Q: What is a CofA?

A CofA (Certificate of Analysis) contains information for the batch (or lot) of chromatographic media (or sorbent) that is packed into an HPLC column.

Each batch of media is manufactured by Phenomenex as a distinct entity (batch), very similar to how an active pharmaceutical ingredient (API) might be. To maintain batch traceability, each batch undergoes extensive testing both during and after the manufacturing process. These tests ensure that the physical and chemical properties of the batch of media conform to the manufacturing specifications set forth during the development and technology transfer process for that material.

The range of tests include both destructive (e.g., % Carbon) and non-destructive (e.g., particle size, surface area) testing, as well as chromatographic testing. The chromatographic testing is typically carried out by running a series of "chromatographic use" tests under a range of conditions typical to those that a customer may use.

The batch of HPLC media is packed into columns of standard size, often 150 x 4.6 mm for 3 and 5 µm materials, or 50 x 4.6 mm for 2.6 µm core-shell materials, then tested under different chromatographic conditions. These conditions range from acidic pH to neutral (higher pH for media that is stable at higher pH), isocratic and gradient mobile phase conditions, and with various types of chemical compounds as analytes. Media containing different bonded phases will be tested under different conditions and analytes to thoroughly probe the surface chemistry of the chromatographic sorbent.

Q: What information does the CofA contain?

The CofA contains the commercial description of the media (e.g., Kinetex 2.6 µm C18) and the batch number).

The CofA also contains a summary of the:

- physical properties of the silica (particle size, particle size distribution, and pore size)
- chemical properties (surface area and, for some media, % carbon)
- chromatographic test results, with the specification range where appropriate

It should be noted that most media undergo multiple chromatographic tests, but space limits the amount of chromatographic test results that can shared on the CofA to 1 or 2. Listed below are the most common tests together with an explanation as to why they are run.

Q: What does this tell me about the column associated with the CofA?

This is essentially a batch record for the batch of media packed into the HPLC column you have received.

Q: Does PHX provide a CofA for each column I purchase?

No, we typically only provide CofA for the following HPLC columns:

- Kinetex™, Luna™ Omega, Luna, Gemini™, bioZen™, Aeris™, Jupiter™ and Yarra™

Q: What should I do with the CofA?

It is good practice to retain the CofA for your records. In the event of a problem, one can revert to the CofA and compare to other CofA for the same media to jump-start the troubleshooting process.

Description of CofA tests

Phenomenex QC Mix 870

This is a Phenomenex variation on the NIST SRM 870 (view here) test mixture that is used to help characterize every HPLC reversed phase media Phenomenex manufactures.

The isocratic HPLC conditions are the same as outlined in NIST SRM 870 (view here).

Uracil is used as an indicator of the void volume (unretained volume) in an LC column.

Toluene and Ethyl benzene are hydrophobic test probes; the alpha value ($\alpha = k'_{\text{Ethyl benzene}} / k'_{\text{Toluene}}$) is a measure of selectivity between these two neutral compounds.

Amitriptyline is a basic pharmaceutical drug (tricyclic antidepressant) that is commonly used to probe silanol activity by measuring peak tailing. Therefore, elution of basic compounds with symmetrical peak shape is an indication of column inertness. Under neutral pH 7 mobile phase conditions, the amitriptyline is positively charged (pK_a 9.4), and any unbonded silanols on the silica surface will be deprotonated and negatively charged, providing ideal conditions for ion exchange interactions resulting in peak tailing for basic analytes.

ABN7 (Acid, Base, Neutral 7-component mix)

This test mixture is run under gradient conditions at low pH (with mobile-phase solutions of 0.1% (v/v) formic acid).

Formic acid is used to maintain the pH of the mobile phase; at pH <3, the unbonded silanol groups will remain protonated with neutral charge, thereby minimizing any secondary interactions with the positively charged basic analytes. Formic acid is also a better alternative to phosphate, for which the bulky nature of the phosphate anion mitigates acid/base interactions at the surface of the silica.

The test mixture contains acidic, basic, and neutral probes.

Acidic: 3-Methyl-4-Nitrobenzoic acid.

Basic: Chlorpheniramine, Nortriptyline, and Pindolol.

Neutral: 2-Hydroxy-5-Methylbenzaldehyde, Hexanophenone.

Uracil = void volume marker (unretained compound).

Basic Drugs

This test mixture is run under isocratic conditions at alkaline pH (adjusted to pH 9.75 with 20 mM sodium phosphate (Na_2HPO_4).

This test is limited to our HPLC media stable at high pH (Kinetex EVO C18 and Gemini).

The test mixture contains 4 basic drug compounds:

1. Thiourea

2. Pindolol

3. Metoprolol

4. Propranolol

Q: The column dimensions on the CofA are different than those of my column. Do I have the correct CofA?

Yes. The batch of media in your column was simply tested using a column of a different dimension.

Q: Can I get the CofA test mixes anywhere?

No. Only the CQA test mixes are available, as tests with these would give you data more closely related to the quality of your specific column.

Q: What if the QC testing specifications change?

In those very rare cases that there is any change to any specification, you will be notified ahead of time through a letter emailed to you.

Certificate of Analysis

PHASE: Kinetex 2.6µm C18

BATCH NO.: 5569-0363

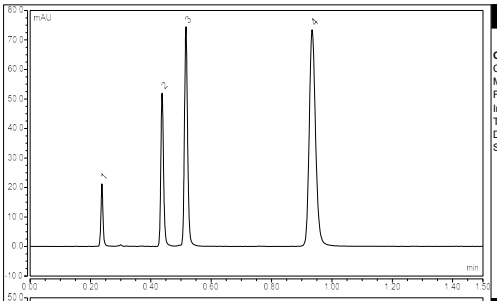
SILICA ANALYSIS

PARAMETER:	SPECIFICATION:	BATCH RESULT:
Pore Diameter(Å)	82 - 102	91
Particle Size (µm)	2.4 - 2.6	2.4
Particle Size Distribution: dp90/10	<= 1.2	1.1

BONDED PHASE ANALYSIS

PARAMETER:	BATCH RESULT:
Surface Coverage(µmole/m2)	2.9

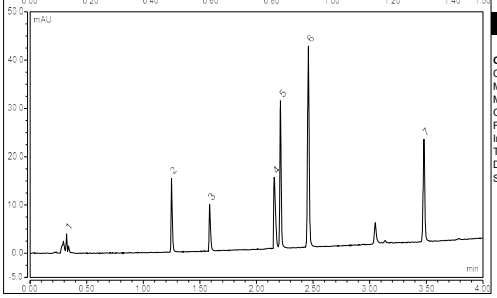
CHROMATOGRAPHIC RESULTS



PHENOMENEX QC MIX 870

CONDITIONS:
Column dimensions: 50 x 4.6 mm
Mobile Phase: 20 mM KH2PO4 pH7.0/ MeOH (20:80)
Flow Rate: 2.0 mL/min
Injection Volume: 1µL
Temperature: 40 °C
Detector: UV @ 254 nm
Sample:
1. uracil
2. toluene
3. ethyl benzene
4. amitriptyline

PARAMETER:	SPECIFICATION:	BATCH RESULT:
Asym4	<= 1.65	1.20
a2,3	1.35 - 1.43	1.40



ABN7

CONDITIONS:
Column Dimensions: 50 x 4.6 mm
Mobile Phase A= 0.1% Formic Acid in H2O
Mobile Phase B= 0.1% Formic Acid in ACN
Gradient: 5-95%B in 4.00 min
Flow Rate: 1.85 mL/min
Injection Volume: 0.4 µL
Temperature: 30 °C
Detector: UV @ 254 nm
Sample:
1. uracil
2. pindolol
3. chlorpheniramine
4. nortriptyline
5. 3-Methyl, 4-Nitrobenzoic acid
6. 2-hydroxy, 5-methylbenzaldehyde
7. hexanophenone

PARAMETER:	SPECIFICATION:	BATCH RESULT:
a3,7	2.35 - 2.65	2.50
a5,7	1.61 - 1.69	1.67

QUALITY ASSURANCE

Approved By: _____ **Date Approved:** December 19, 2023

PHASE: Kinetex 2.6 µm EVO C18

BATCH NO.: 5725-0093

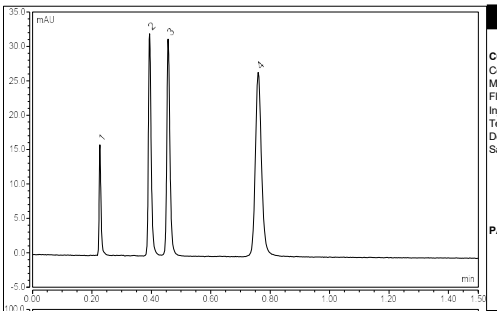
SILICA ANALYSIS

PARAMETER:	SPECIFICATION	BATCH RESULT
Pore Diameter (A)	82 - 102	97
Particle size (µm)	2.4 - 2.6	2.5
Particle Size Distribution: dp90/10	<= 1.2	1.2

BONDED PHASE ANALYSIS

PARAMETER	BATCH RESULT
Total Carbon (%)	5.0

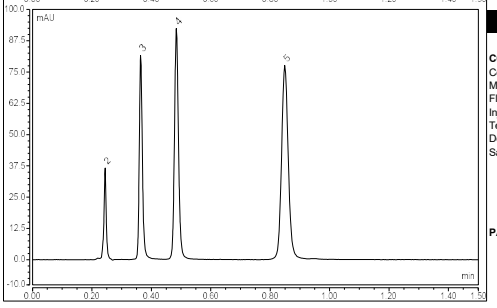
CHROMATOGRAPHIC RESULTS



PHENOMENEX QC MIX 870

CONDITION:
Column Dimensions: 50 X 4.6 mm
Mobile Phase: 20 mM KH2PO4 pH7.0/ MeOH (20:80)
Flow Rate: 2.0 mL/min
Injection Volume: 0.5 µL
Temperature: 30 °C
Detector: UV @ 254 nm
Sample:
1. uracil
2. toluene
3. ethyl benzene
4. amitriptyline

PARAMETER:	SPECIFICATION:	BATCH RESULT:
Asym4	<= 1.35	1.16
a2,3	1.36 - 1.40	1.37
k'3	0.95 - 1.17	1.01



BASIC DRUGS

CONDITIONS:
Column Dimensions: 50 X 4.6 mm
Mobile Phase: 20 mM Na2HPO4 pH 9.75/MeOH/ACN (35:55:10)
Flow Rate: 1.85 mL/min
Injection Volume: 1.0 µL
Temperature: 30 °C
Detector: UV @ 230 nm
Sample:
1. thiourea
2. pindolol
3. metoprolol
4. propranolol

PARAMETER:	SPECIFICATION:	BATCH RESULT:
Asym4	<=1.40	1.10
a2,3	1.90 -2.10	2.01
Rs2,3	>=5.3	5.7

QUALITY ASSURANCE

May 25, 2023