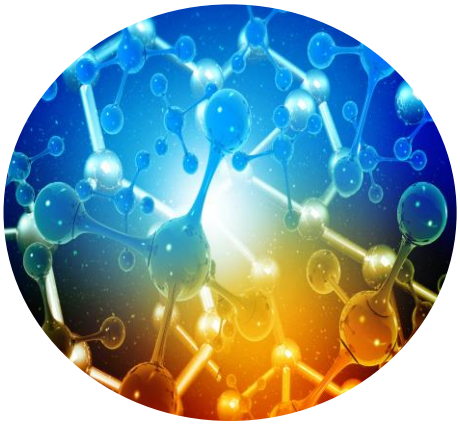

LC-MS/MS Separation of Diclofenac and its Metabolite 4'-Hydroxydiclofenac using Luna® Omega Polar C18 and Kinetex® Biphenyl

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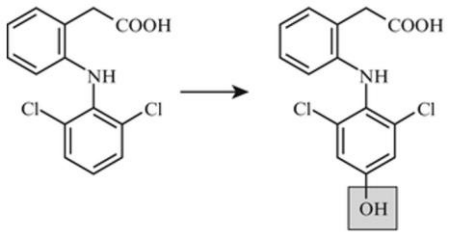
Overview

Diclofenac is a phenylacetic acid derivative and non-steroidal anti-inflammatory drug. Diclofenac is completely absorbed from the GI tract but undergoes significant metabolism. The primary and prevalent metabolite is 4'-Hydroxydiclofenac, which has significantly lower pharmacologic activity than Diclofenac. This drives the interest in developing a reversed phase HPLC method able to separate and analyze Diclofenac and its metabolites successfully and in a short amount of time.

This application illustrates the rapid separation of Diclofenac and 4'-Hydroxydiclofenac using Luna Omega Polar C18 and Kinetex Biphenyl.

In this application note we utilized these different stationary phases to highlight the selectivity differences and the benefits to be realized in selecting an appropriate stationary phase chemistry for the separation challenge.

Under the same mobile phase conditions, we can see that the Kinetex Biphenyl provided increased retention for the Diclofenac and its hydroxy metabolite through a mixture of pi-pi and polar interactions (**Figure 1b**); and the higher efficiency provided by the core-shell particle morphology also results in narrower peaks and increased MS sensitivity.



Diclofenac 4'-Hydroxydiclofenac

LC-UV Conditions

Columns: Luna Omega 1.6 µm Polar C18 (00B-4748-AN)
Kinetex 2.6 µm Biphenyl (00B-4622-AN)
Dimensions: 50 x 2.1 mm
Mobile Phase: A = 0.1 % Formic Acid in Water
B = 0.1 % Formic Acid in Acetonitrile
Injection: 1 µL
Gradient:

Time (min)	%B
0	5
4	95
5	95
5.1	5
7	5

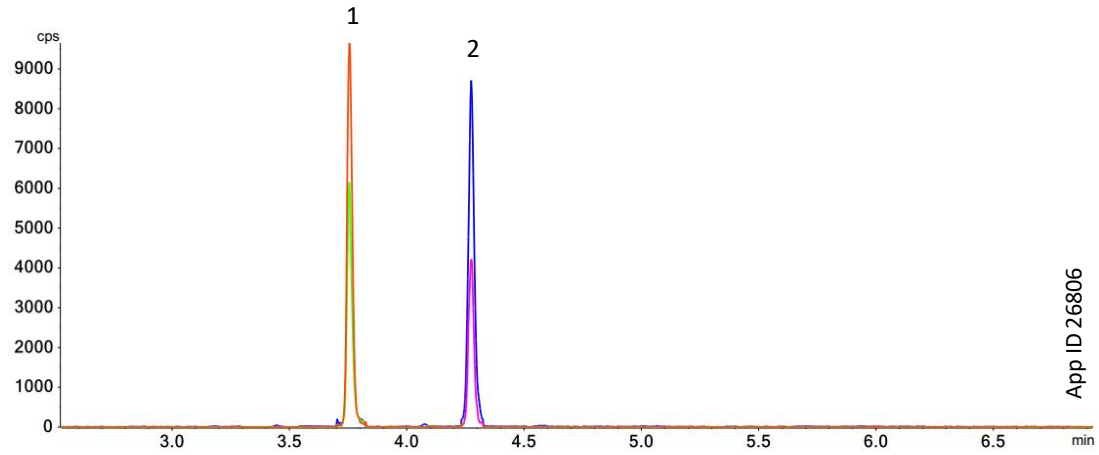
Pressure (bar): 400 (Luna Omega 1.6 µm Polar C18)
300 (Kinetex 2.6 µm Biphenyl)
Flow Rate: 0.4 mL/min
Column Temp.: 40 °C
Detection: MS/MS
Detector: SCIEX® 4500
System: Agilent® 1260 Infinity UHPLC

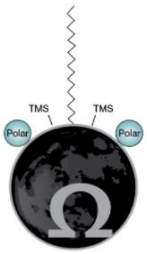
MRM Transitions

Peak #	Analyte	Q1	Q3	CXP
1	Diclofenac-1	294	250	-10
1	Diclofenac-2	294	214	-8
2	4-Hydroxydiclofenac-1	310	230	-13
2	4-Hydroxydiclofenac-2	310	194	-13

Figure 1. Results and Observations

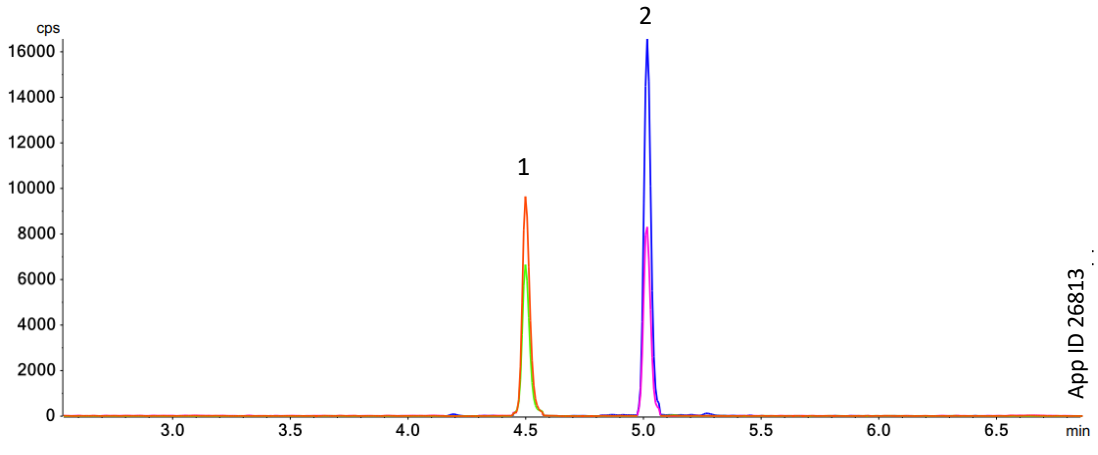
(a) Luna® Omega 1.6 µm Polar C18

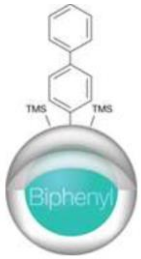




The Luna Omega Polar C18 incorporates a polar surface treatment to offer increased retention for polar analytes, in addition to hydrophobic retention via the C18 bonded phase.

(b) Kinetex® 2.6 µm Biphenyl





The Kinetex Biphenyl stationary phase offers multiple potential interactions to increase retention for polar aromatic compounds. These include pi-pi interactions and increased hydrogen bond accepting capacity.

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