#VOCALFORLOCAL

ADHOC

ALPHA

HPLC COLUMN

AD HOC SCIENTIFIC PVT LTD

CONTENTS

PRODUCT INFORMATION

The ALPHA series represents a high performance C18, C8, CN., Phenyl and Amino phase based on a new type of silica gel developed to show better retention, greater selectivity and high loadability, caused by its exceptionally high surface area. The bonding density is chosen with respect to optimal selectivity for both hydrophilic and hydrophobic compounds. enabling even the use of 100% aqueous eluents. The end capping technology minimizes residual silanol groups to an amount which is below the detectable level. Silanol groups have negative effects on peak symmetry, particularly in case of basic compounds, and on chemical phase robustness.

ALPHA ANALYTICAL AND PREPARATIVE COLUMNS OFFER

- **EXCELLENT MECHANICAL STRENGTH**

Our columns are made of ultra-pure silica with completely spherical and totally porous particle.

- **OUTSTANDING CHEMICAL STABILITY**

Our cutting-edge technology ensures high bonding density with minimal silanol activity.

- **GREATER SURFACE AREA**

Our product is optimized for particle size, surface area, pore volume and pore diameter for better surface morphology.

- **EXCELLENT HOMOGENEITY & REPRODUCIBILITY**

Our quality is carefully monitored and very tightly controlled at each step of the manufacturing process.

- **FINEST RESOLUTION**

Our product provides better resolution with higher peak efficiencies due to optimal particle size distribution. Accomplish higher performance with enhanced selectivity, retention capacity and efficiency.

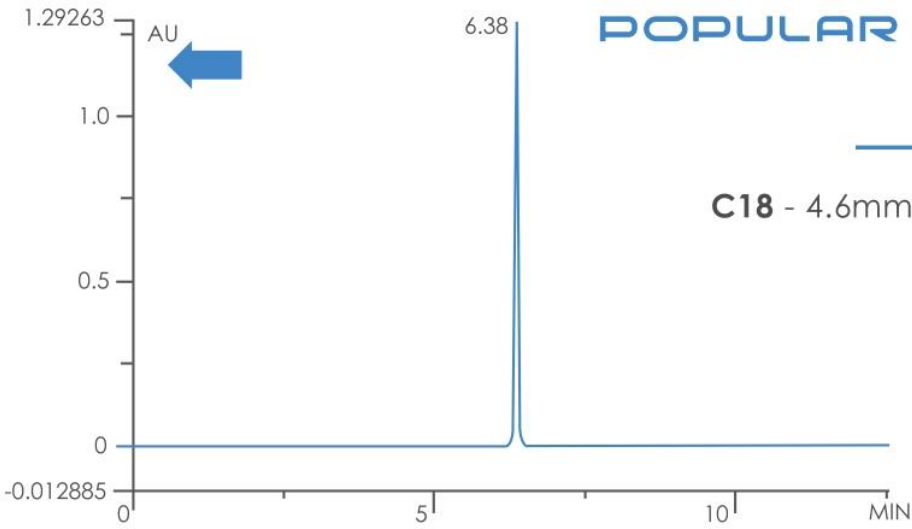
TECHNICAL SPECIFICATION

Bonding Phase	C18	C8	CN	Phenyl	Amino
Average Particle Size (µm)	5 µm & 3 µm	5 µm	5 µm	5 µm	5 µm
Average Pore Size (Å)	100	120	120	120	120
Specific Surface Area (m.sq/g)	450	300	287	340	300
Average Pore Volume (ml/g)	1.1	1.0	1.06	1.07	1.0
Carbon Load (%)	17	10	7.1	8.3	4
pH Range	1.5-10.5	2-8	2-8	2-8	2-8
USP Listing	L1	L7	L10	L11	L18

Brand	Bonded Phase	D50	D90/D10	Carbon load (%)	Surface area (m^2/g)	Pore size Å	pH range
ALPHA	**C18**	**4.3**	**1.23**	**17**	**450**	**100Å**	**1.5-10.5**
Luna	C18	-	-	17.5	400	100Å	2-10
Oyster	C18	-	-	17.5	450	100Å	2-10
YMCPRo	C18	-	-	16	335	120Å	2-8
Inertsil ODS3	C18	-	-	15	450	100Å	2-7.5
Hypersil ODS	C18	-	-	10	170	100Å	2-8
Symmetry	C18	-	-	19.1	335	100Å	2-8

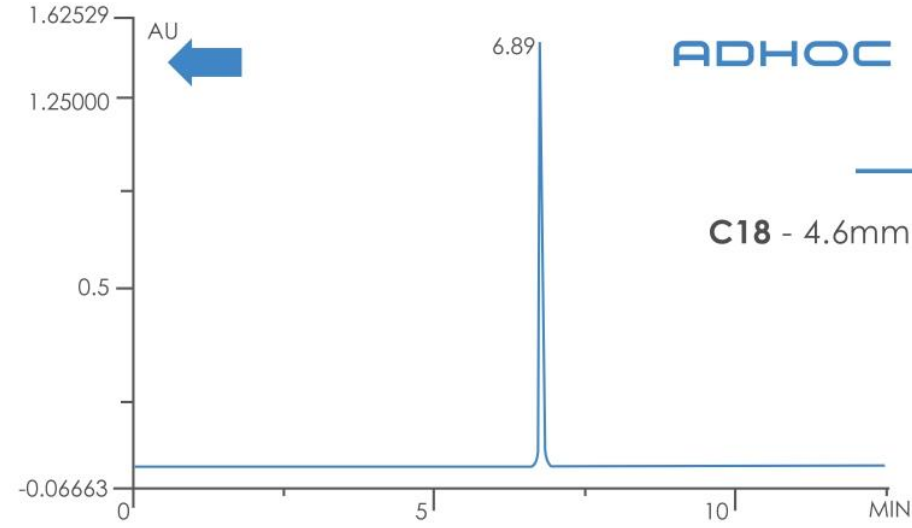
EFFICIENCY COMPARISON

(CAFFEINE)



POPULAR AMERICAN COLUMN

C18 - 4.6mm X 250mm, 100Å, 5µm



ADHOC SCIENTIFIC ALPHA

C18 - 4.6mm X 250mm, 100Å, 5µm

Every alpha column is tested for efficiency and passed only if it shows more than 80,000 plates per meter for toluene.

This is practical data generated in the lab and we do not mean to degrade another product. Comparitive separations may not berepresentative of all applications

OVERLAY OF SAMPLES

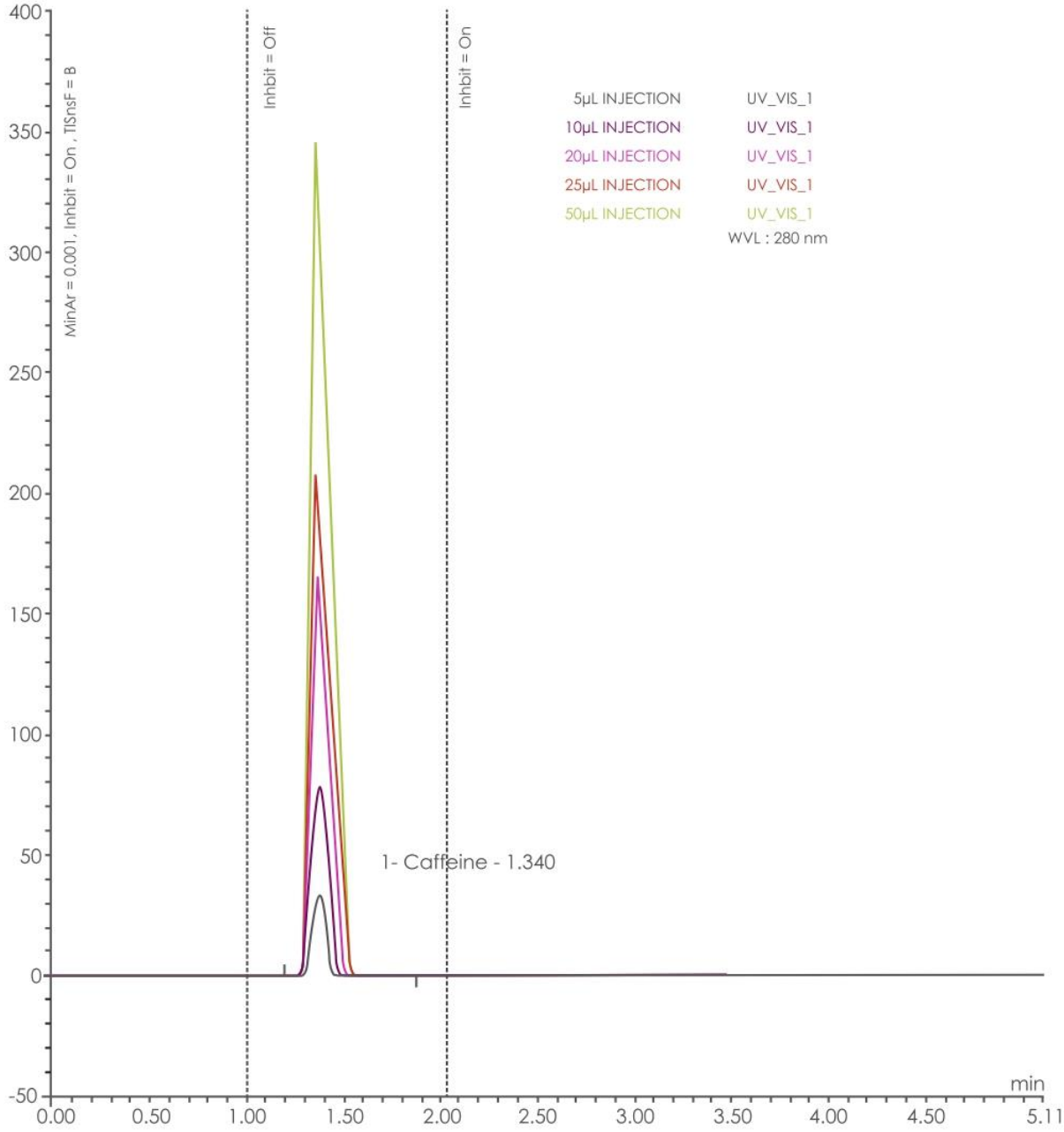


PLATE COUNT ON ALPHA

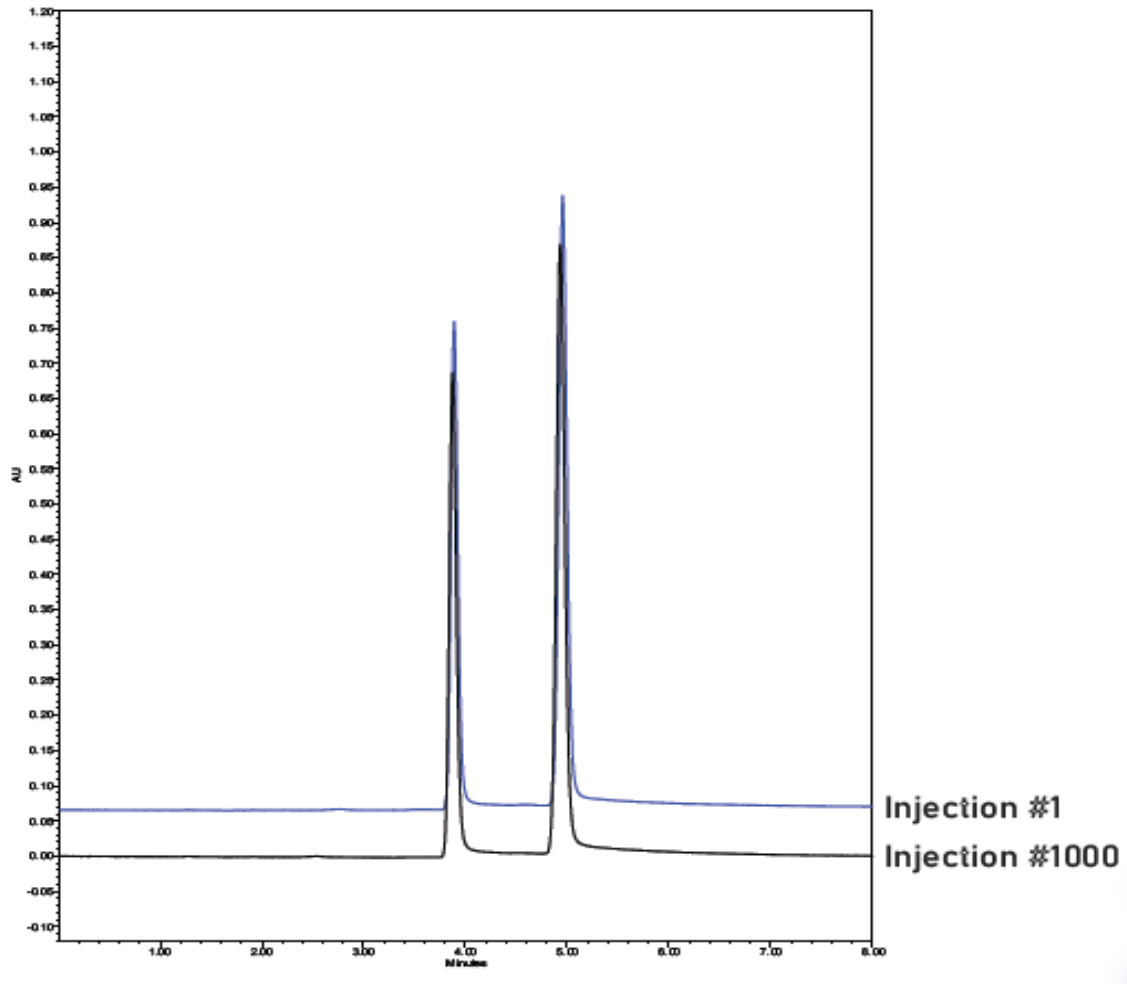
Sample No.	Sample Name	Ref.Time min Caffeine UV_VIS_1	Area mAU*min Caffeine UV_VIS_1	Height mAU Caffeine UV_VIS_1	Amount ppm Caffeine UV_VIS_1	Type Caffeine UV_VIS_1	Plates (EP) Caffeine UV_VIS_1
1	Blank	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	Blank	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	Blank	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4	5µl Injection	1.340	2.7629	33.51	n.a.	BMB	2162
5	5µl Injection	1.347	2.7469	33.50	n.a.	BMB	2183
6	5µl Injection	1.347	2.7418	33.49	n.a.	BMB	2194
7	Blank	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
8	10µl Injection	1.347	6.4991	78.26	n.a.	BMB	2050
9	10µl Injection	1.347	6.5024	78.44	n.a.	BMB	206 0
10	10µl Injection	1.347	6.5024	78.24	n.a.	BMB	2050
11	Blank	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
12	20µl Injection	1.353	14.1285	165.08	n.a.	BMB	1904
13	20µl Injection	1.353	14.12073	164.59	n.a.	BMB	1895
14	20µl Injection	1.353	14.1390	161.33	n.a.	BMB	1828
15	Blank	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
16	25µl Injection	1.360	17.8995	198.73	n.a.	BMB	1759
17	25µl Injection	1.360	17.9220	199.27	n.a.	BMB	1759
18	25µl Injection	1.360	17.9147	199.14	n.a.	BMB	1759
19	Blank	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
20	50µl Injection	1.36 7	36.958 3	346.90	n.a.	BMB	1306
21	50µl Injection	1.373	36.9444	347.17	n.a.	BMB	1319
22	50µl Injection	1.373	36.9644	346.57	n.a.	BMB	1314
23	Blank	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Averege		1.355	15.649	164.283	n.a.		1836
Rel.Std.Dev		0.755%	78.902%	68.471%	n.a.		16.870%

ALPHA COLUMN
LIFE TIME

ACHIEVE IMPROVED COLUMN LIFE TIME

Column Details: Alpha C18 150mm x 4.6mm 3u
Elution Type: Isocratic
Mobile Phase: Acetonitrile:Water (70:30)

Flow Rate: 1.0 ml/min
Column Temperature: Ambient
Detection: UV 254nm

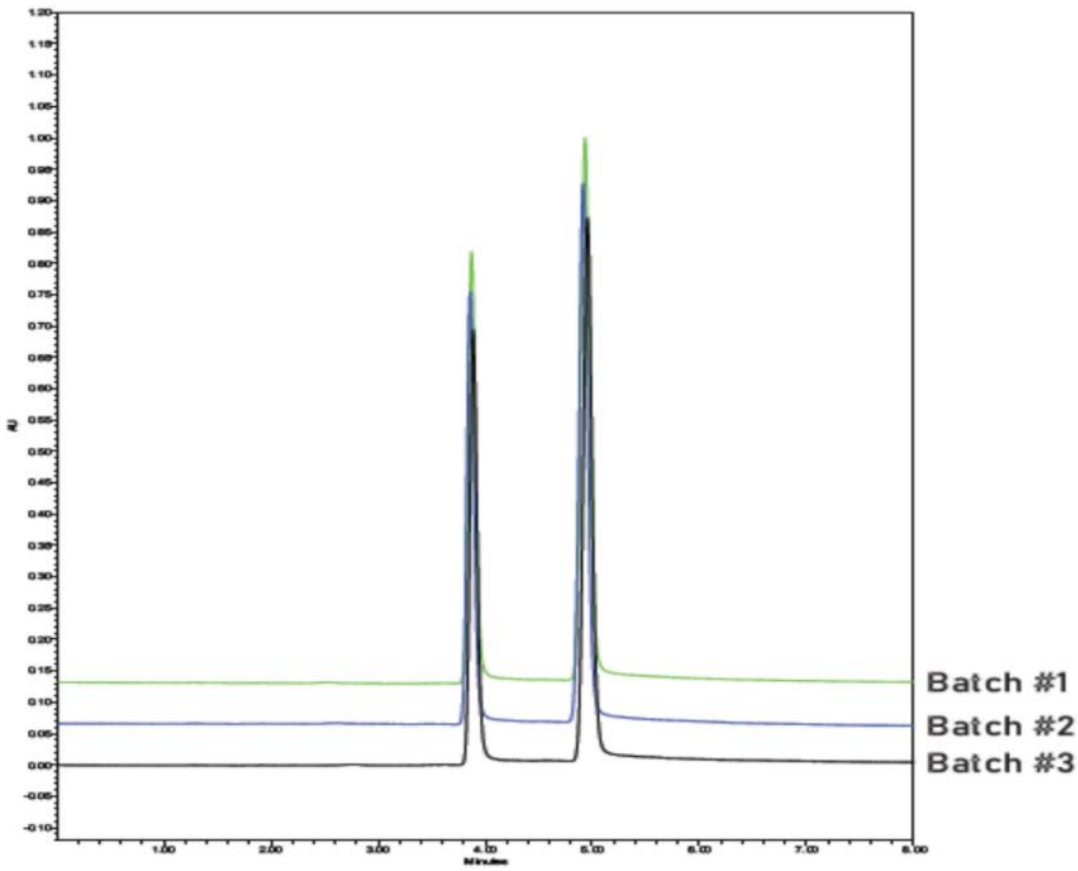


ALPHA COLUMN
B2B REPRODUCIBILITY

BATCH TO BATCH REPRODUCIBILITY ON ALPHA COLUMN

Column Details: Alpha C18 150mm x 4.6mm 3u
Elution Type: Isocratic
Mobile Phase: Acetonitrile:Water (70:30)

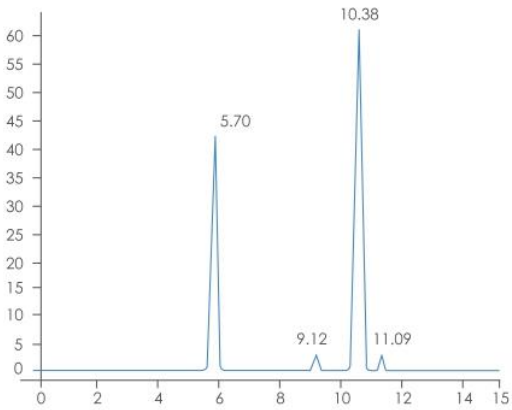
Flow Rate: 1.0 ml/min
Column Temperature: Ambient
Detection: UV 254nm



FLUCONAZOLE & IMPURITIES

POPULAR JAPANESE COLUMN

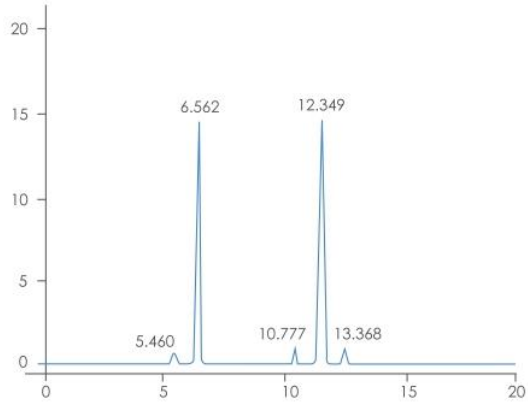
C18 - 4.6mm X 150mm, 100Å, 3µm



NAME	RT (min)	Area	Height	Area%	Resolution (usp)
Fluconazole Impurity A	5.70	448.96	40.05	32.73	
Fluconazole Impurity B	9.12	39.34	2.94	2.87	11.15
Fluconazole Impurity C	10.38	830.31	58.91	60.53	3.58
Fluconazole	11.09	53.22	2.98	3.88	1.73
	Sum	1371.84	104.85	100.00	

ADHOC SCIENTIFIC ALPHA

C18 - 4.6mm X 150mm, 100Å, 3µm



NAME	RT (min)	Area	Theoretical	Area%	Tacling factor	Resolution (usp)
Fluconazole Impurity A	6.562	118552	12637	32.733	1.106	
Fluconazole Impurity B	10.777	9977	15415	2.755	0.946	14.516
Fluconazole Impurity C	12.349	220779	14485	60.959	0.998	4.149
Fluconazole	13.368	12866	15869	3.552	0.975	2.441
	Sum	362174		100.00		

This is practical data generated in the lab and we do not mean to degrade another product. Comparitive separations may not berepresentative of all applications

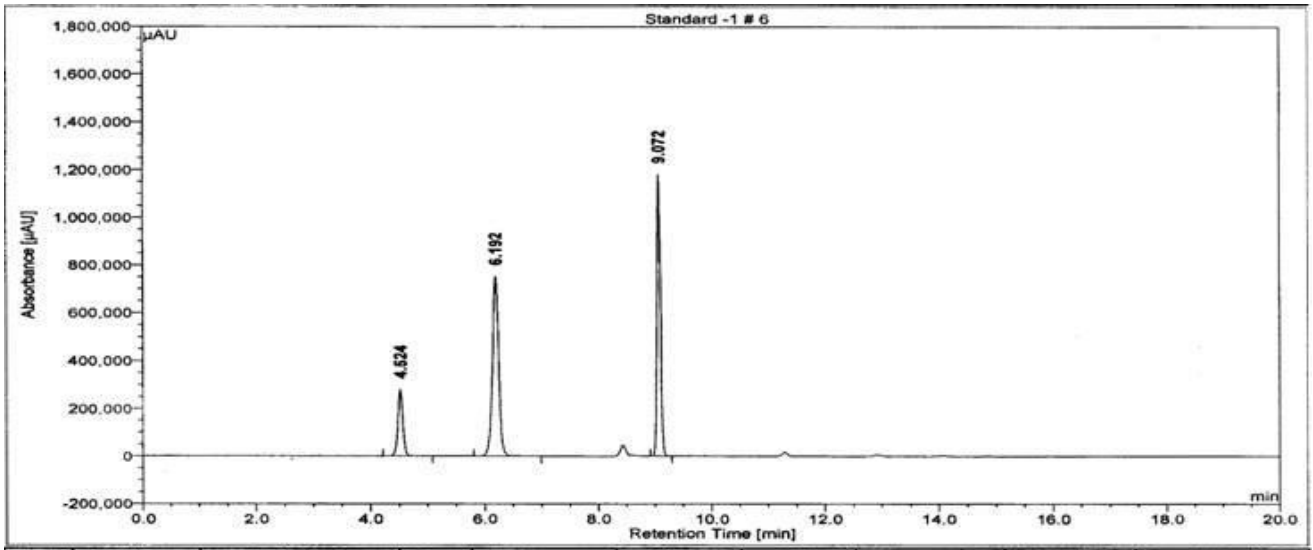
HPLC APPLICATION
ASSAY METHOD

ASSAY METHOD FOR RIFAMPIN, ISONIAZID, PYRAZINAMIDE

Column Details: Alpha C18 250x4.6 5u
Elution Type: Gradient
Buffer: 1.4g/L anhydrous dibasic sodium phosphate (pH 6.8)
Elution A: Buffer: Acetonitrile (96:04)
Elution B: Buffer: Acetonitrile (45:55)
Method conditions: Gradient

Flow Rate: 1.5 ml/min
Column Temperature: Ambient
Detection: UV 238 nm

Time	0	5	6	15
Mobile Phase A	100	100	0	0
Mobile Phase B	0	0	100	100



Peak No.	Ret.Time (min)	Peak Name	Type	Area µAU*sec	%Area %	RRT %	Asymmetry (Tailing)	Plates(USP) (USP)	Resolution(USP)
1	4.524		BMB	1683748	13.476	0.73	1.01	13719	9.51
2	6.192		BMB	5745895	45.988	1.00	1.00	15966	18.64
3	9.072		BMB	5064683	40.536	1.47	1.18	102826	n.a.
Total				12494326	100.00				

COLUMN INDEX

Sr. No.	PRODUCT CODE	COLUMN DIMENSIONS	PARTICLE SIZE	BONDING PHASE
1	A18-30040 -5	300 mm x 4.0 mm	5µm	C18
2	A18-30039 -5	300 mm x 3.9mm	5µm	C18
3	A18-25046 -5	250 mm x 4.6 mm	5µm	C18
4	A18-25040 -5	250 mm x 4.0 mm	5µm	C18
5	A18-20046 -5	200 mm x 4.6 mm	5µm	C18
6	A18-15046 -5	150 mm x 4.6 mm	5µm	C18
7	A18-15040 -5	150 mm x 4.0 mm	5µm	C18
8	A18-12546 -5	125 mm x 4.6 mm	5µm	C18
9	A18-12540 -5	125 mm x 4.0 mm	5µm	C18
10	A18-10046 -5	100 mm x 4.6 mm	5µm	C18
11	A18-10040 -5	100 mm x 4.0 mm	5µm	C18
12	A18-5046 -5	50 mm x 4.6 mm	5µm	C18
13	A18-30040 -3	300 mm x 4.0 mm	3µm	C18
14	A18-30039 -3	300 mm x 3.9mm	3µm	C18
15	A18-25046 -3	250 mm x 4.6 mm	3µm	C18
16	A18-25040 -3	250 mm x 4.0 mm	3µm	C18
17	A18-20046 -3	200 mm x 4.6 mm	3µm	C18
18	A18-15046 -3	150 mm x 4.6 mm	3µm	C18
19	A18-15040 -3	150 mm x 4.0 mm	3µm	C18
20	A18-12546 -3	125 mm x 4.6 mm	3µm	C18
21	A18-12540 -3	125 mm x 4.0 mm	3µm	C18
22	A18-10046 -3	100 mm x 4.6 mm	3µm	C18
23	A18-10040 -3	100 mm x 4.0 mm	3µm	C18
24	A18-5046 -3	50 mm x 4.6 mm	3µm	C18
25	A8-30040 -5	300 mm x 4. 0 mm	5µm	C8
26	A8-30039 -5	300 mm x 3.9mm	5µm	C8
27	A8-25046 -5	250 mm x 4.6 mm	5µm	C8
28	A8-25040 -5	250 mm x 4.0 mm	5µm	C8
29	A8-20046 -5	200 mm x 4.6 mm	5µm	C8
30	A8-15046 -5	150 mm x 4.6 mm	5µm	C8
31	A8-15040 -5	150 mm x 4.0 mm	5µm	C8

33	A8-12540 -5	125 mm x 4.0 mm	5um	C8
34	A8-10046 -5	100 mm x 4.6 mm	5um	C8
35	A8-10040 -5	100 mm x 4.0 mm	5um	C8
36	A8-5046 -5	50 mm x 4.6 mm	5um	C8
37	A8-30040 -3	300 mm x 4.0 mm	3um	C8
38	A8-30039 -3	300mm x 3.9mm	3 um	C8
39	A8-25046 -3	250 mm x 4.6 mm	3um	C8
40	A8-25040 -3	250 mm x 4.0 mm	3um	C8
41	A8-20046 -3	200 mm x 4.6 mm	3um	C8
42	A8-15046 -3	150 mm x 4.6 mm	3um	C8
43	A8-15040 -3	150 mm x 4.0 mm	3um	C8
44	A8-12546 -3	125 mm x 4.6 mm	3um	C8
45	A8-12540 -3	125 mm x 4.0 mm	3um	C8
46	A8-10046 -3	100 mm x 4.6 mm	3um	C8
47	A8-10040 -3	100 mm x 4.0 mm	3um	C8
48	A8-5046 -3	50 mm x 4.6 mm	3um	C8
49	ACN-30040 -5	300mmx4.0mm	5um	Cyano
50	ACN-30039 -5	300mm x 3.9mm	5um	Cyano
51	ACN-25046 -5	250mmx4.6mm	5um	Cyano
52	ACN-25040 -5	250mmx4.0mm	5um	Cyano
53	ACN-20046 -5	200mmx4.6mm	5um	Cyano
54	ACN-15046 -5	150mmx4.6mm	5um	Cyano
55	ACN-15040 -5	150mmx4.0mm	5um	Cyano
56	ACN-12546 -5	125mmx4.6mm	5um	Cyano
57	ACN-12540 -5	125mmx4.0mm	5um	Cyano
58	ACN-10046 -5	100mmx4.6mm	5um	Cyano
59	ACN-10040 -5	100mmx4.0mm	5um	Cyano
60	ACN-5046 -5	50mmx4.6mm	5um	Cyano
61	APH-30040 -5	300mmx4.0mm	5um	Phenyl
62	APH-30039 -5	300mmx3.9mm	5um	Phenyl
63	APH-25046 -5	250mmx4.6mm	5um	Phenyl
64	APH-25040 -5	250mmx4.0mm	5um	Phenyl
65	APH-20046 -5	200mmx4.6mm	5um	Phenyl
66	APH-15046 -5	150mmx4.6mm	5um	Phenyl
67	APH-15040 -5	150mmx4.0mm	5um	Phenyl
68	APH-12546 -5	125mmx4.6mm	5um	Phenyl
69	APH-12540 -5	125mmx4.0mm	5um	Phenyl
70	APH-10046 -5	100mmx4.6mm	5um	Phenyl
71	APH-10040 -5	100mmx4.6mm	5um	Phenyl
72	APH-5046 -5	50mmx4.6mm	5um	Phenyl

ALPHA COLUMN
CARE & INSTALLATION

Ad Hoc Scientific columns are individually manufactured and tested to meet strict specification criteria. The following measures will maintain their performance and lifetime:

COLUMN INSTALLATION

- Set flow rate to 0.1 mL/min (for 2.1-4.6 mm ID) and install the column making sure that the arrow is in the direction of flow.
- Stop flow and wipe outlet end of column to remove any particulates before connecting to detector.
- Install fitting/tubing into outlet end and run minimum 10 column volumes at low flow (~0.2 mL/min) while monitoring the backpressure.
- A steady pressure should indicate a constant flow while pressure fluctuation will indicate air in the system. Wide fluctuations in pressure may shock and damage the column so it's important to monitor the pressure as well as signal from the detector, when both are steady, the column is ready for use.

EQUILIBRATION

The storage solvent in a new column is the mobile phase used to evaluate the column unless otherwise specified on the chromatogram. Initially, care should be taken not to pass any material through the column that may precipitate in the storage solvent. Ensure that the column is fully equilibrated to the mobile phase prior to starting an analysis. A normal phase silica column usually requires more conditioning than a reverse phase column.

SOLVENTS

Use only HPLC grade solvents and freshly prepared buffer solutions to minimize bacteria Growth. Always filter the mobile phase using a 0.2um filter. This will ensure maximum column life.

MOBILE PHASE PH STABILITY

Alpha HPLC columns are stable within a pH range of 1.5 to 10.5 for C18 and 2-8 for other chemistries. Although, using the column at extreme pH will reduce the column lifetime.

BACKPRESSURE LIMIT

Alpha HPLC columns can safely be used up to 4500 psi. However, please avoid pressure shocks on the column. Pressure shocks lead to channeling in the column, which results in peak splitting in the corresponding chromatogram.

MOBILE PHASES (ELUENTS)

Alpha's stationary phases are compatible with all organic solvents in the above-mentioned pH range. Please use the highest quality solvents available (HPLC grade).

TEMPERATURE

The column temperature should not exceed 60^0C.

STORAGE

For short term storage, i.e., overnight, columns can be stored in the eluent used in last analysis. For middle term storage, i.e., 2 days or over the weekend, columns should be flushed with pure water to prevent microbial growth. For long term storage, silica-based columns should be stored in an aprotic solvent.

Caution! Please make sure that all buffers are washed out of the column.

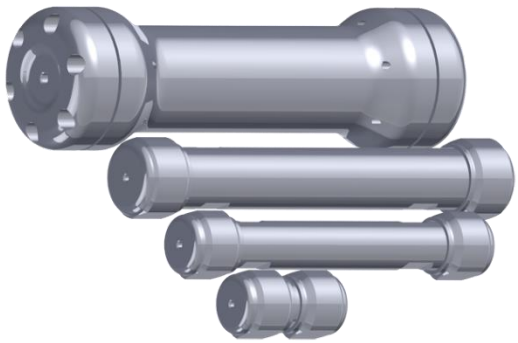
MECHANICAL DAMAGE

Protect the column from mechanical shock, dropping or banging.

ALPHA PREPARATIVE COLUMNS

HIGHER LOADING CAPACITIES

BETTER SEPARATIONS



BETTER RESOLUTIONS

REPRODUCIBLE RESULTS

ALPHA ANALYTICAL AND PREPARATIVE COLUMNS ARE AVAILABLE IN ALL STANDARD AND SPECIALIZED DIMENSIONS.

APC DESCRIPTION

BASICS OF SCALING UP FROM ANALYTICAL LC TO PREPARATIVE LC

A recommended method-development strategy is to develop and optimize the initial separation on an analytical size column, overload the column while maintaining adequate separation of components of interest, then scale-up accordingly to a preparative column of appropriate dimensions based upon the amount of purified compound needed.

The choice of analytical column is often dictated by the availability of preparative columns containing the same column-packing material.

The scale-up should be linear with perhaps only minor adjustments required to finalize the preparative method. It is highly recommended that one ensures that both analytical and preparative columns from the same line of packing material be readily available before beginning the preparative method development and optimization process.

CONTACT US FOR CUSTOMISED COLUMN DIMENSIONS TO SUFFICE YOUR NEEDS.

HPLC APPLICATION

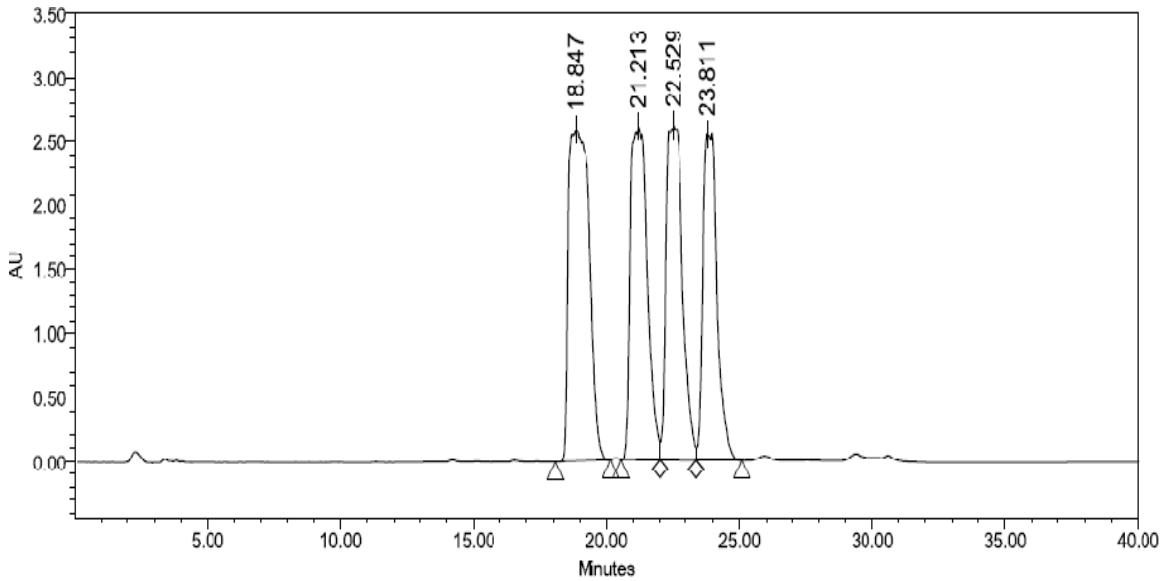
DIACEREIN IMPURITIES

DIACEREIN IMPURITIES

Column Details: Alpha C18 250mm x 20mm, 10um
Elution Type: Gradient
Mobile Phase A: 10mM Ammonium Formate
Mobile Phase B: Mixture of Acetonitrile (95%) & Mobile Phase A (5%)

Flow Rate: 20 ml/min
Injection Volume: 2 mL

Time	0	5	10	15	20	25	30	35
Mobile Phase A	70	70	60	50	40	40	70	70
Mobile Phase B	30	30	40	50	60	60	30	30



	Retention Time (min)	Area	% Area	Height	
1	18.847	144672294	31.04	2581732	Diacerein
2	21.213	113199827	24.29	2600248	Diacerein Rhein-I, Impurity-D
3	22.529	115366694	24.75	2614521	Diacerein Rhein-II, Impurity-E
4	23.811	92856558	19.92	2545317	Diacerein Rhein, Impurity-C

ALPHA PREPARATIVE COLUMN INDEX

Sr. No.	PRODUCT CODE	COLUMN DIMENSIONS	PARTICLE SIZE	BONDING PHASE
1	A18-10020-10	100mm x 20mm	10 µm	C18
2	A18-15020-10	150mm x 20mm	10 µm	C18
3	A18-25020-10	250mm x 20mm	10 µm	C18
4	A18-10030-10	100mm x 30mm	10 µm	C18
5	A18-15030-10	150mm x 30mm	10 µm	C18
6	A18-25030-10	250mm x 30mm	10 µm	C18
7	A18-25050-10	250mm x 50mm	10 µm	C18
8	A18-10020-5	100mm x 20mm	5 µm	C18
9	A18-15020-5	150mm x 20mm	5 µm	C18
10	A18-25020-5	250mm x 20mm	5 µm	C18
11	A18-10030-5	100mm x 30mm	5 µm	C18
12	A18-15030-5	150mm x 30mm	5 µm	C18
13	A18-25030-5	250mm x 30mm	5 µm	C18
14	A8-10020-10	100mm x 20mm	10 µm	C8
15	A8-15020-10	150mm x 20mm	10 µm	C8
16	A8-25020-10	250mm x 20mm	10 µm	C8
17	A8-10030-10	100mm x 30mm	10 µm	C8
18	A8-15030-10	150mm x 30mm	10 µm	C8
19	A8-25030-10	250mm x 30mm	10 µm	C8
20	A8-25050-10	250mm x 50mm	10 µm	C8
21	APH-10020-10	100mm x 20mm	10 µm	Phenyl
22	APH-15020-10	150mm x 20mm	10 µm	Phenyl
23	APH-25020-10	250mm x 20mm	10 µm	Phenyl
24	APH-10030-10	100mm x 30mm	10 µm	Phenyl
25	APH-15030-10	150mm x 30mm	10 µm	Phenyl
26	APH-25030-10	250mm x 30mm	10 µm	Phenyl
27	APH-25050-10	250mm x 50mm	10 µm	Phenyl

ALPHA COLUMN
CARE & MAINTENANCE

pH STABILITY

Alpha HPLC Columns are stable within a pH range of 1.5 to 10.5 for C18 and 2-8 for C8. Although, using the column at extreme pH will reduce the column lifetime.

BACKPRESSURE LIMIT

Alpha HPLC Columns can safely be used upto 4500 psi. However, please avoid pressure shocks on the column. Pressure shocks lead to channelling in the column, which results in peak spliting in the corresponding chromatogram.

MOBILE PHASES (ELUENTS)

Alpha's stationary phases are compatible with all organic solvents in the above mentioned pH range. Please use the highest quality solvents available (HPLC grade) also, please filter all prepared buffer through a 0.5 um filter before using them in your HPLC system. Always keep in mind; your column is the best filter!

PROPER STORAGE OF HPLC COLUMNS

•For short term storage, i.e. overnight, columns can be stored in the eluent used in last analysis.

•For middle term storage, i.e. 2 days or over the weekend, columns should be flushed with pure water to prevent microbial growth.

•For long term storage, silica based columns should be stored in an aprotic solvent. The water content sholud not be higher than 50%. The best storing solvent is Acetonitrile.

•Caution!!! Please make sure that all buffers are washed out of the columns before flushing with Acetonitrile. Buffer salts are mainly not soluble in Acetonitrile and can block the capillaries & the column.

EQUILIBRATION TIME

The equilibration time of a column depends on the column dimensions. In general, a column is equilibrated after flushing with 20 column volumes. The equilibration time for the most important column dimensions is summarized in the following table.

Column Dimension	Column Volume (ml)	Flow Rate (ml/min)	Equilibration Time (min)
250X4.6 mm	2.91	1.0	58
150X4.6 mm	1.74	1.0	35
100X4.6 mm	1.16	1.0	23
50X4.6 mm	0.58	1.0	12

Shorter equilibration times are possible if you simply increase the flow rate. It is no problem to do that if no chromatography is done. However, 20 column volumes are necessary to ensure a 100% equilibration.

ADHOC

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