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Analytical Products

Application Note

HILIC Analysis of 12 Nucleosides on a Ascentis®Express ZIC®-cHILIC Column

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Introduction

Nucleosides are classified as glycosylamines and consist of a nucleobase covalently linked to a five-carbon sugar moiety (pentose). Various nucleoside analogues have been developed and are employed in clinical practice as therapeutic agents, specifically in the treatment of cancer and viral infections. Structural modifications in these analogues have been introduced to enhance their efficacy and specificity toward pathways involved in cellular proliferation and viral replication.[1,2]

This application focusses on the qualitative analysis of a standard mixture containing twelve nucleosides (**Figure 1**) by HPLC with UV detection. The separation was performed on a HILIC column with a zwitterionic phase (phosphorylcholine) on superficially porous particles (SPP), the Ascentis® Express ZIC®-cHILIC.

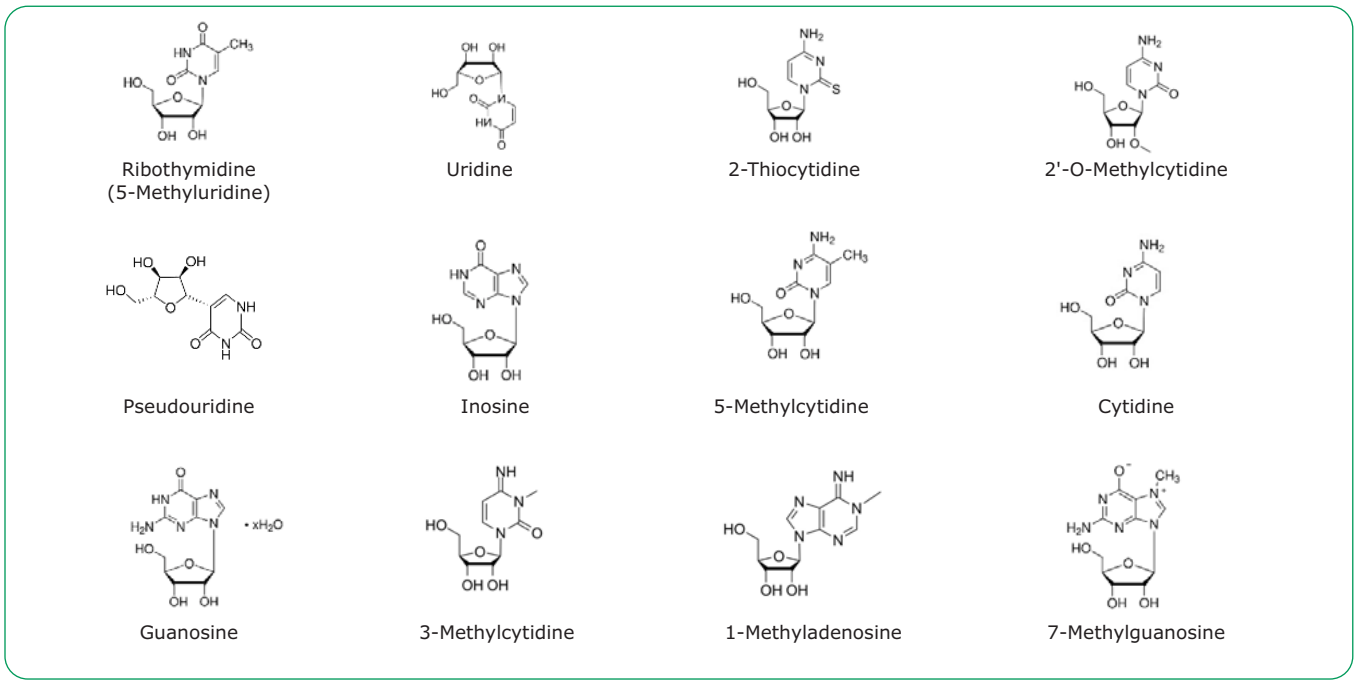

Figure 1. Compounds in Nucleoside Test Mix (**47310-U**).

Experimental

The Nucleoside Test Mix (**47310-U**; matrix: 1% sodium formate in water) was diluted at a ratio of 1:10 with acetonitrile, to prepare a standard solution (**Table 1**) with a matrix composition similar to the initial mobile phase condition of the used HILIC method, which consisted of buffer and acetonitrile in a 3:97 ratio (**Table 2**). The qualitative analysis of the twelve nucleosides at varying concentrations in the standard mixture was carried out using UV detection on an Ascentis® Express 160 Å ZIC®-cHILIC 2.7 µm SPP column (150 x 2.1 mm I.D.).

Table 1. Compounds and concentrations in the twelve nucleoside standard mixture prepared from a 1:10 acetonitrile dilution of the Nucleoside Test Mix**, 47310-U)**

Compound	Conc. (µg/mL)
Cytidine	5.0
Guanosine	2.5
Inosine	25.0
1-Methyladenosine	2.5
5-Methylcytidine	10.0
2′-O-Methylcytidine	2.0
3-Methylcytidine methosulfate	10.0
7-Methylguanosine	2.5
5-Methyluridine (ribothymidine)	5.0
β-Pseudouridine	2.5
2-Thiocytidine dihydrate	1.0
Uridine	2.5

Table 2. HPLC conditions for the analysis of twelve nucleotides in a standard mixture

LC Conditions			
Column:	Ascentis® Express 160 Å ZIC®-cHILIC, 2.7 µm, 150 x 2.1mm I.D. (**50504-U**)		
Mobile phase:	[A] 5 mM ammonium acetate buffer, adjusted to pH 5 with acetic acid [B] Acetonitrile		
Gradient:	Time (min)	A%	B%
	0.0	3	97
	1.5	3	97
	16.5	20	80
	25.0	20	80
Flow rate:	0.3 mL/min		
Pressure drop:	64-98 bar (928-1421 psi)		
Column temp.:	40° C		
Detection:	DAD, UV at 250 nm (LP flow cell; 2.2 µL/light pipe); 20 Hz		
Injection:	2 µL		
Sample:	Nucleoside-Test Mix (**47310-U**) diluted 1:10 with acetonitrile		

Results

The qualitative analysis of the standard mixture containing twelve nucleosides at varying concentrations was carried out by U/HPLC-UV on an Ascentis®Express 160 Å ZIC®-cHILIC, 2.7 µm SPP column (150 x 2.1 mm I.D.). The separation (**Figure 2**) showed good chromatographic performance, providing baseline separation with sharp and symmetrical peaks (**Table 3**).

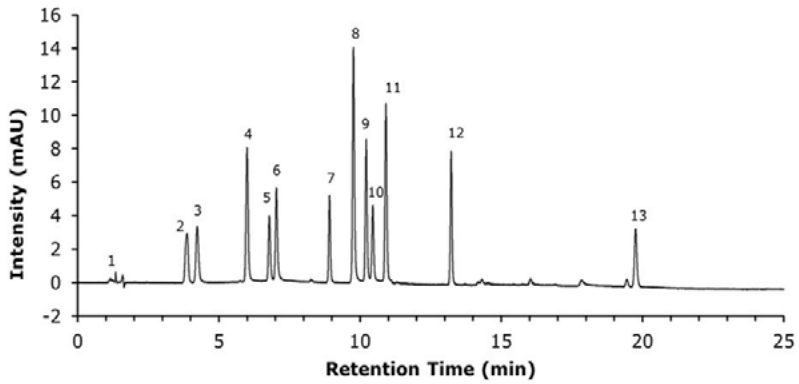

Figure 2. U/HPLC-UV chromatogram obtained for the standard mixture containing twelve nucleosides using an Ascentis® Express 160 Å ZIC®-cHILIC column (2.7 µm, 150 x 2.1 mm).

Table 3. Chromatographic data for the injection of the standard solution of twelve nucleosides at varying concentrations

Peak no.	Compound	Retention time (min)	Area (mAU*min)	Tailing factor (USP)	Resolution
1	t_0 void volume	1.3			
2	Ribothymidine	3.9	0.3568	0.93	
3	Uridine	4.2	0.3570	1.20	2.01
4	2-Thiocytidine	6.0	0.7027	1.12	11.89
5	2'-O-Methylcytidine	6.8	0.2983	1.07	6.49
6	Pseudouridine	7.0	0.4432	1.19	1.88
7	Inosine	8.9	0.3601	1.06	17.53
8	5-Methylcytidine	9.8	1.1033	1.16	8.47
9	Cytidine	10.2	0.6011	1.09	3.91
10	Guanosine	10.5	0.3117	1.10	2.09
11	3-Methylcytidine	10.9	0.7970	1.05	4.40
12	1-Methyladenosine	13.2	0.5492	1.05	21.81
13	7-Methylguanosine	19.6	0.3295	1.06	52.94

Conclusion

The developed simple HPLC-UV HILIC method enabled the determination of twelve nucleosides with baseline separation and good peak symmetry on an Ascentis® Express 160 Å ZIC®-cHILIC 2.7 µm SPP column (150 x 2.1 mm). The zwitterionic phosphorylcholine phase on the superficially porous particles (SPP) provided excellent retention and efficiency, enabling optimized HILIC separations.

References

1. Van Rompay AR, Johansson M, Karlsson A. Substrate specificity and phosphorylation of antiviral and anticancer nucleoside analogues by human deoxyribonucleoside kinases and ribonucleoside kinases. Pharmacology & therapeutics. 2003;100(2):119–139. https://doi.org/10.1016/j.pharmthera.2003.07.001.
2. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases. 2012. Nucleoside Analogues. [Updated 2020 May 1]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK548938/

Product List

Description	Cat. No.
HPLC Column	
Ascentis® Express 160 Å ZIC®-cHILIC, 2.7µm, 150x2.1 mm I.D.	**50504-U**
Reagents, Solvents, and Standard	
Acetonitrile gradient grade for liquid chromatography LiChrosolv®	**1.00030**
Water for chromatography (LC-MS grade) LiChrosolv®	**1.15333**
Tap fresh water from an appropriate Milli-Q® system	**ZIQ7000T0C**
Acetic acid, 96%, for analysis EMSURE®	**1.00062**
Ammonium acetate for analysis EMSURE® ACS,Reag. Ph Eur	**1.01116**
Ammonium acetate for HPLC LiChropur™	**5.43834**
Nucleoside Test Mix, in 1% sodium formate (varied conc.), analytical standard	**47310-U**

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