TECHNICAL NOTE

Liquid Chromatography

Retention of Salbutamol Using Various C18 Phases

Introduction

Salbutamol (also known as albuterol) is a bronchodilating agent which is ubiquitously used in the treatment of asthma and chronic obstructive pulmonary disorder (COPD).[1] It is a polar hydrophilic compound (see Figure 1) which can be problematic when analyzing using reverse phase HPLC. Polar compounds can elute very close to the solvent front. It is therefore ideal for an eluting compound to have a capacity factor (k) between 1 and 10.[2] Polar compounds with poor retention can have k values close to zero. Analyses with this level of retention are not reproducible and so it is important to increase the affinity that polar compounds have for the stationary phase.

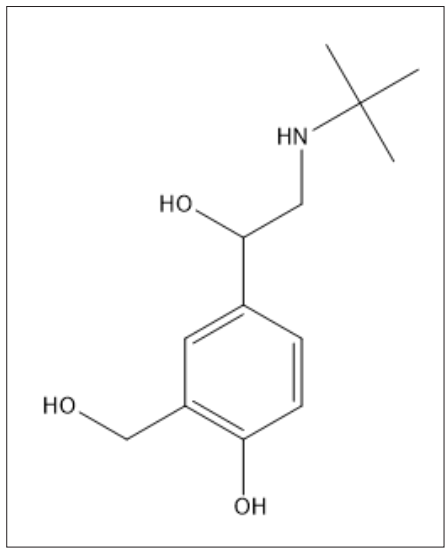

Figure 1. Chemical structure of salbutamol.

The technical note investigates the impact on retention of salbutamol by changing the chemistry of the stationary phase. When analyzing salbutamol in accordance with the British Pharmacopeia, the monograph stipulates the use of 'end-capped octadecylsilyl silica gel for chromatography (5 µm)'. This definition does not specify the type of end capping giving scope to the evaluation of different stationary phase whilst still adhering to BP requirements.

Salbutamol sulfate was analyzed according to the British Pharmacopeia monograph: *Salbutamol Pressurised Inhalation, Suspension*.[3] This analysis was performed on four Quasar columns: C18, AQ, AQ Plus and SPP C18/PFP in order to obtain the maximum improvement in the retention of salbutamol.

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Experimental Conditions

Method Parameters

All HPLC method parameters are shown in Table 1.

Table 1. HPLC method parameters for analysis.

Instrument	PerkinElmer Flexar™ with PDA Plus™ Detector			
Quasar C18	100 mm	3.0 mm	3 µm	N9308809
Quasar AQ C18				N9308848
Quasar AQ Plus				N9304414
Quasar C18/PFP SPP	100 mm	3.0 mm	5 µm	N9304433
Mobile Phase	**A:** Ammonium Acetate Solution (0.1 % w/v) **B:** Methanol 23.8 % **A,** 76.2 % **B**			
Flow Rate	0.85 mL/min			
Temp	50 °C			
Wavelength	276 nm			
Injection Volume (Fully Porous)	50 µL			
Injection Volume (SPP)	5 µL			
Analyte	Salbutamol sulfate			

Solvents and Samples

All solvents were HPLC grade and samples were filtered using a 0.45 µm nylon filter, P/N 02542880.

Two standard solutions of salbutamol sulfate (0.0054 % and 0.00054 % w/v) were prepared in methanol. The injection volumes were lowered in comparison with the volume specified by the BP monograph due to the smaller capacity injection loop fitted on the Flexar system. Therefore, the concentration of the standards differ from the monograph specification of 0.00018 % w/v. Injections on fully porous columns were reduced to 50 µL and reduced to 5 µL on the SPP column to account for reduced sample loading capacities. To match the concentrations of sample loaded onto the column, the 0.00054 % w/v standard and the 0.0054 % standard were injected onto the fully porous columns and SPP column, respectively.

Results and Discussion

Salbutamol was first analyzed under BP conditions using a Quasar C18 phase. This C18 phase simply has C18 alkyl ligands bonded to the surface of the silica, with hydrophobic end capping, as shown in Figure 2A. Table 2 shows that the retention of salbutamol is poor. Salbutamol elutes so shortly after the solvent front (Figure 3) that capacity factor cannot be accurately calculated. It can be inferred that salbutamol has a very low affinity for the non-polar C18 phase and is, therefore, not significantly retained on the column. Increasing the aqueous component in the mobile phase yielded no significant improvements in retention.

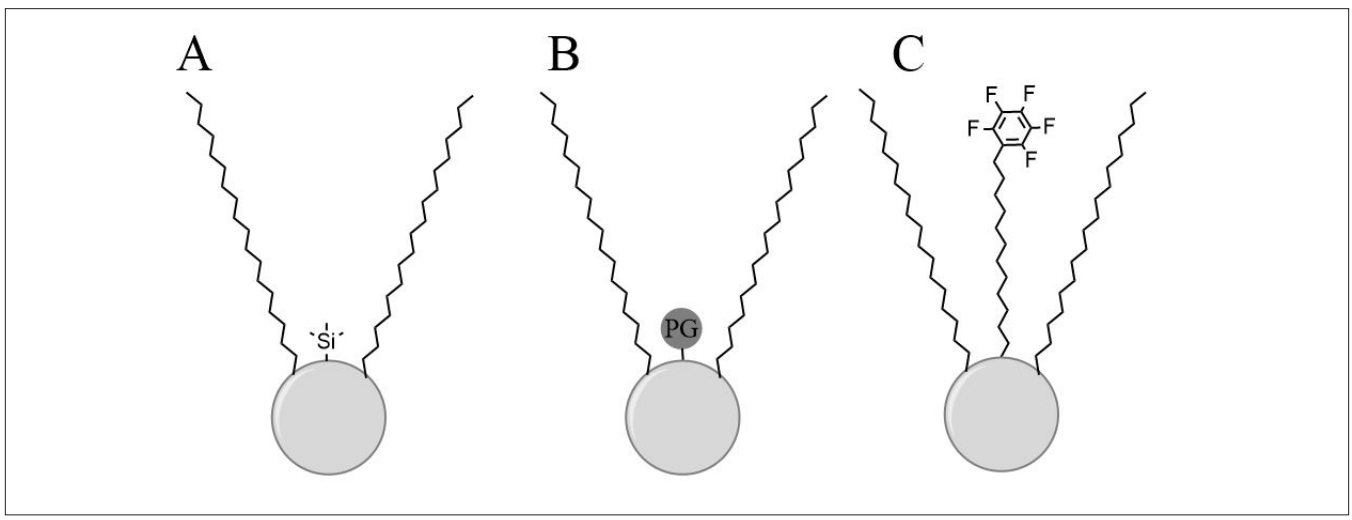

Figure 2. Schematic representation of phase chemistries A) C18 phase, B) AQ and AQ Plus phase where PG = Polar End capping, C) C18/PFP phase.

The Quasar AQ and AQ Plus phases utilize polar end-capping (Figure 2B). The resulting increase in polarity of the stationary phase could theoretically improve salbutamol's affinity, increasing its retention. Analysis of salbutamol on the Quasar AQ column, Figure 4, shows that the retention was improved but not to a satisfactory degree (see Table 2). Increasing the aqueous component of the mobile phase did not improve retention. Although the affinity of the analyte to the stationary phase was slightly improved with this column compared to the C18 column, the use of the AQ phase was not suitable for the reproducible analysis of salbutamol.

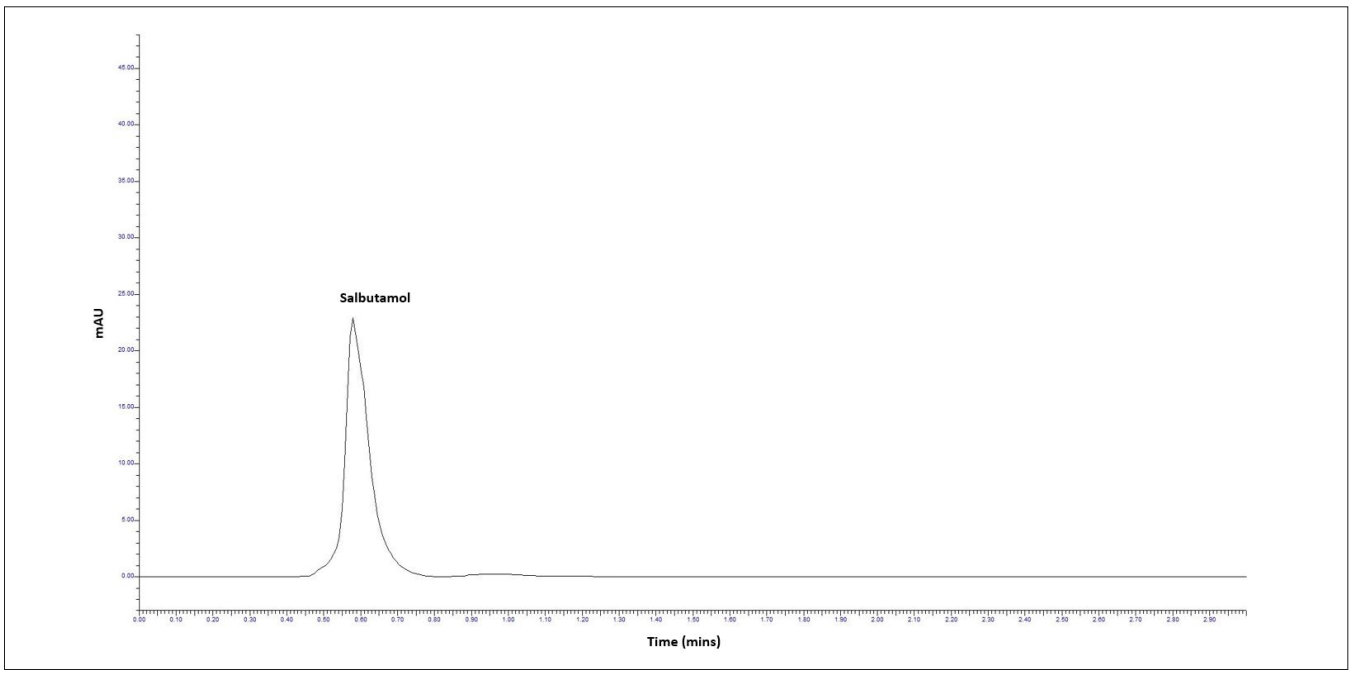

Figure 3. Analysis of salbutamol using the Quasar C18 column.

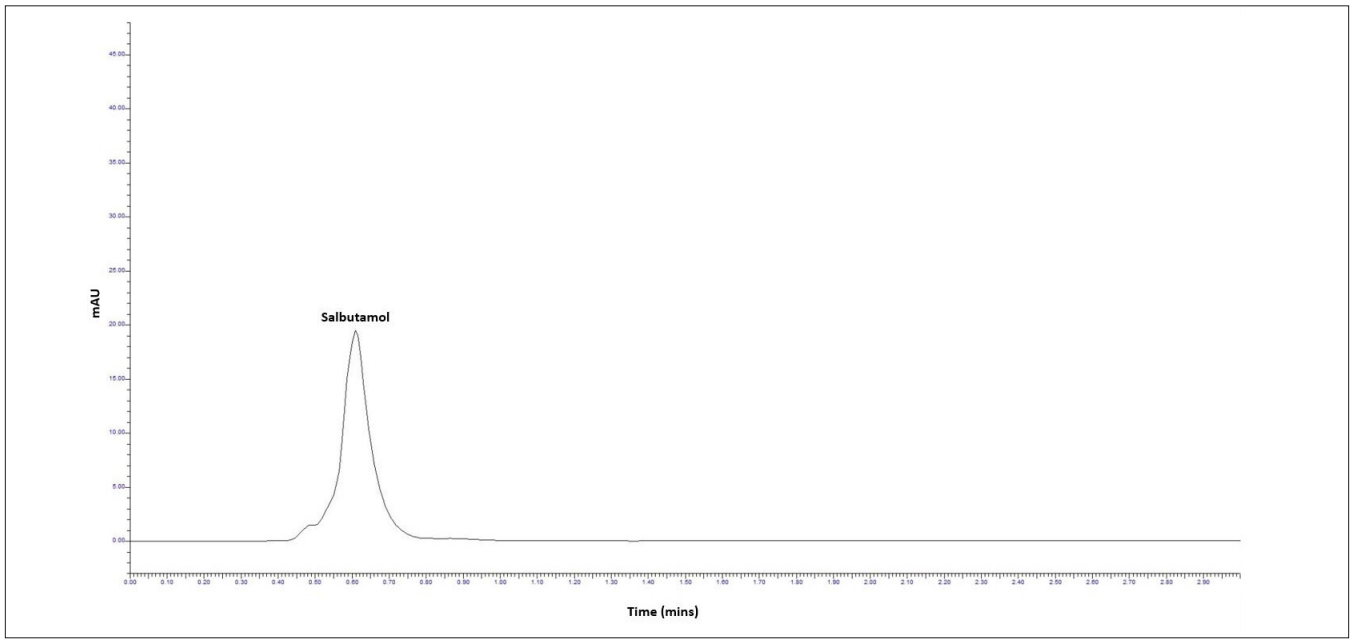

Figure 4. Analysis of salbutamol using the Quasar AQ column.

Salbutamol was then analyzed under the same conditions, Figure 5, using the Quasar AQ Plus phase. The retention time of salbutamol increased relative to the C18 and AQ column (Table 2). Unfortunately, the retention was still insufficient. Increasing the polarity of the stationary phase through more polar end capping moieties was not a suitable methodology for increasing retention.

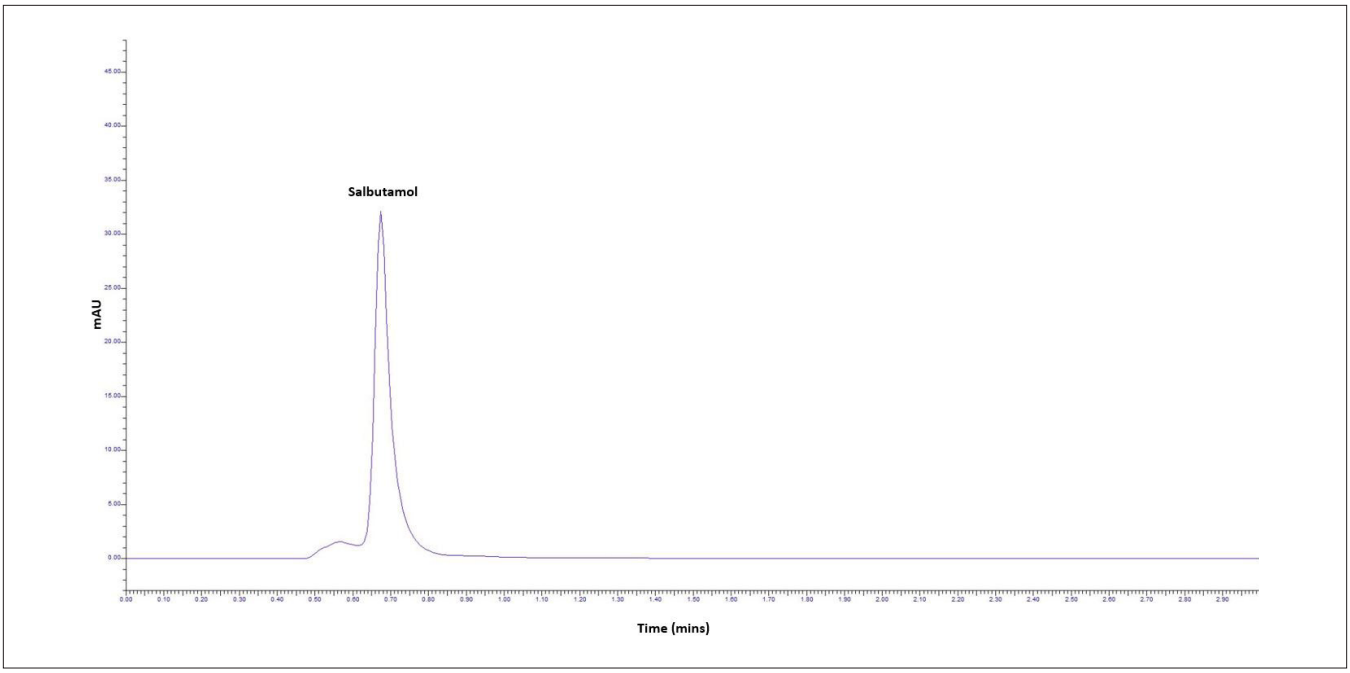

Figure 5. Analysis of salbutamol using the Quasar AQ Plus column.

Finally, salbutamol was analyzed using the Quasar SPP C18/PFP phase (Figure 6) which utilizes a mixture of pentafluorophenyl (PFP) ligands and C18 alkyl ligands (Figure 2C). The aromatic moiety of salbutamol has synergy with the PFP moiety on the stationary phase through π-π interactions and thus has a greater retention than the standard C18 phase (Table 2). The C18/PFP phase gave the most retentive separation for salbutamol out of all C18 phases screened. Unlike the previous analyses, salbutamol did not elute with the solvent front, but had a k value of 0.85. The suitability conditions in the BP monograph specify a symmetry factor less than 2.5. The symmetry factor for this peak was 1.20 (measured at 5 % peak height as required by the BP), well within the requirements of the monograph.

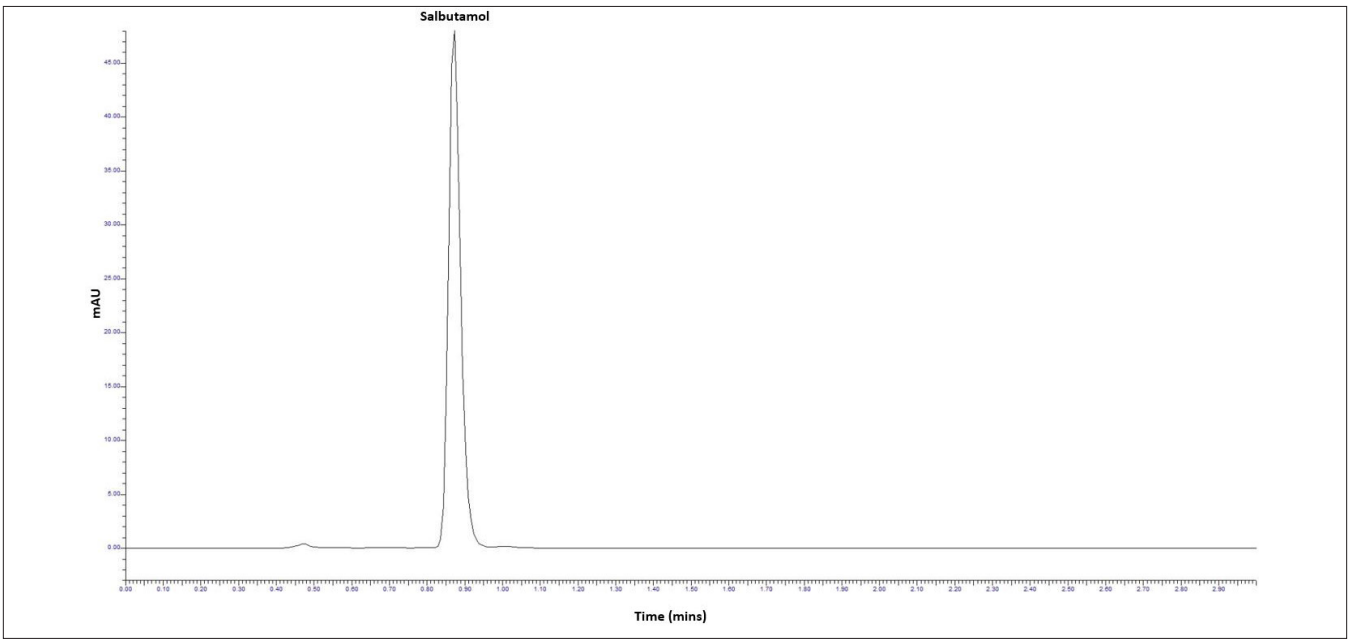

Figure 6. Analysis of salbutamol using the Quasar SPP C18/PFP column.

Table 2. Retention for the analysis of salbutamol.

Column	Retention Time (min)
Quasar SPP C18/PFP	0.872
Quasar AQ Plus	0.675
Quasar AQ	0.609
Quasar C18	0.582

Conclusion

Different C18 phases were screened to investigate the impact on retention of salbutamol sulfate on C18 phases, with the aim to increase retention to an acceptable level. The reason for the poor retention of salbutamol on standard C18 phases is a result of its high polarity compared to that of the stationary phase. Increasing the polarity of the stationary phase through the use of polar end capping of the AQ and AQ Plus columns failed to prevent salbutamol from eluting with the solvent front.

Targeting the aromatic region of salbutamol with π-π interactions was investigated using a SPP C18/PFP phase. The increased affinity provided successful retention of salbutamol with excellent peak shape. The use of this Quasar SPP C18/PFP phase also gave a symmetry factor of 1.20 which is well within the limits of the BP requirements. The separation was repeatable, the RSD of the peak area from five replicate injections was 0.92 %. This study shows how the variations in C18 phases can be strategically chosen to increase the synergy between the stationary phase and functionalities in the analyte for increased retention.

References

1. Drugbank Salbutamol, https://www.drugbank.ca/drugs/DB01001, (accessed 01/07/2020).
2. HPLC Chromatographic Parameters, https://www.chromacademy.com/channels/hplc/principles/hplc-chromatographic-parameters-retention-factor/, (accessed 21/07/2020).
3. British Pharmacopeia. Salbutamol pressurized inhalation, suspension. [Internet]. 2019 [revised 24 September 2019; cited 2020 June 01]. Available from: https://www.pharmacopoeia.com/.

Consumables Used

Component	Description	Part Number
Columns	Quasar C18 (100 x 3.0 mm, 3 µm)	N9308809
	Quasar AQ (100 x 3.0 mm, 3 µm)	N9308848
	Quasar AQ Plus (100 x 3.0 mm, 3 µm)	N9304414
	Quasar SPP C18/PFP (100 x 3.0 mm, 5 µm)	N9304433
HPLC Vials	2 mL, 9 mm Screw Top Vial with Write-on Patch and Fill Lines (100/Pack)	N9307802
HPLC Vial Caps	9 mm Screw Top Blue (polypropylene) Cap with PTFE/Silicone pre-slit Septa (100/pack)	N9306203
Syringes	Syringe 1 mL BD Luer-Lok Disposable, Pack of 100	02542890
Syringe Filters	0.45 µm nylon syringe filter	02542880
PEEK Fittings	Fingertight for 1/16" OD PEEK tubing	09920513

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