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Application Note

Comprehending COVID-19: Fast Analysis of Ribavirin and Related Compounds Using Hydrophilic Interaction Chromatography

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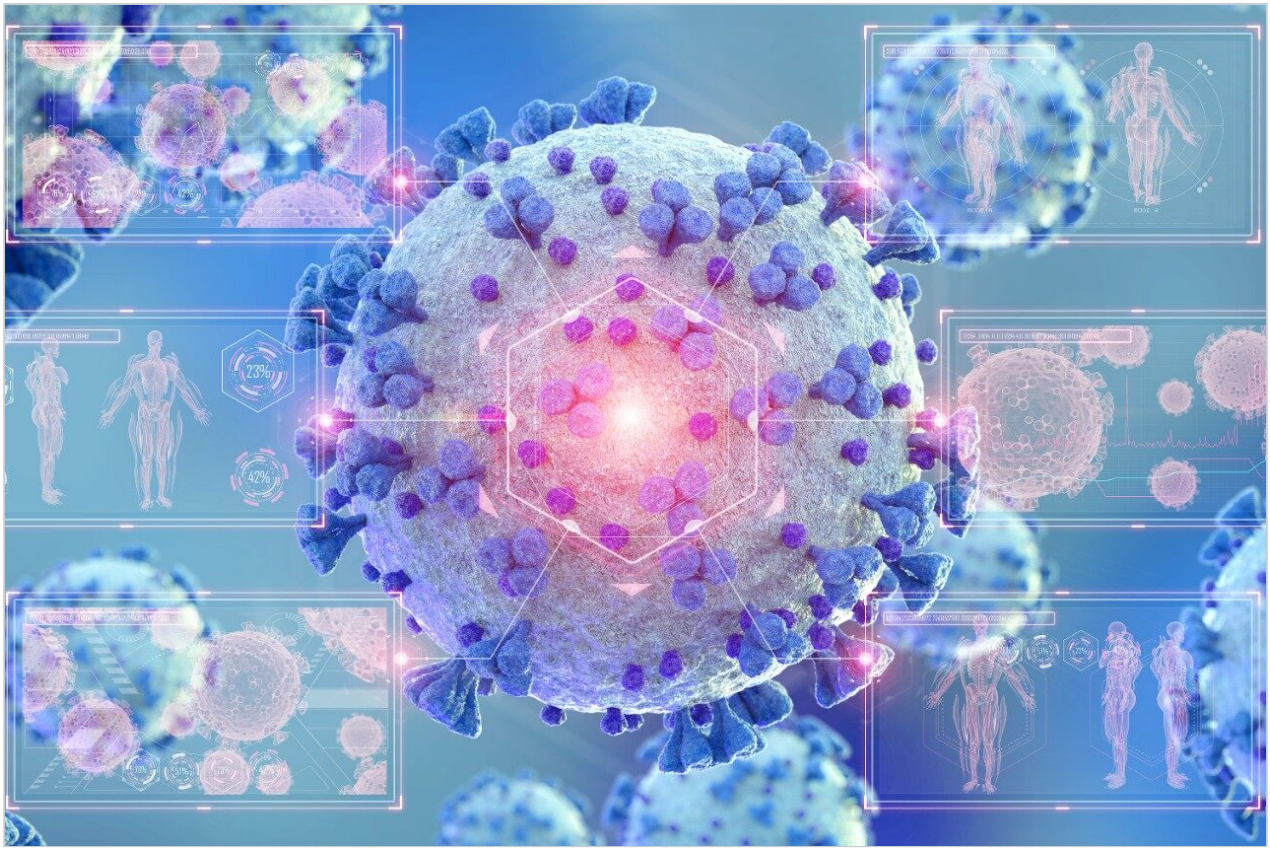

Abstract

Motivated by the current COVID-19 pandemic, a separation method for ribavirin and four related compounds was developed using Hydrophilic Interaction Chromatography (HILIC) with an ACQUITY UPLC BEH Amide Column. While separation of these compounds can be achieved using reversed phase chromatography with specific conditions as outlined in the USP monograph, HILIC is also suitable for the retention of these polar analytes. The benefits of using HILIC include high MS sensitivity and better compatibility with sample preparation techniques such as SPE.[1,2]

Benefits

- Baseline separation of isobaric and structurally similar compounds
- Full separation of analytes with a 6-minute analysis time

Introduction

Ribavirin is an antiviral prodrug prescribed to treat infections including hepatitis C, human respiratory syncytial virus, and some viral hemorrhagic fevers. Moreover, as a result of the COVID-19 pandemic, clinical studies of ribavirin in combination with other antiviral drugs were initiated. One such study reported that a therapeutic combination containing ribavirin shortened the time of SARS-CoV-2 viral shedding.[3] Irrespective of the clinical outcome for ribavirin as a treatment for the novel coronavirus, analysis of ribavirin in cells is important to understand the mechanism of action as well as its treatment course. Endogenous compounds like uridine can interfere with the analysis of ribavirin since the two are structurally similar and are isobaric. This means that the compounds must be separated chromatographically if ribavirin is to be quantified. In this application note we report a method for the separation of ribavirin and related compounds using HILIC.

Experimental

Sample Description: Neat standards were purchased from Sigma Aldrich. Stock solutions were created at 1 mg/mL in 50:50 acetonitrile:water. The stock solutions were combined and diluted to the final composition of

90:10 acetonitrile:water. The concentration of ribavirin, uridine, and ribavirin Related Compound A in the mixture was 100 µg/mL. The concentration of uracil was 30 µg/mL, and that of ribavirin Related Compound D was 10 µg/mL.

Method Conditions:

LC Conditions

LC system:	ACQUITY UPLC H-Class with CHA, PDA
Detection:	UV @ 230 nm
Vials:	TruView LCMS Certified Max Recovery Vials
Column(s):	ACQUITY UPLC BEH Amide, 2.1 x 50 mm 1.7 µm
Column temp.:	30 °C
Sample temp.:	15 °C
Injection volume:	2 µL
Flow rate:	0.5 mL/min
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase D:	100 mM Ammonium Bicarbonate pH 10.0

Gradient Table:

Time (min)	Flow (mL/min)	%A	%B	%D	Curve
0.00	0.5	5	90	5	6
1.50	0.5	5	90	5	6
3.00	0.5	25	70	5	6
4.00	0.5	25	70	5	6
4.01	0.5	5	90	5	6
6.00	0.5	5	90	5	6

Data Management

Chromatography software:	Empower 3 Feature Release 4
MS software:	N/A
Informatics:	N/A

Results and Discussion

The analysis of ribavirin and related compounds is challenging because all the analytes are hydrophilic and poorly retained on traditional reversed phase columns. As shown in Figure 1, the cLogD values (pH 10) of these compounds are less than -1.5, indicating very high solubility in water.[4] While ribavirin and related compounds have been separated using reversed phase chromatography, HILIC is better suited for retaining these highly polar analytes. In HILIC, analytes interact with the stationary phase in multiple ways, including partitioning and through ionic interactions.[5-7] This provides the opportunity to adjust the interactions to optimize retention, resolution, and peak shape.

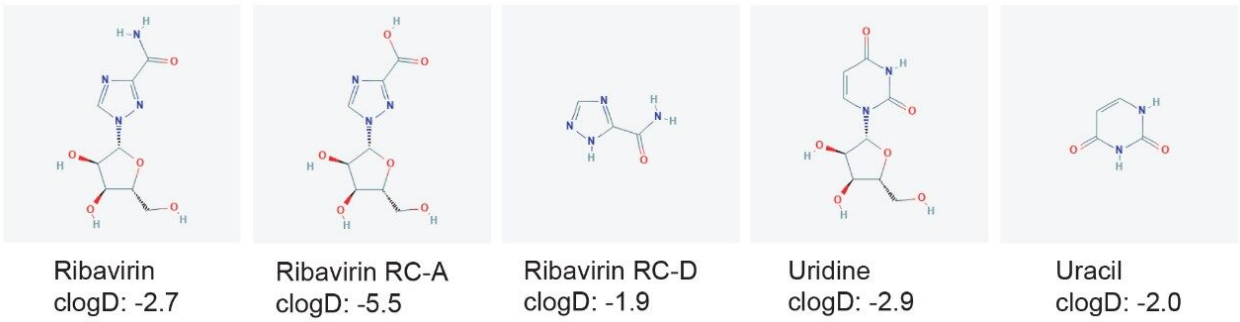

Figure 1. Structure and cLogD values of ribavirin and related compounds. RC (related compound) A and D are either structurally similar or are fragments of ribavirin.

An additional challenge arises when quantifying ribavirin in biological matrices like plasma. The endogenous compound uridine is isobaric with ribavirin thus preventing identification of ribavirin by mass. In order to accurately quantify ribavirin it must be fully separated from uridine allowing identification of ribavirin by retention time. In some method development cases uridine is also included to ensure separation from ribavirin even if uridine is not detected in the final sample.[8] Both compounds contain a ribose group attached to a heterocyclic structure providing them with similar chemical properties and therefore similar interactions with the stationary phase.

A BEH Amide Column was selected for the analysis of these compounds. This stationary phase is based on a hybrid particle which is resistant to high pH mobile phases and has a neutral ligand structure which has been shown to provide greater retention than unbonded hybrid and silica particles. Figure 2 shows the separation of the compounds with UV detection at 230 nm.

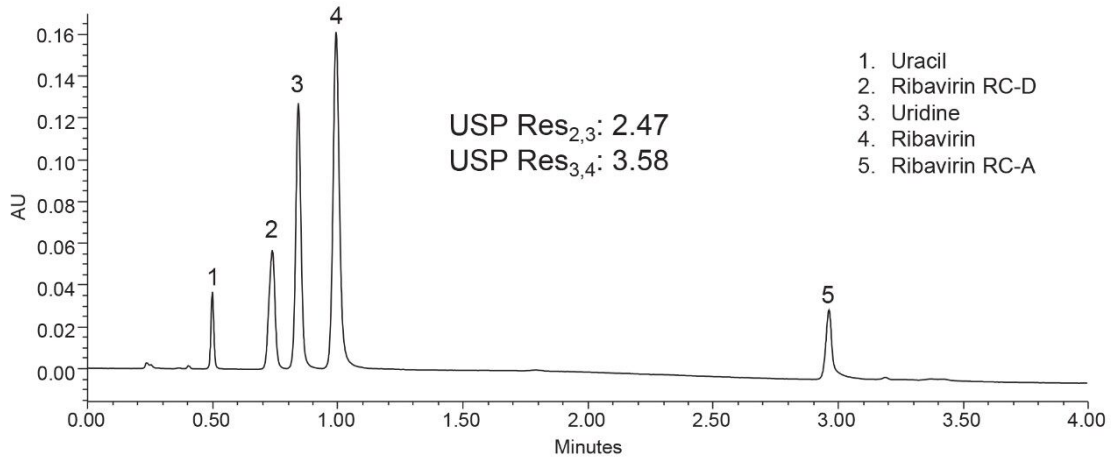

Figure 2. Separation of ribavirin and related compounds using an ACQUITY BEH Amide Column 2.1 x 50 mm, 1.7 µm.

The baseline separation of all analytes was achieved with an analysis time of 6 minutes. Compounds 3 and 4 (uridine and ribavirin, respectively) are well resolved with a USP resolution of 3.58. Good peak symmetry and narrow peaks were also achieved for these compounds allowing for accurate integration of the peaks and reliable quantitation. It should be noted that ribavirin related compound A is retained considerably longer than the other compounds. Examining the cLogD values, this is the most hydrophilic of these compounds, and is therefore expected to have the greatest retention from the partitioning mechanism. Understanding the dominant retention mechanism allows for better method optimization and development if needed.

Conclusion

A rapid method for the separation of ribavirin and related compounds was demonstrated using HILIC with an ACQUITY UPLC BEH Amide Column. Excellent resolution of ribavirin and uridine was achieved, which is critical for the quantitation of ribavirin using LC-MS because uridine is isobaric. This method may be useful for a range of applications, including purity determination of ribavirin and therapeutic drug monitoring. The COVID-19 pandemic further motivates the need for advances in ribavirin analysis, however, improvements in chromatographic methods for ribavirin analysis remains useful regardless of the clinical outcome for ribavirin as a treatment COVID-19.

References

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