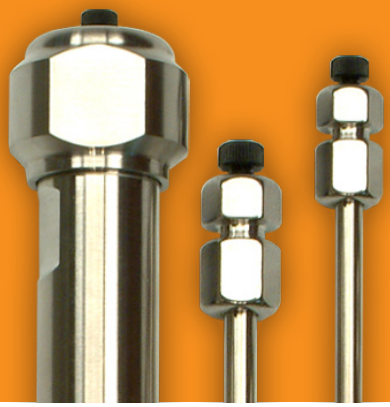
TECHNICAL NOTE

Liquid Chromatography



Clone Columns: Sonoma C18(2) and Luna C18(2)

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. Better lot-to-lot reproducibility is also achieved due to more stable production methods especially when compared to older brands.

Our Sonoma™ line is an alternative to Phenomenex Luna®. Excellent efficiencies, peak shape and resolution are obtained for virtually all Luna HPLC applications from high quality Sonoma HPLC columns. A range of particle sizes offers versatility from analytical and LC/MS to prep and process scale applications. Sonoma C18(2), the most popular phase, is equivalent to Luna C18(2). Available in 3 µm and 5 µm particle sizes, with bulk and preparative material available. Other Sonoma phases available include C18, C5, C8, C8(2), Cyano, HILIC, NH_2 (amino), PFP(2), Phenyl-Hexyl and Silica(2).

This technical note displays examples of comparative studies between our Sonoma C18(2) (150 x 4.6 mm, 5 µm) column and the Phenomenex Luna C18(2) (150 x 4.6 mm, 5 µm) column, for the following pharmaceutical drug applications:

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

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Application: HPLC Analysis of Finasteride

Finasteride (Figure 1) is an antiandrogenic compound or 'testosterone blocker' which is used as a treatment for an enlarged prostate and can treat male hair loss.[2] The method described in this application is in accordance with the official United States Pharmacopoeia (USP) finasteride tablets method for the assay. The monograph prescribes a 100 x 4.6 mm column, the different dimensions of the columns used here are within the USP <621> allowed changes. Both the Sonoma C18(2) and the Luna C18(2) are classified as L1 columns and herein the Sonoma C18(2) is shown to be a viable alternative to the Luna C18(2).

All HPLC method parameters are shown in Table 1.

Instrument	PerkinElmer LC 300 HPLC system with LC 300 multi-wavelength UV/Vis (MWD) Detector
Columns	PerkinElmer Sonoma C18(2) 150 x 4.6 mm, 5 µm P/N: 135221-SMA-C18(2)
	Phenomenex Luna C18(2) 150 x 4.6 mm, 5 µm
Mobile Phase	**A:** 2.5 mM phosphoric acid **B:** ACN **A:** 50% **B:** 50%
Flow Rate	1.5 mL/min
Tempature	45 °C
Wavelength	240 nm
Injection Volume	20 µL
Analyte	Finasteride

Table 1: Method parameters for the analysis of finasteride using Sonoma C18(2) and Luna C18(2).

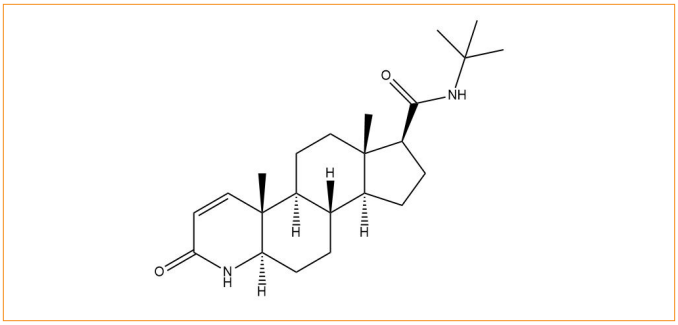

Figure 1: Chemical structure of finasteride.

Results and Discussion

The analysis of finasteride was carried out on a Sonoma C18(2) and a Luna C18(2) as shown in Figure 2. The suitability parameters are displayed in Table 2. The Sonoma phase is suited to the analysis of finasteride, providing a very similar retention time to the Luna, and in addition better peak shape. The analyte on the Luna column displays fronting, whereas the Sonoma column exhibited no fronting and very little peak tailing. The USP monograph requires the tailing factor be ≤2 and the number of plates be ≥1000 N. Both columns pass these requirements and show that the more cost-effective Sonoma phase is a viable alternative to the Luna phase.

Column	Retention Time (min)	Efficiency (N)	Tailing Factor
Sonoma C18(2)	3.80	5808	1.02
Luna C18(2)	3.88	6234	0.95

Table 2: Suitability parameters for the analysis of finasteride using Sonoma C18(2) and Luna C18(2) Columns.

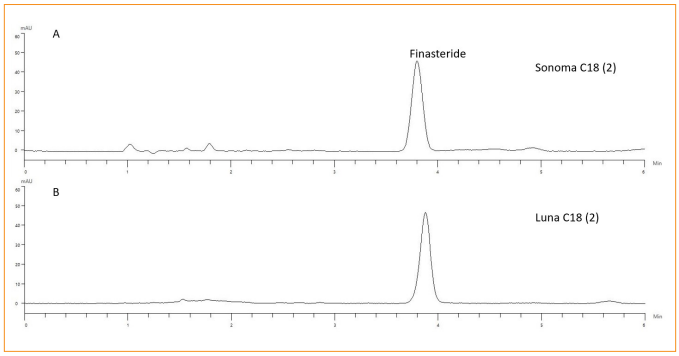

Figure 2: Analysis of finasteride using Sonoma C18(2) (A) and Luna C18(2) (B).

Application: HPLC Analysis of Betamethasone Dipropionate and Beclomethasone Dipropionate

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] 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 Sonoma C18 (2) and the Luna C18(2) are L1 columns and display similar results. Therefore, the benefits of switching to the more cost-effective Sonoma phase can be realized.

All HPLC method parameters are shown in Table 3.

Instrument	PerkinElmer LC 300 HPLC system with LC 300 multi-wavelength UV/Vis (MWD) Detector
Columns	PerkinElmer Sonoma C18(2) 150 x 4.6 mm, 5 µm P/N: 135221-SMA-C18(2)
	Phenomenex Luna C18(2) 150 x 4.6 mm, 5 µm
Mobile Phase	**A:** Acetonitrile **B:** Water **A:** 65% **B:** 35%
Flow Rate	1.0 mL/min
Temp	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)

Table 3: Method parameters for analysis of betamethasone dipropionate using Sonoma C18(2) and Luna C18 (2).

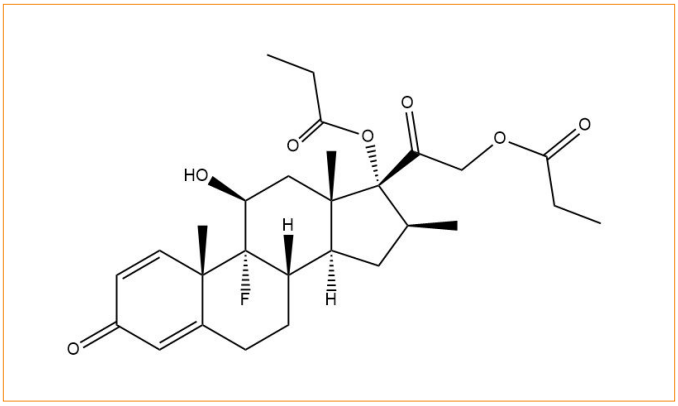

Figure 3: Chemical structure of betamethasone dipropionate.

Results and Discussion

The analysis of betamethasone dipropionate and beclomethasone dipropionate were carried out on a Sonoma C18(2) and a Luna C18(2) as shown in Figure 4. The suitability parameters are displayed in Table 4. The Sonoma phase performs well in this analysis and displays excellent peak shape with very little tailing (tailing factors below 1.05). The Luna phase again displays peak fronting for both peaks. Both columns display similar selectivity and similar efficiencies.

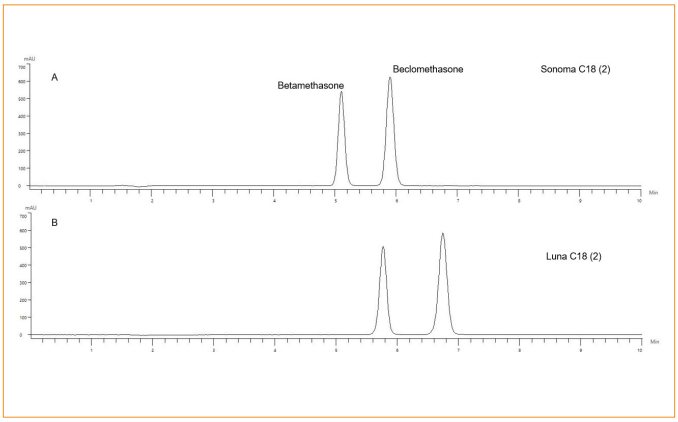

Figure 4: Analysis of betamethasone dipropionate and beclomethasone dipropionate using Sonoma C18(2) (A) and Luna C18(2) (B).

	Retention Time (minutes)		Efficiency (N)		Tailing Factor		Resolution	
	Sonoma C18(2)	Luna C18(2)	Sonoma C18(2)	Luna C18(2)	Sonoma C18(2)	Luna C18(2)	Sonoma C18(2)	Luna C18(2)
Betamethasone	5.11	5.77	10141	10717	1.04	0.98	n/a	n/a
Beclomethasone	5.90	6.74	10017	10471	1.03	0.98	3.76	4.18

Table 4: Suitability results for the analysis of betamethasone dipropionate and beclomethasone dipropionate using Sonoma C18 (2) and Luna C18(2) columns.

Application: HPLC Analysis of Acetaminophen, Aspirin, Caffeine and Benzoic Acid

Acetaminophen, aspirin and caffeine (Figure 5) are commonly used in combination to treat acute headaches and migraines.[4] This method is in accordance with the USP assay method for 'Acetaminophen, Aspirin, and Caffeine Tablets'. An L1 column must be used as per the method. The Sonoma C18(2) and Luna C18(2) both fit the USP monograph requirement of an L1 column, with the Sonoma C18(2) offering the potential to reduce the cost of analysis.

All HPLC method parameters are shown in Table 5.

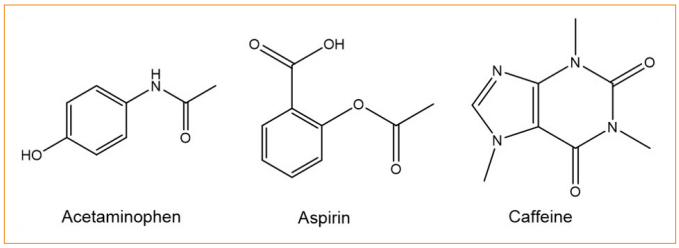

Figure 5: Chemical structure of acetaminophen, aspirin and caffeine.

Instrument	PerkinElmer LC 300 HPLC system with LC 300 multi-wavelength UV/Vis (MWD) Detector
Columns	PerkinElmer Sonoma C18(2) 150 x 4.6 mm, 5 µm P/N: 135221-SMA-C18(2)
	Phenomenex Luna C18(2) 150 x 4.6 mm, 5 µm
Mobile Phase	Pre-mixed (methanol: glacial acetic acid: water, 28:3:69)
Flow Rate	1.0 mL/min
Temp	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)

Table 5: Method parameters for analysis of acetaminophen, aspirin and caffeine using Sonoma C18(2) and Luna C18(2).

Result and Discussion

The analysis of acetaminophen, aspirin, caffeine and a benzoic acid internal standard was carried out as shown in Figure 6. The suitability parameters are shown in Table 6. The Sonoma C18(2) provides better peak shape than the Luna C18(2) column. The Sonoma phase displays very little peak tailing for all peaks whereas the last two eluting peaks both front on the Luna phase. The Sonoma phase also displays greater efficiency than the Luna phase with increases of 38%, 20%, and 12% for caffeine, aspirin and benzoic acid respectively. Both columns meet the USP suitability requirements displaying a tailing factor ≤ 2 and therefore are suitable for the analysis.

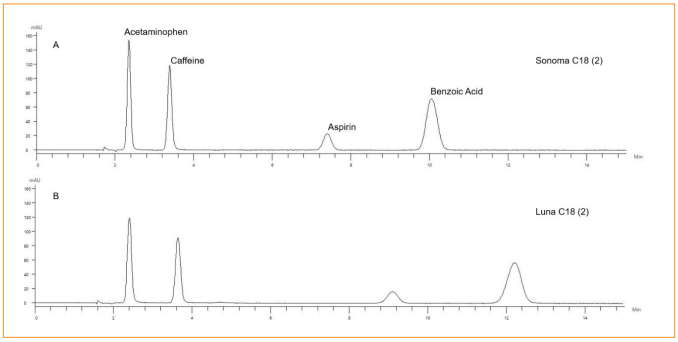

Figure 6: Analysis of acetaminophen, aspirin, caffeine and benzoic acid on Sonoma C18 (2) (A) and Luna C18 (2) (B).

	Retention Time (minutes)		Efficiency (N)		Tailing Factor		Resolution	
	Sonoma C18(2)	Luna C18(2)	Sonoma C18(2)	Luna C18(2)	Sonoma C18(2)	Luna C18(2)	Sonoma C18(2)	Luna C18(2)
Acetaminophen	2.35	2.41	3063	2233	1.02	1.07	N/A	N/A
Aspirin	3.39	3.64	4542	3288	1.07	1.08	5.72	5.43
Caffeine	7.40	9.10	5439	4523	1.01	0.96	13.64	14.04
Benzoic acid	10.06	12.21	5444	4858	1.03	0.95	5.70	5.11

Table 6: Suitability Results for the analysis of acetaminophen, aspirin, caffeine and benzoic acid using Sonoma C18 (2) and Luna C18 (2) columns.

Conclusion

These 3 applications have demonstrated the validity of using a Sonoma C18(2) column as an alternative to the Phenomenex Luna C18(2) column when using the same column dimensions. Sonoma C18(2) offers similar selectivity and better peak shapes with comparable efficiencies. The Sonoma C18(2) can act as a viable alternative to the Phenomenex Luna C18(2) column, providing similar or improved results.

References

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