USP METHOD MODERNIZATION USING "EQUIVALENT L/d_P" AND "EQUIVALENT N" ALLOWED CHANGES WITH CORTECS C_8 COLUMNS

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INTRODUCTION

Many *United States Pharmacopeia* (USP) monograph LC methods were created years ago when longer columns, packed with larger fully porous particle sorbents, were the norm. Methods using these columns can now be considered "outdated" in resolving power and speed. Switching the stationary phase particles from larger to smaller and from fully porous to solid-core can greatly improve method resolution and speed. Better resolution arises from the narrower peaks (higher efficiency) that these particles provide. Quicker methods originate in the ability to use shorter columns with such particles without sacrificing efficiency.

Here we illustrate how an analyst can use solid-core CORTECS columns on the ACQUITY UPLC to modernize a USP method.[1] We selected the dofetilide USP assay method for improvement and demonstrate two different allowed USP isocratic LC method changes to achieve much higher analysis speed and lower solvent consumption.

BACKGROUND

USP General Chapter <621> specifies the alterations to an LC method that are permissible without revalidation. For isocratic methods, the analyst can change the stationary phase particle in one of two allowed ways. The first approach maintains an equivalent ratio of the column length, ***L***, to the particle diameter, d_p, in the range of -25% to +50% of the L/d_p ratio specified in the USP method. The second way employs other combinations of ***L*** and d_p that provide an equivalent plate count, ***N***, within -25% to +50% of that measured for the original column specified in the method.

The "equivalent L/d_p" guideline is based on **Eq. 1** where, for isocratic LC methods, the column plate count can be estimated from the column length, particle size and reduced plate height, ***h***. For chromatography of small molecules on well packed columns with fully porous particles, ***h*** is approximately equal to 2. With ***h*** ≈ constant for fully porous particles, it is justified to scale a USP method by L/d_p to get an equivalent plate count. However, when solid-core particles replace fully porous particles, ***h*** can decrease (in the range of 1.4 to 1.6), which increases the efficiency. In such cases, the "equivalent ***N***" guideline may replace "equivalent L/d_p" in modernizing USP methods.

$$N = \frac{L}{h d_p} \quad \text{Eq. 1} \qquad \Delta P = \frac{180 F L \eta}{\pi r^2 d_p^2} \cdot \frac{(1-\varepsilon_e)^2}{\varepsilon_e^3} \quad \text{Eq. 2}$$

During chromatography, the pressure drop across a column, ***ΔP***, is given by **Eq. 2** where ***F*** is the mobile phase flow rate, ***η*** is the mobile phase viscosity, and ***r*** is the column radius. Parameter ε_e is the column external porosity which has an inverse effect on the column backpressure. With similar packing conditions, solid-core particle columns tend to have higher external porosities compared to fully porous particle columns. The gain in analysis speed by using the shorter column during "equivalent L/d_p" method modernization has a cost, in the form of higher column pressure. Holding constant all parameters of **Eq. 3** except for external porosity allows a plot of ***ΔP*** vs. ε_e. **Figure 1** shows one such plot. One can calculate the efficiency vs. pressure (***N***/***ΔP***) relationship for optimally packed fully porous and solid-core columns from **Eq. 1** and **Eq. 2**. **Table 1** gives some examples of this calculation with different column particles. The pattern is clear; a solid-core particle will give more efficiency benefit for a given pressure cost.

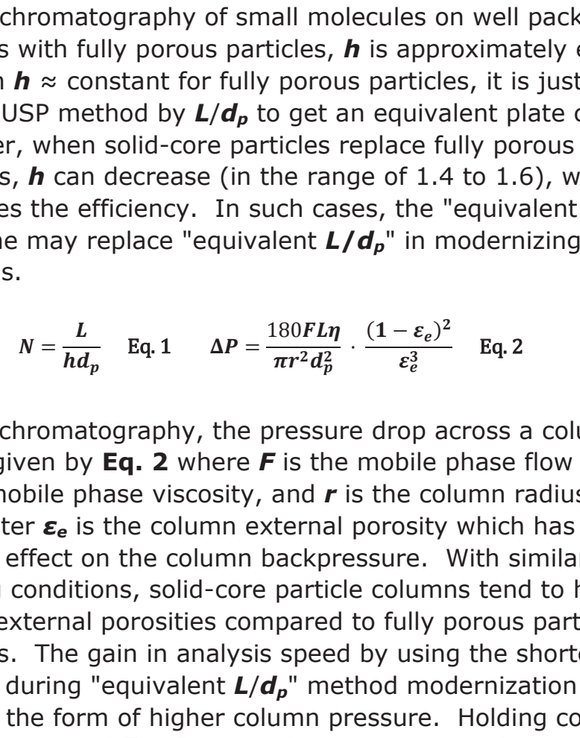

*Figure 1. Typical column pressure drop, **ΔP**, vs. external porosity, ε_e, relationship from **Eq. 2** (▲ fully porous; ● solid-core for a 3.0 x 100 mm column with 2.7 µm particles at 1.0 mL/min with 1:1 acetonitrile/water).*

Column (3.0 x 100 mm)	d_p (µm)	*h*	ε_e	Calculated *N*	Calculated ΔP (psi)	*N*/ΔP (plates/psi)
Solid-Core 2.7 µm	2.7	1.5	0.39	24,691	4,290	5.76
Fully Porous 2.5 µm	2.5	2.0	0.37	20,000	6,251	3.20
Solid-Core 1.6 µm	1.6	1.5	0.39	41,667	12,217	3.41
Fully Porous 1.7 µm	1.7	2.0	0.37	29,412	13,518	2.18

*Table 1. Calculated Efficiency, **N**, vs. Column Pressure, **ΔP**, for columns at 1.0 mL/min with 1:1 acetonitrile/water.*

METHODS

Sample Preparation

An assay sample solution containing dofetilide, **1**, (25 µg/mL) and dofetilide related compound A, **2**, (0.5 µg/mL) was prepared with mobile phase as the diluent.

Instruments & Data Management

Alliance HPLC and ACQUITY UPLC H-Class using Empower 3 CDS.

Original Compendial Method Conditions

A Nova-Pak™ C_8 4 µm, 3.9 x 150 mm column was used under isocratic conditions with a 1:3 mixture of acetonitrile and buffer solution (1.36 g monobasic potassium phosphate and 5 mg ascorbic acid in 1 L water, adjusted with 0.01M potassium hydroxide solution to pH 7.0). The flow rate was 1.00 mL/min at a column temperature of 30 °C. Detection was by UV (230 nm). Triplicate injections (50 µL) were made.

Modernized Replacement Method Conditions
(only changes are listed)

CORTECS C_8 2.7 µm columns (3.0 x 100 mm, 75 mm, 50 mm) and CORTECS UPLC C_8 1.6 µm columns (3.0 x 50 mm, 30 mm) were used. The flow rates were 0.88 mL/min (2.7 µm columns) and 1.30 mL/min (1.6 µm columns). Triplicate injections (19.8 µL, 14.8 µL, 9.9 µL and 5.9 µL for the 100 mm, 75 mm, 50 mm and 30 mm columns, resp.) were made.

RESULTS AND DISCUSSION

The USP assay method uses both dofetilide, **1**, and its related desmethyl compound, **2**, for analysis, **Figure 2**.

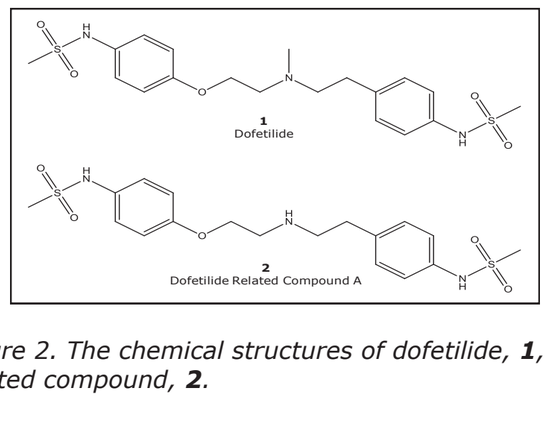

*Figure 2. The chemical structures of dofetilide, **1**, and its related compound, **2**.*

1. Original Compendial Method

An L7 column, specifically a Waters Nova-Pak C_8 4 µm, 3.9 x 150 mm, with an L/d_p of 37,500 was originally used. **Figure 3** and **Table 2** show the chromatograms and the results for the original compendial method, against which all comparisons are made, and the method modernizations. The flow rates and injection volumes were altered, as provided in USP General Chapter <621>, to account for the modernization changes in column diameter and particle size from the original compendial method.

2. Modernization Using "Equivalent L/d_p"

Two CORTECS C_8 columns that cover both particle sizes and meet the "equivalent L/d_p" guideline were selected. For the larger 2.7 µm solid-core particle, dofetilide elutes at 2.33 minutes, which is a 67% decrease in run time and thus a 71% decrease in solvent consumption compared to the compendial method. There is also a large increase in both resolution (90%) and efficiency (136%) for dofetilide.

With the sub 2 µm CORTECS column, the smaller particles allow use of a still shorter column, resulting in even faster analysis and further reduction in solvent use. This is seen by the rapid elution of dofetilide at 0.91 minutes, an 87% run time decrease with still a 74% efficiency increase relative to the original method. There is an associated 83% reduction in solvent consumption.

3. Modernization Using "Equivalent *N*"

Shorter CORTECS C_8 columns that fall outside of the "equivalent L/d_p" range can be used under the "equivalent ***N***" guideline if it can be demonstrated that the measured plate count is in the range of 5,651 (-25%) to 11,302 (+50%) for analyte **1** and 3,222 (-25%) to 6,444 (+50%) for analyte **2**. Two 2.7 µm CORTECS C_8 columns in shorter lengths (75 and 50 mm) with L/d_p values beyond the lower limit of 28,125 (-25%) were tested for "equivalent ***N***" modernization.

The longer CORTECS C_8 2.7 µm column (75 mm) gives chromatography meeting all USP method requirements and provides a shorter dofetilide retention time of 1.80 min. However, the plate counts for both compounds are outside the upper end of the guideline efficiency range for the original method (+76% for dofetilide, **1**, and +72% for the related compound, **2**). Most analysts would welcome such higher efficiency methods. The ACQUITY UPLC is an example of a very high performance LC instrument with a low dispersion fluid path, a small detector cell volume and a high detector sampling rate, all designed to handle the reduced peak width and volume caused by modern high efficiency columns such as the CORTECS family. A case could therefore be made that, for UPLC class instruments, efficiencies beyond the +50% guideline are acceptable when modernizing USP methods.

The shorter CORTECS C_8 2.7 µm column (50 mm), trades some efficiency to gain still more analysis speed (**1** elutes at 1.22 min). This places the resulting modernized method in the USP General Chapter <621> "equivalent ***N***" range while meeting USP method requirements.

Finally, the CORTECS UPLC C_8 1.6 µm, 3.0 x 30 mm column has an L/d_p of 18,750, also below the "equivalent L/d_p" criteria range. Here, the dofetilide USP method criteria are achieved, the analysis is very fast with dofetilide eluting at 0.55 min and the measured plate counts meet the "equivalent ***N***" criteria. This demonstrates that even the shortest CORTECS UPLC C_8 1.6 µm column can meet the USP General Chapter <621> "equivalent ***N***" guideline and also meet the dofetilide USP method requirements. The result is an almost 13 times faster method (92% decrease in analysis time) with a 90% reduction in solvent consumption, compared to the original compendial method.

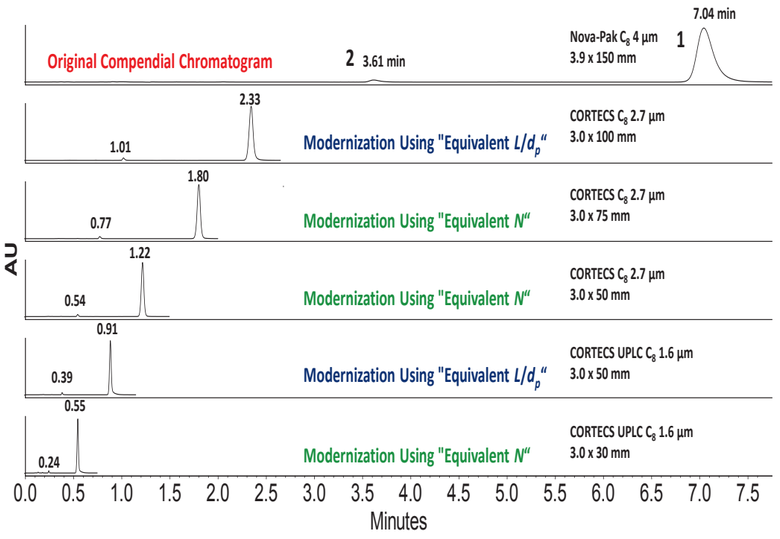

Figure 3. Dofetilide USP Assay Method Chromatograms.

Column	L/d_p	Resolution NLT 8.0	%RSD Retention Time (min) NMT 2.0 Compound **1**	USP Plate Count (***N***) Compound **1**	USP Plate Count (***N***) Compound **2**
1. Original Compendial Dofetilide USP Method Results *(Alliance HPLC)*					
Nova-Pak C_8 4µm 3.9 x 150 mm	**37,500**	**13.08**	**0.17**	**7,535**	**4,296**
2. Modernization Using "Equivalent L/d_p" Results *(ACQUITY UPLC H-Class)*					
CORTECS C_8 2.7 µm 3.0 x 100 mm	**37,037**	**24.90**	**0.03**	**17,813**	**12,798**
CORTECS UPLC C_8 1.6 µm 3.0 x 50 mm	**31,250**	**20.67**	**0.00**	**13,075**	**7,057**
3. Modernization Using "Equivalent *N*" Results *(ACQUITY UPLC H-Class)*					
CORTECS C_8 2.7 µm 3.0 x 75 mm	**27,778**	**20.89**	**0.06**	**13,261**	**7,375**
CORTECS C_8 2.7 µm 3.0 x 50 mm	**18,519**	**16.84**	**0.09**	**9,484**	**5,251**
CORTECS UPLC C_8 1.6 µm 3.0 x 30 mm	**18,750**	**14.84**	**0.00**	**7,094**	**4,469**

Table 2. Dofetilide USP Assay Method Results

CONCLUSION

- **Modernizing isocratic USP methods with the "equivalent L/d_p" guideline, CORTECS columns and the ACQUITY UPLC is easy and affords large analysis speed increases.**
- **Using the "equivalent *N*" guideline with CORTECS columns and the ACQUITY UPLC to modernize isocratic USP methods will fully leverage the efficiency of these solid-core particles. This presents a maximum analysis speed boost over the original USP method.**

References

1. See Waters Application Note #APNT134886223 for additional information.

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