Translating Monograph Chromatography Methods to Smaller Dimension Columns with Porous and Solid Core particles Using new Guidance from USP <621>

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Compendial tests and other product specific tests are necessary to assure the safety and quality of human medicinal products manufactured around the world. Monographs include a variety of tests and may include an LC method for quantitative or qualitative purposes. Many such LC methods tend to be based upon 'traditional' column formats and technology such as 250 x 4.6 mm 10 µm porous particles. Recognising the recent advances in LC technology (smaller particles and smaller column formats) including the widespread use of solid core technology, an update of USP <621> (general chapter on chromatography) came into effect in 2014. This short article highlights the key features and gives an overview of the major changes to USP <621>. Improved productivity and solvent cost savings are demonstrated by applying the constant column length to particle size ratio option for method translations to example USP monographs using both porous and solid core particle based columns.

Introduction

Compendial and product specific testing is necessary within the pharmaceutical industry and independent testing laboratories to ensure the safety and quality of medicines produced by a variety of manufacturers for countries around the world. LC methods within monographs are common and used for a variety of quantitative and qualitative tests such as potency, purity and identification. Most pharmacopeia contain a general chapter on chromatography outlining broad guidance for performing LC methods listed within monographs. USP <621> describes general chromatography information for USP based procedures and includes the allowable changes to LC methods within USP monographs without revalidation [1]. The recent update to USP <621> came into effect on August 1st 2014 and describes new options for allowed changes to LC methods for analysts seeking to utilise modern rapid column formats that include smaller column dimensions, smaller particles and solid core technology. Considerable increases in productivity through reduced analysis times are therefore possible. Substantial reductions in solvent consumption are also possible with these new rapid column format, translated methods, highlighting further attractive cost savings. In this work, the new USP <621> guidance for translating LC methods to small dimension, rapid analysis format columns containing both porous and solid core particles is reported.

Experimental

Chemicals, reagents, analytes, solvents and water were purchased from Sigma-Aldrich (Poole, UK) and were suitable grade for LC separations. All analyses were performed on either Agilent 1100 or 1200 series LC instruments. ACE Excel and ACE UltraCore columns were provided by Advanced Chromatography Technologies Ltd (Aberdeen, UK) and are all commercially available formats.

Results and Discussion

USP <621> 2014 update

The recent changes to USP <621> have provided analysts with clearer (and new) options for modifying compendial isocratic LC methods to fully utilise modern high pressure-based LC instrumentation employing smaller dimension column formats with sub-2 µm particles or solid core particles. There have been three major LC method parameter changes (for isocratic separations – there has been little change for gradient methods) in USP <621>. These are particle size (d_p), column length (L) and flow rate (F). Guidance now offers an L/d_p ratio approach when changing column dimensions to maintain separation performance. Further changed text now helpfully describes an accurate equation for flow rate changes to account for the potential influence of particle size effects on peak efficiency. Table 1 compares the previous USP 36 / NF31 <621> chapter allowed changes with the recent USP 37 / NF32 <621> chapter allowed changes.

As can be seen from Table 1, the major change to USP <621> (for isocratic LC methods) is the flexibility to simultaneously alter specified method column dimensions, particle size and flow rate to improve speed and productivity without the need for method revalidation. There is further helpful explanatory text throughout the USP <621> update to guide the analyst. This includes cautionary notes under the particle size parameter section on the possible deleterious effects of instrument extra column band broadening on smaller peak volumes of translated methods. Additionally there is commentary under the flow rate parameter guiding the analyst to reflect on the effects of linear velocity on efficiency via particle size changes and van Deemter considerations. Finally there is a useful table listing the approximate changes to practical parameters such as back pressure and analysis time for popular column lengths, diameters and particle sizes (with the L/d_p ratios included). Readers are strongly encouraged to seek out the USP <621> primary text as an overview is only provided in this short article.

Table 1. An overview of previous and current USP <621> allowable changes to LC methods

	USP 36 / NF31 <621>	USP 37 / NF 32 <621>
Mobile phase		
Composition	Isocratic & gradient: - Minor components can be changed by ±30% relative or ±10% absolute	Isocratic: - Minor components can be changed by ±30% relative or ±10% absolute Gradient: - No changes
pH	Isocratic & gradient: - ±0.2 units (1% for neutrals)	Isocratic & gradient: ±0.2 units
Ionic strength	Isocratic & gradient: - ±10% if the permitted pH variation is met	Isocratic & gradient: - ±10% if the permitted pH variation is met
Column		
Length	Isocratic & gradient: - ±70%	Isocratic: - Particle size (d_p) and length (L) may be changed if a) L/d_p is constant or varies -25% to +50% OR b) number of plates (N) is -25% to +50% Gradient: - No changes
Particle size	Isocratic & gradient: -50%	
Internal diameter	Isocratic & gradient: - Any changes if linear velocity kept constant - ±25%	Isocratic: - Any changes if linear velocity kept constant Gradient: - No changes
Method		
Flow rate	Isocratic & gradient: $F_2 = F_1 \times \left(\frac{{d_{c_2}}^2}{{d_{c_1}}^2}\right)$ (Where d_c = column diameter and F = flow rate) - Or, flow rate may change ±50%	Isocratic: - If particle size has changed use following equation for similar performance: $F_2 = F_1 \times \left(\frac{{d_{c_2}}^2 \times d_{p_1}}{{d_{c_1}}^2 \times d_{p_2}}\right)$ (Where d_c = column diameter, F = flow rate and d_p = particle size) Column efficiency should not drop below 20%. - Or, flow rate may change ±50% Gradient: - No changes
Injection volume	Isocratic & gradient: - Any reduction	Isocratic & gradient: Any change as long as peak repeatability is satisfactory
Temperature	Isocratic & gradient: - ±10°C when temperature is listed	Isocratic & gradient: ±10°C when temperature is listed
Detection wavelength	Isocratic & gradient: - No change permitted. ±3 nm between detectors	Isocratic & gradient: No change permitted. ±3 nm between detectors

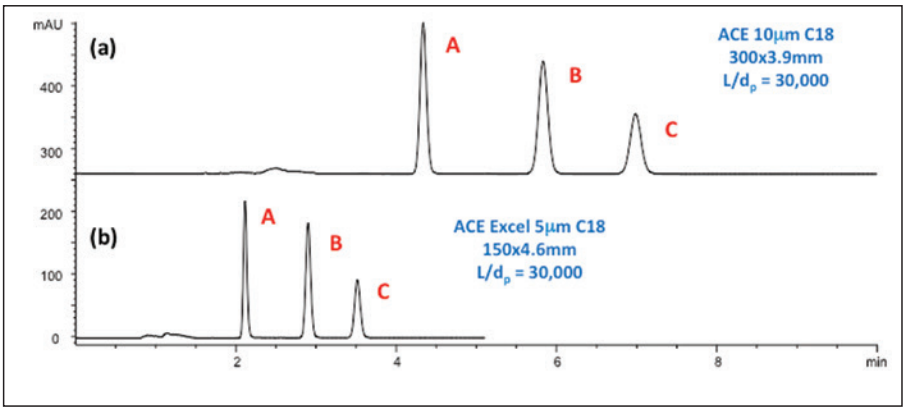

Figure 1. USP Estradiol assay system suitability chromatograms (a) original monograph method (b) translated method. Conditions: 45:55 v/v water:acetonitrile, 205 nm, 22°C (a) 1 mL/min, 25 µL injection (b) 1.4 mL/min, 17 µL injection. Peaks: A= Ethyl paraben B= Estrone C= Estradiol.

For isocratic methods, reducing column dimensions but keeping the column length (L) to particle size (d_p) ratio the same will result in similar chromatographic performance (efficiency, selectivity and resolution) but with a shorter analysis time. Whilst an over simplification and not absolute, this general principle is reasonable for method translations. With translated methods, the analyst should always consider sources of error and approximation with this generalised approach which may include adjusting flow rate for similar efficiency, the deleterious effects of instrument extra column volume on efficiency (specifically mentioned in the USP <621> guidance) and the impact on selectivity of the scalability (or not) of stationary phases across the particle size range being explored.

Table 2 describes various commonly available column lengths and particle sizes that include older, traditional formats to modern fast analysis formats and the resulting calculated L/d_p ratios for all combinations.

The new equation included in USP <621> for a more accurate translation of flow rate for simultaneous changes in column internal diameter and particle size is important. Significant changes to particle size (i.e. >3 µm to <2 µm or 10 µm to <3 µm) should ideally include an adjustment in flow rate to allow for changes in efficiency that may be observed due to operating on different sections of van Deemter curves. The option for maintaining a constant linear velocity for the translated method is also still available but changes in separation performance may be noted if this option is applied to translations that include a large particle size change. It is recommended to use the now specified more accurate equation.

USP Estradiol assay

As an example, the current LC method for USP Estradiol assay states that an L1 (C18), 300 x 3.9 mm column must be used. No particle size is defined so a default of 10 µm (largest particle size for L1 classification) must be used according to guidance. This give L/d_p = 30,000. Translating this method to an ACE Excel 5 µm C18, 150 x 4.6 mm column (porous particles) provides L/d_p = 30,000 which is the same and therefore acceptable. The flow rate was adjusted for the change in column internal diameter to maintain a constant linear velocity. Injection volumes were adjusted accordingly. Figure1 shows the current LC method and the translated, faster LC method.

Table 2. L/d_p ratios for traditional column formats and modern fast analysis column formats including current commercially available solid core particle sizes

Particle Size (µm)	Column Length (mm)							
		50	75	100	125	150	250	300
	1.5	33,333	50,000	66,667				
	1.6	31,250	46,875	62,500				
	1.7	29,412	44,118	58,824				
	1.8	27,778	41,667	55,556				
	1.9	26,316	39,474	52,632				
	2	25,000	37,500	50,000	62,500	75,000		
	2.5	20,000	30,000	40,000	50,000	60,000		
	2.6	19,231	28,846	38,462	48,077	57,692		
	2.7	18,519	27,778	37,037	46,296	55,556		
	3	16,667	25,000	33,333	41,667	50,000	83,333	
	5	10,000	15,000	20,000	25,000	30,000	50,000	
	10	5,000	7,500	10,000	12,500	15,000	25,000	30,000

The run time has changed from approximately 8 minutes in the original LC method to <4 minutes with the new method representing a >50% reduction in analysis time. The solvent consumption has reduced by approximately 30% with the new translated method. The system suitability criteria require that resolution between estrone (peak B) and estradiol (peak C) is > 2.0 and that the %RSD of multiple injections is <2.0. Satisfactory data that met the criteria were achieved with the translated, faster LC method indicating no loss in method performance and so the method would be suitable for use. This first example translation illustrates the scope to improve method speed and productivity (whilst significantly saving solvent) by moving away from legacy column configurations and particle sizes to modern formats with the new L/d_p option in the USP <621> guidance.

Exploring the flexibility of the L/d_p option from USP <621>

Translating methods from larger column format and particles sizes to exactly the same L/d_p values with smaller columns formats and particle sizes is not always possible or provides limited options. Using the USP Estradiol assay original method which gives L/d_p = 30,000 it is clear from Table 2 that there are only two other common options of column length and particle size which give exactly 30,000 (150 mm column length and 5 µm particle OR 75 mm column length and 2.5 µm particle size). Recognising this limitation, USP <621> therefore offers further flexibility and allows the analyst to work within an L/d_p range -25% to +50% relative to the original monograph L/d_p value. Table 3 shows common monograph column lengths and particle sizes with their resulting L/d_p values and the allowable L/d_p range based upon the -25% to +50% guide.

Taking the USP Estradiol assay method example already discussed, an L/d_p ratio range of 22,500 to 45,000 is acceptable based upon the specified range. This allows far more column length and particle size combinations to be considered (Table 2) and gives the analyst flexibility.

Whilst exploring the fully allowable -25% to +50% L/d_p range is an option, it should be noted that compared to the reference L/d_p value, lower L/d_p ratios will typically give a reduced method performance and higher L/d_p ratios typically an improved method performance. Method performance differences from the reference L/d_p ratio may be observed as changes in efficiency, selectivity or resolution for the translated method. The analyst must determine whether the different L/d_p ratio used from the reference value (that are an allowable and acceptable change) are suitable and meet the system suitability criteria for the monograph. Additionally, the column and particle size format must be practically acceptable (i.e. pressure, flow rate, run time, *etc.*).

As an example for acceptable lower L/d_p values, it is possible to translate the original USP Estradiol assay method (L/d_p=30,000) to an even smaller column and particle size format for further speed gains. Thus, an ACE Excel 2 µm C18, 50 x 3 mm column (porous particles) provides L/d_p = 25,000 which is still within the allowable L/d_p range and with a back pressure of 145 bar is satisfactory for routine HPLC use. The chromatogram can be seen in Figure 2(b) and compared to the original LC method chromatogram in Figure 2(a). As an example for higher L/d_p values, it is possible to translate the original USP Estradiol assay method to an ACE UltraCore 2.5 µm SuperC18, 100 x 4.6 mm column (solid core particles) which provides L/d_p = 40,000. This solid core column provides high performance with a moderate back pressure of 222 bar which would be acceptable for routine HPLC use. The chromatogram can be seen in Figure 2(c). Flow rates and injection volumes were scaled accordingly for these two new method translations. The system suitability criteria, acceptance limits and results for these lower and higher L/d_p format translations along with the all 3 methods performance data can be seen in Table 4.

Both of these optional translated methods use different L/d_p values to the reference L/d_p value (within the allowable range) and meet all system suitability requirements. They are also practically acceptable (e.g.

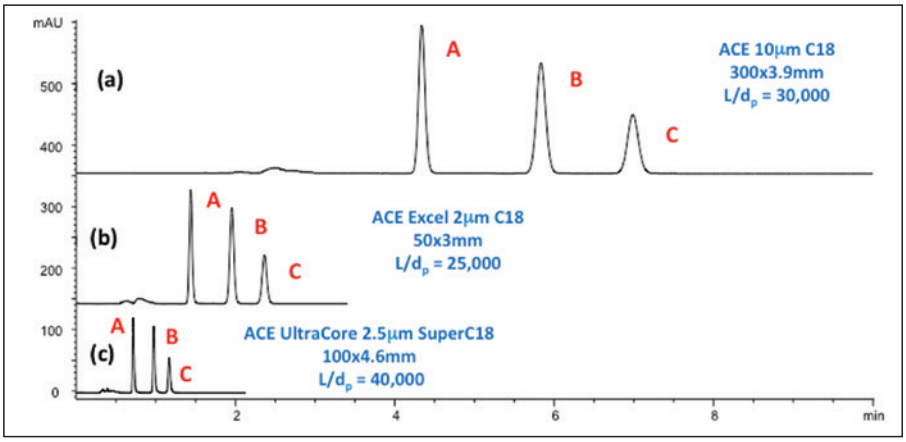

Figure 2. USP Estradiol assay system suitability chromatograms (a) original porous particle monograph method (b) porous particle translated method (c) solid core particle translated method. Conditions: 45:55 v/v water:acetonitrile, 205 nm, 22°C (a) 1 mL/min, 25 µL injection (b) 0.6 mL/min, 2.5 µL injection (c) 1.4 mL/min, 10.1 µL injection. Peaks: A= Ethyl paraben B= Estrone C= Estradiol.

Table 3: Common monograph column and particle size combinations, their subsequent L/d_p values and allowable L/d_p ranges calculated from from USP <621> guidance

Column Length, L (mm) and Particle Size, d_p (µm)							
	L	100	150	250	150	250	300
	d_p	5	5	5	10	10	10
-25%	Lower L/d_p	15,000	22,500	37,500	11,250	18,750	22,500
	L/d_p	20,000	30,000	50,000	15,000	25,000	30,000
+50%	Higher L/d_p	30,000	45,000	75,000	22,500	37,500	45,000

Table 4. USP Estradiol assay results including system suitability data and savings in time and solvent for translated methods

	Original Method L/d_p=30,000	Translated Method L/d_p=25,000	Translated Method L/d_p=40,000
System suitability requirement 1: Resolution between estradiol and estrone > 2.0	4.8	4.0	5.1
System suitability requirement 2: %RSD multiple injections <2.0	0.1	0.2	<0.1
Analysis time per injection	10 min	3.5min (65% reduction)	2min (80% reduction)
Solvent used per injection	10 mL	2.1mL (79% reduction)	2.8mL (72% reduction)

Table 5. USP Guaifenesin Tablets assay results including system suitability data and savings in time and solvent for translated methods using porous and solid core particle columns.

	Original Porous Method 250 x 4.6 mm, 10 µm	Translated Porous Method 100 x 4.6 mm, 5 µm	Translated Solid Core Method 50 x 3 mm, 2.5 µm
System suitability requirement 1: Resolution between guaifenesin and benzoic acid > 3.0	10.8	9.8	10.6
System suitability requirement 2: %RSD multiple injections <2.5	<0.1	0.1	0.1
Analysis time per injection	10 min	4 min (60% reduction)	2 min (80% reduction)
Solvent use per injection	20 mL	8 mL (60% reduction)	1.7 mL (>90% reduction)

run time and back pressure are satisfactory) whilst providing further cost savings through reduced solvent use. This example successfully demonstrates the flexibility to the analyst of method translations within the 25% to +50% L/d_p range.

The ACE UltraCore translated method additionally illustrates in practice that solid core particle based columns are just as suitable for L/d_p conversions as their porous counterparts for monograph testing. The additional solid core particle benefit of a low back pressure is also helpful for robust, routine analyses and actually could offer further increased speed of analysis with elevated flow rates. These choices for the analyst are useful when translating methods.

USP Guaifenesin Tablets assay

As a second example, the original LC method for USP Guaifenesin Tablets assay specifies a 10 µm L1 (C18), 250 x 4.6 mm column which gives an L/d_p ratio of 25,000. However, there are few common column length and particle size combinations that give L/d_p of 25,000. Using the L/d_p range flexibility in USP <621> (see Table 3), it is possible to identify column length and particle size options that fall within L/d_p of 18,750 to 37,500 (Table 2). Translating this method to an ACE Excel 5 µm C18, 100 x 4.6 mm column provides L/d_p = 20,000 which is towards the lower end of the L/d_p ratio range, but acceptable. In fact it is possible to optionally translate this method to an even faster format using an ACE UltraCore 2.5 µm SuperC18, 50 x 3 mm solid core column which also provides L/d_p of 20,000. Flow rates were scaled for constant linear velocities as required. For comparison, the original method chromatogram can be seen in Figure 3(a) with the ACE Excel porous method translation chromatogram at Figure 3(b) and the ACE UltraCore solid core method translation chromatogram at Figure 3(c).

Significant improvements in analysis speed and reductions in solvent consumption for both method translation options were observed compared to the original LC monograph method conditions. The system suitability criteria, acceptance limits and results as well as analysis time and solvent use data for the original LC method and the two translated LC methods can be seen in Table 5.

Clearly all acceptance criteria for system suitability have been met indicating the two translated methods are satisfactory. The translated ACE UltraCore method is seen to give a back pressure of 370 bar, which may not be acceptable for routine use with standard HPLC instrumentation and a 400 bar pressure limitation. If using standard HPLC instruments with a 400 bar limit, it may be better to choose the translated ACE Excel porous method with a back pressure of 260 bar or change the solid core column configuration (following L/d_p rules) to reduce the pressure. Reducing flow rate is also a possibility. USP <621> offers options to reduce flow rate (and concomitantly reduce back pressure) but this would also increase analysis time. Thus a compromise must be reached.

Using allowable peak efficiency changes for method translation

Analysts are recommended to follow the clear and simple L/d_p guidance when translating monograph methods to maintain performance and increase productivity. However, USP <621> offers an alternative approach (which can be applied for instance to the high performing solid core columns) where other ratios of L/d_p outside those in Table 2 are allowable provided that the peak efficiency (N, measured number of theoretical plates for the peak) is within

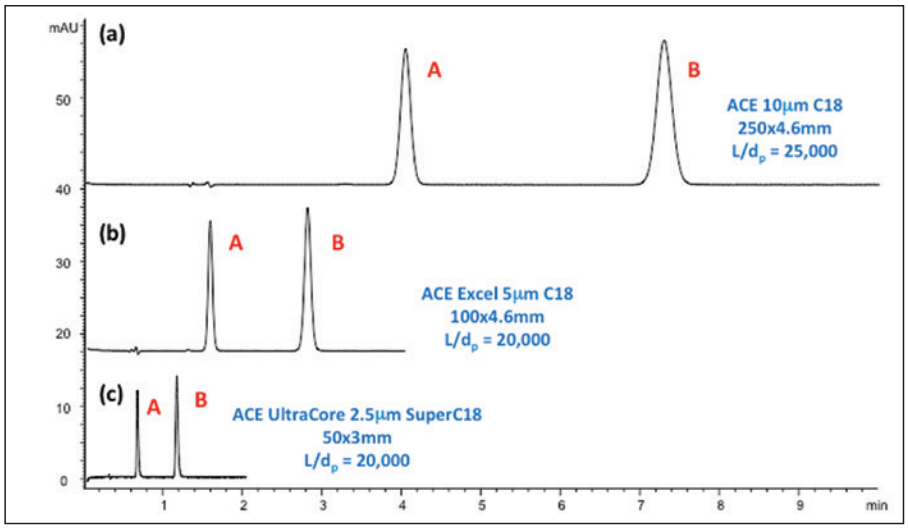

Figure 3. USP Guaifenesin Tablets assay system suitability chromatograms (a) original porous particle monograph method (b) porous particle translated method (c) solid core translated method. Conditions: 60:40:1.5 v/v/v water: methanol: glacial acetic acid, 276 nm, 22°C (a) 2 mL/min, 20 µL injection (b) 2 mL/min, 8 µL injection (c) 0.85 mL/min, 1.5 µL injection. Peaks: A= Guaifenesin B= Benzoic acid.

-25% to +50% relative to the peak efficiency from the prescribed or reference column. This option gives further flexibility to analysts using solid core technology in particular, expanding and welcoming the use of solid core technology to monograph testing.

Conclusions

The changes implemented to USP <621> offer considerable flexibility for the analyst to apply modern chromatography technologies within laboratories. The use of small particles, solid core technologies and ultra high pressure instrumentation to improve productivity and reduce costs (through decreased analysis time and reduced solvent consumption) is helpful. The examples shown within this brief article use the L/d_p approach and show how both porous and solid core technologies can be successfully applied to speed up testing. The expanded guidance and ancillary text in USP <621> in particular provide analysts with a far clearer understanding of allowable changes to LC methods within monographs. The flexibility of USP <621> provides real options for the analyst. Looking forward, it will be useful to see further flexibility in gradient LC method parameters within monographs to allow analysts to apply modern chromatography technology and enjoy similarly improved productivity and cost savings across all LC methods used for monograph testing.

References

1. www.usp.org