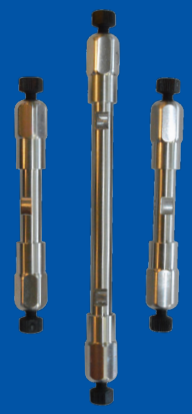
TECHNICAL NOTE

Liquid Chromatography



Clone Columns: Harmony C18 and Waters Symmetry C18

Introduction

Legacy methods, by their very nature, often use older column technologies. These older phases can be accompanied by larger variations in batch-to-batch performance which can lead to inconsistent results and cause out of specification (OOS) occurrences. With routine analysis, often completed with compliant procedures, any unplanned downtime to investigate OOS instances can impact productivity. Our range of clone phases offer a cost-effective comparable alternative to many of the older leading brands, whilst ensuring consistency and stability in analysis. Improved lot-to-lot reproducibility is also achieved due to more stable production methods especially when compared to older brands.

The Harmony™ C18 provides equivalent selectivity to the Waters Symmetry® C18 HPLC column and many chromatographers use Harmony when looking for a Symmetry equivalent. Harmony offers excellent column lifetime and reproducibility, and is engineered to the tightest specifications. It is also available in large scale bulk. Other Harmony phases are also available including C8 and C4.

This technical note provides examples of comparative studies between our Harmony C18 and the Waters Symmetry C18 phases for the following pharmaceutical drug applications:

- Fluconazole
- Betamethasone dipropionate and beclomethasone dipropionate
- Acetaminophen, aspirin, caffeine and benzoic acid

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Application: Analysis of Fluconazole using Harmony C18 and Symmetry C18 Columns

Fluconazole (Figure 1) is an azole anti-fungal medication used primarily in the treatment of a wide range of fungal infections. It is the only anti-fungal agent available for the treatment of cryptococcal meningoencephalitis in many parts of the world.[1] The method described in this application is adapted from the official USP monograph for fluconazole. The monograph specifies an L1 column with a 3 µm particle size be used, whilst the method shown here uses a 5 µm particle size (outside of the USP <621> allowed changes). A C18 column is used for the analysis of fluconazole, as per the USP method, and as such the Harmony C18 is shown to be a viable alternative to the Symmetry C18 column.

All HPLC method parameters are shown in Table 1.

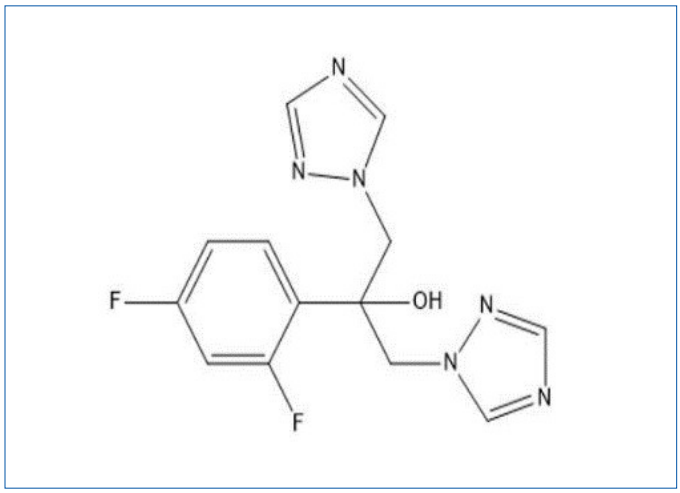

Figure 1: Chemical structure of fluconazole.

Table 1: Method parameters for Analysis of Fluconazole using Harmony C18 and Symmetry C18.

Instrument	Flexar™ with PDA Plus™ Detector
Columns	Harmony C18 150 x 4.6 mm, 5 µm (P/N: 135221-HRM-C18)
	Waters Symmetry C18 150 x 4.6 mm, 5 µm
Mobile Phase	A: Water B: Acetonitrile A: 80% B: 20%
Flow Rate	0.75 mL/min
Temperature	40 °C
Wavelength	260 nm
Injection Volume	20 µL
Analyte	Fluconazole (0.5 mg/mL in mobile phase)

Results and Discussion

The analysis of fluconazole was carried out on both Harmony and Symmetry C18 phases as shown in Figure 2. The suitability parameters are detailed in Table 2. Both columns are suited to the analysis of fluconazole and would pass the USP suitability requirement of a tailing factor < 2. However, the Harmony C18 column offers sharper peak shape, with an increase in efficiency of 36% in comparison with the Symmetry C18 as well as reduced peak tailing.

Table 2: Suitability parameters for the analysis of fluconazole using Harmony C18 and Symmetry C18 columns.

Column	Retention time (minutes)	Peak Efficiency (N)	Tailing factor
Harmony C18	6.60	9691	1.1
Symmetry C18	6.53	7143	1.3

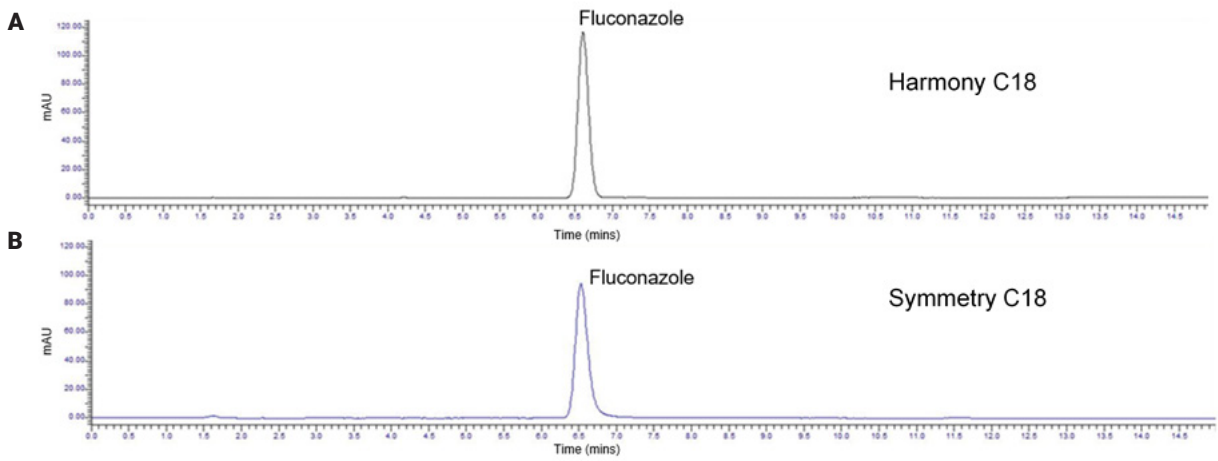

Figure 2: Analysis of fluconazole using Harmony C18 (A) and Symmetry C18 (B) columns.

Application: HPLC Analysis of Betamethasone Dipropionate and Beclomethasone Dipropionate Using Harmony C18 and Symmetry C18 Columns

Betamethasone dipropionate (Figure 3) is a glucocorticoid steroid which suppresses various aspects of the human immune system in conditions where hyperactivity can cause poor health through allergies, inflammation and autoimmune dysfunction.[3] Beclomethasone dipropionate is another common glucocorticoid steroid commonly used in the treatment of asthma.[4] This method is based on the USP method for the analysis of betamethasone dipropionate, which specifies an L1 column be used. L1 is defined as octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 3 to 10 µm in diameter. Both the Harmony C18 and Symmetry C18 phases fit this description and should display similar results. Therefore, the benefits of switching to the more cost effective Harmony phase can be realized.

All HPLC method parameters are shown in Table 3.

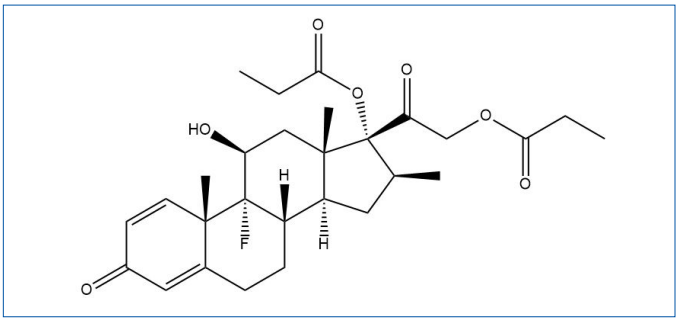

Figure 3: Chemical structure of betamethasone dipropionate.

Table 3: Method parameters for analysis of betamethasone dipropionate using Harmony C18 and Symmetry C18.

Instrument	LC 300 HPLC system with LC 300 multi-wavelength UV/Vis (MWD) Detector
Columns	Harmony C18 150 x 4.6 mm, 5 µm (P/N: 135221-HRM-C18)
	Waters Symmetry C18 150 x 4.6 mm, 5 µm
Mobile Phase	A: Acetonitrile B: Water A: 65% B: 35%
Flow Rate	1.0 mL/min
Temperature	23 °C
Wavelength	254 nm
Injection Volume	10 µL
Analyte and internal standard	Betamethasone dipropionate and beclomethasone dipropionate (0.3, 0.9 mg/mL in acetic acid and methanol, 1 in 1,000)

Results and Discussion

The analysis of betamethasone dipropionate was carried out on both the Harmony and Symmetry C18 phases as illustrated in Figure 4. The suitability parameters are detailed in Table 4. The Harmony C18 displays equivalent selectivity and similar retention times to the Symmetry C18 in this application. The Harmony column shows greater efficiency with an increase of 51% for betamethasone and 44% for beclomethasone, as well as reduced tailing for both peaks and improved resolution.

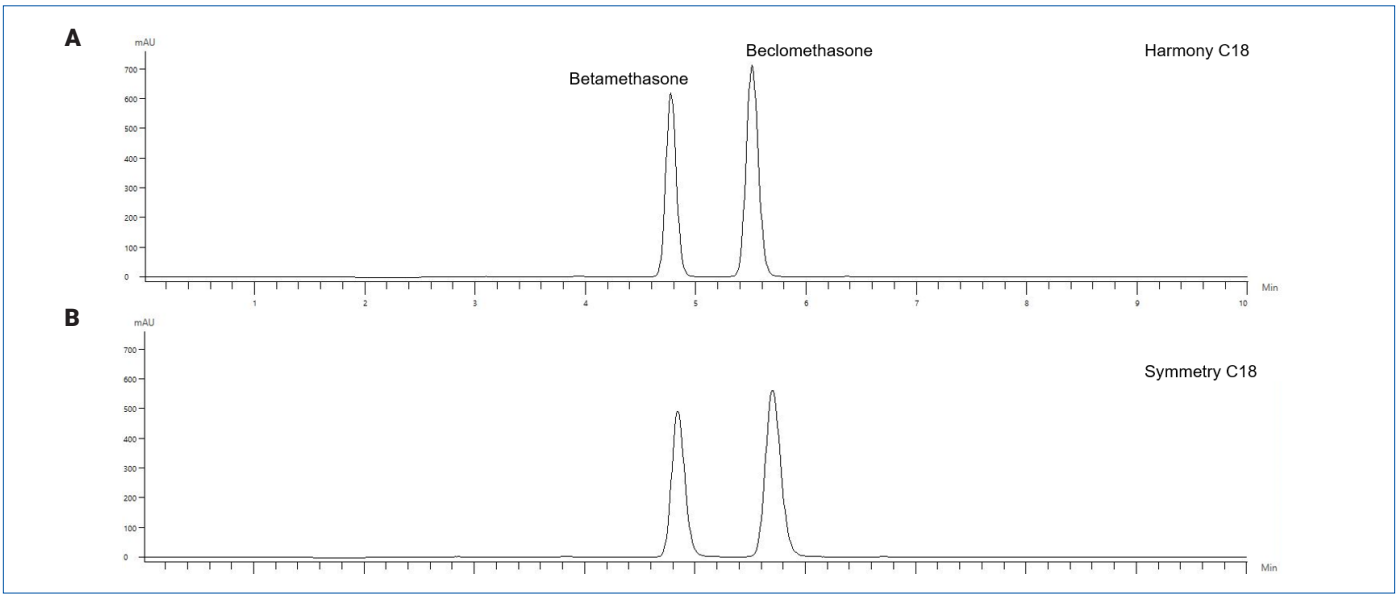

Figure 4: Analysis of betamethasone dipropionate using Harmony C18 (A) and Symmetry (B) columns.

Table 4: Suitability Results for the analysis of betamethasone dipropionate using Harmony C18 and Symmetry C18 columns.

	Retention Time		Efficiency (N plates)		Tailing Factor		Resolution	
	Harmony C18	Symmetry C18	Harmony C18	Symmetry C18	Harmony C18	Symmetry C18	Harmony C18	Symmetry C18
Betamethasone	4.78	4.84	11113	7339	1.08	1.14	n/a	n/a
Beclomethasone	5.51	5.70	10715	7427	1.09	1.15	3.85	3.60

Application: HPLC analysis of Acetaminophen, Aspirin, Caffeine and Benzoic Acid using Harmony C18 and Symmetry C18 Columns

Acetaminophen, aspirin and caffeine (Figure 5) are commonly used in combination to treat acute headaches and migraines, when used in combination, these three drugs are more effective than when used individually.[5] Benzoic acid is used as an internal standard. This method is USP compliant and so an L1 column must be used as per the method. Harmony C18 and Symmetry C18 are both L1 columns and so are suitable for this analysis, with the clone phase, Harmony, offering the potential to reduce the cost of analysis.

All HPLC method parameters are shown in Table 5.

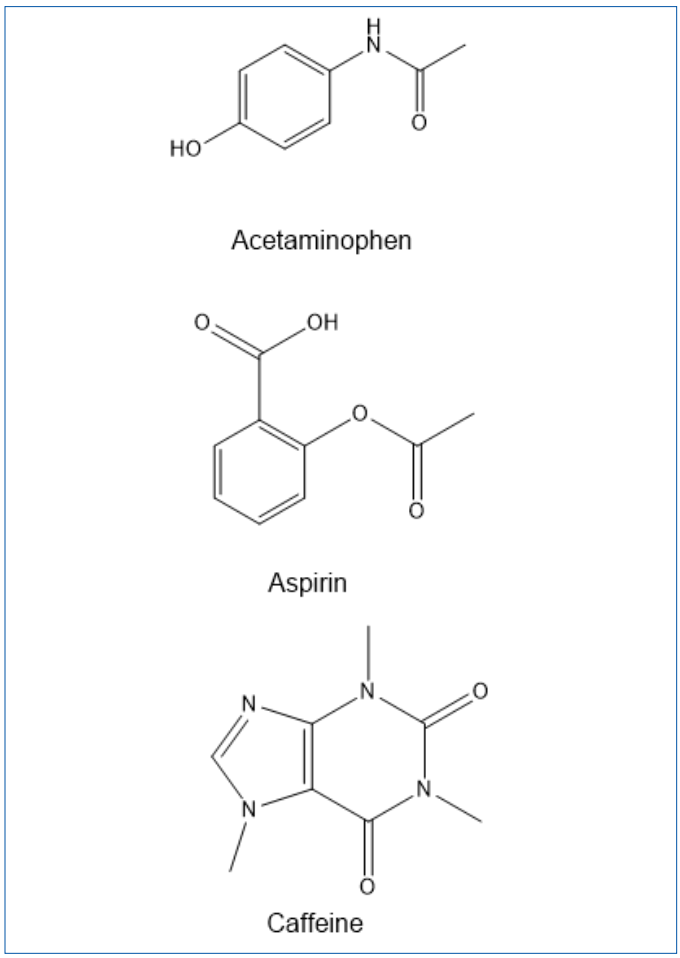

Figure 5: Chemical structure of acetaminophen, aspirin and caffeine.

Table 5: Method parameters for Analysis of Acetaminophen, Aspirin and Caffeine using Harmony C18 and Symmetry C18.

Instrument	LC 300 HPLC system with LC 300 multi-wavelength UV/Vis (MWD) Detector
Columns	Harmony C18 150 x 4.6 mm, 5 µm (P/N: 135221-HRM-C18)
	Waters Symmetry C18 150 x 4.6 mm, 5 µm
Mobile Phase	A: Pre-mixed (methanol: glacial acetic acid: water, 28:3:69)
Flow Rate	1.0 mL/min
Temperature	45 °C
Wavelength	275 nm
Injection Volume	10 µL
Analyte and internal standard	Acetaminophen, aspirin, caffeine and benzoic acid (0.1, 0.1, 0.026, 0.36 mg/mL in 95:5 MeOH:glacial acetic acid)

Results and Discussion

The analysis of acetaminophen, aspirin, caffeine and a benzoic acid internal standard was carried out and shown in Figure 6. The Harmony C18 column displays similar selectivity to the Symmetry C18 column with a slight increase in retention. Both columns meet the USP suitability requirements of tailing factors no more than 2 and resolutions >1.4. However, the Harmony C18 displays greater efficiency than the Symmetry C18 column with increases of 15%, 23% and 11% for the caffeine, aspirin and benzoic acid peaks respectively. Additionally, the Harmony C18 demonstrates reduced tailing for all compounds.

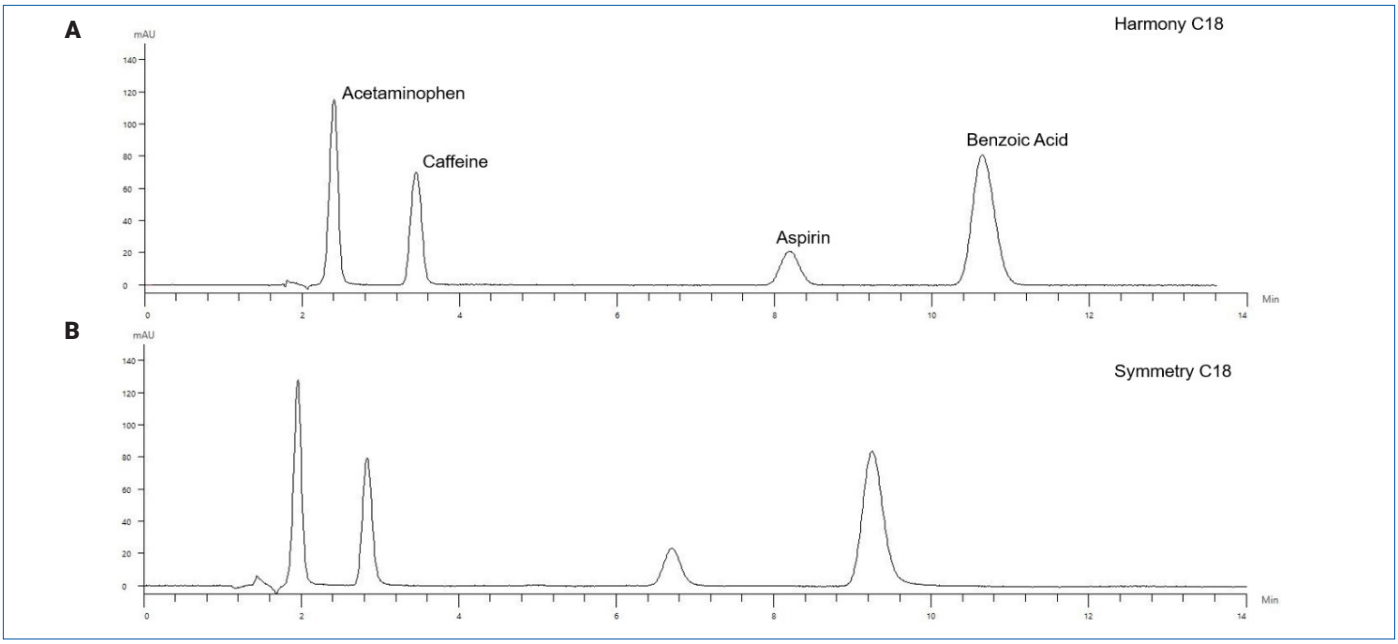

Figure 6: Analysis of acetaminophen, aspirin and caffeine using Harmony C18 (A) and Symmetry C18 (B) columns.

Table 6: Suitability results for the analysis of acetaminophen, aspirin and caffeine using Harmony C18 and Symmetry C18 columns.

	Retention Time		Efficiency (N plates)		Tailing Factor		Resolution	
	Harmony C18	Symmetry C18	Harmony C18	Symmetry C18	Harmony C18	Symmetry C18	Harmony C18	Symmetry C18
Acetaminophen	2.43	1.97	2239	1670	0.95	0.99	n/a	n/a
Aspirin	3.54	2.88	2668	2314	1.03	1.10	4.62	2.30
Caffeine	8.52	6.84	5241	4276	1.06	1.16	13.49	12.26
Benzoic acid	11.01	9.40	6272	5627	1.14	1.18	4.94	5.66

Conclusion

These three applications have shown the validity of using our Harmony C18 column as an alternative to the Waters Symmetry C18 column when using the same dimensions. It has been shown that the Harmony C18 provides similar selectivity to the Symmetry C18 for all applications, whilst offering increases in efficiency and reduced peak tailing. The Harmony C18 can clearly act as a direct replacement for the Symmetry C18, providing similar or improved results.

References

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