Strategies to Purify Carbohydrate-Based Compounds

with Flash Chromatography

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Chromatography Application Note AN75

Abstract

This technical review provides a summary of methods for purifying compounds containing carbohydrate moieties and detection/fractionation of these compounds. Chromatographic techniques include silica gel, C18, and ion pairing. Peak-cutting techniques include All-Wavelength Collection, UV detection, Evaporative Light Scattering Detection (ELSD), and monitoring fractions with TLC plates. The purification and detection of carbohydrate based compounds is strongly influenced by the groups attached to the carbohydrate groups. Synthesized compounds and natural products are examined with examples from the published literature using Combi*Flash*® systems or Redi*Sep*® columns.

Introduction

Carbohydrate compounds are synthesized for a variety of purposes. They are used as probes in life sciences such as mechanism studies. Carbohydrate compounds are studied as potential antibiotics and to modulate the solubility of small molecules.

Carbohydrate compounds are purified in a similar manner as other small molecules. Carbohydrates are often reacted with protecting groups that greatly modify their polar character and allow purification with silica gel.

Carbohydrates themselves are UV transparent, but groups attached to the carbohydrate generally absorb UV light allowing the use of UV detectors or *All-Wavelength Collection*.

All-Wavelength Collection (abbreviated as "AWC" in the tables herein) monitors the absorbance across a user-specified wavelength range. The program applies a signal processing algorithm that suppresses interference from solvent absorbance that causes drifting baselines that can "hide" peaks or interfere with fraction collection. Detecting all wavelengths ensures that no compound absorbing light in a single wavelength range is missed during collection. All-Wavelength detection converts the absorbance across a range of wavelengths into a single signal for the fraction collector while suppressing absorbance changes due to the eluting solvent.

Purification Strategies

Silica Gel Strategies

The first examples demonstrate the purification of protected carbohydrates. These compounds are relatively easy to purify on Redi*Sep* silica columns because the protecting groups mask the hydroxyl groups which greatly reduce the polarity of the compound and allow solubility in organic solvents. Normal phase solvents are easily removed from the purified compounds due to their low boiling points.

The first group of examples (Table 1) are compounds where the hydroxyl groups are protected, rendering the carbohydrates non-polar.

Compounds **1** and **2** were detected by TLC plates in the original research paper[1]. Compound 3 was purified using all three collection methods and served as a model to demonstrate that other carbohydrates with acetyl protecting groups could be detected in this fashion. Compound 4 was fractionated using TLC plates or ELSD.

Table 1: Purification Data: Non-polar carbohydrate compounds

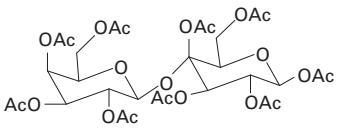
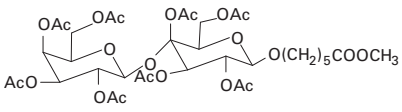
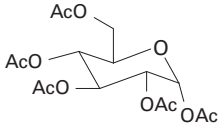
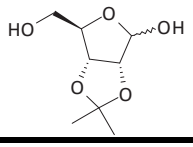
Id	Structure	Name	Solvent System	Column	Detection
1[1]		2,3,4,6-Tetra-O-acetyl-β-D-galactopyransyl-(1->4)-1,2,3,6-tetra-O-acetyl-β-D-glucopyranose	methylene chloride : methanol	silica gel	TLC, AWC[a], ELSD
2[1]		Methoxycarbonylpentyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyransyl-(1->4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside	hexane : acetone	silica gel	TLC, AWC[a], ELSD
3		Glucose pentaacetate	methylene chloride : methanol	silica gel	TLC, AWC[a], ELSD

Table 1: Purification Data: Non-polar carbohydrate compounds (Continued)

Id	Structure	Name	Solvent System	Column	Detection
4		2,3-O-Isopropylidene-D-Ribofuranose	hexane : ethyl acetate	silica gel	TLC, ELSD

a. AWC = All Wavelength Collection

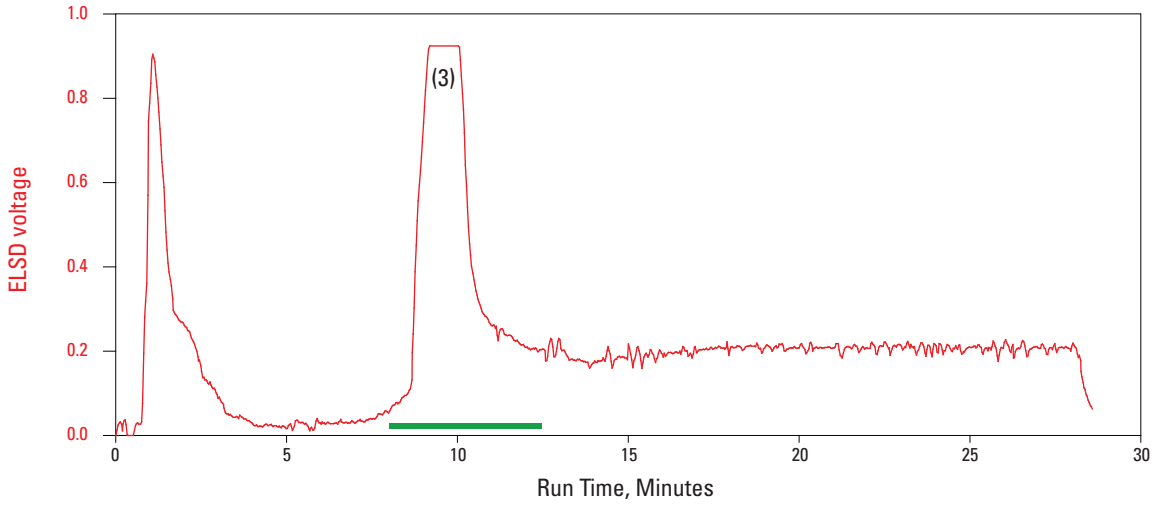


Figure 1: Glucose pentaacetate fractionated with ELSD

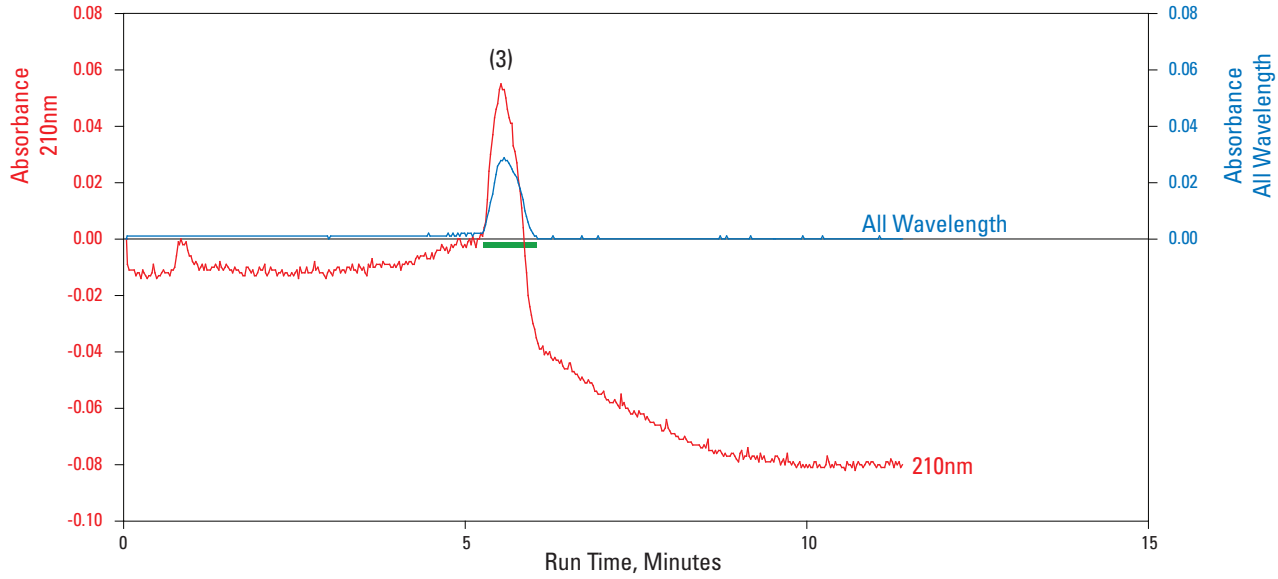


Figure 2: Glucose pentaacetate fractionated with All Wavelength Collection
Note that All-Wavelength Collection maintains a flat baseline allowing proper operation of the fraction collecting system.

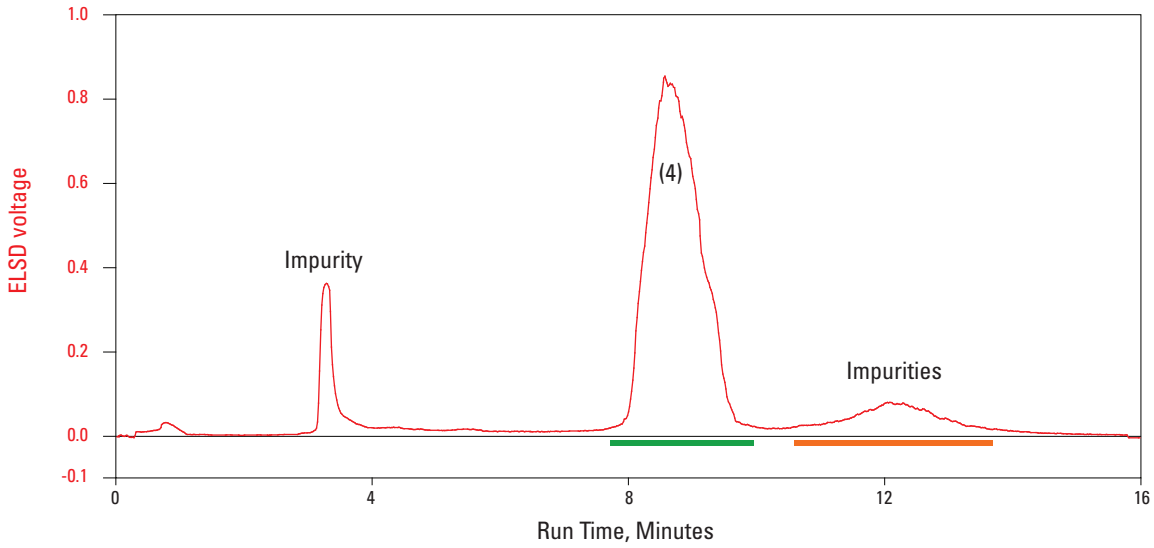
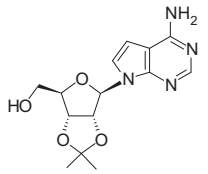
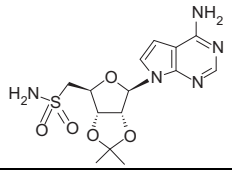
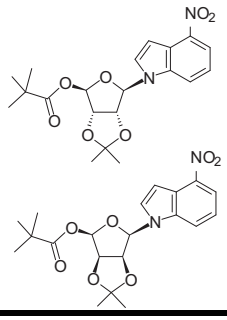


Figure 3: 2,3-O-Isopropylidene-D-Ribofuranose fractionated by ELSD

Table 2: Purification Data: Intermediate polarity carbohydrate compounds

Id	Structure	Name	Solvent System	Column	Detection
5[2]		7-Deaza-2′,3′-O-isopropylideneadenosine	99:1 ethyl acetate : methanol	silica gel	260 nm
6[2]		7-Deaza-2′,3′-O-isopropylidene-5′-O-(sulfamoyl)adenosine	200:1 ethyl acetate : methanol	silica gel	260 nm
7[2]		(1S,2R,3S,5S)-1-{1,2-Dihydroxy-1,2-O-isopropylidene-5-[(pivaloyloxy)methyl]cyclopent-3-yl}-4-nitroindole (7 upper) *and* (1R,2S,3S,5S)-1-{1,2-Dihydroxy-1,2-O-isopropylidene-5-[(pivaloyloxy)methyl]cyclopent-3-yl}-4-nitroindole (7 lower)	methylene chloride : methanol gradient	silica gel	260 nm

The acetonide protecting groups reduced the polarity of the compounds[2] in Table 2 to enable them to be purified on silica gel. Although not specifically mentioned in the paper, the adenine and nitroindole groups have UV absorbance at 260 nm and can be fractionated by UV detection on the Combi*Flash* system.

As more hydroxyl groups are available for interaction with the silica gel, the solvent polarity needs to be increased, or the silica requires deactivation. Compounds **8** and **9** in Table 3[1] require a very polar solvent system of methylene chloride and methanol modified with water or ammonium hydroxide for their purification. The water in both solvent systems deactivates the silica. Compound **9** required ammonium hydroxide due to the amine groups.

Table 3: Purification Data: Carbohydrate compounds with many exposed hydroxyl or other polar groups

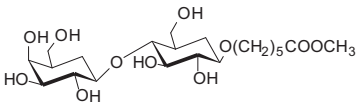
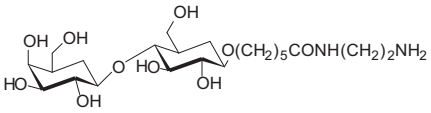
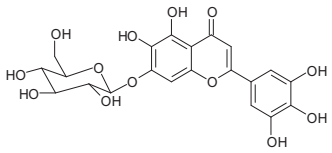
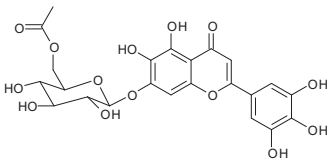
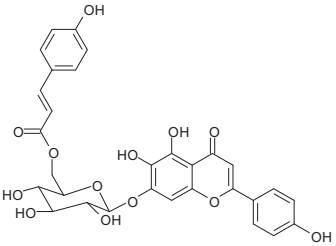
Id	Structure	Name	Solvent System	Column	Detection
8[1]		5-Methoxycarbonylpentyl β-D-galactopyranosyl-(1->4)-β-D-glucopyranoside	4 : 1 : 0.1 CH_2Cl_2 : MeOH : H_2O	silica gel	TLC
9[1]		(2-Aminoethylamido)carbonylpentyl β-D-galactopyranosyl-(1->4)-β-D-glucopyranoside	6 : 2 : 1 MeOH : CH_2Cl_2 : NH_4OH	silica gel	TLC

Table 4: Purification Data: Carbohydrates with large substituents

Id	Structure	Name	Solvent System	Column	Detection
10[3]		5,6,7,30,40,50-Hexahydroxyflavone-7-O-β-glucopyranoside	methanol : water	C18	283 nm
11[3]		5,6,7,3′,4′,5′-Hexahydroxyflavone-7-O-[6″-O-acetyl]-β-glucopyranoside	methanol : water	C18	283 nm
12[3]		5,6,7,4′-Tetrahydroxyflavone-7-O-(6″-O-[E]-coumaroyl)-β-glucopyranoside	methanol : water	C18	286 nm

C18 Reverse Phase Strategies

There are several reasons to use C18 flash chromatography to purify carbohydrate compounds. One reason is the increased polarity of the carbohydrate after removal of protecting groups requires solvent systems that are incompatible with silica gel. The solvents used for reverse phase chromatography also allow detection at wavelengths masked by the solvents used for silica gel. However, removal of the solvents is more difficult due to their higher boiling point. The use of water often requires lyophilization to provide a dry product.

Table 4 lists purifications[3] of carbohydrate containing natural products using Redi*Sep* C18 columns. In this case, all compounds showed absorbance at ~285 nm from the attached flavones groups allowing easy detection by the UV detector. The carbohydrate moiety is a small portion in these molecules. Thus the chromatography choices are more greatly influenced by the flavone substituents.

Table 5: Purification Data: Intermediate polarity on C18

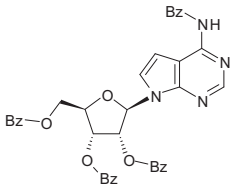
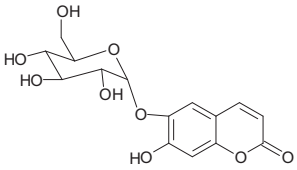
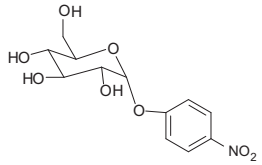
Id	Structure	Name	Solvent System	Column	Detection
13[4]		[(2R,3R,4R,5R)-5-(4-benzamidopyrrolo[2,3-d]pyrimidin-7-yl)-3,4-dibenzoyloxy-tetrahydrofuran-2-yl] methyl benzoate	90 : 10 methylene chloride : methanol	C18	260 nm

Table 6: Purification Data: Reversed phase on C18

Id	Structure	Name	Solvent System	Column	Detection
14		Esculin	water : acetonitrile	C18	214 nm
15		4-nitrophenyl-α-glucopyranoside	water : acetonitrile	C18	214 nm

Protected sugar compounds can be purified using reverse phase as well as silica gel (Table 5). In the purification of compound **13**[4], the protecting groups render the compound non-polar so methylene chloride was used as the solvent.

Reverse phase purification of carbohydrate compounds is also run with acetonitrile:water systems (Table 6).

Compounds **14** and **15** were purified using the standard method (5–95% acetonitrile) on a Combi*Flash* system. The Redi*Sep* Gold C18 column, with a smaller spherical particle, exhibits near-baseline purification compared to the standard Redi*Sep* C18 column without a large backpressure increase (Figure 4).

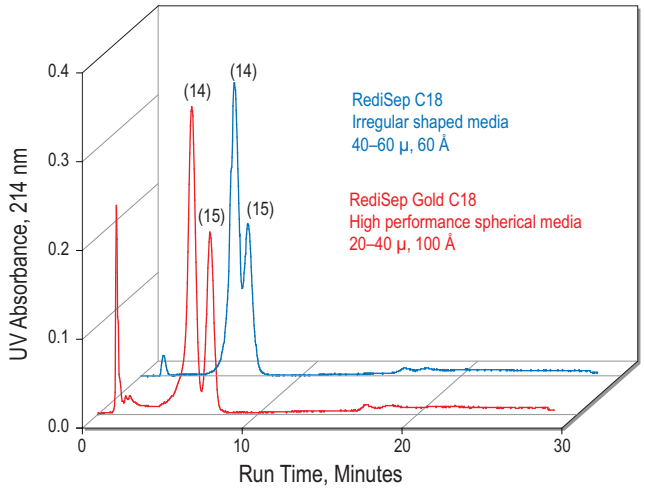
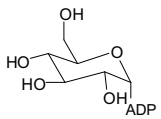
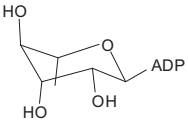


Figure 4: Purification of esculin monohydrate (14) and 4-nitrophenyl-α-glucopyranoside (15) on Redi*Sep* C18 (blue trace, back) and Redi*Sep* Gold (red trace, front).

Table 7: Purification Data: Polar carbohydrates purified with ion pairing

Id	Structure	Name	Solvent System	Column	Detection
16[5]		Adenosine diphosphate-α-D-glucose	A= 10 mM tributyl amine and 30 mM glacial acetic acid in water; B = MeOH **Gradient:** 2 CV 100:0 A:B 5CV linear to 70:30 A:B 8 CV 50:50 A:B	C18	215 & 254 nm
17[5]		Adenosine diphosphate-α-L-rhamnose	A = aqueous tributylammonium bicarbonate buffer (10 mM, pH 6); B = MeOH **Gradient:** 2CV 100:0 A:B 15 CV linear to 60:40 A:B 2CV 60:40 A:B	C18	215 & 254 nm

Carbohydrate containing compounds are purified by ion-pair chromatography. Examples include the nucleotides[5] in Table 7. The ADP group induces a high charge causing the need for ion pairing.

Ion pair reagents have several disadvantages for preparative chromatography. The ion pair reagent needs to be removed from the purified material. In addition, the Combi*Flash* system needs to be washed to remove the ion pair reagent and any associated buffers to avoid the precipitation of salts in the pumps. The pump seals may require more frequent maintenance due to the formation of crystals over time. However, ion pairing provides a useful means of purifying sugar nucleotides.

Conclusion

Flash chromatography is a useful technique for purifying carbohydrate compounds.

Protected carbohydrates can be purified using normal phase techniques on silica gel while carbohydrates with free hydroxyl groups are more easily purified on C18 columns. Ion pairing can be an effective technique for highly polar compounds, however the instrument needs to be cleaned well to remove the buffer.

The compounds are generally detected by UV absorbance although ELSD is employed if there are no UV absorbing groups. All-Wavelength Collection can be employed if the compound absorbs at the same wavelength as the solvent or if the UV absorbance is unknown.

References

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5 Timmonsa, S.C. and Jakeman, D.L. Stereospecific synthesis of sugar-1-phosphates and their conversion to sugar nucleotides *Carbohydrate Research*, **2008**, *343*, 865-874

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