

## Liquid Chromatography



## Clone Columns: Aviator C18 and ACE 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. Better lot-to-lot reproducibility is also achieved due to more stable production methods especially when compared to older brands.

Our Aviator™ line exhibits similar selectivity to Avantor ACE® HPLC columns. Aviator C18 provides a rugged, reproducible starting point for method development and applications with analytes differing in hydrophobicity, polar, moderately polar and non-polar analytes, uncharged acids and bases, and ionized acids or bases using ion-pairing. Other Aviator phases are also available including AQ, C8, C4, Cyano and Phenyl.

This technical note provides examples of comparative studies between the Aviator C18 (150 x 4.6 mm, 5 µm) column and the ACE C18 (150 x 4.6 mm, 5 µm) column, for the following pharmaceutical drug applications:

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

## Application: Analysis of Fluconazole

Fluconazole (Figure 1) is an azole anti-fungal medication used primarily in the treatment of a wide range of fungal infections.<sup>1</sup> This method is based on the USP monograph 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 Aviator C18 and ACE C18 fit this description and should display similar results whilst also highlighting the advantage of using the more cost-effective Aviator C18.

All HPLC method parameters are shown in Table 1.

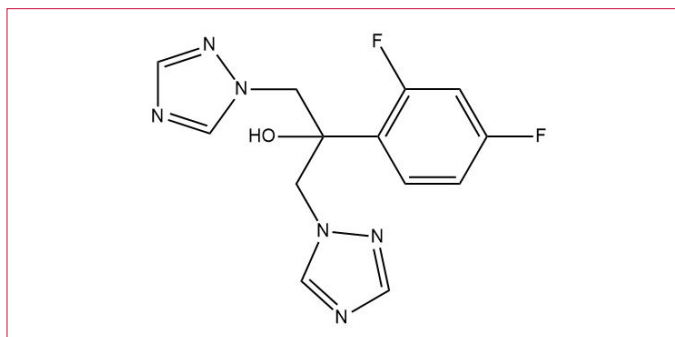


Figure 1: Chemical structure of fluconazole.

<b>Instrument</b>	PerkinElmer LC 300 HPLC system with LC 300 multi-wavelength UV/Vis (MWD) Detector
<b>Columns</b>	PerkinElmer Aviator C18 150 x 4.6 mm, 5 µm P/N: 135221-AV-C18 Avantor ACE C18 150 x 4.6 mm, 5 µm
<b>Mobile Phase</b>	<b>A:</b> Water <b>B:</b> Acetonitrile <b>A:</b> 80% <b>B:</b> 20%
<b>Flow Rate</b>	0.75 mL/min
<b>Temperature</b>	40 °C
<b>Wavelength</b>	260 nm
<b>Injection Volume</b>	20 µL
<b>Analyte</b>	Fluconazole (0.5 mg/mL in Mobile Phase)

Table 1: Method parameters for the analysis of fluconazole using Aviator C18 and ACE C18 columns.

## Results and Discussion

The analysis of fluconazole was carried out on an Aviator C18 and ACE C18 as shown in Figure 2. The suitability parameters are detailed in Table 2. The Aviator C18 is suited to the analysis of fluconazole as it displays excellent peak shape with low tailing. Both columns give very similar retention times, with only 0.1 minutes difference. The ACE C18 column exhibits greater peak tailing than the Aviator C18, demonstrating that switching to the Aviator C18 can yield better peak shape.

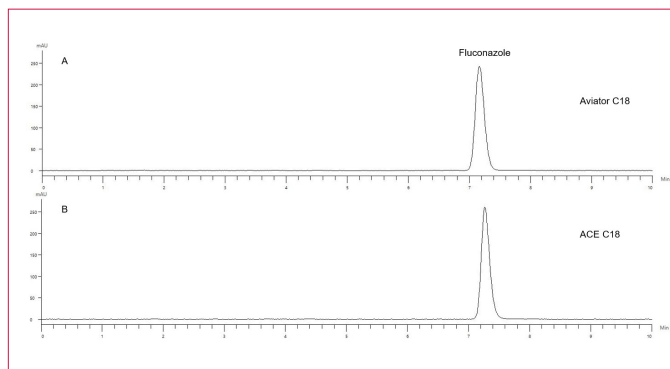


Figure 2: Analysis of fluconazole using Aviator C18 150 x 4.6 mm, 5 µm (A) and ACE C18 150 x 4.6 mm, 5 µm (B).

Instrument	Retention time (min)	Peak efficiency (N)	Tailing factor
Aviator C18	7.17	10429	1.16
ACE C18	7.27	12807	1.26

Table 2: Suitability parameters for the analysis of fluconazole using Aviator C18 and ACE C18 columns.

## 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.<sup>3</sup> As prescribed by the USP method, a C18 column is used. Both the Aviator C18 and ACE C18 meet this requirement and the Aviator C18 is shown to be a viable alternative.

All method parameters are shown in Table 3.

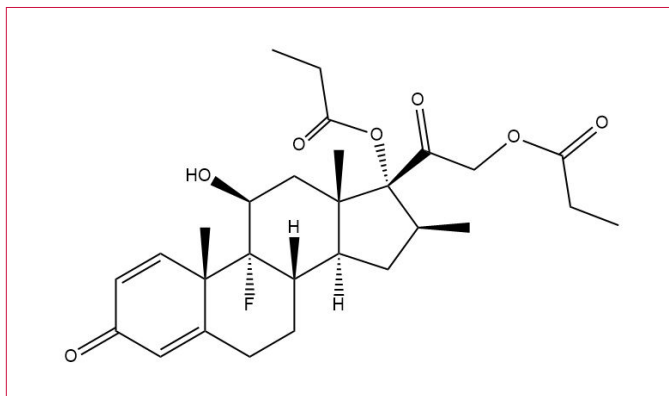


Figure 3: Chemical structure of betamethasone dipropionate.

<b>Instrument</b>	PerkinElmer LC 300 HPLC system with LC 300 multi-wavelength UV/Vis (MWD) Detector
<b>Columns</b>	PerkinElmer Aviator C18 150 x 4.6 mm, 5 µm P/N: 135221-AVI-C18
	Avantor ACE C18 150 x 4.6 mm, 5 µm
<b>Mobile Phase</b>	<b>A:</b> Acetonitrile <b>B:</b> Water <b>A:</b> 65% <b>B:</b> 35%
<b>Flow Rate</b>	1.0 mL/min
<b>Temperature</b>	23 °C
<b>Wavelength</b>	254 nm
<b>Injection Volume</b>	10 µL
<b>Analyte and Internal Standard</b>	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 the analysis of betamethasone dipropionate and beclomethasone dipropionate using Aviator C18 and ACE C18.

## Results and Discussion

The analysis of betamethasone dipropionate and beclomethasone dipropionate was carried out on an Aviator C18 and an ACE C18 as shown in Figure 4. The suitability parameters are displayed in Table 4. The Aviator C18 column has a similar retention profile, yields similar efficiencies and less peak tailing than the ACE C18 column.

The Aviator C18 column shows similar retention times, with less than 0.15 minutes difference for either peak, and greater efficiency for the betamethasone peak. It also yields improved tailing factors for both peaks in comparison with the ACE C18.

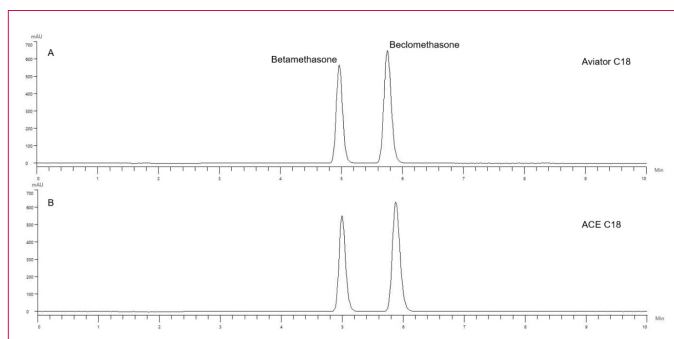


Figure 4: Analysis of betamethasone dipropionate and beclomethasone dipropionate on Aviator C18 150 x 4.6 mm, 5 µm (A) and ACE C18 150 x 4.6 mm, 5 µm (B).

	Retention time (min)		Efficiency (N plates)		Tailing factor		Resolution	
	Aviator C18	ACE C18	Aviator C18	ACE C18	Aviator C18	ACE C18	Aviator C18	ACE C18
Betamethasone	4.97	5.00	10233	10150	1.13	1.18	n/a	n/a
Beclomethasone	5.75	5.88	9974	9995	1.14	1.19	3.83	4.18

Table 4: Suitability results for the analysis of betamethasone dipropionate and beclomethasone dipropionate using Aviator C18 and ACE C18.

## Application: HPLC Analysis of Acetaminophen, Aspirin, Caffeine and Benzoic Acid on Aviator C18 and ACE C18

Acetaminophen, aspirin and caffeine (Figure 5) are commonly used in combination to treat acute headaches and migraines.<sup>4</sup> Benzoic acid has also been analysed as an internal standard. This method is in accordance with the USP assay method for 'Acetaminophen, Aspirin, and Caffeine Tablets'. A column with packing L1 is required. Both the Aviator C18 and ACE C18 columns are suitable for this analysis. However, the cost effective option of the Aviator phase can significantly reduce cost per analysis.

All HPLC method parameters are shown in Table 5.

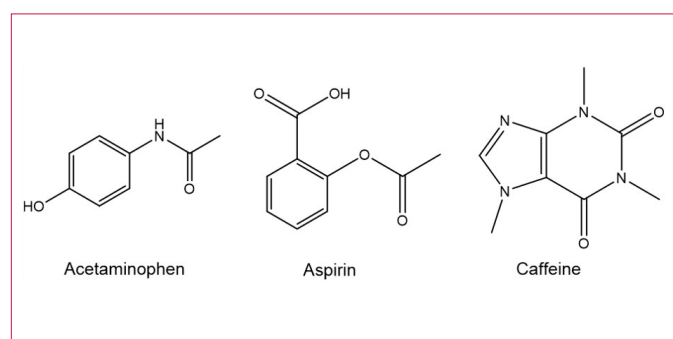


Figure 5: Chemical structure of Acetaminophen, Aspirin and Caffeine.

<b>Instrument</b>	PerkinElmer LC 300 HPLC system with LC 300 multi-wavelength UV/Vis (MWD) Detector
<b>Columns</b>	PerkinElmer Aviator C18 150 x 4.6 mm, 5 µm P/N: 135221-AVI-C18
	Avantor ACE C18 150 x 4.6 mm, 5 µm
<b>Mobile Phase</b>	Pre-mixed (methanol: glacial acetic acid: water, 28:3:69)
<b>Flow Rate</b>	1.0 mL/min
<b>Temperature</b>	45 °C
<b>Wavelength</b>	275 nm
<b>Injection Volume</b>	10 µL
<b>Analyte and Internal Standard</b>	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 the analysis of acetaminophen, aspirin and caffeine using Aviator C18 and ACE C18.

## Results 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 Aviator and ACE C18 phases exhibit similar selectivities and peak shapes.

	Retention time (min)		Efficiency (N plates)		Tailing factor		Resolution	
	Aviator C18	ACE C18	Aviator C18	ACE C18	Aviator C18	ACE C18	Aviator C18	ACE C18
Acetaminophen	2.23	2.18	2040	1869	0.96	0.95	n/a	n/a
Aspirin	2.81	3.13	2303	2707	1.11	1.07	2.67	4.27
Caffeine	5.83	7.38	4057	5461	1.04	1.03	10.12	13.33
Benzoic acid	7.92	9.98	5502	8658	1.11	1.05	5.41	6.39

Table 6: Suitability results for the analysis of acetaminophen, aspirin, caffeine and benzoic acid using Aviator C18 and ACE C18.

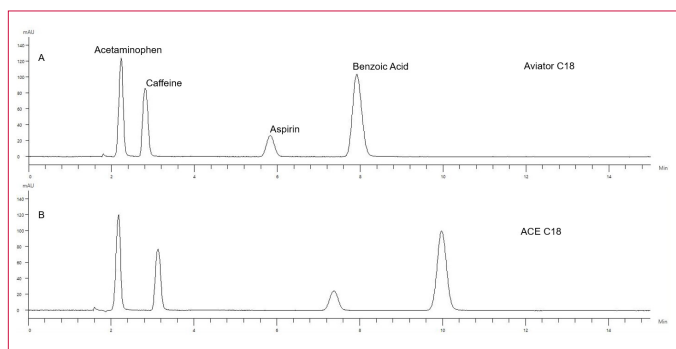


Figure 6: Analysis of acetaminophen, aspirin, caffeine and benzoic acid on Aviator C18 150 x 4.6 mm, 5  $\mu$ m (A) and ACE C18 150 x 4.6 mm, 5  $\mu$ m (B).

## Conclusion

The three applications demonstrated in this technical note showcase the validity of switching from an ACE C18 column to an Aviator C18 column. Similar selectivities can be achieved while displaying comparable or greater efficiencies and similar or less peak tailing. The Aviator C18 is a more cost-effective column allowing for greater financial savings with no significant drop in performance.

## References

1. A. Sudan et al., Antimicrobial Agents and Chemotherapy, 2013, 57, 2793-2800.
2. DrugBank Database, <https://www.drugbank.ca/drugs/DB01216>, (accessed 30/04/2021)
3. Drugbank database, <https://go.drugbank.com/drugs/DB00443>, (accessed 28/04/21)
4. National Center for Biotechnology Information, <https://www.ncbi.nlm.nih.gov/books/NBK513274/>, (accessed 28/04/21)