Supelco®
Analytical Products

Application Note

UHPLC Analysis of Diclofenac in Gel using a Monolithic Silica Column

A comparison to a sub-2 µm fully porous particulate (FPP) hybrid silica column with UV & MS detection

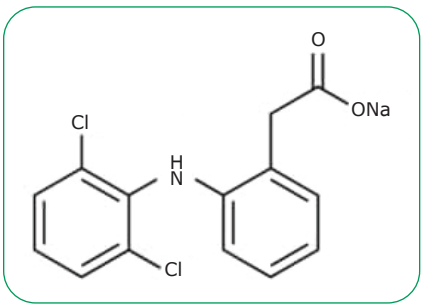
Introduction

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) used to treat pain and inflammatory diseases. Diclofenac was patented in 1965 and came into medical use in the United States in 1988. It is available as a sodium or potassium salt.

This report focuses on the analysis of diclofenac sodium salt in a gel under UHPLC conditions. Matrix-rich formulations such as gel, typically require extensive sample preparation in particular when using sub-2 µm UHPLC columns. In this work the sample preparation was kept simple as extensive sample preparation significantly contributes to time and cost spent per analysis.

A Chromolith® HighResolution RP-18 endcapped column 100 x 2 mm I.D. is compared with a fully porous particulate (FPP) 1.7 µm hybrid silica C18 column 100 x 2.1 mm I.D. from a competitor using HPLC-MS and HPLC-UV methods.

For both columns a stability test was performed with repeated sample overloading in order to stress both columns to a maximum.



Diclofenac sodium (2-[(2,6-Dichlorophenyl) amino]benzeneacetic acid sodium salt, Diclofenac sodium salt)

Diclofenac Sodium in Gel with MS Detection

Experimental HPLC-MS

Experimental Conditions	
Column 1:	Chromolith® HighResolution RP-18 endcapped 100 x 2 mm I.D.
Column 2:	FPP Competitor column (hybrid silica C18 1.7 µm, 100 x 2.1 mm I.D.)
Mobile phase:	[A] Water + 0.1% formic acid; [B] Methanol + 0.1% formic acid
Isocratic:	A/B 34/66 (v/v)
Flow Rate:	200 µL/min for 2.0 mm I.D. and 221 µL/min for 2.1 mm I.D
Temperature:	30 °C
Pressure Drop	86 bar (1247 psi) for Chromolith® 468 bar (6786 psi) for FPP 1.7 µm hybrid silica column
Injection volume:	1 µL
Detection:	Shimadzu LCMS 20; ESI+
Standard solution (50 µg/mL):	0.05% (w/v) of Diclofenac sodium were dissolved in methanol, then further diluted taking 1 volume of the resulting solution to 10 volumes using the mobile phase.
Sample preparation:	A quantity of 6 g of the gel containing 5 mg/g of Diclofenac sodium was shaken with 50 mL acetone for 10 minutes. The solution was filtered and the filtrate evaporated to dryness under a gentle nitrogen flow. The residue was dissolved in 100 mL of a mixture of 40 volumes of water and 60 volumes of methanol, 1 volume of this solution was diluted to 10 volumes with the mobile phase and then filtered through a glass fiber filter.

LC/MS Settings	
Detection:	ESI+
DL temperature:	250 °C
Heating block temperature:	200 °C
Interface voltage:	4.5 V
Detector voltage:	0.9 V
Dry gas:	1.5 L/h

Results HPLC-MS

Standard solutions

Chromolith® HighResolution

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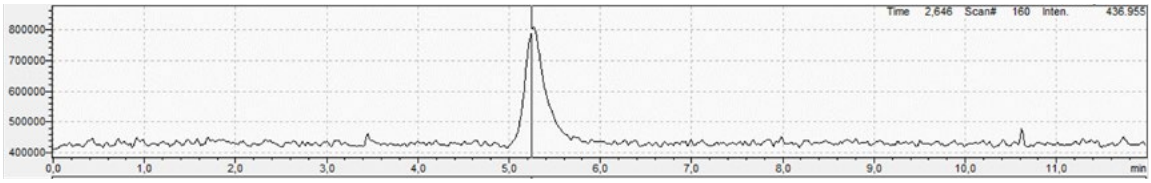
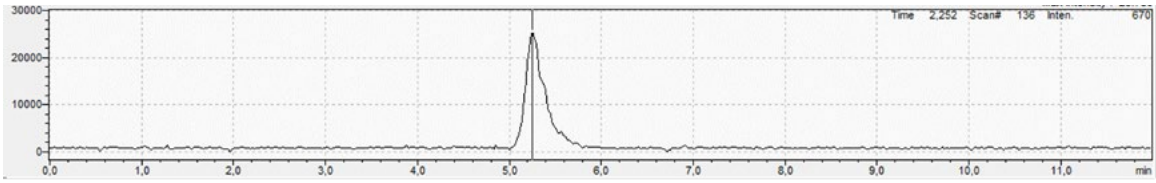


BPC 297 m/z



MS 297 m/z

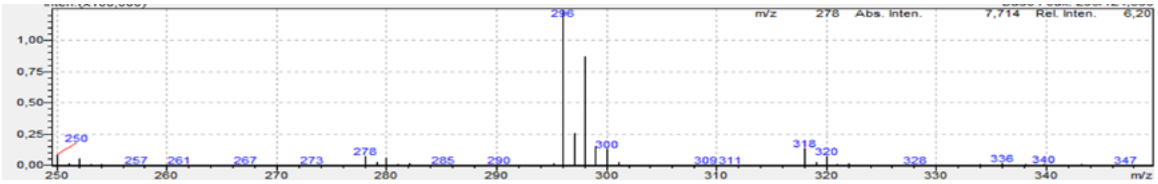


Figure 1. LC-MS Data on separation of Diclofenac sodium standard solution on Chromolith® HighResolution RP-18 endcapped 100 x 2 mm I.D. column.

Chromatographic Data - Standard solution (Figure 1.)

No.	Compound	Retention Time (min)	Quantitation Ion m/z	Area (Counts)
1	Diclofenac sodium	5.3	296	378161

FPP 1.7 µm Hybrid Silica Column

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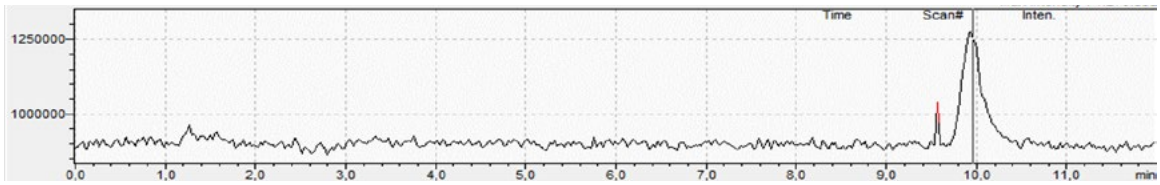

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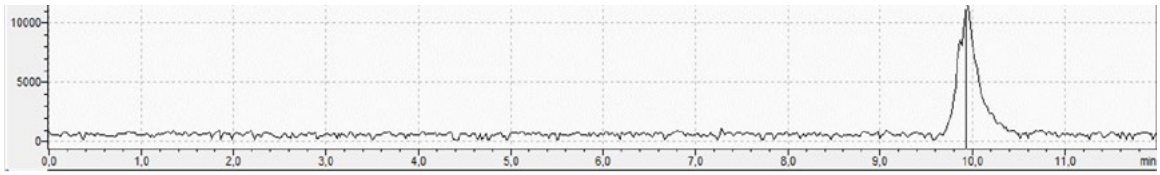

MS 297 m/z

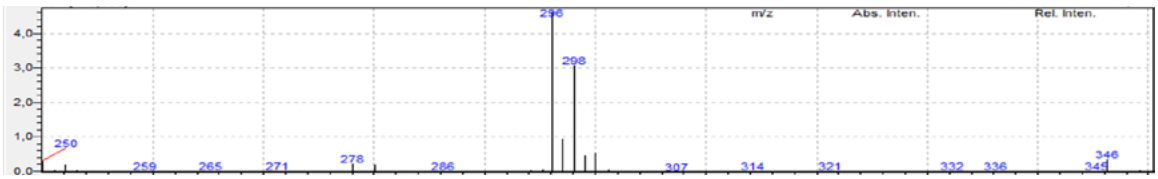

Figure 2. LC-MS Data on separation of Diclofenac sodium standard solution on FPP 1.7 µm hybrid silica C18 column 100 x 2.1 mm I.D.

Chromatographic Data - Standard solution (Figure 2.)

No.	Compound	Retention Time (min)	Quantitation Ion m/z	Area (Counts)
1	Diclofenac sodium	9.9	296	376893

Chromolith® HighResolution

1. Specificity: Inject standard solution of Diclofenac sodium and determine the retention time and content of desired analyte with MS detection

		Retention Time (min)	Quantitation Ion m/z	Area (Counts)
1	Diclofenac sodium	5.3	296	378161

2. Sample repeatability of Diclofenac sodium gel with MS detection

Sample	Area (Counts)
STD 1	265764
STD 2	264397
STD 3	265396
STD 4	265121
STD 5	264351
Mean	265006
Standard Deviation	620
(%) RSD	0.2

3. LOD & LOQ Diclofenac sodium with MS detection

Conc. (µg/mL)	Mean Area, n=3 (Counts)
5	42097
10	82103
25	207676
50	410393
75	614103
100	817291
STEYEX	1234.8
Slope	8161.7
LOD (µg/mL)	0.5
LOQ (µg/mL)	1.5

4. Linearity Diclofenac sodium with MS detection

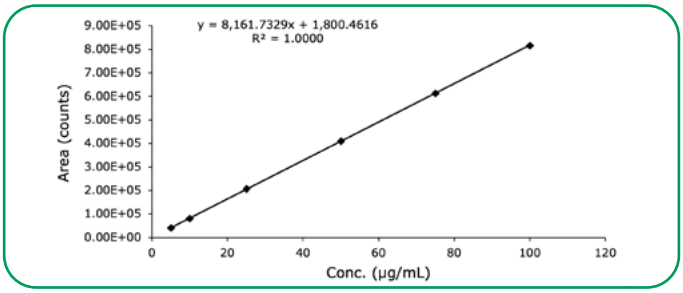

Figure 3. Linearity data with LC-MS on separation of Diclofenac sodium standard solution on Chromolith® HighResolution RP-18 endcapped 100 x 2 mm I.D. column.

FPP 1.7 µm Hybrid Silica Column

1. Specificity: Inject standard solution of Diclofenac sodium and determine the retention time and content of desired analyte with MS detection

		Retention Time (min)	Quantitation Ion m/z	Area (Counts)
1	Diclofenac sodium	9.9	296	376893

2. Sample repeatability of Diclofenac sodium gel with MS detection

Sample	Area (Counts)
STD 1	263385
STD 2	261452
STD 3	261853
STD 4	262274
STD 5	261761
Mean	262145
Standard Deviation	753
(%) RSD	0.3

3. LOD & LOQ Diclofenac sodium with MS detection

Conc. (µg/mL)	Mean Area, n=3 (Counts)
5	42874
10	83303
25	209917
50	410865
75	614276
100	815050
STEYEX	2102.7
Slope	8129.5
LOD (µg/mL)	0.9
LOQ (µg/mL)	2.6

4. Linearity Diclofenac sodium with MS detection

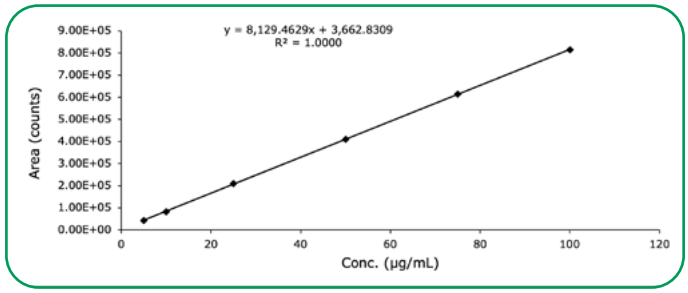

Figure 4. Linearity data with LC-MS on separation of Diclofenac sodium standard solution on FPP 1.7 µm hybrid silica C18 column 100 x 2.1 mm I.D.

Diclofenac gel sample solutions

Chromolith® HighResolution

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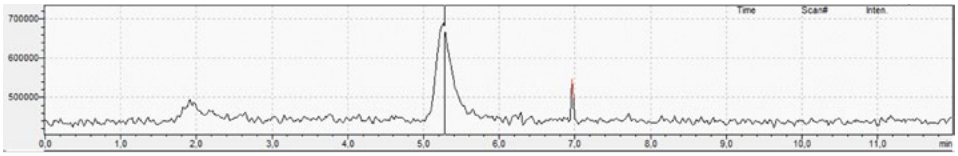
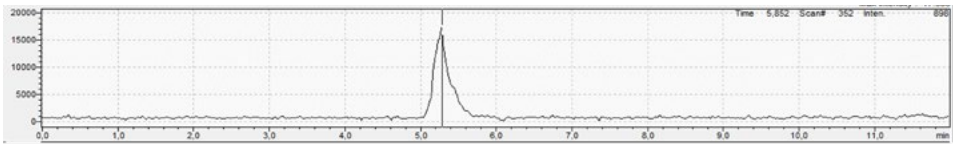


BPC 297 m/z



MS 297 m/z

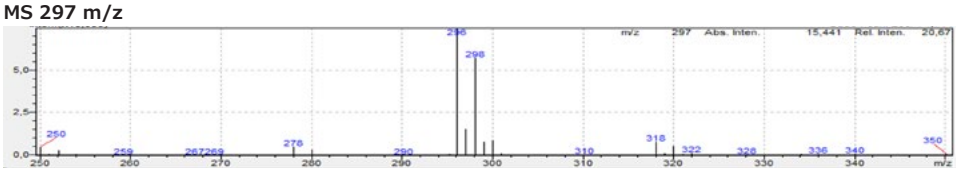


Figure 5. LC-MS Data on separation of Diclofenac gel sample solution on Chromolith® HighResolution RP-18 endcapped 100 x 2 mm I.D. column.

Chromatographic Data - Sample solution (Figure 5.)

No.	Compound	Retention Time (min)	Quantitation Ion m/z	Area (Counts)
1	Diclofenac sodium	5.3	296	264379

Calculation $\omega = \frac{c_{st}}{c_{Pr}}$

$\omega = \frac{31.8\ \mu g/mL}{0.006\ g/mL}$

ω_{Clo} = Concentration of Diclofenac in sample (mg/g)

c_{st} = Concentration calculated from calibration curve (µg/g)

c_{Pr} = Concentration of gel sample in final dilution (g/mL)

ω_{Clo} = 5.3 mg/g → 0.5% Diclofenac sodium in gel

FPP 1.7 µm Hybrid Silica Column

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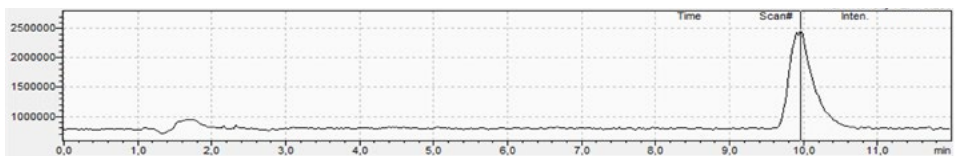

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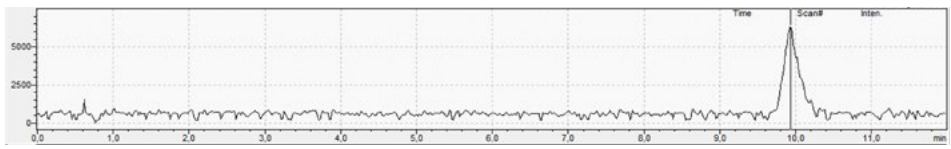

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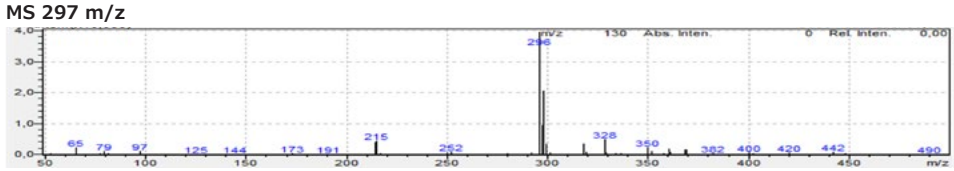

Figure 6. LC-MS Data on separation of Diclofenac gel sample solution on FPP 1.7 µm hybrid silica C18 column 100 x 2.1 mm I.D.

Chromatographic Data - Sample solution (Figure 6.)

No.	Compound	Retention Time (min)	Quantitation Ion m/z	Area (Counts)
1	Diclofenac sodium	9.9	296	261452

Calculation $\omega = \frac{c_{st}}{c_{Pr}}$

$\omega = \frac{31.1\ \mu g/mL}{0.006\ g/mL}$

ω_{Clo} = Concentration of Diclofenac in sample (mg/g)

c_{st} = Concentration calculated from calibration curve (µg/g)

c_{Pr} = Concentration of gel sample in final dilution (g/mL)

ω_{Clo} = 5.2 mg/g → 0.5% Diclofenac sodium in gel

Diclofenac Sodium in Gel with UV Detection

Experimental HPLC-UV

Experimental Conditions	
Column 1:	Chromolith® HighResolution RP-18 endcapped 100 x 2 mm I.D.
Column 2:	FPP Competitor column (hybrid silica C18 1.7 µm, 100 x 2.1 mm I.D.)
Mobile phase:	[A] Water + 0.1% formic acid; [B] Methanol + 0.1% formic acid
Isocratic:	A/B 34/66 (v/v)
Flow Rate:	200 µL/min for 2.0 mm I.D. and 221 µL/min for 2.1 mm I.D.
Temperature:	30 °C
Pressure Drop	86 bar (1247 psi) for Chromolith® 468 bar (6786 psi) for FPP 1.7 µm hybrid silica column
Injection volume:	1 µL
Detection:	PDA @ 254 nm
Standard solution (50 µg/mL):	0.05% (w/v) of Diclofenac sodium were dissolved in methanol, then further diluted taking 1 volume of the resulting solution to 10 volumes using the mobile phase.
Sample preparation:	A quantity of 6 g of the gel containing 5 mg/g of Diclofenac sodium was shaken with 50 mL acetone for 10 minutes. The solution was filtered and the filtrate evaporated to dryness under a gentle nitrogen flow. The residue was dissolved in 100 mL of a mixture of 40 volumes of water and 60 volumes of methanol, 1 volume of this solution was diluted to 10 volumes with the mobile phase and then filtered through a glass fiber filter.

Blank Runs

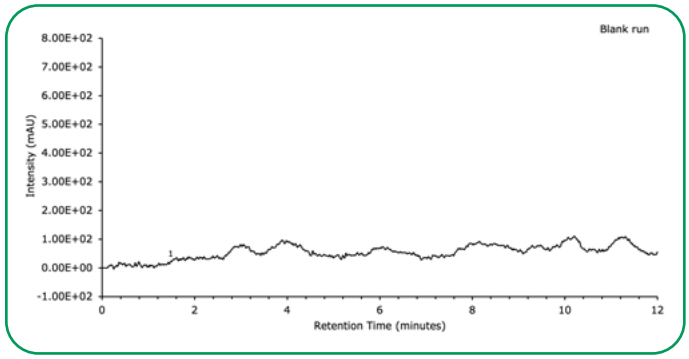

Figure 7. Blank run UV data on Chromolith® HighResolution RP-18 endcapped 100 x 2 mm I.D. column.

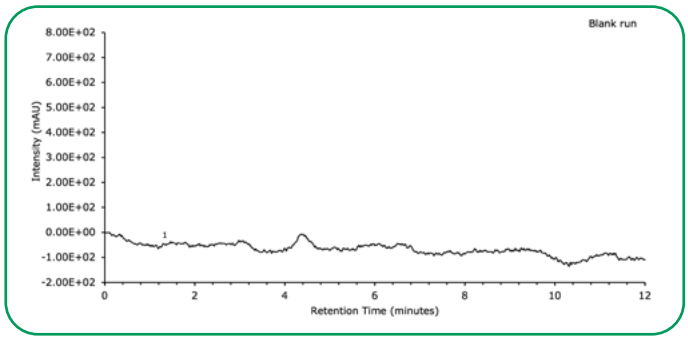

Figure 8. Blank run UV data on FPP 1.7 µm hybrid silica C18 column 100 x 2.1 mm I.D.

Results HPLC-UV

Standard solutions

Chromolith® HighResolution

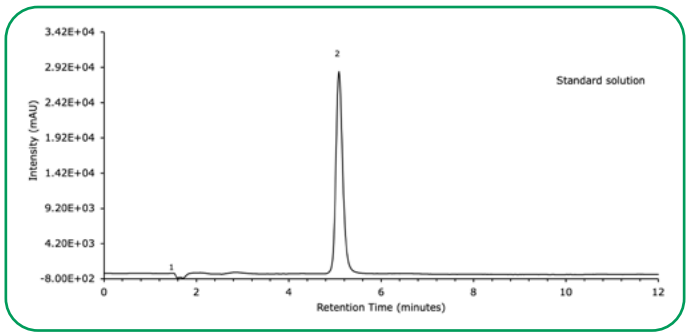

Figure 9. UV Data on separation of Diclofenac sodium standard solution on Chromolith® HighResolution RP-18 endcapped 100 x 2 mm I.D. column.

Chromatographic Data - Sample solution (Figure 9.)

No.	Compound	Retention Time (min)	S/N	Area (Counts)	Tailing Factor
1	t_0	1.5			
2	Diclofenac sodium	5.1	1060.2	296274	1.2

FPP 1.7 µm Hybrid Silica Column

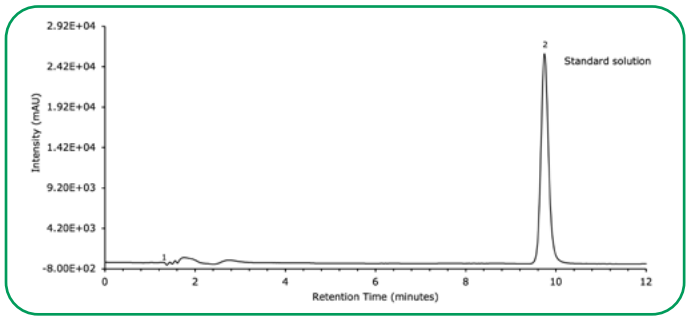

Figure 10. UV Data on separation of Diclofenac sodium standard solution on FPP 1.7 µm hybrid silica C18 column 100 x 2.1 mm I.D.

Chromatographic Data - Sample solution (Figure 10.)

No.	Compound	Retention Time (min)	S/N	Area (Counts)	Tailing Factor
1	t_0	1.4			
2	Diclofenac sodium	9.7	1558.2	308174	1.1

Chromolith® HighResolution

1. Specificity: Inject standard solution of Diclofenac sodium and determine the retention time and content of desired analyte with UV detection

		Retention Time (min)	S/N	Area (Counts)	Tailing Factor
1	Diclofenac sodium	5.1	1060.2	296274	1.2

2. Sample repeatability of Diclofenac sodium gel in area (counts) with UV detection

Sample	Area (Counts)
STD 1	167858
STD 2	167475
STD 3	167718
STD 4	167316
STD 5	167425
Mean	167558
Standard Deviation	223
(%) RSD	0.1

3. LOD & LOQ Diclofenac sodium with UV detection

Conc. (µg/mL)	Mean Area, n=3 (Counts)
5	24882
10	56262
25	146502
50	295264
75	439453
100	583866
STEYEX	2464.6
Slope	5880.3
LOD (µg/mL)	1.4
LOQ (µg/mL)	4.2

4. Linearity Diclofenac sodium with UV detection

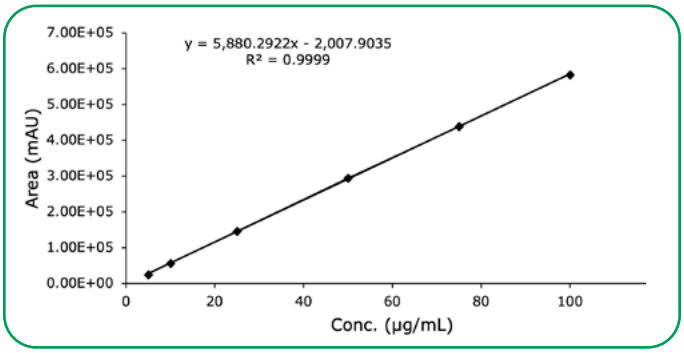

Figure 11. Linearity data with UV on separation of Diclofenac sodium standard solution on Chromolith® HighResolution RP-18 endcapped 100 x 2 mm I.D. column.

FPP 1.7 µm Hybrid Silica Column

1. Specificity: Inject standard solution of Diclofenac sodium and determine the retention time and content of desired analyte with UV detection

		Retention Time (min)	S/N	Area (Counts)	Tailing Factor
1	Diclofenac sodium	9.7	1558.2	308174	1.1

2. Sample repeatability of Diclofenac sodium gel in area (counts) with UV detection

Sample	Area (Counts)
STD 1	174899
STD 2	175642
STD 3	174113
STD 4	174814
STD 5	175721
Mean	175038
Standard Deviation	663
(%) RSD	0.4

3. LOD & LOQ Diclofenac sodium with UV detection

Conc. (µg/mL)	Mean Area, n=3 (Counts)
5	24986
10	56240
25	151867
50	308038
75	462262
100	612579
STEYEX	2438.2
Slope	6197.5
LOD (µg/mL)	1.3
LOQ (µg/mL)	3.9

4. Linearity Diclofenac sodium with UV detection

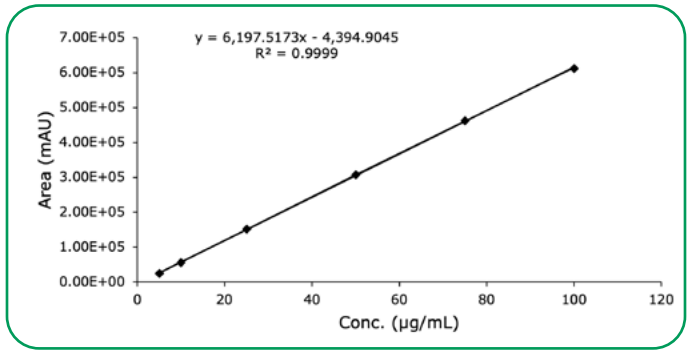

Figure 12. Linearity data with UV on separation of Diclofenac sodium standard solution on FPP 1.7 µm hybrid silica column from competitor 100 x 2.1 mm I.D.

Diclofenac gel sample solutions

Chromolith® HighResolution

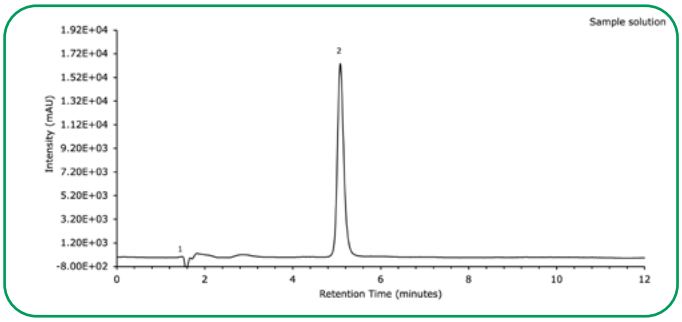

Figure 13. UV Data on separation of Diclofenac gel sample solution on Chromolith® High Resolution RP-18 endcapped 100 x 2 mm I.D. column.

Chromatographic Data - Sample solution (Figure 13.)

No.	Compound	Retention Time (min)	S/N	Area (Counts)	Tailing Factor
1	t_0	1.5			
2	Diclofenac sodium	5.1	916.8	167518	1.2

Calculation $\omega = \frac{C_{st}}{C_{Pr}}$

$$\omega = \frac{28.6\ \mu g/mL}{0.006\ g/mL}$$

ω_{Clo} = 4.8 mg/g → 0.5% Diclofenac sodium in gel

ω_{Clo} = Concentration of Diclofenac in sample (mg/g)

c_{st} = Concentration calculated from calibration curve (µg/g)

c_{Pr} = Concentration of gel sample in final dilution (g/mL)

FPP 1.7 µm Hybrid Silica Column

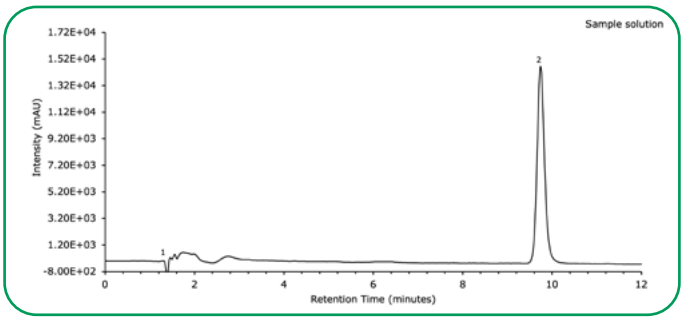

Figure 14. UV Data on separation of Diclofenac gel sample solution on FPP 1.7 µm hybrid silica C18 column 100 x 2.1 mm I.D.

Chromatographic Data - Sample solution (Figure 14.)

No.	Compound	Retention Time (min)	S/N	Area (Counts)	Tailing Factor
1	t_0	1.4			
2	Diclofenac sodium	9.7	722.41	174618	1.1

Calculation $\omega = \frac{C_{st}}{C_{Pr}}$

$$\omega = \frac{28.7\ \mu g/mL}{0.006\ g/mL}$$

ω_{Clo} = 4.8 mg/g → 0.5% Diclofenac sodium in gel

ω_{Clo} = Concentration of Diclofenac in sample (mg/g)

c_{st} = Concentration calculated from calibration curve (µg/g)

c_{Pr} = Concentration of gel sample in final dilution (g/mL)

Stability Test – Diclofenac Gel Sample Solution

The stability test was performed to demonstrate the resistance to column clogging rep. matrix-tolerance and stability of a monolithic silica column in comparison to a 1.7 µm fully porous particulate (FPP) column which is typically used in UHPLC applications. Both columns have been treated the same way with frequent overloading to stress both columns to a maximum. Both columns were tested with three 1 µL injections after stressing the columns 10 times with 10 µL injections. No pre-columns were used for this test.

Experimental Conditions for Stability Test	
Column 1:	Chromolith® HighResolution RP-18 endcapped 100 x 2 mm I.D.
Column 2:	FPP Competitor column (hybrid silica C18 1.7 µm, 100 x 2.1 mm I.D.)
Mobile phase:	[A] Water; [B] Methanol
Isocratic:	A/B 35/65 (v/v)
Flow Rate:	200 µL/min for Chromolith®; 221 µL/min for FPP 1.7 µm column (alignment of linear flow conditions - 2.0 mm I.D. for monolithic vs. 2.1 mm I.D. for FPP)
Temperature:	Ambient
Injection volume:	3 times 1 µL; 10 times 10 µL; 3 times 1 µL; 10 times 10 µL etc.
Detection:	UV @254 nm
Sample preparation:	A quantity of 6 g of the gel containing 5 mg/g of Diclofenac sodium was shaken with 50 mL acetone for 10 minutes. The solution was filtered and the filtrate evaporated to dryness under a gentle nitrogen flow. The residue was dissolved in 100 mL of a mixture of 40 volumes of water and 60 volumes of methanol, 1 volume of this solution was diluted to 10 volumes with the mobile phase and then filtered through a glass fiber filter.

Column backpressure / Injection volume

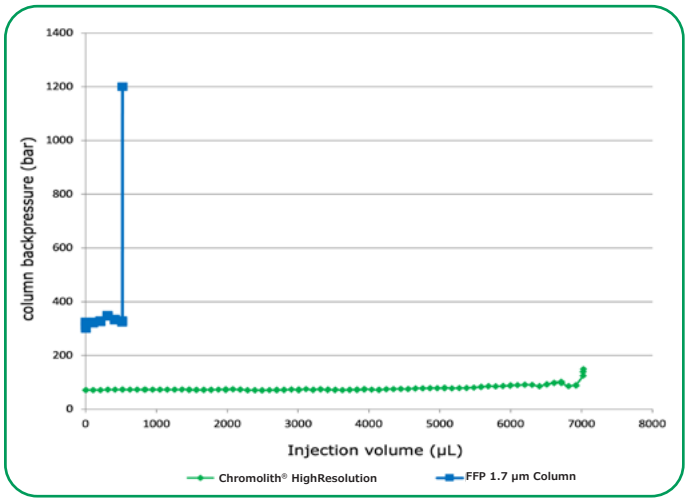

Figure 15. Stability test data on Chromolith® HighResolution RP-18 endcapped 100 x 2 mm I.D. column and FPP 1.7 µm hybrid silica column from competitor 100 x 2.1 mm I.D. Data points with 1 µL injections were used for measurement.

The 1.7 µm FPP column clogged after 520 injection volumes (520 µL sample).
The monolithic Chromolith® HighResolution column started to clog after 7000 injection volumes (7000 µL sample).

Conclusion

It was shown, that a Chromolith® HighResolution RP-18e 100 x 2 mm I.D. column can be utilized for the determination of Diclofenac sodium in a gel with a typical UHPLC instrument. The resulting LOD and LOQ values are comparable to the values of the fully porous particulate (FPP) 1.7 µm hybrid silica C18 column 100 x 2.1 mm I.D. with UV and MS detection.

The limit of detection (LOD) with UV detection was 1.4 µg/mL and the limit of quantitation (LOQ) with UV detection was 4.2 µg/mL for the Chromolith® HighResolution RP-18e 100 x 2 mm column.

By using MS detection, the sensitivity was improved to 0.5 µg/mL (LOD) and 1.5 µg/mL (LOQ).

Due to its large macroporous structure, the Chromolith® monolithic silica column shows high permeability with significant lower column backpressure in comparison to the FPP 1.7 µm column. The bi-modal pore structure of monolithic columns also results in short analysis times.

In addition, monolithic silica columns show an outstanding robustness and high matrix-tolerance. In a stability test, stressing the columns to the limit, the monolithic column was able to allow for 7000 µL of gel sample to be injected. Under same conditions the fully porous particulate (FPP) 1.7 µm hybrid silica C18 column 100 x 2.1 mm I.D. clogged after 520 µL total injected sample volume. As a consequence, the 1.7 µm FPP column will require an extended sample preparation in order to ensure a longer column lifetime. This results in significantly higher cost per sample and additional time spend per analysis.

Featured Products

Description	Cat. No.
Chromolith® HighResolution RP-18 endcapped 100 x 2 mm I.D.	**1.52322**
FPP Hybrid Silica RP-18 1.7 µm, 100 x 2.1 mm I.D. column	**competitor**
Methanol hypergrade for LC-MS LiChrosolv®	**1.06035**
Water for chromatography (LC-MS grade) LiChrosolv®	**1.15333**
or tap fresh from an appropriate Milli-Q® IQ 7000 Ultrapure Water System	**ZIQ7000T0C**
or Water with 0,1 % (v/v) formic acid, hypergrade for LC-MS LiChrosolv®	**1.59013**
Formic acid 98-100 %, for LC-MS LiChropur™	**5.33002**
Acetone for analysis EMSURE® ACS,ISO,Reag. Ph Eu	**1.00014**
Diclofenac Gel, 5 mg/g	**local Pharmacy**
Diclofenac sodium salt, Pharmaceutical Secondary Standard; Certified Reference Material	**PHR1144**

Learn more about Chromolith® monolithic silica columns at **SigmaAldrich.com/chromolith**

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