Waters™

Nota applicativa

Examining Column Selectivity Differences Across Various L1 Designation Columns for Paracetamol EP Impurities Monograph

Kenneth D. Berthelette, Maureen DeLoffi, Kim Haynes

Waters Corporation

Abstract

The United States Pharmacopeia (USP) has designations for all columns stationary phases used in the monograph methods. These designations outline the stationary phase type, ***i.e.*** fully porous or solid-core, and any ligand attachments, ***i.e.*** C_{18} or Phenyl to be used.[1] However, beyond that no column specifics are given. With a multitude of columns that fit into the different designations, understanding that not all columns are the same is vital when selecting a stationary phase for a monograph method. This application note examines three columns that all fit into the L1 designation when analyzing paracetamol impurities. Selectivity differences between the columns are considered in relation to the impurities.

Benefits

- Three L1 designation columns used to analyze the paracetamol EP impurities monograph
- Selectivity differences observed across the three columns
- All columns pass EP monograph requirements for system suitability and resolution
- Full separation of all 16 compounds using the CORTECS™ C_{18}+ Column

Introduction

Monograph methods, whether in the United States Pharmacopeia (USP) or European Pharmacopeia (EP) are used to analyze samples as they are pre-validated and do not require method development before they can be implemented. Monograph methods outline the exact method conditions for analyzing these samples, including but not limited to the mobile phases, flow rates, injection volumes, and column dimensions. However, some ambiguity exists in terms of stationary phase to be used. USP Monograph methods specify an L designation, *i.e.* an L1 column, which is defined in terms of base particle and bonded ligand. However, different L1 columns are designed with varying attributes. For instance, an XSelect™ HSS T3 Column is considerably different than an XBridge™ BEH™ C_{18}, even though both columns are L1 in designation.

For the paracetamol impurities monograph in the EP, an L1 solid core column in 4.6 x 150 mm hardware is called for, and therefore selected in this work.[2] The monograph employs a buffer and methanol gradient to separate the main paracetamol peak from the fifteen identified impurities. While the system suitability requirements are limited to resolution between paracetamol and a single impurity, achieving good separation of all impurities is preferred to allow for adequate quantitation in live samples. For this reason, three different L1 columns were tested, all with solid-core base particles. The CORTECS Premier 5 µm family of columns contains three L1 phases, the T3, C_{18}, and C_{18}+. Each phase is designed for a different chromatographic purpose. The CORTECS Premier C_{18} Column is a full coverage C_{18} phase for general use. The CORTECS Premier C_{18}+ Column is manufactured with a slight positive charge in the base particle which improves the peak shape for basic probes especially under low ionic strength low pH conditions. Finally, the CORTECS Premier T3 Column is a mid-coverage C_{18} phase designed to be 100% aqueous compatible to improve retention for analytes, especially polar analytes. All of these columns take advantage of MaxPeak™ Premier High Performance Surfaces (HPS) technology which mitigates secondary interactions between analytes and the metal surfaces of the column hardware.[3–5]

Experimental

Sample Descriptions

All samples were created in accordance with the European Pharmacopeia (EP) monograph. Test solution created using paracetamol standard at 10 mg/mL using a 85:15 (water:methanol v/v) diluent. Reference solution A

created to a final concentration of 5 µg/mL paracetamol using the sample diluent. Reference solution B created to contain 0.1 µg/mL of Impurity J in diluent. Reference solutions C and D created using Impurity K to a final concentration of 5 µg/mL and 0.5 µg/mL respectively. Reference solution E created to contain 0.5 µg/mL paracetamol and 0.5 µg/mL of Impurity K. Lastly an impurity mixture not required by the EP monograph was created to contain 50 µg/mL Impurity K, 10 µg/mL Impurity J, 10 mg/mL paracetamol, and 5 µg/mL of all the other identified impurities.

LC Conditions

LC system:	Arc™ HPLC System with PDA detector
Detection:	UV @ 254 nm
Columns (Scaling):	CORTECS C_{18}, 4.6 x 150 mm, 5 µm (p/n: 186010792) CORTECS C_{18}+, 4.6 x 150 mm, 5 µm (p/n: 186010806) CORTECS T3, 4.6 x 150 mm, 5 µm (p/n: 186010820)
Column temperature:	30 °C
Sample temperature:	10 °C
Injection volume:	3.0 µL
Flow rate:	0.25 mL/min
Mobile phase A:	1.7g potassium dihydrogen phosphate and 1.8 g dipotassium phosphate per liter of water.
Mobile phase B:	Methanol
Gradient:	Starting at 5% B, hold at 5% for 1.5 minutes. Linear

	gradient to 10% B in 12.9 minutes. Hold at 10% B for 14.4 minutes. Linear gradient to 34% B in 28.8 minutes. Hold at 34% B for 2.4 minutes. Return to starting conditions and re-equilibrate for 4.07 minutes. Total run time per injection: 64.08 minutes.
Detection:	UV @ 254 nm
Injection volume:	50 µL

Data Management

Chromatography software:	Empower™ 3 Service Release 5

Results and Discussion

Selecting a column based on L designation alone can be challenging as not all columns in a particular group behave the same. Slight variations between manufacturing processes, or the design of the actual stationary phase can have a dramatic effect on the performance of the column for a given assay. For this application note L1 solid core columns will be used to analyze paracetamol impurities from the European Pharmacopeia (EP) monograph. L1 columns contain octadecyl silane chemically bonded to porous or non-porous silica particles or superficially porous particles or ceramic micro-particles, 1.5 to 10 µm in diameter or monolithic rod. This details at least the bare minimum requirements for the column however a fair amount of variability can still exist between two different columns that both fall into the L1 designation category.

CORTECS Premier C_{18}, CORTECS Premier C_{18}+, and CORTECS Premier T3 columns are all considered L1 columns as they contain superficially porous particles with a bonded C_{18} ligand. However, the T3 column contains a mid-coverage C_{18}, meaning the ligand density is lower. This positions this column to allow more interaction between the analytes and the base particle, while also being compatible with highly aqueous mobile

phases. The CORTECS Premier C_{18}+ Column is manufactured to contain a slight positive charge on the base particle, along with a full coverage C_{18} ligand. This positive charge improves the peak shape for basic analytes using low pH mobile phases like formic acid and can have some weak ion exchange characteristics. Due to the positive charge on the surface of the particle, some selectivity differences can be realized between the C_{18}+ and the standard CORTECS Premier C_{18}, which is a full coverage C_{18} column without the modifications to the particle.

Figure 1 shows an impurity panel for paracetamol and its fifteen identified impurities using the three CORTECS L1 columns.

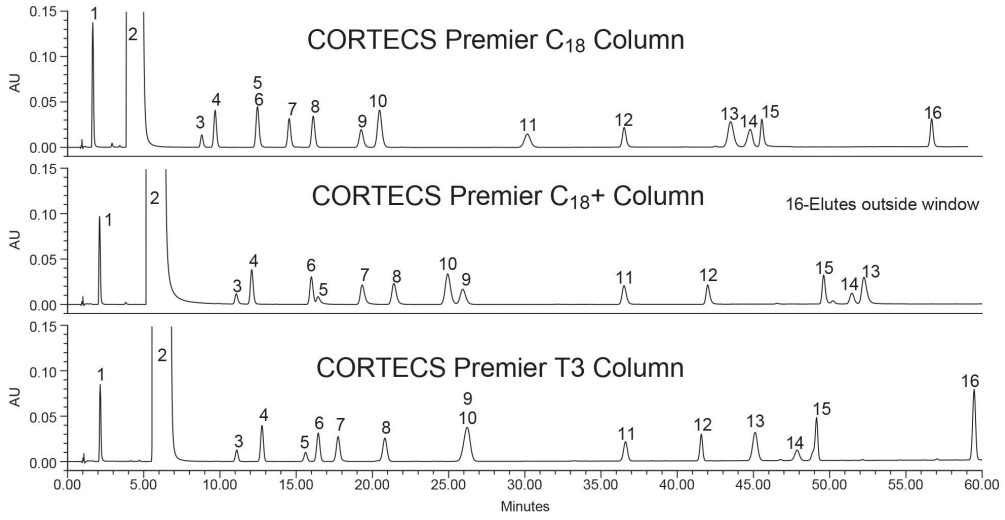

Figure 1. Paracetamol impurities analysis on three CORTECS columns. 1) Imp K, 2) Paracetamol, 3) Imp A, 4) Imp B, 5) Imp F, 6) Imp C, 7) Imp D, 8) Imp E, 9) Imp M, 10) Imp G, 11) Imp H, 12) Imp O, 13) Imp I, 14) Imp J, 15) Imp L, 16) Imp N.

While all three columns have approximately the same elution order there are some selectivity differences noticeable between the three. First it should be noted that all three columns would meet the EP system suitability criteria for the assay. The monograph calls for a resolution of no less than (NLT) 5.0 between paracetamol and impurity K which is achieved for all three columns. No other system suitability requirements exist for this assay, and all other criteria are based on actual sample analysis and determining the amounts of impurities present. To that end, all three columns could be used for analyzing live samples, however the results on the CORTECS Premier C_{18} and CORTECS Premier T3 columns are less than ideal.

Both the CORTECS Premier C_{18} and CORTECS Premier T3 columns have complete co-elution of at least one set of compounds. The C_{18} phase is unable to separate Impurity F and C (5 and 6 respectively) while the T3 column shows total co-elution of Impurity M and G (9 and 10). The CORTECS Premier C_{18}+ Column is able to get separation for all impurities with only a slight co-elution of components 5 and 6. However, the C_{18}+ column retains impurity N, peak 16, beyond the gradient window. It is eluted during re-equilibration of the column. All of these differences can be attributed to the slight differences in column particles.

The CORTECS Premier T3 Column, designed to improve retention, shows the highest retention for paracetamol of the three columns tested. However, the C_{18}+ which can have some ion exchange functionality retains some impurities much better. Namely impurities N, L, J, and I. Additionally, the C_{18}+ phase shows an elution order change for a few compounds. Comparing the C_{18}+ to C_{18} and T3 columns, peaks 5, 6, 9, 10, 15, 14, and 13 all elute differently on the C_{18}+. Since both the C_{18} and T3 phases do not have any charge on the particle, these elution order differences may be due to the presence of that functionality. Figure 2 shows the chemical structures of the impurities which showed the most retention differences across the three columns.

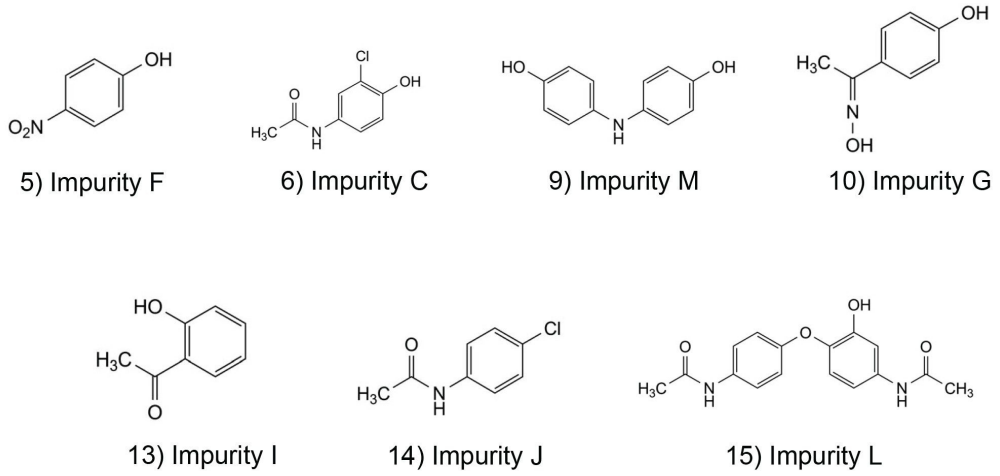

Figure 2. Chemical structures of the impurities highlight the selectivity differences between the three columns tested.

The selectivity differences for these compounds begins to make sense when the structures are examined. Of the compounds shown, five have nitrogen containing groups, whether that be a secondary amine, an amide, or oxime. Impurity F contains a high electron density nitro group, while impurity I only has hydroxy and ketone

groups.

Impurities F and C interact with the stationary phase differently in that they co-elute completely on the C_{18}, but are separated with different elution orders on the C_{18}+ and T3 columns. Impurity F, 4-nitrophenol, contains a nitro group which elevates the pKa of the hydroxyl group to approximately 7. Given the pH of the mobile phase is near neutral, the nitrophenol compound has a partial negative charge. Given that the C_{18}+ phase has a slight positive charge, this would cause the nitrophenol compound to be retained slightly more due to ionic exchange interactions, which is what is seen in the chromatograms.

Similar correlations can be drawn by examining the chemical structures and the resulting chromatograms. For larger panels, like a full impurity analysis it is very important to consider different L designation columns as not all columns are the same, even if they have the same designation. The results shown here don't consider differences between manufacturers either, which can have just as large an impact on the final performance of the column.

Conclusion

The European Pharmacopeia monograph for paracetamol impurities employs a solid-core L1 column to separate the active ingredient and the fifteen impurities. Selectivity differences between three L1 columns were examined when analyzing the impurity mixture for the analysis. Of the three columns tested, the CORTECS Premier C_{18}+ Column showed the best overall separation, even though all three would pass the system suitability criteria. The CORTECS Premier columns have high efficiency solid-core particles designed for HPLC systems while taking advantage of MaxPeak Premier Column Hardware.

References

1. USP Chromatographic Database. https://www.uspchromcolumns.com/chrom/display < https://www.uspchromcolumns.com/chrom/display> Accessed 31-May-2024.
2. Paracetamol EP monograph. European Pharmacopeia 11.0. 04/2022:0049. 3634–3635.

3. Delano M, Walter TH, Lauber M, Gilar M, Jung MC, Nguyen JM, Boissel C, Patel A, Bates-Harrison A, Wyndham K. Using Hybrid Organic-Inorganic Surface Technology to Mitigate Analyte Interactions with Metal Surfaces in UHPLC. *Anal. Chem.* 93 (2021) 5773–5781.

4. Walter TH, Alden BA, Belanger J, Berthelette K, Boissel C, DeLano M, Kizekai L, Nguyen JM, Shiner S. Modifying the Metal Surfaces in HPLC Systems and Columns to Prevent Analyte Adsorption and Other Deleterious Effects. *LCGC Supplements* (2022) 28–34.

5. Jung MC, Lauber MA. Demonstrating Improved Sensitivity and Dynamic Range with MaxPeak High Performance Surfaces (HPS) Technology: A Case Study on the Detection of Nucleotides, Waters Application Note, 720007053, November 2020.

Featured Products

Arc HPLC System <https://www.waters.com/nextgen/in/en/products/chromatography/chromatography-systems/arc-hplc-system.html>

ACQUITY UPLC PDA Detector <https://www.waters.com/nextgen/in/en/products/chromatography/chromatography-detectors/acquity-uplc-pda-detector.html>

Empower Chromatography Data System (CDS) <https://www.waters.com/nextgen/global/products/informatics-and-software/chromatography-software/empower-software-solutions/empower-cds.html>

720008395, June 2024