RESTEK Pure Chromatography

The Evolving Landscape of THC Drug Testing, Delta-8 vs. Delta-9

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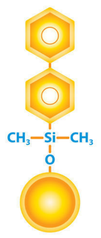
Introduction

In the US, different states have different laws for delta-9-THC products ranging from recreationally legal in some states to illegal in others. This opens the door for other isomers that exist in a legal gray area to be sold on the market for users to get psychoactive effects until the laws are introduced to regulate a specific isomer. A common isomer of delta-9-THC that also has psychoactive effects, is delta-8-THC. For drug testing, the carboxy-THC metabolite is historically the analyte used to determine cannabis usage. This compound has a long half-life and can be detected in urine or blood for several weeks in heavy consumers. This can pose a challenge when determining if a user is intoxicated at the time of incident or just a recent user. Today, labs are interested in the addition of the hydroxy metabolites, the intermediate between THC and the carboxylated metabolite. The intermediate is short lived but is useful in the determination of chronic usage and when determining if a user is under the influence. Several column chemistries were scouted, and a method was developed to include OH-8-THC and OH-9-THC as well as the parent compounds and the carboxy metabolites.

Column Chemistries

Column Description:



Stationary Phase Category:
Phenyl (L11)

Ligand Type:
Biphenyl

Particle:
2.7 µm superficially porous silica (SPP or "core-shell")

Pore Size:
90 Å

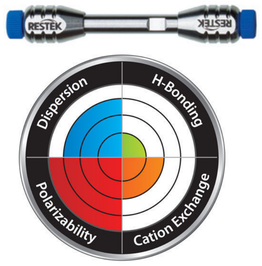
Surface Area:
150 m^2/g

Recommended Usage:
pH Range: 1.5–8.0
Maximum Temperature: 80 °C
Maximum Pressure: 600 bar (8,700 psi)

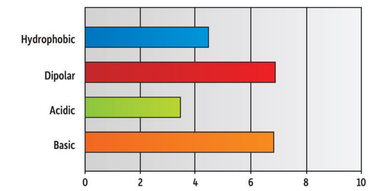
Properties:
- Increased retention for dipolar, unsaturated, or conjugated solutes.
- Enhanced selectivity when used with methanolic mobile phase.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.

Switch to a Biphenyl when:
- Limited selectivity is observed on a C18.
- You need to increase retention of hydrophilic aromatics.

Column Interaction Profile:



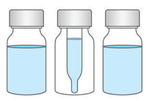
Solute Retention Profile:



Defining Solute Interactions:
- Polarizability
- Dispersion

Complementary Solute Interaction:
- Cation exchange

Target Analyte Structures:
- Aromatic
- Dipolar



Target Analyte Functionalities:
- Hydrophilic aromatics
- Strong dipoles
- Lewis acids
- Dipolar, unsaturated, or conjugated compounds
- Fused-ring compounds with electron withdrawing groups

Figure 1: Biphenyl Retention Properties.

Column Description:

Stationary Phase Category:
C18, octadecylsilane (L1)

Ligand Type:
Sterically protected C18

Particle:
2.7 µm superficially porous silica (SPP or "core-shell")

Pore Size:
90 Å

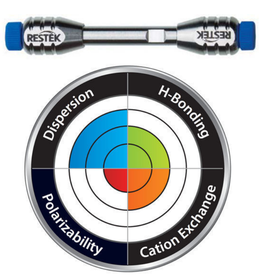
Surface Area:
150 m^2/g

Recommended Usage:
pH Range: 1.0–8.0
Maximum Temperature: 80 °C
Maximum Pressure: 600 bar (8,700 psi)

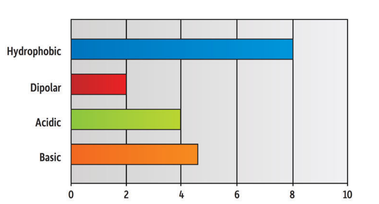
Properties:
- Well-balanced retention profile.
- Sterically protected to resist harsh, low-pH mobile phases.
- Ideal for use with sensitive detectors like mass spec.

Switch to an ARC-18 when:
- You are analyzing large, multiclass lists by LC-MS/MS.
- Strongly acidic (pH 1–3) mobile phases are required.

Column Interaction Profile:



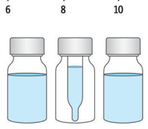
Solute Retention Profile:



Defining Solute Interaction:
- Dispersion

Complementary Solute Interactions:
- Hydrogen bonding
- Cation exchange

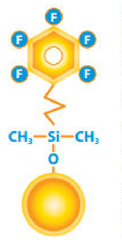
Target Analyte Structure:
- Hydrocarbons



Target Analyte Functionalities:
- Hydrophobic compounds
- Protonated bases

Figure 2: ARC18 Retention Properties.

Column Description:



Stationary Phase Category:
Pentafluorophenyl propyl (L43)

Ligand Type:
Fluorophenyl

Particle:
1.8 µm, 2.7 µm, or 5 µm superficially porous silica (SPP or "core-shell")

Pore Size:
90 Å

Surface Area:
125 m^2/g (1.8 µm),
130 m^2/g (2.7 µm),
or 100 m^2/g (5 µm

Recommended Usage:
pH Range: 2.0–8.0

Maximum Temperature: 80 °C

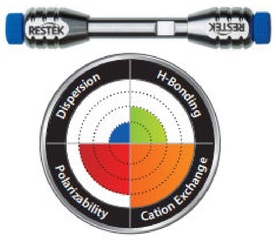
Maximum Pressure: 1,034 bar/15,000 psi* (1.8 µm),
600 bar/8,700 psi (2.7 µm); 400 bar/5,800 psi (5 µm)

** For maximum lifetime, recommended maximum pressure for 1.8 µm particles is 830 bar/12,000 psi.*

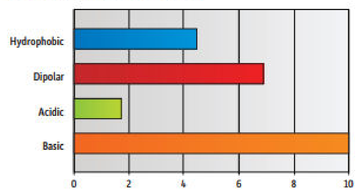
Properties:
- Capable of both reversed-phase and HILIC separations.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.
- Offers increased retention for charged bases.

Switch to a Raptor FluoroPhenyl LC column when:
- You observe limited retention and selectivity on a C18 for basic compounds.
- You need increased retention of hydrophilic compounds.

Column Interaction Profile:



Solute Retention Profile:



Defining Solute Interaction:
- Cation exchange

Complementary Solute Interactions:
- Polarizability
- Dispersion

Target Analyte Structures:
- Nitrogen-containing

Target Analyte Functionalities:
- Protonated amines
- Quaternary ammonium compounds
- Positively charged moieties
- Lewis bases

Figure 3: FluoroPhenyl Retention Properties.

Results

Column:	Raptor Biphenyl 100 x 2.1 mm, 2.7µm
MPA:	0.1% FA in H_2O
MPB:	0.1% FA in Methanol
Column Temp:	30°C
Sample:	100 ppb
Injection Volume:	1 µL
Flow Rate:	0.5 mL/min

Time (min)	%B
0.00	60
6.00	80
9.00	80
9.01	60
11.00	60

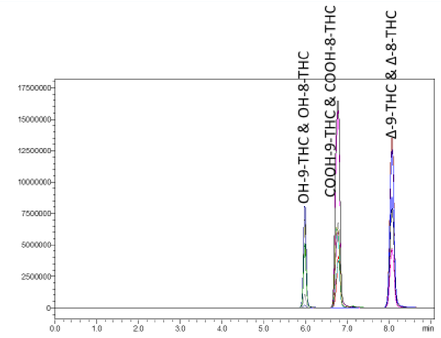


Figure 4: Isomers analyzed on Biphenyl column. No selectivity is observed on this phase for isomer resolution.

Column:	Raptor ARC18 100 x 2.1 mm, 2.7µm
MPA:	0.1% FA in H_2O
MPB:	0.1% FA in Methanol
Column Temp:	30°C
Sample:	100 ppb
Injection Volume:	1 µL
Flow Rate:	0.5 mL/min

Time (min)	%B
0.00	75
6.00	80
8.00	80
8.01	60
10.00	60

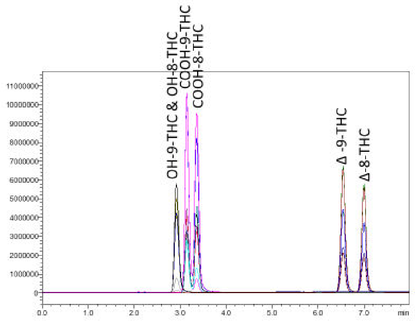


Figure 5: Isomers analyzed on ARC18 column. Delta-8-THC/Delta-9-THC are resolved well on this phase. COOH – isomers show good selectivity but the OH- isomers are coeluted.

Column:	Raptor ARC18 100 x 2.1 mm, 2.7µm
MPA:	0.1% FA in H_2O
MPB:	0.1% FA in Methanol
Column Temp:	30°C
Sample:	100 ppb
Injection Volume:	1 µL
Flow Rate:	0.5 mL/min

Time (min)	%B
0.00	60
6.00	80
8.00+	80

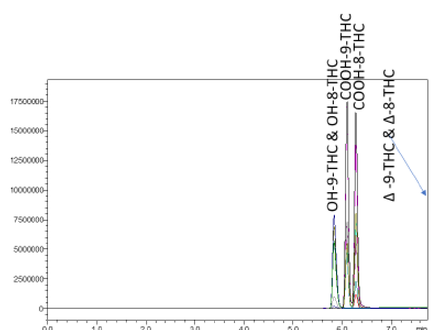


Figure 6: Isomers analyzed on ARC18 column. The starting conditions were weaker to try to help improve the resolution of OH-isomers, but they are still coeluting under these conditions. Delta-8-THC/Delta-9-THC haven't eluted yet and would likely result in a long run time.

Column:	Raptor Fluorophenyl 100 x 2.1 mm, 2.7µm
MPA:	0.1% FA in H_2O
MPB:	0.1% FA in Methanol
Column Temp:	30°C
Sample:	100 ppb
Injection Volume:	1 µL
Flow Rate:	0.6 mL/min

Time (min)	%B
0.00	55
6.00	55
7.00	65
10.00	65
10.01	55
12.00	55

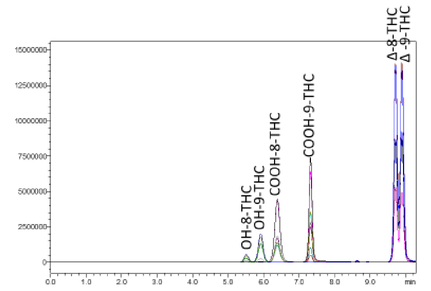


Figure 7: Isomers analyzed on FluoroPhenyl column. All compounds show selectivity on this phase and OH- and COOH- isomers are fully resolved.

Column:	Raptor Fluorophenyl 100 x 2.1 mm, 2.7µm
MPA:	0.1% FA in H_2O
MPB:	0.1% FA in Methanol
Column Temp:	40°C
Sample:	100 ppb
Injection Volume:	1 µL
Flow Rate:	0.6 mL/min

Time (min)	%B
0.00	55
6.00	55
6.50	60
13.00	60
13.01	55
15.00	55

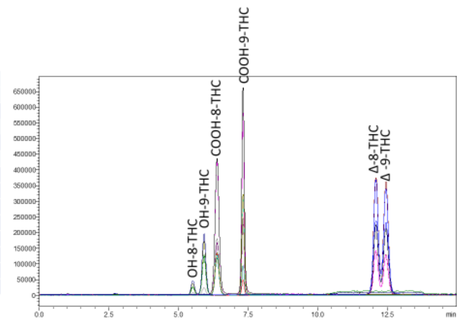


Figure 8: Conditions optimized on a FluoroPhenyl column. Delta-8-THC/Delta-9-THC starting to have better resolution but are not fully resolved.

Column:	Raptor Fluorophenyl 100 x 3 mm, 2.7µm
MPA:	0.1% FA in H_2O
MPB:	0.1% FA in Methanol
Column Temp:	40°C
Sample:	100 ppb
Injection Volume:	1 µL
Flow Rate:	0.9 mL/min

Time (min)	%B
0.00	55
6.50	60
15.00	60
15.01	55
17.00	55

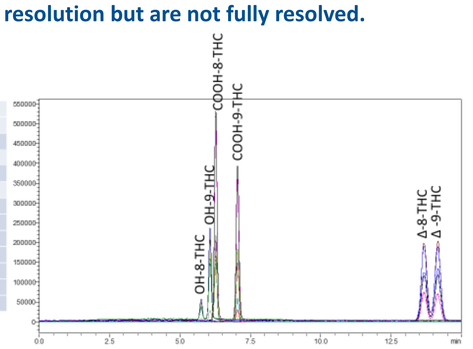


Figure 9: Conditions optimized on a FluoroPhenyl column. Delta-8-THC/Delta-9-THC resolution improved but method run time is 17 minutes.

Column:	Raptor Fluorophenyl 100 x 3 mm, 2.7µm
MPA:	0.1% FA in H_2O
MPB:	0.1% FA in Methanol
Column Temp:	30°C
Sample:	100 ppb
Injection Volume:	1 µL
Flow Rate:	0.9 mL/min

Time (min)	%B
0.00	63
12.00	63

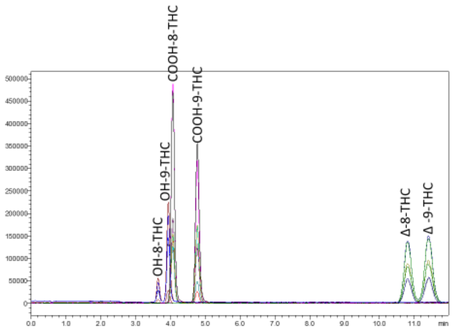


Figure 10: Conditions optimized on a FluoroPhenyl column. All isomers are resolved using isocratic conditions

Conclusions

Three columns were investigated in this study for the analysis of isomers of carboxy and hydroxy metabolites of delta-9-THC & delta-8-THC. The biphenyl ligand is good for the analysis of hydrophilic aromatics but does not possess the interactions for selectivity to resolve the isomer pairs resulting in three coelutions. The ARC18 ligand is good at resolving hydrophobic compounds based on dispersion mechanism. This column is good at resolving delta-8-THC and delta-9-THC and the carboxy metabolites but struggles to resolve the hydroxy metabolites. When the strength of the solvent is reduced to attempt to resolve the carboxy metabolites, the THC isomers become excessively retained, and the carboxy metabolites are still not resolved. The FluoroPhenyl ligand shows selectivity for all compounds under initial starting conditions. The gradient is changed to be a little shallower in order to resolve the THC isomers and full resolution is almost achieved, but the method conditions are resulting in an excessively long method. It was found that dropping the temperature from 40 °C to 30 °C and using isocratic conditions was able to resolve all compounds with a 12-minute cycle tine. In conclusion, the FluoroPhenyl column shows great selectivity for the target analytes and can resolve all three pairs of isomers with great resolution and retention.