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응용 자료

USP Method Modernization Using "Equivalent L/d_p" and "Equivalent N" Allowed Changes with CORTECS C_8 and CORTECS UPLC C_8 Columns

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Abstract

This application demonstrates two approaches for making allowed changes to an isocratic USP method involving modern column particles such as that found in solid-core CORTECS Columns.

Using the "equivalent L/d_p" guideline is an easy way to modernize a USP method and greatly improve the speed of analysis. With the "equivalent N" guideline, the analyst can realize still further analysis speed improvements from even shorter, highly efficient columns in the CORTECS family.

Benefits

- USP method modernization for the analysis of dofetilide, using the "equivalent L/d_p" and "equivalent N" allowed changes to isocratic USP LC compendial methods.
- Up to a 92% decrease in sample analysis time.
- Up to a 90% reduction in solvent consumption.

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 Methods using these columns can now be considered "outdated" in resolving power and speed.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.

This application note illustrates how an analyst can use solid-core CORTECS Columns to modernize a USP method. We selected the dofetilide[1] 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

To change the stationary-phase particles used in a USP method, the analyst must consult USP General Chapter <621>. This section of the USP 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.[2] The first approach maintains an equivalent ratio of the column length, *L*, to the particle size (diameter), d_p, in the range of -25% to +50% of the L/d_p ratio[3] specified in the USP method. The second way employs other combinations of *L* and d_p that provide an equivalent number of theoretical plates (plate count, also called the column efficiency), *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[4] from the column length, particle size, and reduced plate height, *h*.

(1)

$$N = \frac{L}{hd_p}$$

For chromatography of small molecules on well packed columns with fully-porous particles, *h* is approximately[6] equal to 2. With $h \approx$ constant, it is justified to scale a USP method by L/d_p to get an equivalent plate count, *N*, when the original stationary-phase particles and the replacement stationary-phase particles are both fully porous.

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.[7] In such cases, the "equivalent *N*" guideline may replace "equivalent L/d_p" in modernizing USP methods. This requires actual plate count measurements on the USP method comparing the original and replacement columns packed with fully-porous and solid-core particles, respectively. The equation[3] to measure USP plate count, *N*, is:

(2)

$$N = 16\left[\frac{t_R}{W}\right]^2$$

where t_R is the analyte peak retention time and *W* is the analyte peak width at its base. The value of *N*, for each analyte in a USP method, can be easily measured using the System Suitability module of Waters

Empower Chromatography Data Software.

During chromatography, the pressure drop across a column, ΔP, is given[8] by eq 3, where F is the mobile phase flow rate, η is the mobile phase viscosity, r is the column radius, and ε_e is the column external porosity.

(3)

$$\Delta P = \frac{180 F L \eta}{\pi r^2 d_p^2} \cdot \frac{(1-\varepsilon_e)^2}{\varepsilon_e^3}$$

During USP method modernizations using "equivalent L/d_p", the pressure decrease at a given flow rate due to the shorter column length is small compared to the pressure increase from the smaller particles. The gain in analysis speed by using the shorter column with proportionally smaller d_p therefore has a cost, in the form of higher column pressure. The magnitude of the pressure increase can be mitigated, however, if one changes the column external porosity. External porosity is a measure of how much the packed particles in a column resist the flow of liquid. It is a property that does not depend on the other parameters in eq 3. Solid-core particle columns have a higher external porosity (e.g. $\varepsilon_e \approx 0.39$ for CORTECS) whereas fully porous particle columns have a lower external porosity (e.g. $\varepsilon_e \approx 0.37$ for BEH C_{18}). Holding constant all parameters of eq 3, except for external porosity, allows a plot of ε_e vs. ΔP. Figure 1 shows one such plot.

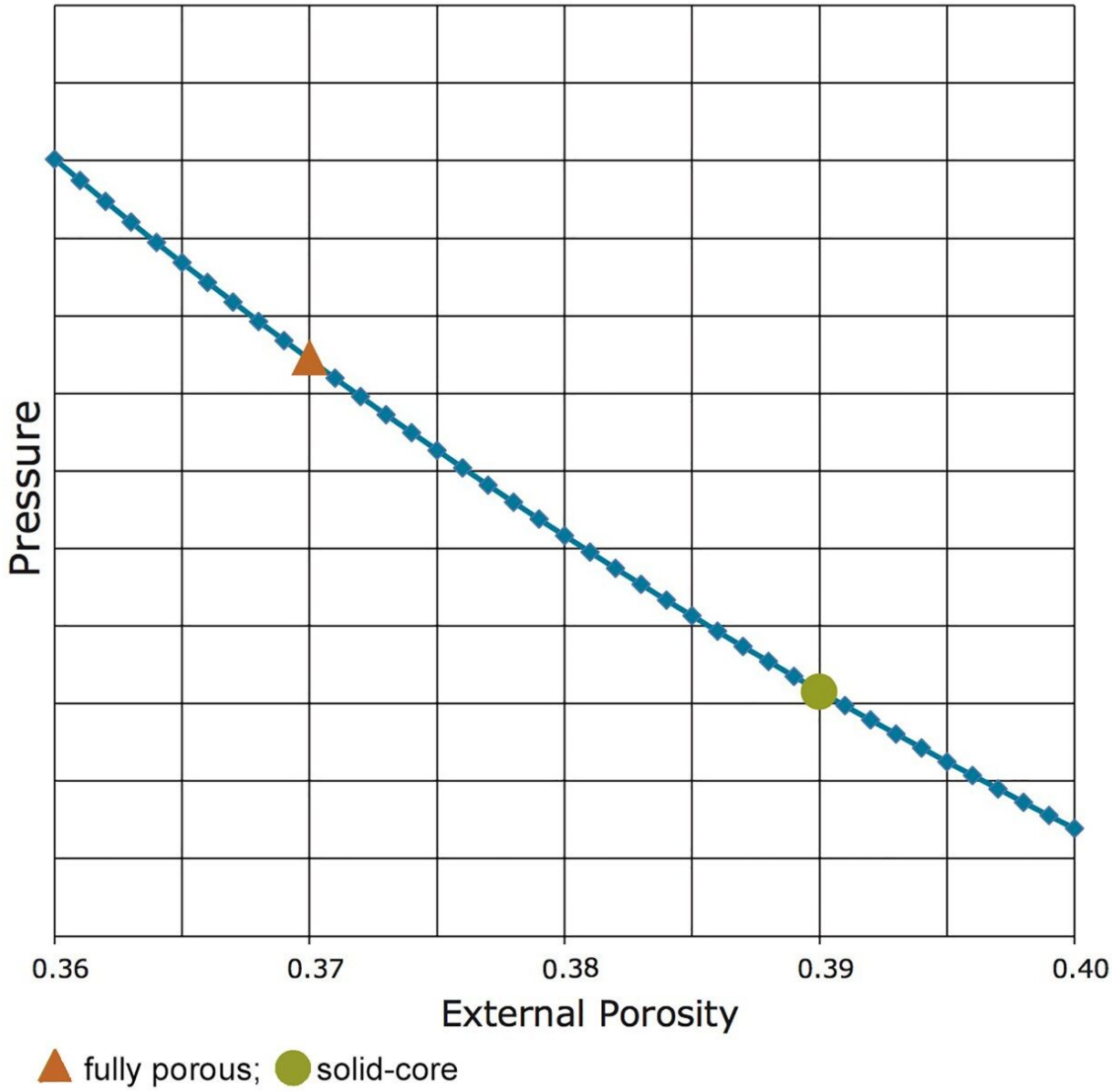

Figure 1. Typical column external porosity, ε_e , vs. pressure, ΔP, relationship from eq 3 with all other parameters held constant.

One can calculate the efficiency vs. pressure *(N/ΔP)* relationship for optimally packed fully-porous and solid-core columns from eqs 1 and 3. Table 1 gives some examples of this calculation with different column particles. The pattern is clear; a solid-core particle will always give more efficiency benefit for a given pressure cost.

Column	d_p (µm)	h	ε_e	Calculated N	Calculated ΔP (psi)	$N/\Delta P$ (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 3.0 x 100 mm columns at 1.0 mL/min with 1:1 acetonitile/water.

Experimental

Sample preparation

System suitability mixture

A sample containing dofetilide (25 µg/mL) and dofetilide related compound A (0.5 µg/mL) was prepared with mobile phase as the diluent.

LC systems:	Alliance HPLC and ACQUITY UPLC H-Class
Data management:	Empower 3 CDS

Method conditions

Original compendial method conditions

Column:	Nova-Pak C_8 Column, 60Å, 4 µm, 3.9 x 150 mm (p/n WAT035876)
Mobile phase:	Acetonitrile:buffer solution (1:3)
Buffer solution:	1.36 g monobasic potassium phosphate and 5 mg ascorbic acid in 1 L water, adjusted with 0.01 M potassium hydroxide solution to pH 7.0
Separation technique:	Isocratic
Flow rate:	1.00 mL/min
Column temp.:	30 °C
Detection (UV):	230 nm
Injection volume:	50 µL

Modernized replacement method conditions

(only changes are listed)

Columns:	CORTECS C_8 Column, 90Å, 2.7 µm, 3.0 x 100 mm (p/n 186008361) CORTECS C_8 Column, 90Å, 2.7 µm, 3.0 x 75 mm (p/n 186008360) CORTECS C_8 Column, 90Å, 2.7 µm, 3.0 x 50 mm (p/n 186008359) CORTECS UPLC C_8 Column, 90Å, 1.6 µm, 3.0 x 50 mm (p/n 186008409) CORTECS UPLC C_8 Column, 90Å, 1.6 µm, 3.0 x 30 mm (p/n 186008408)

Flow rate:	0.88 mL/min (2.7 µm columns) 1.30 mL/min (1.6 µm columns)
Injection volume:[5]	19.8 µL (3.0 x 100 mm columns) 14.8 µL (3.0 x 75 mm columns) 9.9 µL (3.0 x 50 mm columns) 5.9 µL (3.0 x 30 mm columns)

Results and Discussion

Dofetilide is a prescription pharmaceutical given to treat patients with irregular heartbeats. It is a class III antiarrhythmic agent that specifically blocks rapid potassium channels[9] and is manufactured by Pfizer under the brand name TIKOSYN. The USP assay method uses both dofetilide, 1, and its related desmethyl compound, 2, for analysis, Figure 2.

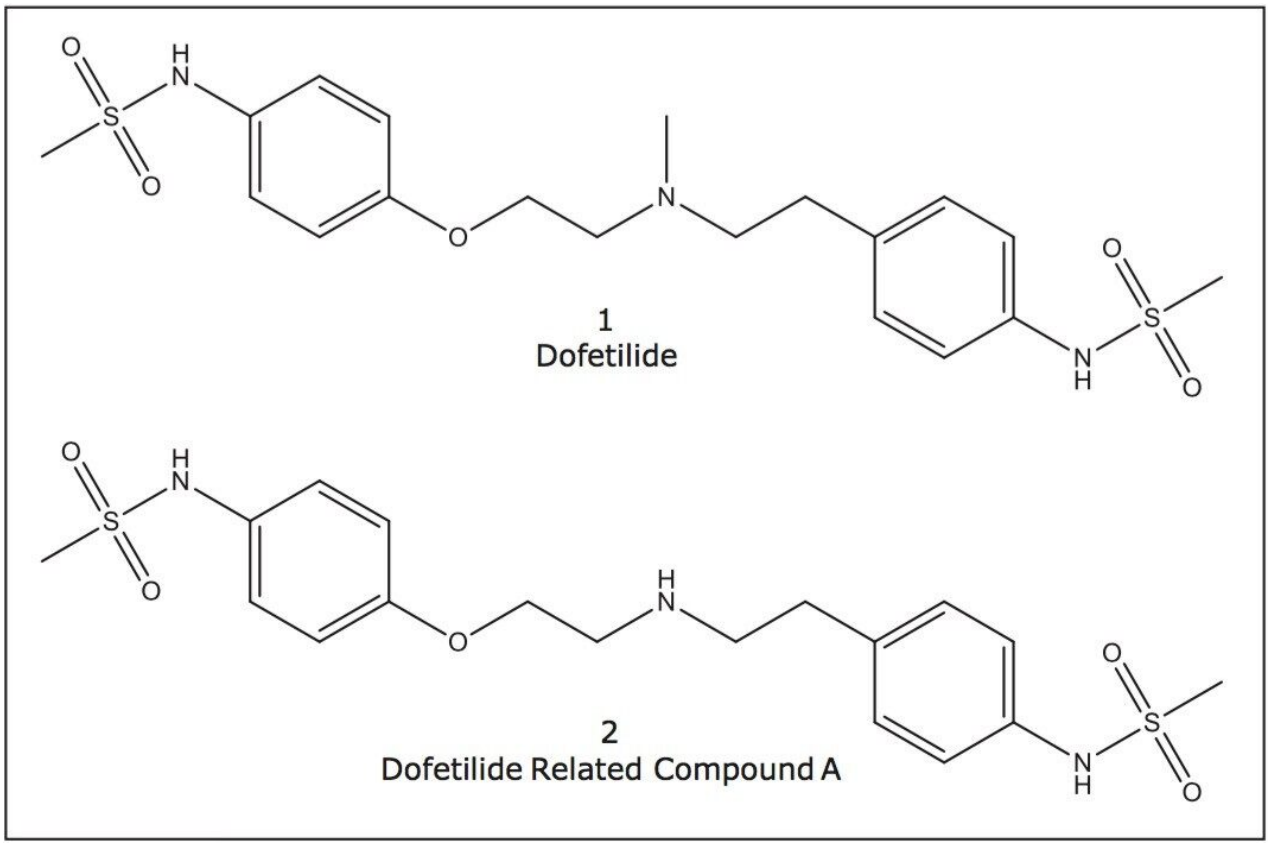

Figure 2. The chemical structures of dofetilide, 1, and its related compound, 2.

This method requires an L7 (C_8) column. In particular, a Waters Nova-Pak C_8 Column, 4 µm,3.9 x 150 mm (p/n WAT035876) with an L/d_p of 37,500 was originally used. Since this original USP method column is designed for HPLC analysis, it was run on the Alliance HPLC System. The chromatogram and results, Figure 3 and Table 2, will be used as the compendial reference for the analytical method modernization.

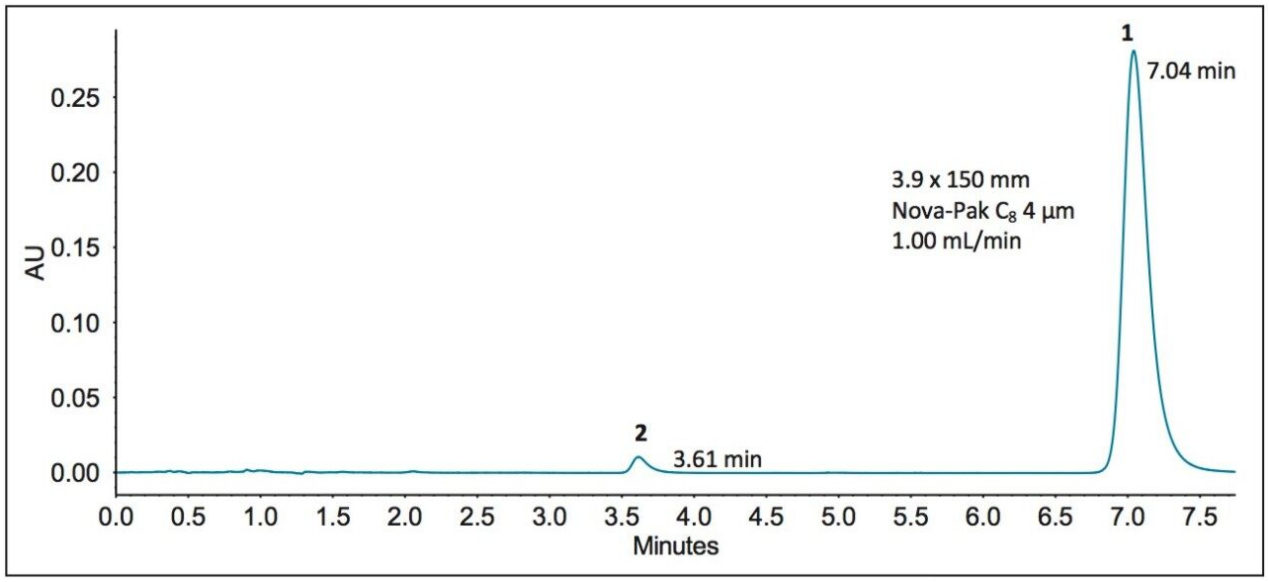

Figure 3. Separation of dofetilide, 1, and its related compound, 2, on the original column using the Alliance HPLC System.

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**
Nova-Pak C_8 4µm 3.9 x 150 mm	37,500	13.08	0.17	7,535	4,296

Table 2. Original dofetilide USP method results on a Nova-Pak C_8 Column, 4 µm, 3.9 x 150 mm (p/n WAT035876) Column using the Alliance HPLC System.

From Table 2, observe that the original column passes the USP method system suitability criteria, which specify that the resolution between the compounds must be not less than (NLT) 8.0 and the %RSD[10] for the retention time of dofetilide must be not more than (NMT) 2.0%. Triplicate injections were performed to calculate the %RSD and report the average results.

Modernization using the "equivalent L/d_p"allowed change

We first used "equivalent L/d_p" from USP General Chapter <621> to modernize the dofetilide USP assay method. Table 2 lists L/d_p = 37,500 for the original column in the compendial method. This required use of CORTECS C_8 Columns with L/d_p between 28,125 (-25%) and 56,250 (+50%). The CORTECS C_8 Column, 2.7

µm, 3.0 x 100 mm (p/n 186008361) and the CORTECS UPLC C_8 Column, 1.6 µm, 3.0 x 50 mm (p/n 186008409) both satisfy this guideline, with L/d_p = 37,037 and L/d_p = 31,250, respectively. We thus equipped an ACQUITY UPLC H-Class System with these columns. The flow rates were scaled using eq 4, where "F_1 and F_2 are the flow rates for the original and modified conditions, respectively; dc_1 and dc_2 are the respective column diameters; and dp_1 and dp_2 are the particle sizes."[3]

(4)

$$F_2 = F_1 \cdot \left[\frac{(dc_2)^2 \cdot dp_1}{(dc_1)^2 \cdot dp_2}\right]$$

From eq 4, we calculate scaled flow rates of 0.88 mL/min for the CORTECS C_8 Column, 2.7 µm, 3.0 x 100 mm (p/n 186008361) and 1.48 mL/min for the CORTECS UPLC C_8 Column, 1.6 µm,3.0 x 50 mm (p/n 186008409). Unfortunately,the latter column and flow rate comb ination exceeds the maximum system pressure. USP General Chapter <621> states that isocratic USP method flow rates may be adjusted by ±50% so we reduc ed the flow rate for this column to 1.30 mL/min.

Figure 4 shows the separation on the CORTECS C_8, 2.7 µm, 3.0 x 100 mm and the CORTECS UPLC C_8, 1.6 µ m, 3.0 x 50 mm Columns. 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 and efficiency for dofetilide at 90% and 136% respectively.

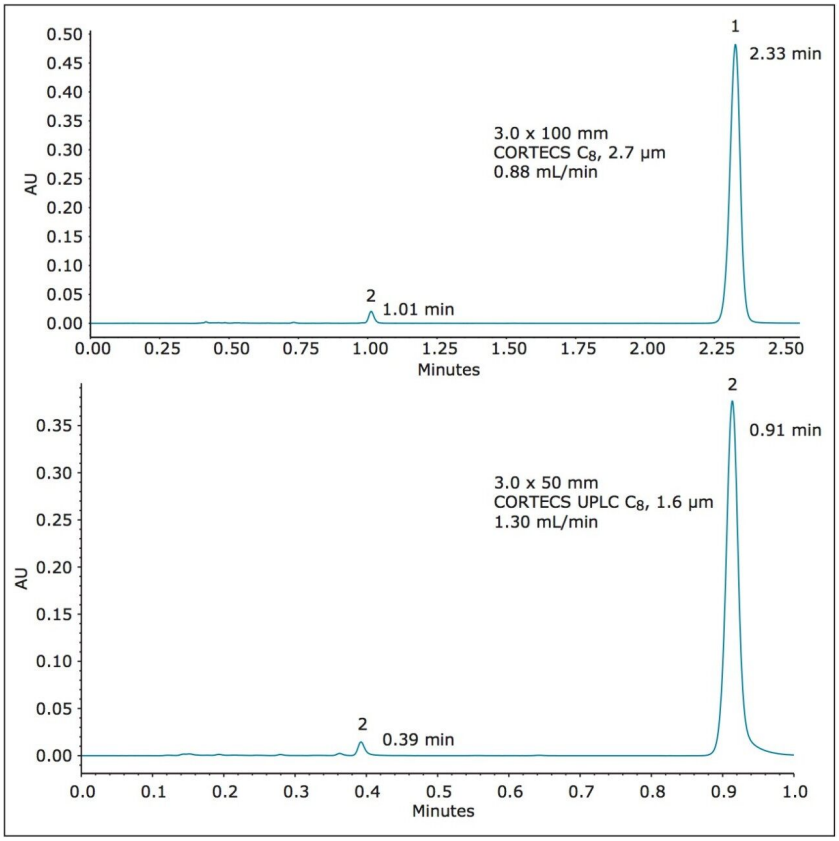

Figure 4. Separation of dofetilide, 1, and its related compound, 2, on CORTECS C_8 Column, 2.7 µm, 3.0 x 100 mm (p/n 186008361) and CORTECS UPLC C_8 Column, 1.6 µm, 3.0 x 50 mm (p/n 186008409) using the ACQUITY UPLC H Class System.

With the sub-2-µm CORTECS Column, the smaller particles allow use of a shorter column resultingin further analysis speed increase and solvent reduction. This is seen by the rapid elution of dofetili de 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. The results of these separations are found in Table 3.

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
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

Table 3. Modernized dofetilide USP method results on CORTECS C_8 , 2.7 µm, 3.0 x 100 mm and CORTECS UPLC C_8 , 1.6 µm, 3.0 x 50 mm Columns using the ACQUITY UPLC H-Class System.

Modernization using the "equivalent N" allowed change

We are at the limit of the modernizations possible using the "equivalent L/d_p" allowed changes with the dofetilide USP method. Next, we examined the "equivalent *N*" guideline. The compendialmethod USP plate count, from Table 2, is $N = 7{,}535$ for dofetilide, 1, and $N = 4{,}296$ for the rela ted compound, 2. Shorter CORTECS C_8 Columns that falloutside of the "equivalent L/d_p" range can be used if it can be demonstrated that the measured plate count is in the range of 5,651 (-25%) to 11,302 (+50%) for 1 and 3,222 (-25%) to 6,444 (+50%) for 2.

Since plate count must be experimentally determined, we first equipped the ACQUITY UPLC H-Class System with 2.7 µm CORTECS C_8 Columns in the shorter lengths of 3.0 x 75 mm and 3.0 x 50 mm. These column configurations offer L/d_p values of 27,778 and 18,519 respectively. Both values lie beyond the lower L/d_p limit of 28,125 (-25%) discussed above. In Figure 5 are the chromatograms obtained from these columns.

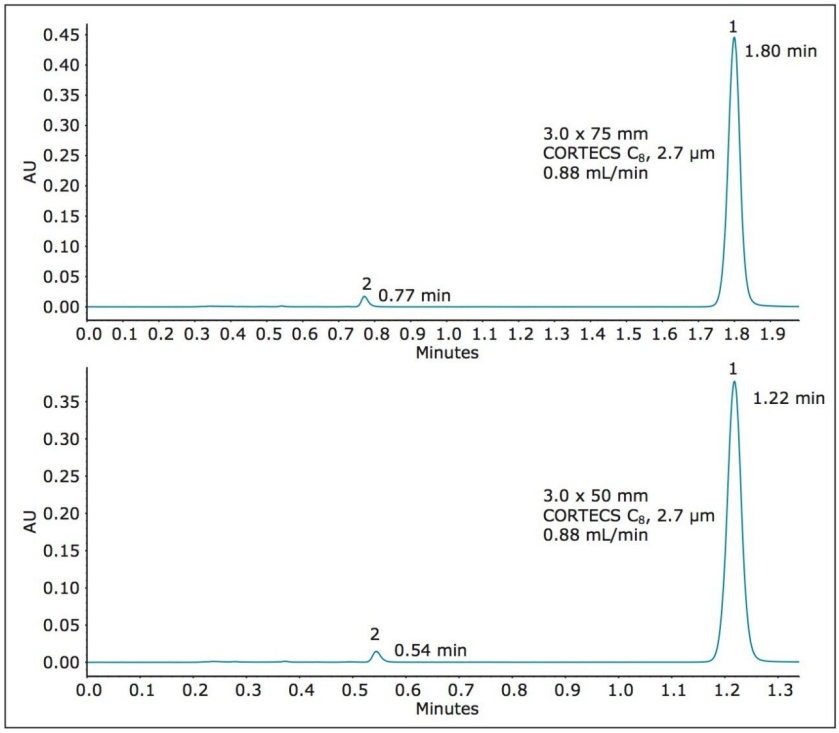

Figure 5. Separation of dofetilide, 1, and its related compound, 2, on CORTECS C_8 Column, 2.7 µm, 3.0 x 75 mm (p/n 186008360) and CORTECS C_8 Column, 2.7 µm, 3.0 x 50 mm (p/n 186008359) using the ACQUITY UPLC H-Class System.

The USP method results for these shorter columns are listed in Table 4. The longer CORTECS C_8 Column, 2.7 µm, 3.0 x 75 mm (p/n 186008360) meets all USP method requirements and gives dofetilide a shorter retention time of 1.80 minutes. However, the plate counts for both compounds are outside the upper end of the efficiency range of the original method (+76% for dofetilide, 1, and +72% for the related compound, 2).

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
CORTECS C_8 2.7 µm 3.0 x 75 mm	27,778	20.89	0.06	13,261	7,375
CORTECS UPLC C_8 2.7 µm 3.0 x 50 mm	18,519	16.84	0.09	9,484	5,251

Table 4. Modernized dofetilide USP method results on CORTECS C_8, 2.7 µm, 3.0 x 75 mm and CORTECS C_8, 2.7 µm, 3.0 x 50 mm Columns using the ACQUITY UPLC H-Class System.

Higher efficiency methods are welcomed by analysts. The USP General Chapter <621> cautions that higher efficiency columns may necessitate use of instruments that "minimize extra-column band broadening by factors as instrument plumbing, detector cell volume and sampling rate, and injection volume."[3] The ACQUITY UPLC H-Class System is an example of an UltraPerformance 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, 3.0 x 50 mm Column, 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.

A 1.6 µm particle CORTECS UPLC C_8 Column in a shorter length was also examined. The CORTECS UPLC C_8 Column, 1.6 µm, 3.0 x 30 mm (p/n 186008408) has an L/d_p of 18,750, below the "equivalent L/d_p" criteria range. When run on the ACQUITY UPLC H-Class System, this column gave the chromatogram in Figure 6.

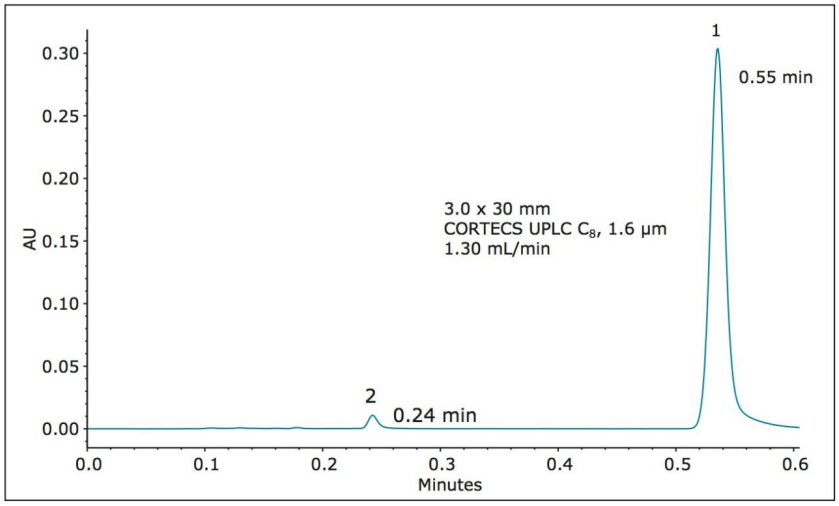

Figure 6. Separation of dofetilide, 1, and its related compound, 2, on a CORTECS UPLC C_8 Column, 1.6 µm, 3.0 x 30 mm (p/n 186008408) using the ACQUITY UPLC H-Class System.

Table 5 summarizes the results. The dofetilide USP method criteria are achieved, the analysis is very fast with dofetilide eluting at 0.55 minutes and the measured plate counts meet the "equivalent *N*" criteria.

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
CORTECS C_8 1.6 µm 3.0 x 30 mm	18,750	14.84	0.00	7,094	4,469

Table 5. Modernized dofetilide USP method results on a CORTECS UPLC C_8 , 1.6 µm, 3.0 x 30 mm Column using the ACQUITY UPLC H-Class System.

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. The dramatic decrease in analysis times across all the modernized methods discussed above is summarized in Figure 7.

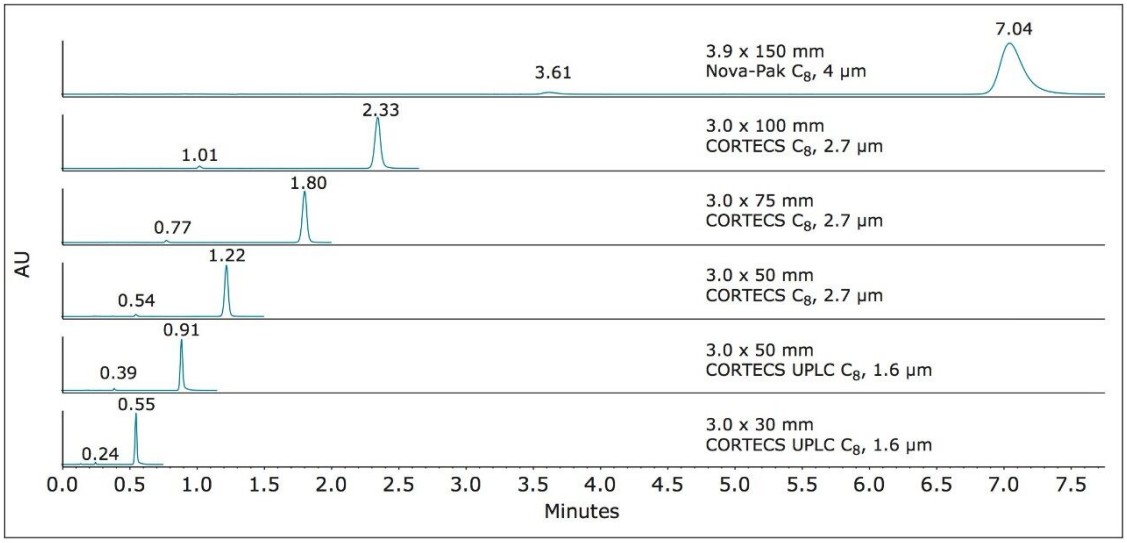

Figure 7. Separation of dofetilide, 1, and its related compound, 2, ordered by decreasing analysis time.

Conclusion

This application illustrates two approaches for making allowed changes to an isocratic USP method involving modern column particles such as that found in solid-core CORTECS Columns. Using the "equivalent L/d_p" guideline is an easy way to modernize a USP method and greatly improve the speed of analysis. With the "equivalent *N*" guideline, the analyst can realize still further analysis speed improvements from even shorter, highly efficient columns in the CORTECS family.

References

1. USP38 - NF33 S2 Monograph: Dofetilide.
2. USP38 – NF33 S2, General Chapter <621>.
3. L/d_p is expressed as a ratio of the same units (e.g. µm).

4. Equation 1 is obtained by combination and rearrangement of eq 2.12 (p 37) and eq 2.18 (p 44) in Snyder, L. R.; Kirkland, J. J.; Dolan, J. W. *Introduction To Modern Liquid Chromatography*, 3rd ed.; John Wiley & Sons: U.S., 2010.

5. Injection volumes were scaled using the relationship: new injection volume = original injection volume X (new column volume/original column volume).

6. Snyder, L.R.; Kirkland, J.J.; Dolan, J.W. *Introduction To Modern Liquid Chromatography*, 3rd ed.; John Wiley & Sons:U.S., 2010, p 205.

7. Fekete, S.; Oláh, E.; Fekete, J. Fast Liquid Chromatography: The domination of core-shell and very fine particles. *Journal of Chromatography A*. 2012, 1228, 57–71.

8. Cramers, C.A.; Rijks, J. A.; Schutjes, C.P.M. Factors Determining Flow Rate in Chromatographic Columns. *Chromatographia.* 1981, 14 (7), 439–444. Column pressures calculated from eq 3 do not include any additional pressure contribution from the instrument.

9. Yang T.; Tande P.M.; Lathrop D.A.; Refsum, H. Class III antiarrhythmic action by potassium channel blockade: dofetilide attenuates hypoxia induced electromechanical changes. Cardiovascular Research. 1992, 26, 1109–1115.

10. %RSD = percent relative standard deviation.

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