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アプリケーションノート

USP Method Transfer and Routine Use Analysis of Budesonide Nasal Spray from HPLC to UPLC

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Abstract

USP monograph for Budesonide was used to successfully transfer the HPLC method to a UPLC platform using appropriate software tools and modern sub-2 µm column chemistry. System suitability requirements for the USP method transferred from the Alliance HPLC system to the ACQUITY UPLC system were met. Optimizing the sample preparation protocol (i.e. increasing centrifugation time), to ensure maximum sample clean-up, adding a pre-column filter to protect the column from particulates and maintaining a constant column temperature, resulted in excellent column performance for over 2800 injections. The ACQUITY UPLC BEH C18 1.7µm column demonstrated robustness under low-pH (3.2) conditions with phosphate buffer. With proper sample preparation, one can successfully transfer and analyze USP methods for generic drugs on UPLC systems. Other benefits realized through this transfer were an 87% reduction in run time and more than 90% reduction in solvent and sample consumption. The ease of transfer using the simplistic workflow design3 helps in the seamless adoption of UPLC technology with conspicuous gains in overall laboratory efficiency and productivity for running USP monographs.

Benefits

- 87% reduction in run time while preserving peak resolution and efficiency
- More than 90% reduction in solvent and sample consumption
- Availability of wide selection of sub-2 µm column chemistries
- Proven column performance of over 2800 injections on the ACQUITY UPLC column

Introduction

Compendial methods originally written, several years ago, do not take advantage of the advancements in chromatographic chemistries and instrumentation. With the advent of UPLC technology, it is desirable to transfer USP methods with the transfer process following accepted guidelines and yielding same analytical results. These migrated methods however are only valuable when they are robust enough for routine use.

In this study we use the USP monograph for budesonide as an example to transfer the long HPLC run to a short UPLC run. Budesonide formulation is available as a nasal spray, analytically separated under low-pH conditions with phosphate buffer. By effectively managing chromatographic variables such as, proper handling of the nasal spray formulation and operating conditions delivered by the instrument, a routine use study of over 2800 injections is shown. Following the process described here will help with the successful adoption of modern UPLC technology and its benefits, to test products described in the Pharmacopeial monographs.

Experimental

HPLC conditions

LC System:	Alliance HPLC with 2489 UV/Visible detector
Run Time:	32 minutes
Column:	XBridge C_{18} 4.6 x 150 mm, 5 µm (USP designation: L1), part number 186003116
Mobile Phase:	Acetonitrile and Solution A (32:68); Solution A: 3.17 mg/mL of monobasic sodium phosphate and 0.23 mg/mL of phosphoric acid; pH 3.2 +/- 0.1
Separation Mode:	Isocratic
Flow Rate:	1.5 mL/min
Injection Volume:	20 µL
Detection:	UV at 254 nm

UPLC conditions

LC System:	ACQUITY UPLC with TUV detector
Run Time:	4 minutes
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7 µm (USP

	designation: L1), part number 186002350
Mobile Phase:	Acetonitrile and Solution A (32:68); Solution A: 3.17 mg/mL of monobasic sodium phosphate and 0.23 mg/mL of phosphoric acid; pH 3.2 +/- 0.1
Wash Solvents:	70:30 water/ acetonitrile (weak wash) 100% acetonitrile (strong wash)
Separation Mode:	Isocratic
Flow Rate:	0.92 mL/min
Injection Volume:	1.4 µL
Detection:	UV at 254 nm
Data Management:	Empower 2 CDS

USP System Suitability Criteria

USP Resolution:	NLT 1.5
USP Plate Count:	NLT 5500

$RT_{epimer\ A} = 1.1 \times RT_{epimer\ B}$

Standard Preparation

A 12.8 µg/mL working standard of budesonide was prepared in a solution of 70:30 Solution A/acetonitrile. (Please refer to Mobile-Phase description in HPLC Conditions for details on Solution A.)

Sample Preparation

The concentration of the working standard and sample specified in the USP monograph, is 0.5 mg/mL.[1] This

monograph, however, is for drug substance analysis. For drug product analysis, the sample preparation protocol adopted use of a lower concentration to ensure that the budesonide formulation dissolves in its diluent. Rhinocort AQUA (budesonide) nasal spray formulation contains a micronized suspension of budesonide in an aqueous medium consisting of microcrystalline cellulose, carboxy-methyl cellulose sodium, dextrose anyhydrous, polysorbate 80, disodium edentate, potassium sorbate, HCl and water.

An amount equivalent to 1.0 g of Rhinocort AQUA (budesonide) nasal spray was accurately weighed and transferred to a 50 mL volumetric flask. 16 mL of acetonitrile was added to this flask. This mixture was mechanically shaken in the Burrell Wrist-Action shaker, Model 75 for 15 minutes. The mixture was diluted with Solution A to volume and mechanically shaken for an additional 10 minutes. This mixture shown in 1(b) was then subjected to centrifugation at 3,220 rcf (4,000 rpm) for 15 minutes. The supernatant shown in Figure 1(c) was aliquoted into a 2 mL Waters Certified Glass Screw Cap Vial with bonded pre-slit PTFE/silicone Septum (Part number 186000307C). Final concentration of the working sample was 12.8 µg/mL.

Figure 1. RHINOCORT AQUA (budesonide) nasal spray sample preparation. (a) RHINOCORT AQUA (budesonide) nasal spray formulation; (b) budesonide formulation in diluent; (c) budesonide sample after centrifugation.

Results and Discussion

The USP monograph for budesonide requires the use of a 4.6 x 150 mm, 5 µm packing (L1) column.[1] As per the USP monograph, the original methodology was developed and submitted using a Supelcosil LC C_{18} column. The Waters XBridge C_{18} column was selected in regards to similar selectivity with the guidance of the Waters Reversed-Phase Column Selectivity chart. The separation of the budesonide standard run using

Waters XBridge C_{18} 4.6 x 150 mm, 5 µm (L1) column on an Alliance HPLC system is as shown in Figure 2.

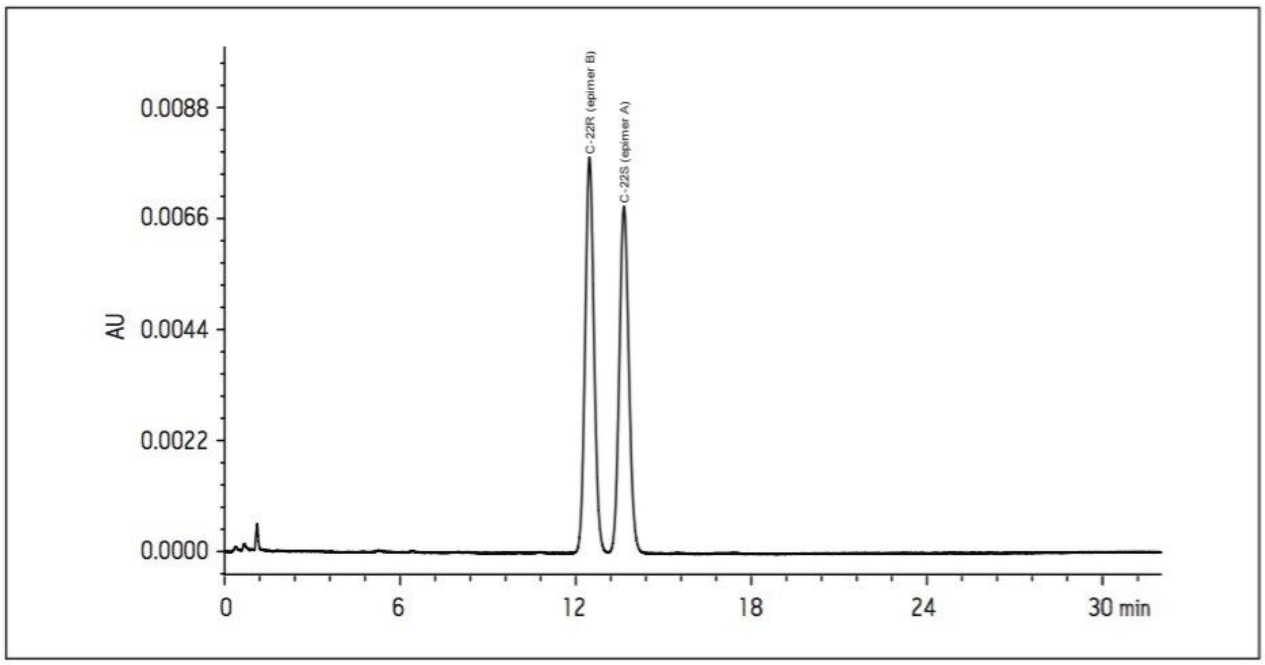

Figure 2. USP budesonide standard method run on XBridge 4.6 x 150 mm, 5 µm column on the Alliance HPLC system.

The USP method was then transferred from HPLC to UPLC using the Waters ACQUITY UPLC Columns Calculator as described in the application note "*Implementation of Methods Translation between Liquid Chromatography Instrumentation*", part number 720003721en.[3] The retention factor (k') of budesonide, calculated from the separation on Alliance, was input into the columns calculator to optimally scale the method and generate UPLC method conditions while preserving the resolving power of the separation. The run time generated by the ACQUITY UPLC Columns Calculator is based on the retention factor (k') and may be adjusted to account for total run time (Figures 3 and 4).

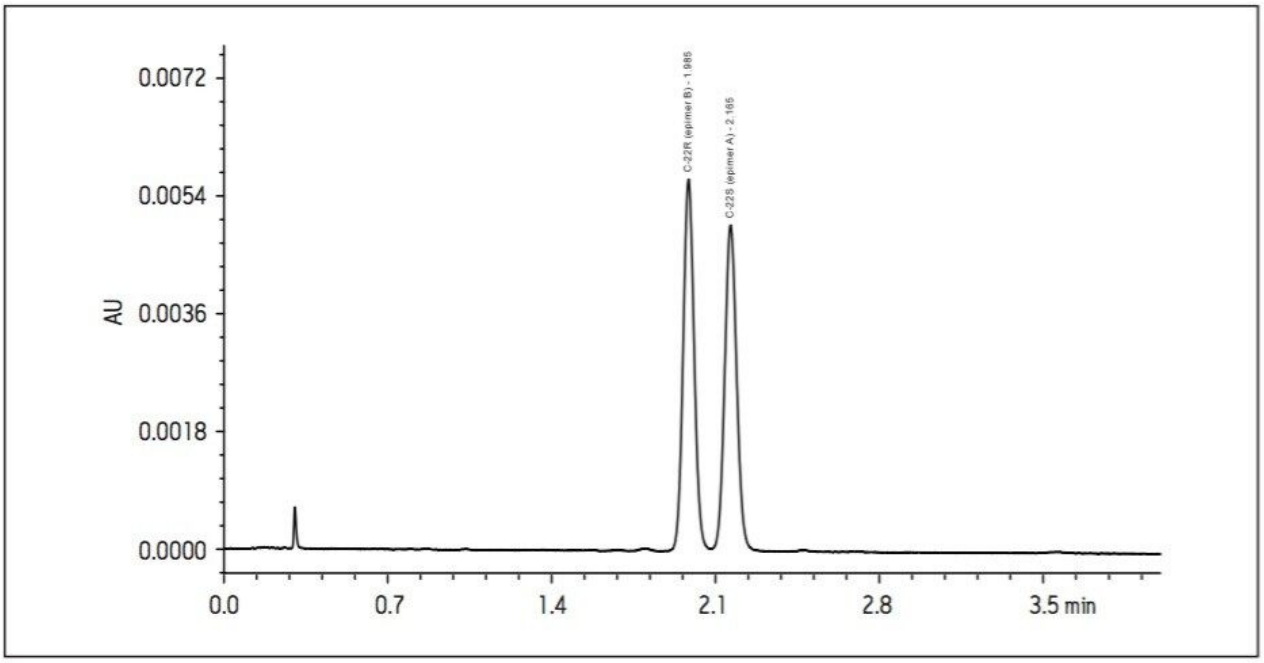

Figure 3. USP budesonide standard run on an ACQUITY UPLC BEH C_{18} column on the ACQUITY UPLC system.

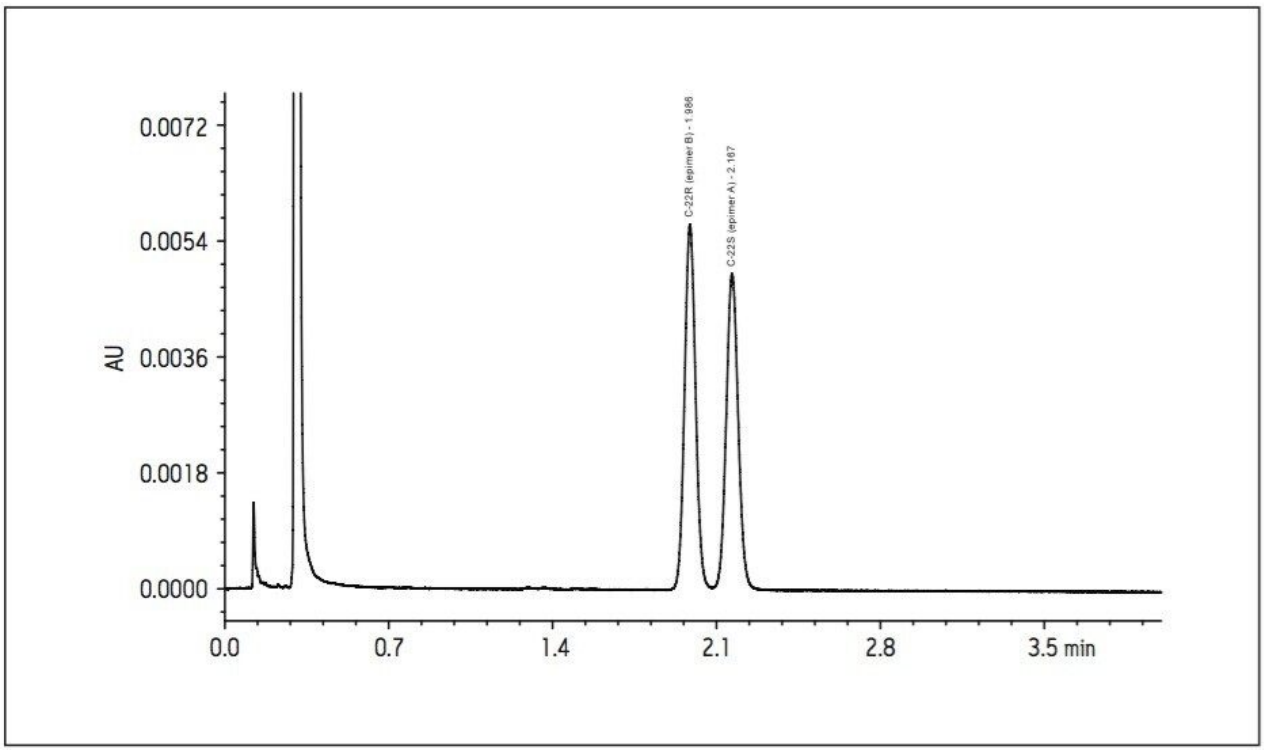

Figure 4. USP budesonide sample run on an ACQUITY UPLC BEH C_{18} column on the ACQUITY UPLC system.

System suitability requirements for the budesonide separation on the Alliance HPLC system and the ACQUITY UPLC system were met (Table 1).

USP Requirements	Budesonide Standard (HPLC)	Budesonide Standard (UPLC)	Budesonide Sample (UPLC)
USP Resolution (≥ 1.5)	2.03	1.96	1.97
USP Plate Count from Epimer B peak (≥ 5500)	7962	8539	8540
Relative Retention Time (Epimer A = 1.1 X Epimer B)	1.1	1.1	1.1

Table 1. System suitability results for six replicate injections of budesonide standard and sample on Alliance HPLC and ACQUITY UPLC systems.

Routine Use Study

Following the protocol that a typical QC lab would use to perform the USP monograph testing both standards and samples were injected to evaluate routine use. The sample set was constructed with two replicate injections of standard bracketing 6 replicate injections of sample.

Two system suitability studies were conducted. In the first study, it was observed that the working standard gave higher plate count values than the sample (figure 5). It should be noted that the plate count and resolution values at the start of the study for this column were only 6477 and 1.78, respectively. System suitability results for the first study are reported in table 2. After about 830 injections, USP plate count values for Epimer B began to fail the USP specification. In an effort to revive the column, the column was washed overnight with 100% acetonitrile at 0.1mL/min for about 8 hours followed by a mobile-phase composition of 50:50 water:acetonitrile, respectively at a flow rate of 0.1mL/min for another 8 hours. USP plate count for Epimer B continued to fail the USP specification. The column inlet frit was replaced to determine if it experienced blockage leading to the reduced column lifetime. This approach alone did not retain USP specifications for budesonide Epimer B (table 2).

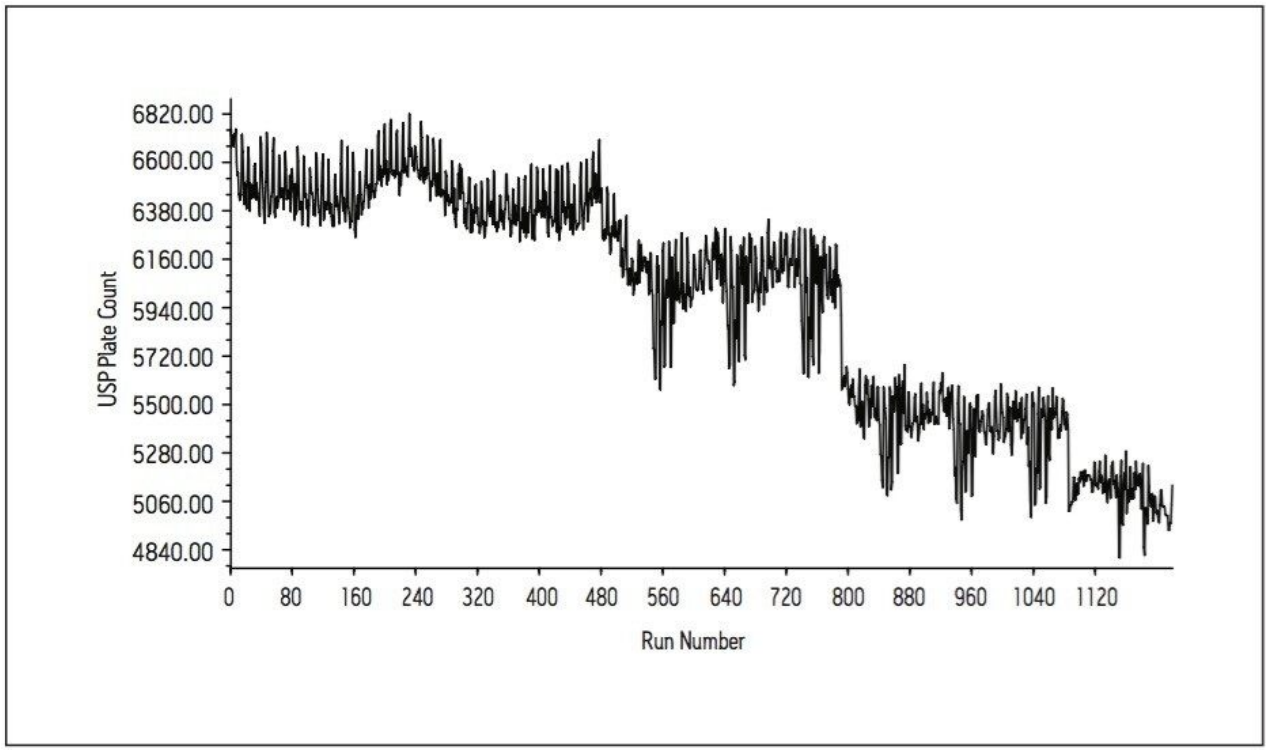

Figure 5. USP plate count trend plot from routine use study 1 on an ACQUITY UPLC BEH C_{18}, 1.7 µm column.

Injection #/ System Suitability Requirements	52 - 57	506 - 511	824 - 829	1199 - 1204	1228 – 1233 (New Column Inlet Frit)
USP Resolution (≥ 1.5)	1.78	1.72	1.62	1.58	1.50
USP Plate Count from Epimer B Peak (≥ 5500)	6477	6137	5508	4992	4897
Relative Retention Time (Epimer A = 1.1 X Epimer B)	1.1	1.1	1.1	1.1	1.1

Table 2. System suitability results from routine use study 1 of only about 1000 injections on ACQUITY UPLC BEH C_{18} column and ACQUITY UPLC system.

A second study was then conducted after making the following three minor modifications. First, within the sample preparation protocol, the time required to centrifuge the budesonide sample was increased from 15 minutes to 25 minutes. This change was made to ensure appropriate filtration of the sample. Next, a pre-column filter (part number 700002775) was connected to the head of the column for preventative maintenance and to prolong column lifetime. Finally, a constant column temperature of 30°C was used in

the method to improve retention time reproducibility. This is an acceptable change according to USP 621.2 With the above mentioned improvements, plate count and resolution values for a new ACQUITY UPLC BEH 2.1 x 50 mm, 1.7 µm column began at 8540 and 1.97 respectively. Both plate count and resolution values were maintained throughout the course of the study, for over 2846 injections. System pressure remained stable at around 11,800 psi throughout the course of the study (figure 6). System suitability results are shown in table 3.

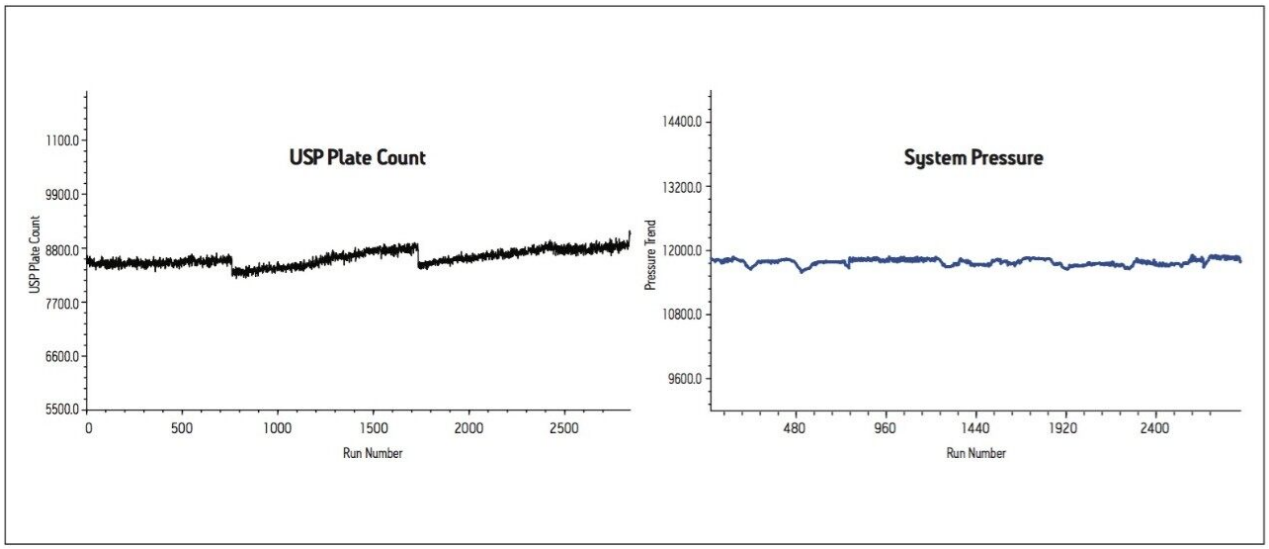

Figure 6. USP plate count and system pressure trend plot from routine use study 2 of over 2800 injections on ACQUITY UPLC BEH C_{18} column.

Injection #/ System Suitability Requirements	10 - 15	506 - 511	1002 - 1007	1502 - 1507	2006 - 2011	2504 - 2509	2840 - 2846
USP Resolution (≥ 1.5)	1.97	1.96	1.95	1.99	1.95	1.97	2.00
USP Plate Count from Epimer B Peak (≥ 5500)	8540	8515	8419	8750	8591	8737	8857
Relative Retention Time (Epimer A = 1.1 X Epimer B)	1.1	1.1	1.1	1.1	1.1	1.1	1.1

Table 3. System suitability results from routine-use study 2 of over 2800 injections on ACQUITY UPLC BEH C_{18} column and ACQUITY UPLC system.

Conclusion

USP monograph for Budesonide was used to successfully transfer the HPLC method to a UPLC platform using appropriate software tools and modern sub-2 µm column chemistry. System suitability requirements for the USP method transferred from the Alliance HPLC system to the ACQUITY UPLC system were met. Optimizing the sample preparation protocol (i.e. increasing centrifugation time), to ensure maximum sample clean-up, adding a pre-column filter to protect the column from particulates and maintaining a constant column temperature, resulted in excellent column performance for over 2800 injections. The ACQUITY UPLC BEH C_{18} 1.7µm column demonstrated robustness under low-pH (3.2) conditions with phosphate buffer. With proper sample preparation, one can successfully transfer and analyze USP methods for generic drugs on UPLC systems. Other benefits realized through this transfer were an 87% reduction in run time and more than 90% reduction in solvent and sample consumption. The ease of transfer using the simplistic workflow design3 helps in the seamless adoption of UPLC technology with conspicuous gains in overall laboratory efficiency and productivity for running USP monographs.

References

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