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UNDERSTANDING HOW UHPLC SYSTEM CHARACTERISTICS EFFECT "GOING GREEN" WITH REGULATED METHODS

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PURPOSE

Liquid chromatography with the use of optical detectors is typically not considered environmentally friendly due to high solvent waste, especially in high-throughput labs. While most validated methods use HPLC, switching to UHPLC can significantly reduce solvent consumption. UHPLC achieves faster analysis with shorter, narrower columns. However, successful implementation requires understanding system parameters like dwell volume and dispersion. In this study, a Untied States Pharmacopeia (USP) method which specifies a 1.7 µm particle column was run on various LC systems (UHPLC and HPLC). From the resulting data, strategies were developed to address specific system-related challenges.

METHOD(S)

System suitability solution was prepared containing 0.5 mg/mL Amlodipine Besylate and 1.5 µg/mL Amlodipine Related Compound F.
The sensitivity and standard solution contained 0.25 µg/mL and 0.5 µg/mL of Amlodipine Besylate, respectively.
The sample solution contained 0.5 mg/mL of Amlodipine Besylate

Amlodipine Besylate, USP Organic Impurities Method			
System	1. ACQUITY™ UPLC™ I-Class System (System 1) 2. ACQUITY UPLC H-Class System (System 2) 3. UHPLC A System (System 3) 4. UHPLC B System (System 4) 5. Alliance™ iS HPLC System (System 5)		
Diluent	Methanol: Water (50:50)		
Column	ACQUITY BEH™ C18 2.1 × 50 mm, 1.7 µm p/n: 186002350		
Column Temp	40°C		
Sample Temp:	10°C		
Solution A	1.36 g/L potassium phosphate monobasic in water		
Solution B	Methanol		
Injection Volume	2.0 µL		
Flow Rate	0.5 mL/min		
Gradient Table	Time (min)	%A	%B
	0	60	40
	0.5	60	40
	8	20	80
	9	20	80
	10	60	40
	11	60	40

RESULTS (UHPLC)

The analysis of amlodipine besylate organic impurities was successfully conducted on UHPLC Systems 1, 2, and 3, as shown in the chromatograms in Figure 1. These systems met the system suitability criteria outlined in the monograph. In contrast, Systems 4 and 5 initially exhibited chromatographic issues, which were resolved prior to meeting the system suitability requirements (Table 1).

Dwell volume – the volume between the pump's mixing point and the column head – can influence peak retention times, as illustrated in Figure 2. Specifically, amlodipine's elution time was inversely correlated with dwell volume: the lowest dwell volume (91 µL) resulted in the earliest elution, while the highest dwell volume (1008 µL) led to the latest elution.

System 4, with a dwell volume of 1008 µL, presented notable challenges. Without dwell volume adjustment, its chromatograms showed a large spike at the start of injection and a small, broad amlodipine peak in the sensitivity solution. Additionally, the system failed to return to initial conditions before the next injection, indicating insufficient re-equilibration time as defined by the method.

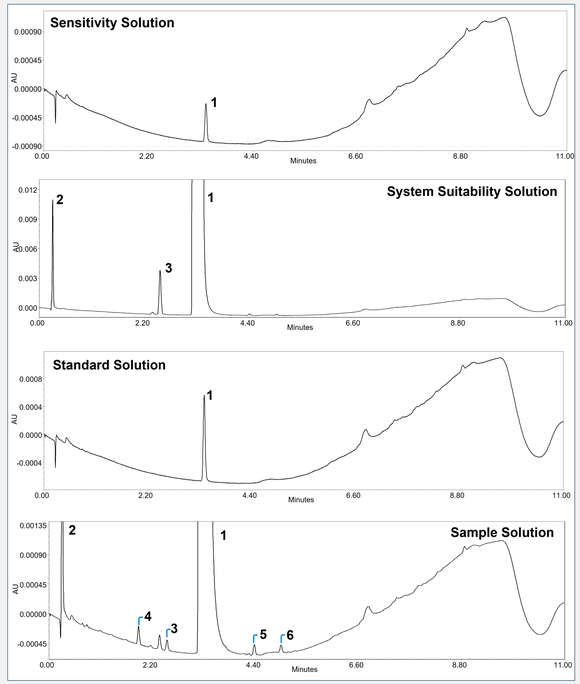

Figure 1: Chromatograms of the amlodipine besylate organic impurities samples analysed on System 2. (1) amlodipine; (2) benzenesulfonic acid; (3) amlodipine related compound F; (4) amlodipine related compound A; (5) amlodipine ethyl analog; (6) hydroxyethyl phthalyl amlodipine

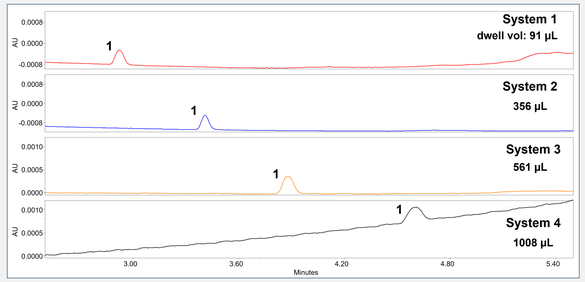

Figure 2: Stacked overlay of amlodipine besylate sensitivity solution on the four UHPLC systems showing amlodipine (1) and the effect of dwell volume on analysis.

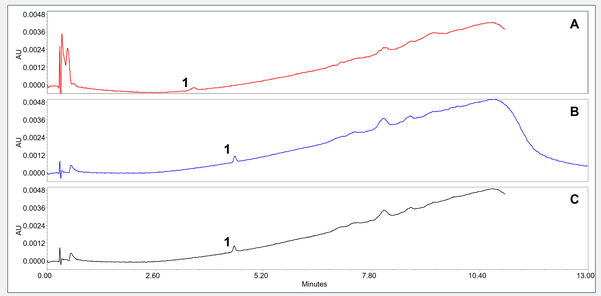

Figure 3: Stacked overlay of amlodipine besylate sensitivity solution under two conditions on System 4 showing impact of delay volume. (A) Original 11-minute method; (B) Final isocratic hold extended to 13 minute; (C) Final method with 2-min next injection delay. (1) amlodipine

To investigate the re-equilibration issue, the gradient was modified by extending the final hold by 2 minutes. This adjustment allowed the system to return to initial conditions by the 13-minute mark, as shown in chromatogram B (Figure 3).

Accounting for System 5's large dwell volume, the "next injection delay" feature was implemented. This approach was preferred over modifying the gradient table, which could necessitate method re-validation. By adding a 2-minute delay between injections, illustrated in chromatogram C (Figure 3), the system achieved proper re-equilibration, ensuring consistent and reliable analysis even when the original method lacked sufficient re-equilibration time.

	System Characteristics	Results				
System	**Dwell Volume (µL)**	**Resolution[1] (NLT 3.0)**	**Tailing[1] (NMT 2.5)**	**USP S:N[2] (NLT 10)**	**Retention Time %RSD[3] (NMT 5.0%)**	**Area %RSD[3] (NMT 5.0%)**
1	91	4.9	2.4	148	0.0	1.3
2	356	5.1	2.4	113	0.0	0.6
3	561	4.8	2.0	47	0.1	0.6
4*	1008	4.4	1.6	21	0.0	2.3
5*	1605	4.2	1.8	108	0.0	0.5

Table 1: Results for Amlodipine Besylate Organic Impurities system suitability om all systems tested. (*) Required dwell volume adjustments (1) Results from System Suitability Solution; (2) Results from Sensitivity Solution; (3) Results from Standard Solution

RESULTS (HPLC)

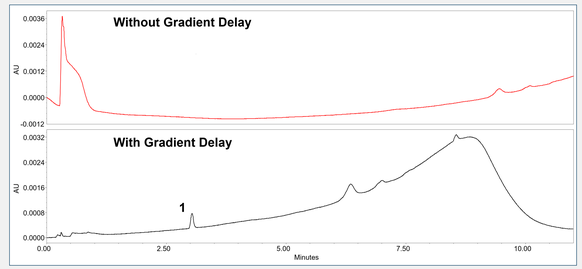

Figure 4: Stacked overlay of amlodipine besylate sensitivity solution on System 5 showing the impact of delay volume and adjusting for it. (1) amlodipine

System 5 was selected for its high-pressure capabilities, enabling the use of sub-2 µm columns that deliver faster separations at lower flow rates. However, its large dwell volume (1650 µL) impacted performance. Specifically, the 1-minute (0.5 mL) equilibration step at the end of the method was insufficient to return the system to initial conditions before the next injection. This led to the amlodipine peak being washed out at the start of the chromatogram, resulting in a pronounced spike (Figure 4).

To resolve this issue, the Gradient Start feature on System 5 was utilized to compensate for dwell volume. Unlike the "next injection delay" feature, which adds a hold after each injection, Gradient Start delays the onset of the gradient change by a specified time or volume before injection. This ensures the system is properly equilibrated to initial conditions prior to sample introduction. The resulting chromatogram demonstrates the effectiveness of this approach, showing successful re-equilibration and accurate peak detection.

CONCLUSION(S)

UHPLC systems offer faster analysis and lower flow rates, making them more efficient and environmentally friendly compared to traditional HPLC methods. However, when transferring between systems with different configurations, it's crucial to understand factors like dwell volume, which can significantly impact chromatographic performance. Method adjustments are often necessary to ensure successful development and transfer.

Advancements in system design now allow some methods, especially those using sub-2 µm columns, to be run on HPLC systems. This capability can eliminate the need to upgrade to UHPLC, enabling HPLC systems to operate more sustainably due to reduced flow rates and shorter runtimes. To ensure comparable results during method transfer, HPLC systems must compensate for their larger dwell volumes.

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