Application Note

Pharma & Biopharma

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

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Abstract

The transfer of the USP Assay 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 4.6 × 250 mm, 5 µm column and requires 13 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 13 to 3.5 minutes (27%) of the original method time, without need for revalidation using the InfinityLab Poroshell 120 EC-C8 column. Pressure is monitored and considered as a factor in instrument transfer. This transfer is not allowed under USP37-NF32S1 (official August 1, 2014), but will be allowed under 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.

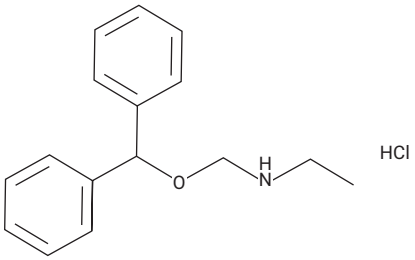
The costs associated with pharmaceutical testing can be reduced using adjustments to chromatography allowed under the general chapters in USP <621>. These costs are associated with chromatographic solvent and time. Of these two considerations, time is the most important.

In this application note, the current assay 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 not allowed adjustments under USP37-NF32S1 (official Aug 1, 2014), but the adjustment of gradients, as shown in this note, will be allowed under USP Stage 4 Harmonization, to be official December 1, 2022.

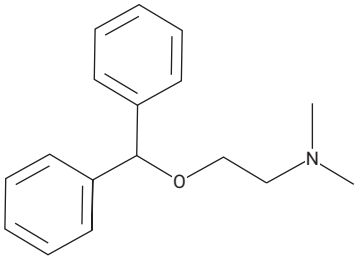
The costs associated with pharmaceutical testing are considerable, and many prudent lab managers are seeking ways to reduce costs by reducing solvent usage and improving productivity, while still using the LC instruments in their lab. Compendial methods from the USP are widely used in drug product and 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 frequently 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. The IUPAC name is 2-(diphenylmethoxy)-N,N-dimethylethanamine hydrochloride. In addition, the structure of diphenhydramine-related Compound A is also shown in Figure 1.

Agilent 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), and L10 (Cyano), as well as many others. The work in this application note will use the L7 phase (Agilent InfinityLab Poroshell 120 EC-C8).



Diphenhydramine related Compound A



Diphenhydramine

Figure 1. Diphenhydramine and diphenhydramine related Compound A.

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 Diphenhydramine RS, and USP Diphenhydramine Related A RS, were purchased from the United States Pharmacopeia. The mobile phase consists 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. Gradient Conditions are listed in Table 3.

Columns used in this work

- Agilent ZORBAX Eclipse Plus C8, 4.6 × 250, 5 µm, part number 959990-906
- Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm, part number 695975-906

Table 1. Instrument configuration.

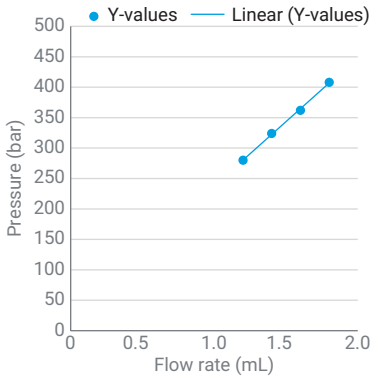
1260 Infinity II LC System	
Agilent 1260 Binary Pump (G7117B)	
Agilent 1260 Multisampler (G7167A)	– Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (p/n 5182-0716) – Cap, screw, blue, PTFE/red silicone septa, 100/pk (p/n 5182-0717)
Agilent G7116A Multicolumn Thermostat (MCT)	– Standard 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)	– 10 mm 1 µl flow cell (p/n 4212-60008) – 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
Mobile Phase	Mobile phase A: Buffer: 5.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0. Mobile phase B: Acetonitrile Diluent: Acetonitrile and buffer (35:65)
Flow Rate	1.2 mL/min
Run Time	13 minutes
Temperature (column)	25 °C (not in method, but under USP recommendations, 25 °C 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 1.5 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, and USP diphenhydramine hydrochloride RS in diluent Tailing factor: NMT 1.8, standard solution relative standard deviation: NMT 0.85% for six replicate injections, standard solution

Table 3. Gradient and pressure table with graph of pressure versus flow rate diphenhydramine assay on 4.6 × 100 mm, 2.7 µm column.

					% Buffer	% ACN
Flow Rate	1.2 mL/min	1.4 mL/min	1.6 mL/min	1.8 mL/min		
Time						
0	0	0	0	0	65	35
4	1.6	1.4	1.2	1.1	65	35
7	2.8	2.4	2.1	1.87	20	80
9	3.6	3.1	2.7	2.4	65	35
13	5.2	4.6	3.9	3.5	65	35
Initial Pressure (max)	280 bar	324 bar	346 bar	408 bar		



Results and discussion

Previously, under allowable adjustment guidelines, no adjustment of gradient conditions was allowed without revalidation. Even slight particle size variance from the USP method (such as 2.7 versus 2.6 µm) were cause for revalidation. With the introduction of the December 2022 Harmonization adjustments from totally porous to superficially porous particles: "combinations of L and dp can be used, provided that the ratio $(t/W_h)^2$ is within −25 to +50%, relative to the prescribed column for all the peaks used to determine the system suitability parameters". These changes are acceptable, provided system suitability criteria are fulfilled, and selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent. Other allowed changes with the method are to proportionately reduce the injection volume to the volume of the column. In this work, no adjustment of other chromatographic conditions is made.

System suitability requirements are the acceptance criteria for adjustments. In the case of the diphenhydramine assay method, there are three criteria to meet. Resolution of not less than (NLT): NLT 1.5 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, and USP diphenhydramine hydrochloride RS in diluent, with tailing factor of not more than (NMT) 1.8 using the standard solution. Finally, relative standard deviation must be NMT 0.85% for six replicate injections using the standard solution.

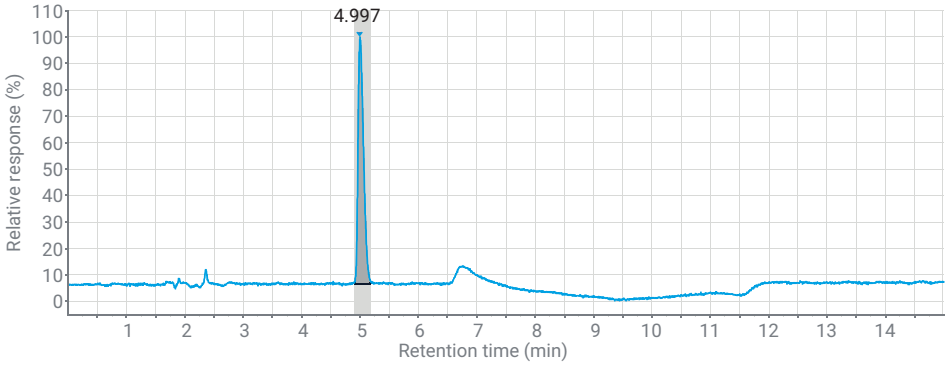


Figure 2. Diphenhydramine assay 1.2 mL/min Agilent ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm.

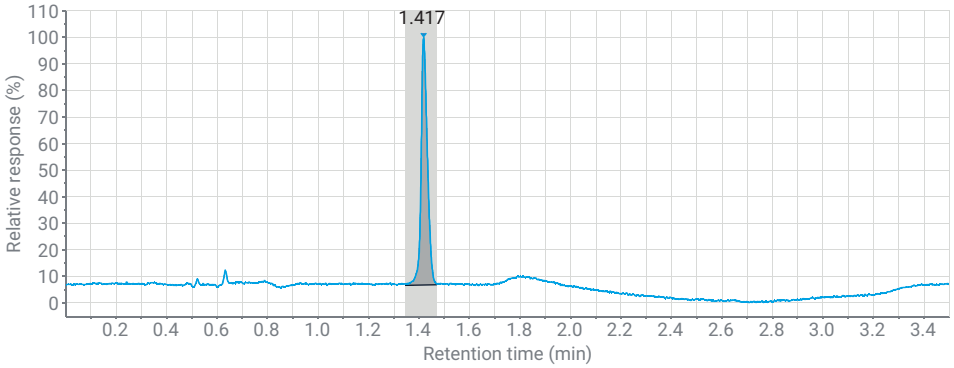


Figure 3. Diphenhydramine assay 1.8 mL/min Agilent Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm.

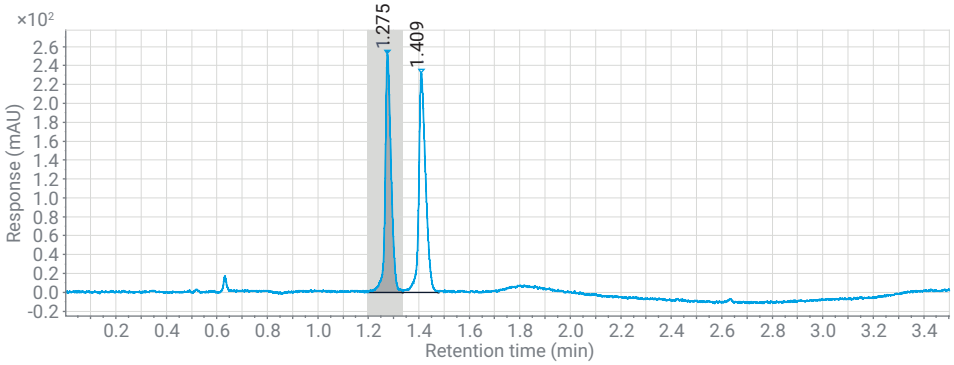


Figure 4. Diphenhydramine assay 1.8 mL/min Agilent Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm system suitability sample.

The gradient was adjusted using the formula:

$$t_{G2} = t_{G1} \times \left(\frac{F_1}{F_2}\right) \times \frac{[L_2 \times dc_{c2}{}^2]}{[L_1 \times dc_{c1}{}^2]}$$

Where the t_G is gradient segment time, F is flow rate, L is column length, and d refers to column diameter.

The gradient is adjusted as the flow rate is increased from 1.2 to 2.0 mL/min. The ratio $(t/W_h)^2$ is calculated for the diphenhydramine peak at each flow rate, to determine if the ratio is within the ratio of the original USP method. The retention time and peak width for the original USP assay method are determined to be 5.069 and 0.103 minutes, respectively. The ratio $(t/W_h)^2$ is calculated to be 2,410, with a range within −25 to +50%, (1,808 and 3,615). The flow rate was varied between 1.2 and 1.8 mL/minute, and evaluated with the diphenhydramine sample. The $(t/W_h)^2$ ratio was calculated for each condition. All four conditions were within the allowable range. It was decided to use the 1.8 mL/min gradient. The values are summarized in Table 4. System suitability was run for both the resolution solution and the standard solution, with six replicates, as stated in the requirements. A resolution of 2.96 with an %RSD of 0.28% was determined with the resolution solution, and a tailing factor of 1.2 was determined with an area %RSD of 0.68%, meeting the system suitability requirements.

Table 4. Flow rate optimization.

Column	Flow Rate (mL/min)	t_R (min)	(w_h) (min)	$(t_R/(w_h))^2$	Acceptable Range
Eclipse Plus C8 4.6 × 250, 5 µm	1.2	5.069	0.103	2,410	1,805 to 3,615
Poroshell 120 EC-C8 4.6 × 100, 2.7 µm	1.2	2.015	0.037	2,897	meets
	1.4	1.755	0.0323	2,863	meets
	1.6	1.563	0.0309	2,804	meets
	1.8	1.411	0.0268	2,778	meets

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.

Table 5. System suitability summary.

	Rs	Area	Tf
1	2.95	305	1.108
2	2.97	309.6	1.103
3	2.95	307.5	1.108
4	2.96	306.5	1.1117
5	2.96	308.2	1.11
6	2.95	310.8	1.12
Average	2.96	307.9	2.96
SD	0.008165	2.093482	0.008165
% std dev	0.28	0.68	0.28

References

1. USP NF Monographs Home Page. USP Diphenhydramine HCl, Impurity Method, *United States Pharmacopeia 43(4)*.
2. USP General Chapter<621>, USP 37-NF32, First supplement.
3. USP Harmonized Standards Home Page. Supplement USP Stage 4 Harmonization, Official, December 1, **2022**.

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