable, if system suitability criteria are fulfilled, and selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent.

Previously, superficially porous columns could be adjusted using the L/dp rule, but under new guidance, only the N rule is acceptable. This means a direct comparison of efficiency (N) must be made using the active pharmaceutical ingredient (API) under investigation in both the original method and the final adjusted method when using superficially porous columns.

The initial column used in this method is a 250 mm, 5 µm column. The L/dp for this column is calculated at 50,000. Following the L/dp rule of −25 to +50%, and using a base of 50,000, there is a range of between 37,500 and 75,000 where the ratio is acceptable for

adjustment without revalidation. This would still be a valid adjustment for totally porous columns to totally porous columns after December 2022. Following the L/dp rule, an adjustment from a 5 µm, 250 mm column to a 2.7 µm, 100 mm column would not be an allowed adjustment, as the L/dP is slightly lower than the allowed range.

When running the USP method, note that N for diphenhydramine is 10,334. A representative chromatogram showing the original USP method using ZORBAX Eclipse Plus C8 chromatograms is shown in Figure 2. Since the L/dp for a 100 mm, 2.7 µm was just below the acceptable range for an allowed adjustment, that column was used to experimentally determine diphenhydramine efficiency. Columns containing superficially porous particles have higher efficiency than those with similar sized totally porous particles. An allowed adjustment to the injection volume was also made geometrically, reducing the volume proportional to the column volume from 10 µL to 4 µL. An experimental value of 12,873 was determined for diphenhydramine when using the column at a slightly higher flow rate of 1.5 mL/min. This value was within the N –25 to +50% range. It was also found that the pressure generated was higher than the original 5 µm, 250 mm column method. As it was below 400 bar, a representative chromatogram showing the original USP method using InfinityLab Poroshell 120 EC- C8 chromatograms is shown in Figure 3.

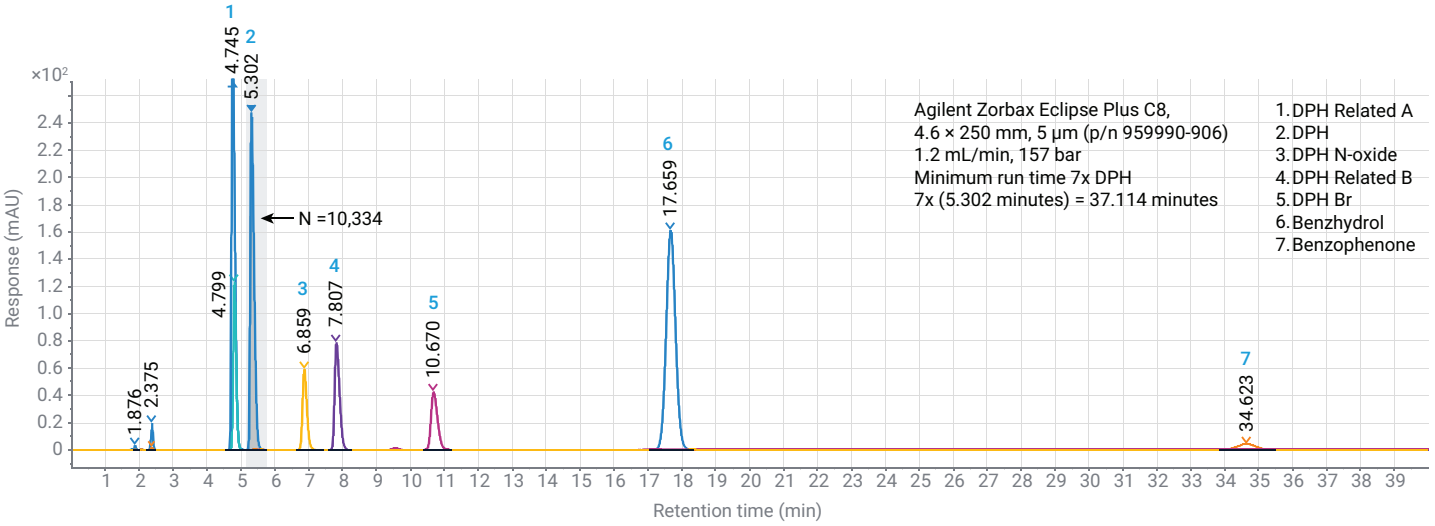

Figure 2. Agilent 1260 Infinity II Binary HPLC System, premix (buffer: 5.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0. Mobile phase: acetonitrile and buffer (35:65) 1.2 mL/min 220 nm, 10 µL injection.

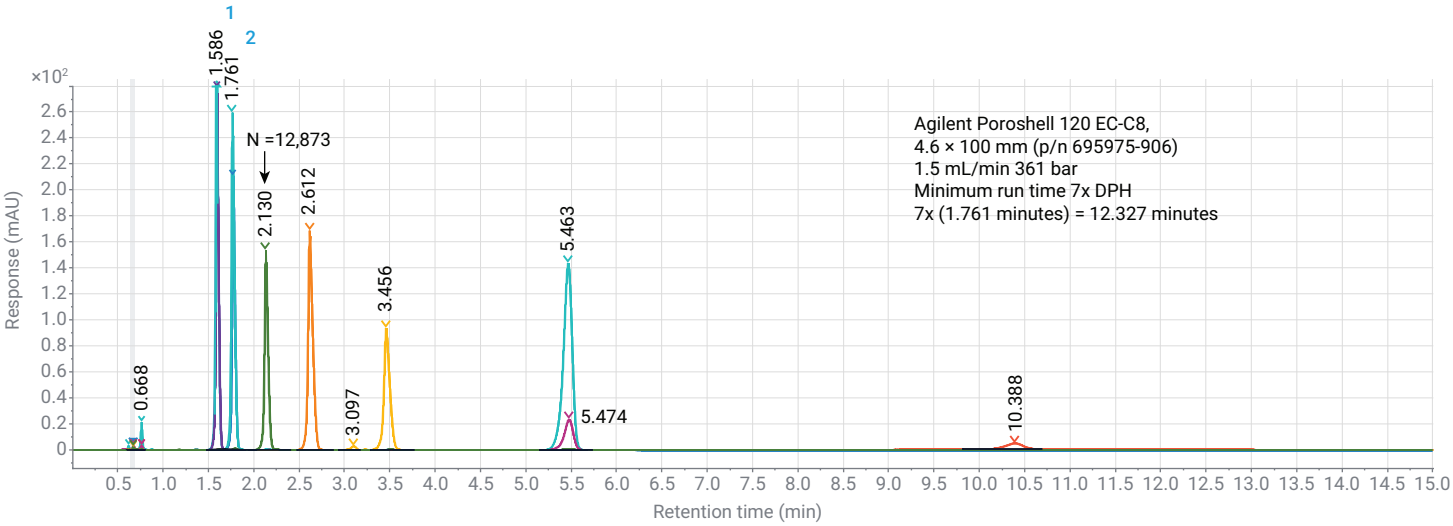

Figure 3. Agilent 1260 Infinity II Binary HPLC System, premix (buffer: 5.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0. Mobile phase: acetonitrile and buffer (35:65) 1.5 mL/min 220 nm, 4 µL injection.

System suitability requirements are the acceptance criteria for adjustments. In the case of the diphenhydramine impurity method, there are two criteria to meet. The first requirement is that the method run time be based on the diphenhydramine retention, which must be greater than seven times its retention. In the original method, the retention time was 5.302 minutes. The run time for the method must be at least 37.114 minutes. In the adjusted method, the diphenhydramine retention time was found to be 1.761 minutes, with a required run time of 12.327 minutes. This leads to a time savings of 66%. The second requirement of the method is that a minimum resolution of 2.0 between diphenhydramine-related Compound A and diphenhydramine must exist. The original method resolution was 2.85, and on the fast adjusted method, it was 3.02. This slight improvement in resolution will lead to increased useful column lifetime.

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. 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. *US PNF Monographs Home Page*. USP Diphenhydramine HCl, Impurity Method, *United States Pharmacopeia 43(4)*.
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3. *USP Harmonized Standards Home Page*. Supplement USP Stage 4 Harmonization, Official, December 1, 2022.

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