Analytical®
Technologies Limited

HyperCHROM

Liquid Chromatography Columns

EPCC / PRODUCTS / APPLICATION / SOFTWARE / ACCESSORIES / CONSUMABLES / SERVICES

Analytical Technologies Limited

An ISO 9001 Certified Company

www.analyticalgroup.net

HPLC Phases and Apllications

Common Name	Functional Group	Normal Phase	Reversed Phase	Ion Exchange	Applications
ODS	$-C_{18}H_{17}$				The least polar in the alkyl-bonded phases. Used widely in pharmaceutical, environmental etc.
C8	$-C_5H_{17}$		√		Recommended for compounds strongly retained on C18 phases.
C4	$-C_4H_9$		√		Shorter retention than C8 and C18.
C1	$-(CH_3)_3$		√		The least retentive in all alklyl group bonded phases. Typically used for moderate polar and multi-functional compounds.
NH_2	$-(CH_2)_9NH_2$	√	√	√	Alternative selectivity to silica. Analysis of saccharide or other polar compounds in reversed phase some organic acid can be separated using buffers and organic modifiers.
SiO_2	-OH				Separation of non-polar and moderate polar compounds.

Column Selection Guide

Description	Polar Size (A°)	Particle Sizes (μm)	Pore Volume (mL/g)	Surface Area (m^2/g)	% of Carbon	End-Capping
HyperCHROM ODS-BP	120	3,5,10,15	1.0	300	15	Yes
HyperCHROM ODS-AP	120	3,5,10,15	1.0	300	17	Yes
HyperCHROM300A ODS-AP	300	3,5,10,15	0.9	100	3	Yes
HyperCHROM C8	120	3,5,10,15	1.0	300	11	Yes
HyperCHROM 300A C8	300	3,5,10,15	0.9	100	5	Yes
HyperCHROM C4	120	3,5,10,15	1.0	300	8	Yes
HyperCHROM C4	300	3,5,10,15	0.9	100	3	Yes
HyperCHROM C1	120	3,5,10,15	1.0	300	5	/
HyperCHROM 300A C1	300	3,5,10,15	0.9	100	2	/
HyperCHROM NH_2	120	3,5,10,15	1.0	300	4	No

Ordering Information

Description	Particle Size (µm)	Size I.D. (mm)	Part No.
	5	4.6 x 150	0103-244
Analytical ODS -BP	5	4.6 x 200	0103-245
	5	4.6 x 250	0103-247
	5	6 x 150	0103-264
	10	4.6 x 150	0103-344
	10	4.6 x 200	0103-345
	10	4.6 x 250	0103-347
	10	10 x 250	0103-387
	10	20 x 250	0103-397
	10	30 x 250	0103-3A7
	10	40 x 250	0103-3C7
	10	50 x 250	0103-3E7
Analytical ODS -AP	5	4.6 x 150	0102-244
	5	4.6 x 200	0102-245
	5	4.6 x 250	0102-247
	5	6.0 x 150	0102-264
	10	4.6 x 150	0102-344
	10	4.6 x 200	0102-345
	10	4.6 x 250	0102-347
	10	10 x 250	0102-387
	10	20 x 250	0102-397
	10	30 x 250	0102-3A7
	10	40 x 250	0102-3C7
	10	50 x 250	0102-3E7
Analytical 300A ODS -AP	5	4.6 x 150	0112-244
	5	4.6 x 200	0112-245
	5	4.6 x 250	0112-247
	10	10 x 250	0112-387
	10	20 x 250	0112-397
	10	30 x 250	0112-3A7
	10	40 x 250	0112-3C7
	10	50 x 250	0112-3E7

Technical information contained in this publication is for reference purposes
Only and is subject to change without notice.

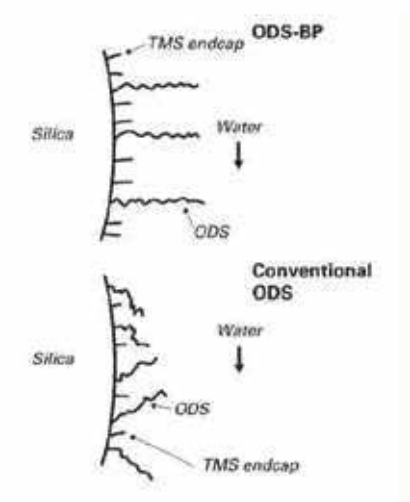
Analytical C8	5	4.6 x 150	0105-244
	5	4.6 x 200	0105-245
	5	4.6 x 250	0105-247
	5	6.0 x 150	0105-264
	10	4.6 x 150	0105-344
	10	4.6 x 200	0105-345
	10	4.6 x 250	0105-347
Analytical C4	5	4.6 x 150	0106-244
	5	4.6 x 200	0106-245
	5	4.6 x 250	0106-247
	10	4.6 x 150	0106-344
	10	4.6 x 200	0106-345

Analytical C4	5	4.6 x 150	0106-244
	5	4.6 x 200	0106-245
	5	4.6 x 250	0106-247
	10	4.6 x 150	0106-344
	10	4.6 x 200	0106-345
Analytical C1	10	4.6 x 250	0106-347
	5	4.6 x 150	0107-244
	5	4.6 x 200	0107-245
	5	4.6 x 250	0107-247
	10	4.6 x 200	0107-345
Analytical NH_2	10	4.6 x 250	0107-347
	5	4.6 x 150	0104-244
	5	4.6 x 200	0104-245
	5	4.6 x 250	0104-247
	10	4.6 x 150	0104-344
	10	4.6 x 200	0104-345
Analytical SiO_2	10	4.6 x 250	0104-347
	5	4.6 x 150	0101-244
	5	4.6 x 200	0101-245
	5	4.6 x 250	0101-247
	10	4.6 x 150	0101-344
	10	4.6 x 200	0101-345
	10	4.6 x 250	0101-347

Other pore sizes, column dimensions are available. please call Customer Service for more informatoion.

HyperCHROM ODS-BP

HyperCHROM ODS-BP phases are designed to show extended selectivity for hydrophilic and polar compounds which are either not or poorly retained on other phases. A proprietary modification technique avoids the collapse of the C18 chains which conventional ODS-phases show at high water contents in the mobile phase, even if pure water is used. typical applications are separations of biomolecules and mtabolites such as oligosaccharides,amino acids, small peptides, nucleotides and organic acids.



HyperCHROM ODS-BP phases are fully end-capped and show smilar selectivity as conventional C18 phases when being used for separation of hydrophobic compounds with typical reversed phase eluents.

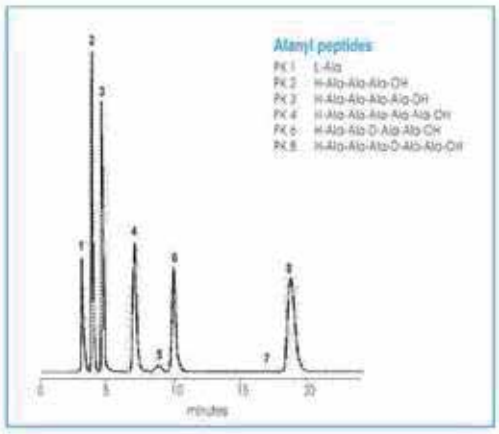
HyperCHROM ODS-BP phases show stable baselines and high sensitivity even under neutral pH conditions and without buffer or counterion additives, which makes them appear especially suited for hyphemated techniques like LC-MS, where such additives disturb the detection.

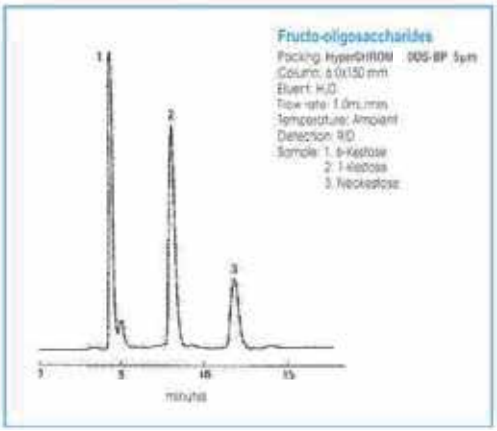
- Suitable hydrophilic compounds separation
- Strong retention in aqueous condition
- Longer lifetime in aqueous eluents
- Different selectivity from ODS-AP
- Enhanced mechanical stability
- Suitable for Dynamic Axial Compression Columns

Alanine and its oligopeptides are separated on analytical ODS-BP using 100% water as eluent. The elution sequence corresponds with the number of amino acid units included in the each peptide.

The diastereoisomer which contains the unnatural D-Ala in its structure shows a different retention time from the corresponding all-L-Ala peptide with the same number of amino acid residue.

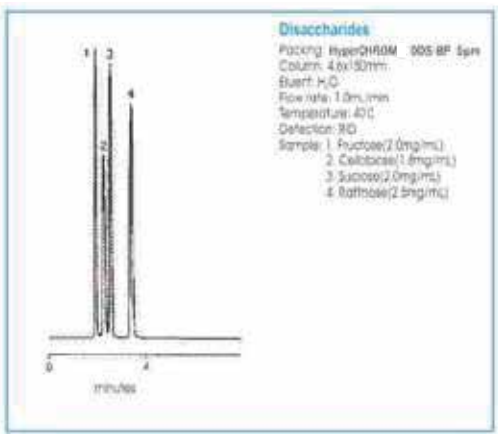
peptides were eluted on Analytical ODS-BP (6.0x150mm) with H_2O+ Flow are: 1mL/min, Detection: UV @ 214nm



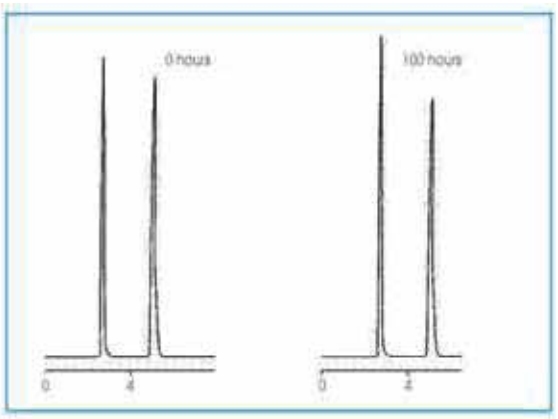


1-Kestose, 6-Kestose and Neokestose are position diastereoisomers which have the same molecular weight and are built up by the same monosaccharides, but they differ in the bonding position between sucrose and fructose. Analytical ODS-BP is sensitive to such small differences.

Disaccharides such as cellobiose and sucrose can be efficiently separated by Analytical ODS-BP. These disac charides are composed of different monosaccharide units and exhibit different hydrophobicity. Ananlytical ODS-BP is capable of recognizing such a small differences.



Intensity of Hydrophobic Interaction of ODS-BP

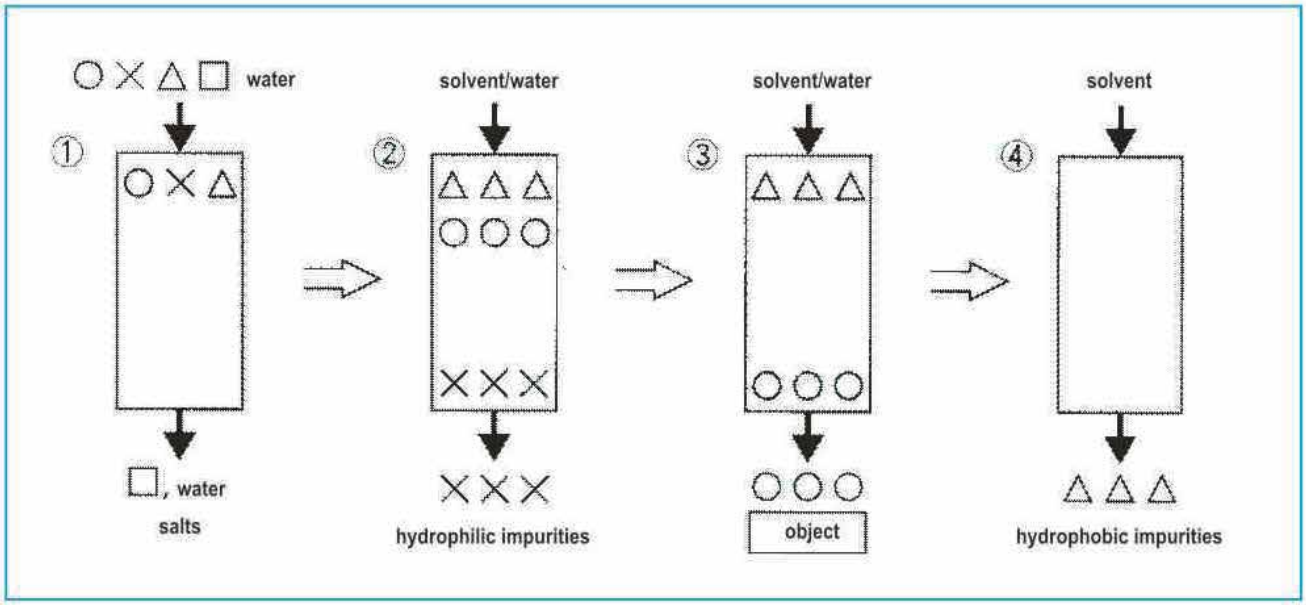


	0 hours	After 100 hours
Pyridine kpy	0.704	0.686
Phenol kph	2.129	2.080
Separation α	3.024	3.032

There is no evidence of phase collapse of Analytical ODS-BP with pure water as eluent. The test chtomatogram of the pyridine/phenol separations shows that after 100 hours washing with water there was no change in selectivity or retention behavior.

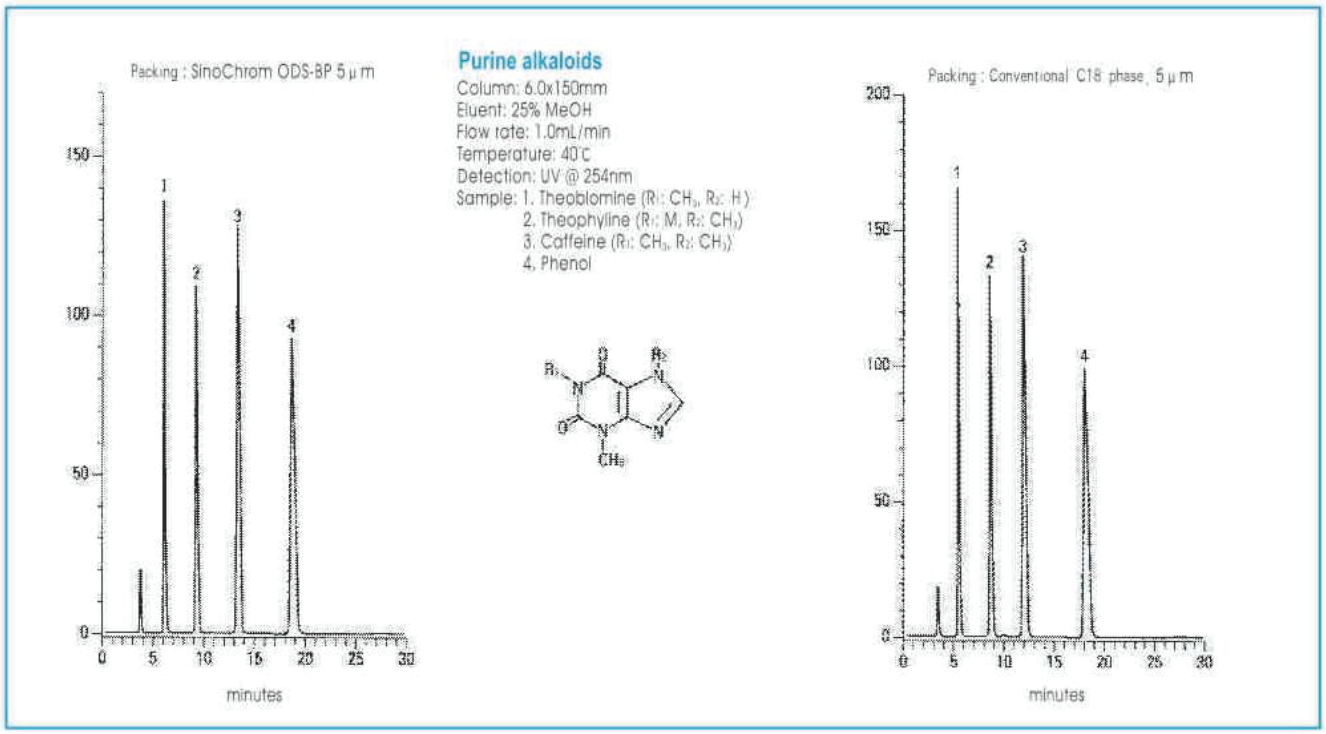
ODS-BP Packings are Useful for Desaltation of Aqueous Solution

ODS-BP packings, as an alternative to ion exchangers are useful for desaltation of aqueious solutions. Desaltation, concentration and subsequent reversed phase separation can be done on one column. Water and inorganic compounds are eluted without retention and organic compounds including the object substance are strongly retained. The object and its impurities are eluted with a water / organic modifier setp gradient.

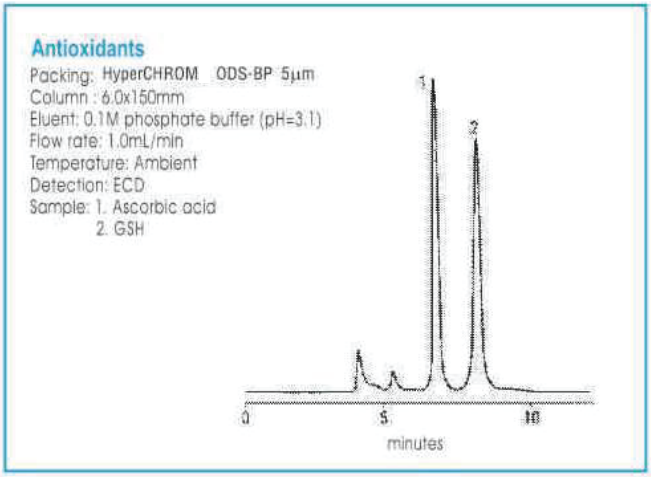


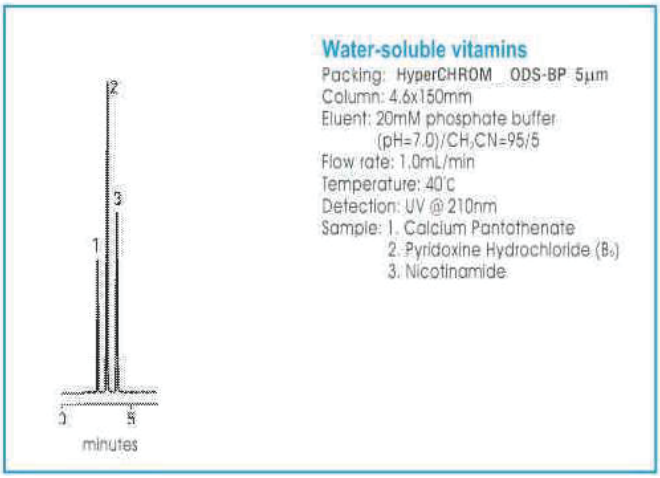
Difference between ODS-BP and Conventional C18 Phases

When typical reversed phase eluents are used ODS-BP phase in comparison to other conventional C18 phases show incresed retention for hydrophilic compounds duo to hydrophilic interaction, where as hydrophobic compounds are less retained. This effect can be used for the separation of two compounds with incidentally similar retention properties on convertional C18 phases, but different polarity.



Application





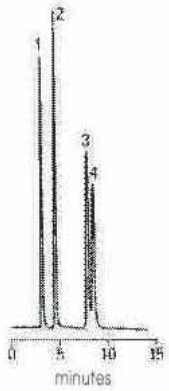
Application

Glycolic acid, Lactic acid

Packing: HyperCHROM ODS-BP 5μm
Column: 4.6x150mm
Eluent: 0.1% H_3PO_4
Flow rate: 1.0mL/min
Temperature: 40°C
Detection: UV @ 210nm
Sample: 1. Glycolic acid (2.0mg/mL)
2. Lactic acid (3.3mg/mL)

Amino acids

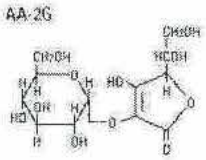
Packing: HyperCHROM ODS-BP 5μm
Column: 6.0x150mm
Eluent: H_2O
Flow rate: 1.0mL/min
Temperature: 40°C
Detection: RID
Sample: 1. Alanine
2. Valine
3. Isoleucine
4. Leucine

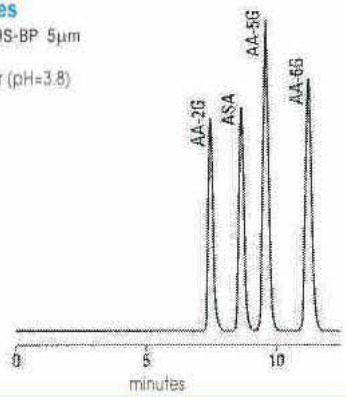


Ascorbic acid glycosides

Packing: HyperCHROM ODS-BP 5μm
Column: 6.0x150mm
Eluent: KH_2PO_4 - H_3PO_4 buffer (pH=3.8)
Flow rate: 0.7mL/min
Temperature: Ambient
Detection: UV @ 240nm

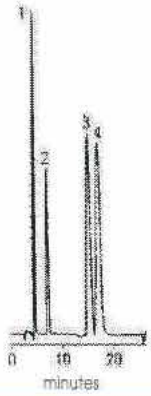
AA-2G





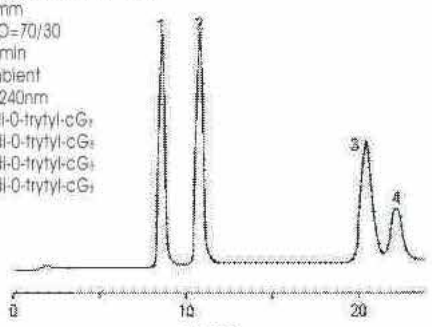
Alcohols

Packing: HyperCHROM ODS-BP 5μm
Column: 6.0x150mm
Eluent: H_2O
Flow rate: 1.0mL/min
Temperature: 40°C
Detection: RID
Sample: 1. Methanol
2. Ethanol
3. iso-Propanol
4. n-Propanol



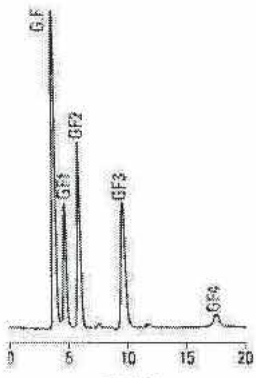
Cyclodextrin derivatives

Packing: HyperCHROM ODS-BP 5μm
Column: 6.0x150mm
Eluent: MeOH/H_2O=70/30
Flow rate: 1.0mL/min
Temperature: Ambient
Detection: UV @ 240nm
Sample: 1. $6^1,6^3$-di-O-trytyl-cG$_3$
2. $6^1,6^4$-di-O-trytyl-cG$_3$
3. $6^1,6^2$-di-O-trytyl-cG$_3$
4. $6^1,6^5$-di-O-trytyl-cG$_3$



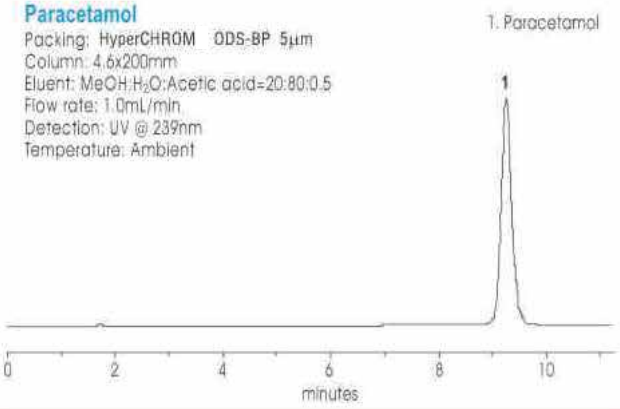
Fructo-oli osaccharides

Packing: HyperCHROM ODS-BP 5μm
Column: 6.0x150mm
Eluent: H_2O
Flow rate: 1.0mL/min
Temperature: Ambient
Detection: RID



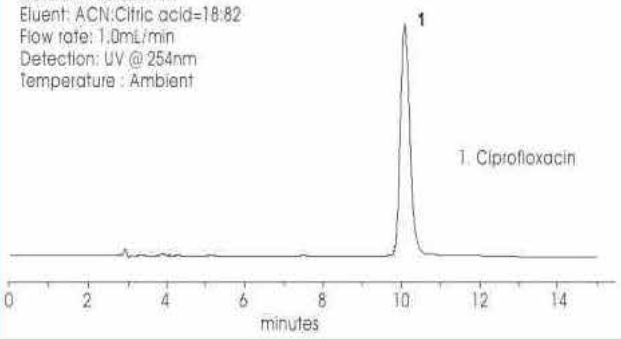
Paracetamol

Packing: HyperCHROM ODS-BP 5μm
Column: 4.6x200mm
Eluent: MeOH:H_2O:Acetic acid=20:80:0.5
Flow rate: 1.0mL/min
Detection: UV @ 239nm
Temperature: Ambient



Ciprofloxacin hydrochloride

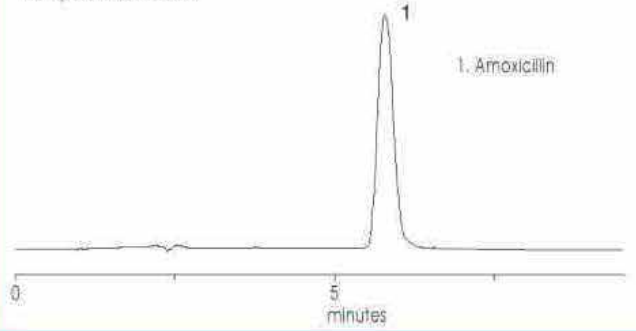
Packing: HyperCHROM ODS-BP 5μm
Column: 4.6x250mm
Eluent: ACN:Citric acid=18:82
Flow rate: 1.0mL/min
Detection: UV @ 254nm
Temperature : Ambient



Application

The assay of amoxicillin

Packing: HyperCHROM ODS-BP 5μm
Column: 4.6x250mm
Eluent: MeOH:H_2O=25:75
Flow rate: 1.0mL/min
Detection: UV @ 254nm
Temperature: Ambient

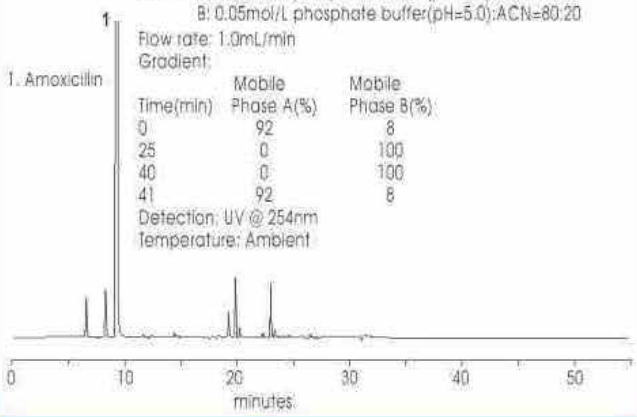


The related substance of amoxicillin sodium for injection

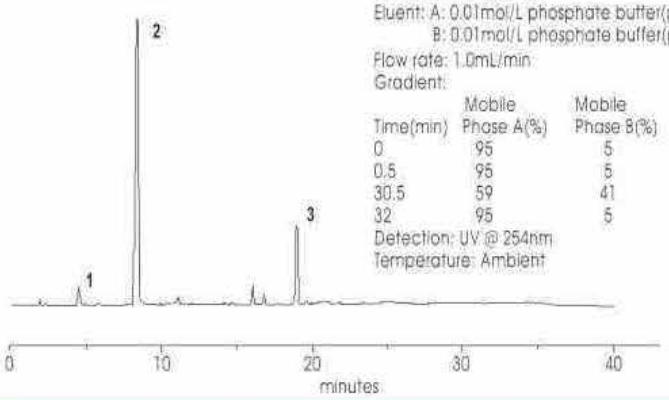
Packing: HyperCHROM ODS-BP 5μm
Column: 4.6x200mm
Eluent: A: 0.05mol/L phosphate buffer(pH=5.0):ACN=99:1
B: 0.05mol/L phosphate buffer(pH=5.0):ACN=80:20
Flow rate: 1.0mL/min
Gradient:

Time(min)	Mobile Phase A(%)	Mobile Phase B(%)
0	92	8
25	0	100
40	0	100
41	92	8

Detection: UV @ 254nm
Temperature: Ambient



1. Clavulanate Potassium
2. Amoxicillin
3. amoxicillin dimer



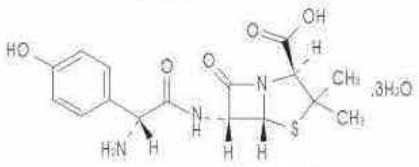
The related substance of amoxicillin sodium-clavulanate potassium for injection

Packing: HyperCHROM ODS-BP 5μm
Column: 4.6 x 200mm
Eluent: A: 0.01mol/L phosphate buffer(pH=6.0)
B: 0.01mol/L phosphate buffer(pH=6.0):ACN=20:80
Flow rate: 1.0mL/min
Gradient:

Time(min)	Mobile Phase A(%)	Mobile Phase B(%)
0	95	5
0.5	95	5
30.5	59	41
32	95	5

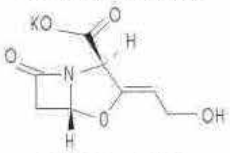
Detection: UV @ 254nm
Temperature: Ambient

Amoxicillin

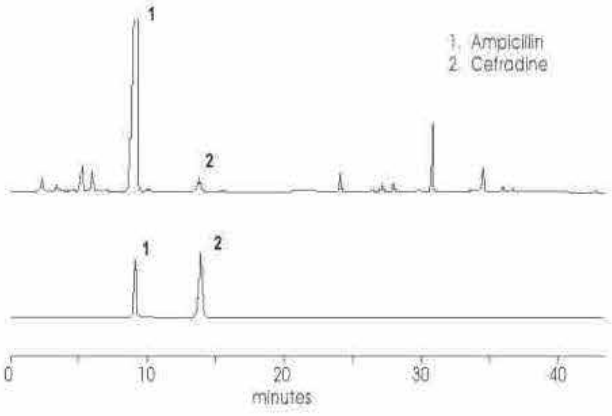


$C_{16}H_{19}N_3O_5S \cdot 3H_2O$ 419.46

Clavulanate Potassium



$C_8H_8KNO_5$ 237.25



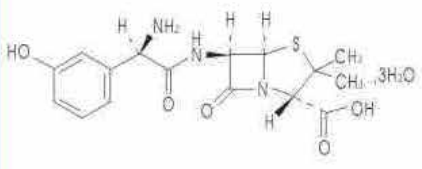
The related substance of ampicillin sodium for injection

Packing: HyperCHROM ODS-BP 5μm
Column: 4.6 x 200mm
Eluent: A: 12% acetic acid:0.2mol/L phosphate buffer:ACN:H_2O=0.5:50:50:900
B: 12% acetic acid:0.2mol/L phosphate buffer:ACN:H_2O=0.5:50:400:550
Flow rate: 1.0mL/min
Gradient:

Time(min)	Mobile Phase A(%)	Mobile Phase B(%)
0	85	15
10	85	15
40	0	100
50	0	100
51	85	15

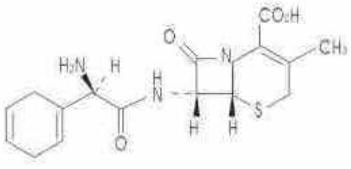
Detection: UV @ 254nm
Temperature: Ambient

Amoxicillin



$C_{16}H_{19}N_3O_5S \cdot 3H_2O$ 403.45

Clavulanate Potassium

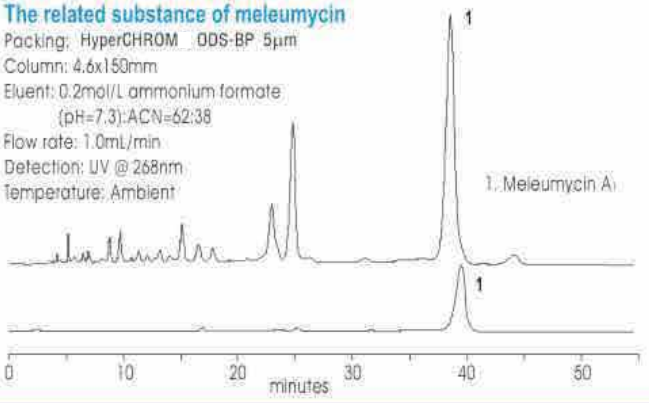


$C_8H_9N_2O_5S$ 349.40

Application

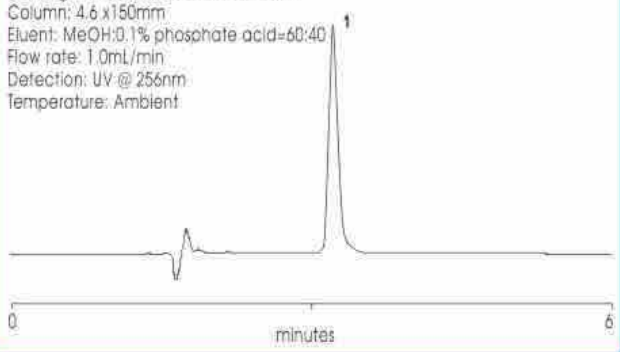
The related substance of meleumycin

Packing: HyperCHROM ODS-BP 5μm
Column: 4.6x150mm
Eluent: 0.2mol/L ammonium formate (pH=7.3):ACN=62:38
Flow rate: 1.0mL/min
Detection: UV @ 268nm
Temperature: Ambient



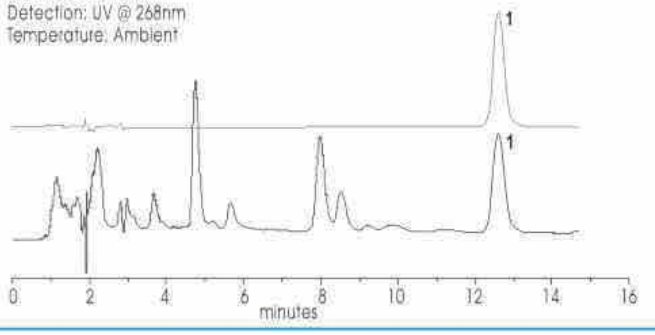
Quercetin

Packing: HyperCHROM ODS-BP 5μm
Column: 4.6 x150mm
Eluent: MeOH:0.1% phosphate acid=60:40
Flow rate: 1.0mL/min
Detection: UV @ 256nm
Temperature: Ambient



Radix Et rhizoma salviae miltiorrhizae

Packing: HyperCHROM ODS-BP 5μm
Column: 4.6x150mm
Eluent: ACN:MeOH:$CHCl_3$:H_2O=40:40:1:20
Flow rate: 1.0mL/min
Detection: UV @ 268nm
Temperature: Ambient

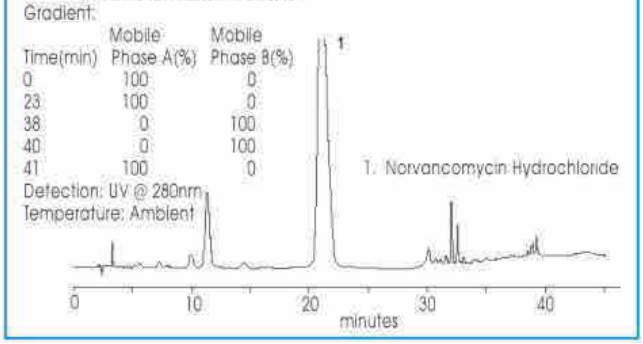


The related substance of norvancomycin hydrochloride

Packing: HyperCHROM ODS-BP 5μm
Column: 4.6 x 200mm
Eluent: A :TEA buffer:ACN:THF=96:3:1
B :TEA buffer:ACN:THF=70:29:1
Gradient:

Time(min)	Mobile Phase A(%)	Mobile Phase B(%)
0	100	0
23	100	0
38	0	100
40	0	100
41	100	0

Detection: UV @ 280nm
Temperature: Ambient

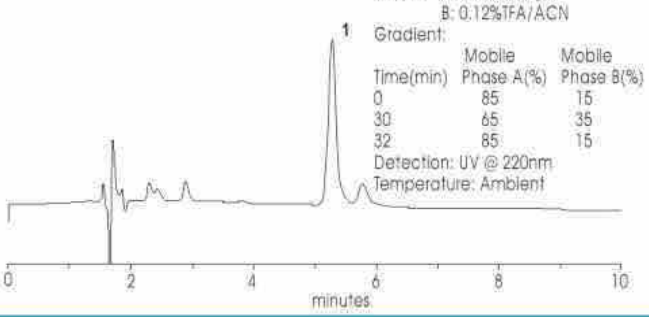


Kaempferol

Packing: HyperCHROM ODS-BP 5μm
Column: 4.6 x 250mm
Eluent: A: 0.1%TFA/H_2O
B: 0.12%TFA/ACN
Gradient:

Time(min)	Mobile Phase A(%)	Mobile Phase B(%)
0	85	15
30	65	35
32	85	15

Detection: UV @ 220nm
Temperature: Ambient

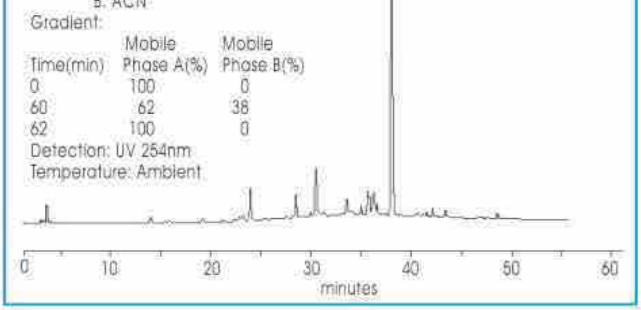


Lilicium henryi diels

Packing: HyperCHROM ODS-BP 5μm
Column: 4.6x150mm
Eluent: A: 0.1% phosphate acid:ACN=98:2
B: ACN
Gradient:

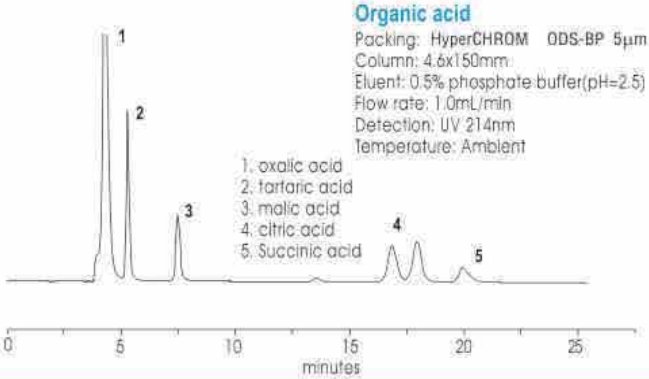
Time(min)	Mobile Phase A(%)	Mobile Phase B(%)
0	100	0
60	62	38
62	100	0

Detection: UV 254nm
Temperature: Ambient



Organic acid

Packing: HyperCHROM ODS-BP 5μm
Column: 4.6x150mm
Eluent: 0.5% phosphate buffer(pH=2.5)
Flow rate: 1.0mL/min
Detection: UV 214nm
Temperature: Ambient

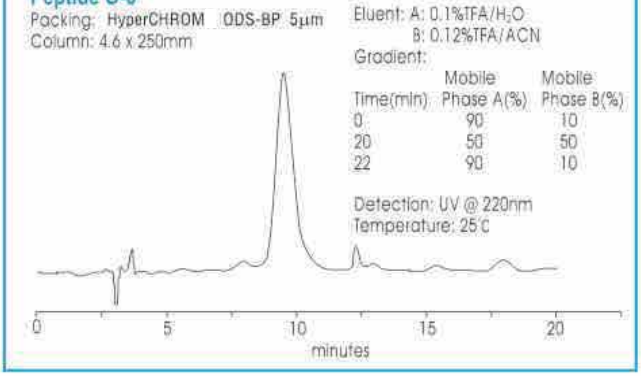


Peptide O-8

Packing: HyperCHROM ODS-BP 5μm
Column: 4.6 x 250mm
Eluent: A: 0.1%TFA/H_2O
B: 0.12%TFA/ACN
Gradient:

Time(min)	Mobile Phase A(%)	Mobile Phase B(%)
0	90	10
20	50	50
22	90	10

Detection: UV @ 220nm
Temperature: 25°C



Hyprev CHROM HPLC Column

Dimension: ID 4.6 mm, Length 150-300mm
Connection: 1/16'' (Standard)
Packing Material: World famous brand material, such as Kromasil, Luna, YMC, Daiso etc.

HyperCHROM Semi-Preparative HPLC Column

Dimension: ID 10-40mm, Length 150-300mm
Connection: 1/16'' (Standard)
Packing Material: World famous brand material, such as Kromasil, Luna, YMC, Daiso etc.

HyperCHROM Preparative HPLC Column

Dimension: ID 50-500mm, Length 150-600mm
Connection: 1/8'' or 1/4'' (Standard)
Packing Material: World famous brand material, such as Kromasil, Luna, YMC, Daiso etc.
we can produce all kinds of hplc columns basis of customers requirement.
Preparative hplc column re-packing service
Preparative hplc cloumn application support.
We promise that each column must be tested strictly and attached with test report.

DAC50 PREPARATIVE HPLC COLUMN

DAC150 PREPARATIVE HPLC COLUMN

DAC800 PREPARATIVE HPLC COLUMN

DAC stands for dynamic axial compression. It combines the preparative column and packing system together. It is very sample to operate. The colimn can used online when it is packed well. Don't need to take the column down. It prevents mechanical degradation of the particles. Bed compression is maintained constant, independent of swelling or shrinking of the bed. If the solvent conditions are such that particle swelling takes place that the piston automatically let the bed expand to maintain constant compression.

HyperCHROM-PACK DAC columns can be consistently packed with small particulate media (10um and less) to high levels of performance: column efficiencies of up to 50,000 theoretical plates per meter have been reported for columns of 50 and 300 mm internal diameter (i.d.) This requires and even distribution of the liquid flow oven the column cross-section. Our engineers have modeled the flow patten at the column ends and designed efficient flow distribution systems for optimum performance. For even better control of chromatographic conditions. DAC columns are delivered with a temperature jacket and/or insulation.

Specification:

Column:	Internal diameter: ID30mm-ID1000mm
	Tube length:650mm
	Tube internal suffice finish: Ra <0.8m
	Material: SST316 sintered
Filter:	Material: SST316 sintered
	pore size: 3.5um
Piston:	Material:SST316L
Hydraulic cylinder:	Maximum working pressure: 50MPa
	Viscosity range: 46-68cst.
Pressure Indicator:	Air pressure meter: 0-10bar, 0.01 degree
	Hydraulic meter: 0-400bar, 0.1 degree
Working pressure:	120bar max. Piston act on the column bed
Air supply requirement:	Rated air out pressure 0.5-1 MPa

Verification report appendix

All experimental data were obtained under the same equipment, mobile phase, and sample conditions.

I. HyperChromZ Sugar Determination Procedure and Result Analysis

1.1 Chromatographic conditions

chromatographic column	HyperChromZ Sugar 4.6×20mm		
Flow rate	1.0mL/min		
mobile phase	NaOH gradient elution, see Table 1 below for details.		
Injection volume	10ÿL		
Column	30ÿ	Detector temperature	35ÿ
temperature	Au electrode	reference	Ag/AgCl
working	ampere detector	electrode	Pulse Integral
electrode detector filter time constant	5S	mode manual gain configuration	1
Waveform selection	Gold electrodes, carbohydrates, four potentials, see Table 2 below for details.		

Table 1. NaOH Gradient Elution Table					B:200mM
NaOH gradient elution table					NaOH
Serial	time	Linear flow rate	(mL/min) A%	A: Pure water	B%
1	0	linear	1	88	12
2	26	linear	1	88	12
3	26.1	linear	1	0	100
4	40	linear	1	0	100
5	40.1	linear	1	88	12
6	65	linear	1	88	12

Table 2 Waveform Selection			
Serial Number	Time	Cell voltage (V)	Start- end integration interval
1	0	0.1	/
2	200	0.1	ÿ
3	400	0.1	ÿ
4	410	-2	/
5	420	-2	/
6	430	0.6	/
7	440	-0.1	/
8	500	-0.1	/

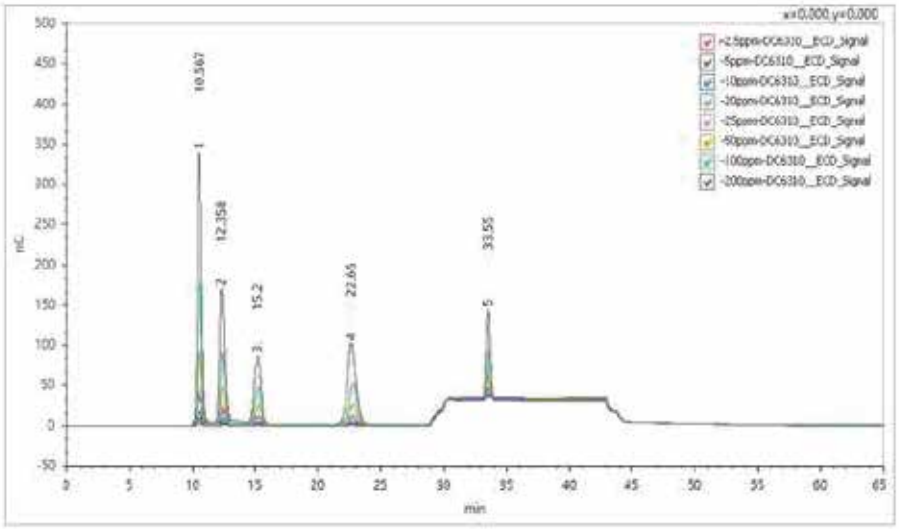
1.2 Test Spectra and Results

1.2.1 Linearity Test

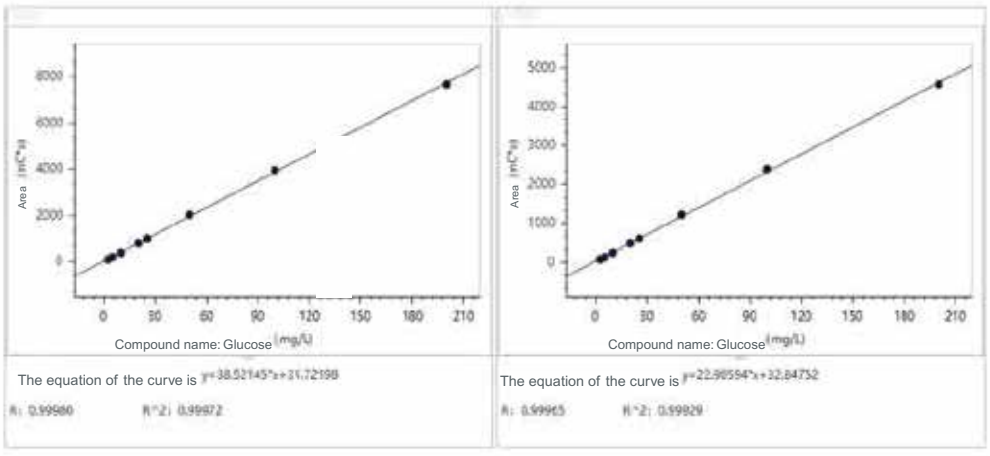
Standard curve series concentration gradient table (mg/L)

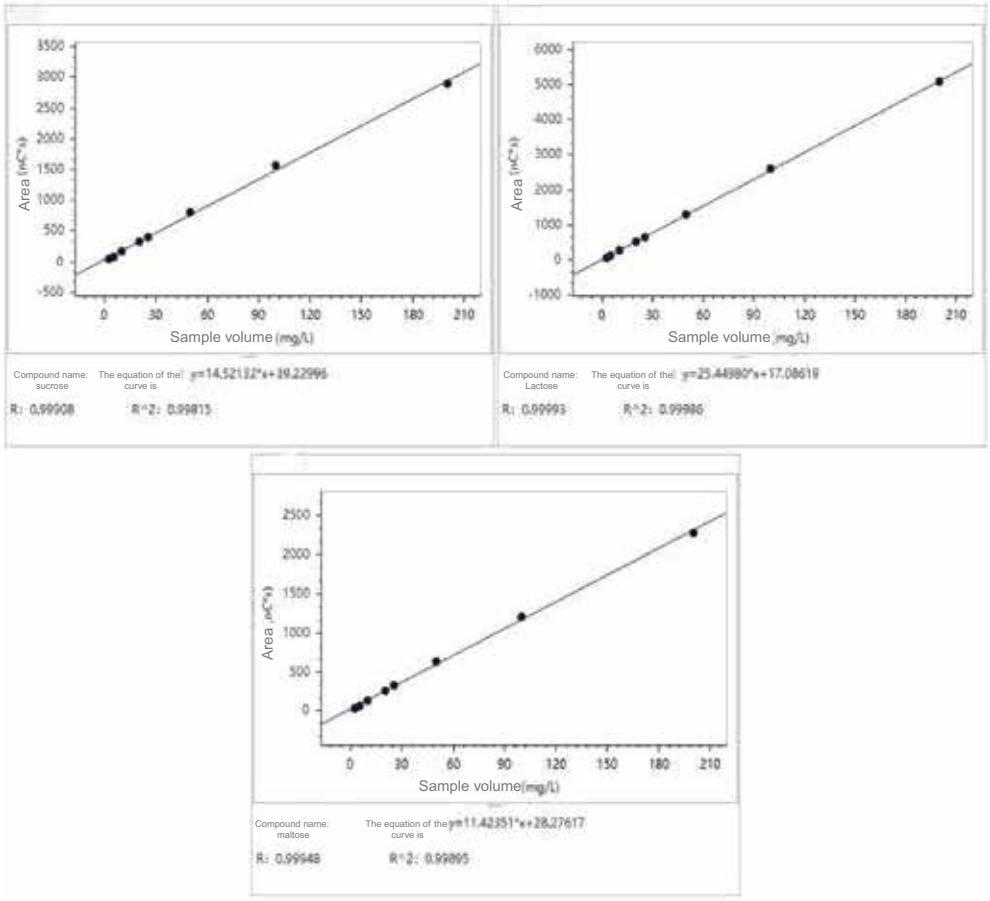
Compound Name	Track 1	Track 2	Track 3	Track 4	Track 5	Track 6	Track 7	Track 8
Glucose 2.5		5	10	20	25	50	100	200
Sucrose 2.5		5	10	20	25	50	100	200
Fructose	2.5	5	10	20	25	50	100	200
and lactose	2.5	5	10	20	25	50	100	200
Maltose 2.5		5	10	20	25	50	100	200

Standard curve test spectrum overlap plot



Five sugars linear

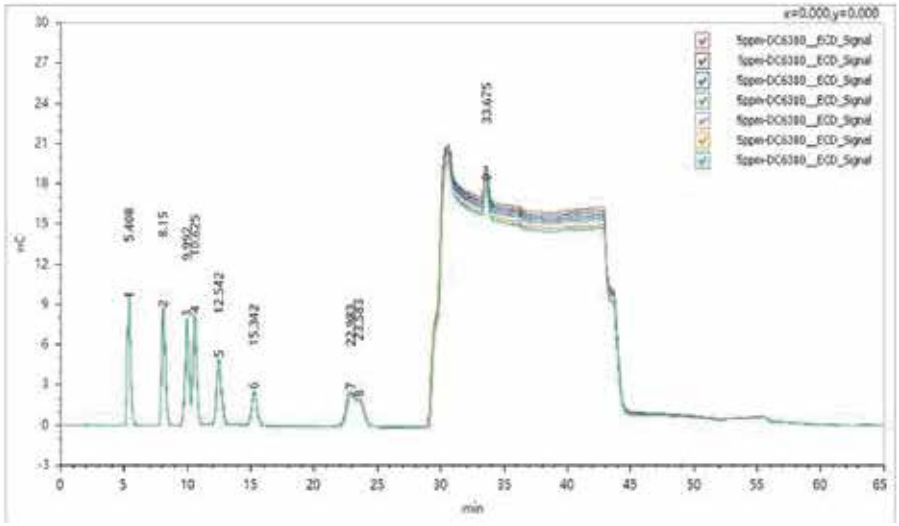




Note: The linear correlation coefficients of the five sugars within the concentration range of 2.5 mg/L to 200 mg/L were all greater than 0.999, meeting the test requirements.
beg.

1.2.2 Nine-sugar mixed standard test (refer to HyperCHROMZ column instruction manual)

Spectrum of nine sugar mixed standard test



Test data

CompoundName	Retention Time(min) Peak Area(nC*s)	Resolution Tailing	Factor	Theoretical	Plate Number
Fucose	5.400	152.073	6.364	1.332	2954
Arabic sugar	8.125	158.975	3.833	1.188	5014
Galactose	9.967	130.288	1.232	0.974	6307
glucose	10.600	141.344	3.177	1.178	6493
fructose	12.517	131.092	3.74	1.092	5412
sucrose	15.308	78.961	8.66	1.052	5684
lactose	22.908	44.716	0.625	0.859	9443
lactulose	23.508	13.974	13.645	2.799	9237
maltose	33.608	50.539	n.a.	1.1	71704

Note: The HyperChromZ Sugar column was used to test a mixed standard of 9 sugars. The resolution between galactose and glucose was 1.232, while that between lactose and lactose was...

The separation degree of sugar was 0.625, while the separation degrees of other sugars were all greater than 3; the tailing factors of the nine sugars, except for lactose and lactulose, ranged from...

The peak values are between 0.974 and 1.332, with a symmetrical shape.The abnormal tailing factors of lactulose and lactose may be due to poor separation between the two sugars.

To.

Compound name	Fucose peak		Storage time of		Galactose	
Number	Retention time (min)	area (nC*s)	arabinose (min)	Peak area (nC*s)	Retention time (min)	Peak area (nC*s)
1	5.400	152.073	9.967	130.288	9.967	130.288
2	5.400	150.836	9.983	129.798	9.983	129.798
3	5.408	149.394	9.992	129.394	9.992	129.394
4	5.400	148.131	9.975	128.687	9.975	128.687
5	5.400	147.259	9.983	128.527	9.983	128.527
6	5.400	146.954	9.967	127.524	9.967	127.524
7	5.408	145.296	9.992	127.679	9.992	127.679
average	5.402	148.563	9.980	128.842	9.980	128.842
RSD(%)	0.072	1.586	0.106	0.809	0.106	0.809

Compound name	Glucose		Fructose		sucrose	
Serial Number	Retention time (min)	Peak area (nC*s)	retentiotime (min)	Peak area (nC*s)	Retention time (min)	Peak area (nC*s)
1	10.600	141.344	12.517	131.092	15.308	78.961
2	10.617	141.239	12.533	130.601	15.317	79.024
3	10.625	141.304	12.542	130.935	15.333	78.434
4	10.608	140.101	12.525	128.266	15.325	78.369

5	10.617	139.495	12.517	127.707	15.308	77.802
6	10.600	138.648	12.508	128.416	15.308	78.049
7	10.625	138.702	12.542	126.822	15.342	77.422
Average	10.613	140.119	12.526	129.12	15.320	78.294
RSD(%)	0.101	0.860	0.105	1.337	0.089	0.749

Compound name	Lactose		Lactulose		maltose	
Serial Number	retention time (min)	Peak area (nC*s)	retention time (min)	Peak area (nC*s)	Retention time (min)	Peak area (nC*s)
1	22.908	44.716	23.508	13.974	33.608	50.539
2	22.933	44.673	23.550	14.025	33.650	50.175
3	22.958	44.307	23.600	14.257	33.625	49.801
4	22.933	43.109	23.550	14.357	33.617	49.584
5	22.900	43.816	23.475	14.289	33.592	49.250
6	22.908	43.613	23.508	14.149	33.592	48.841
7	22.983	42.776	23.583	14.330	33.675	48.389
Average	22.932	43.859	23.539	14.146	33.623	49.511
RSD(%)	0.131	1.715	0.189	1.419	0.091	1.512

Note: The RSD values for retention time are between 0.072% and 0.189%, and the RSD values for peak area are between 0.749% and 1.715%.

II. HyperCHROMZ Measurement Process and Result Analysis

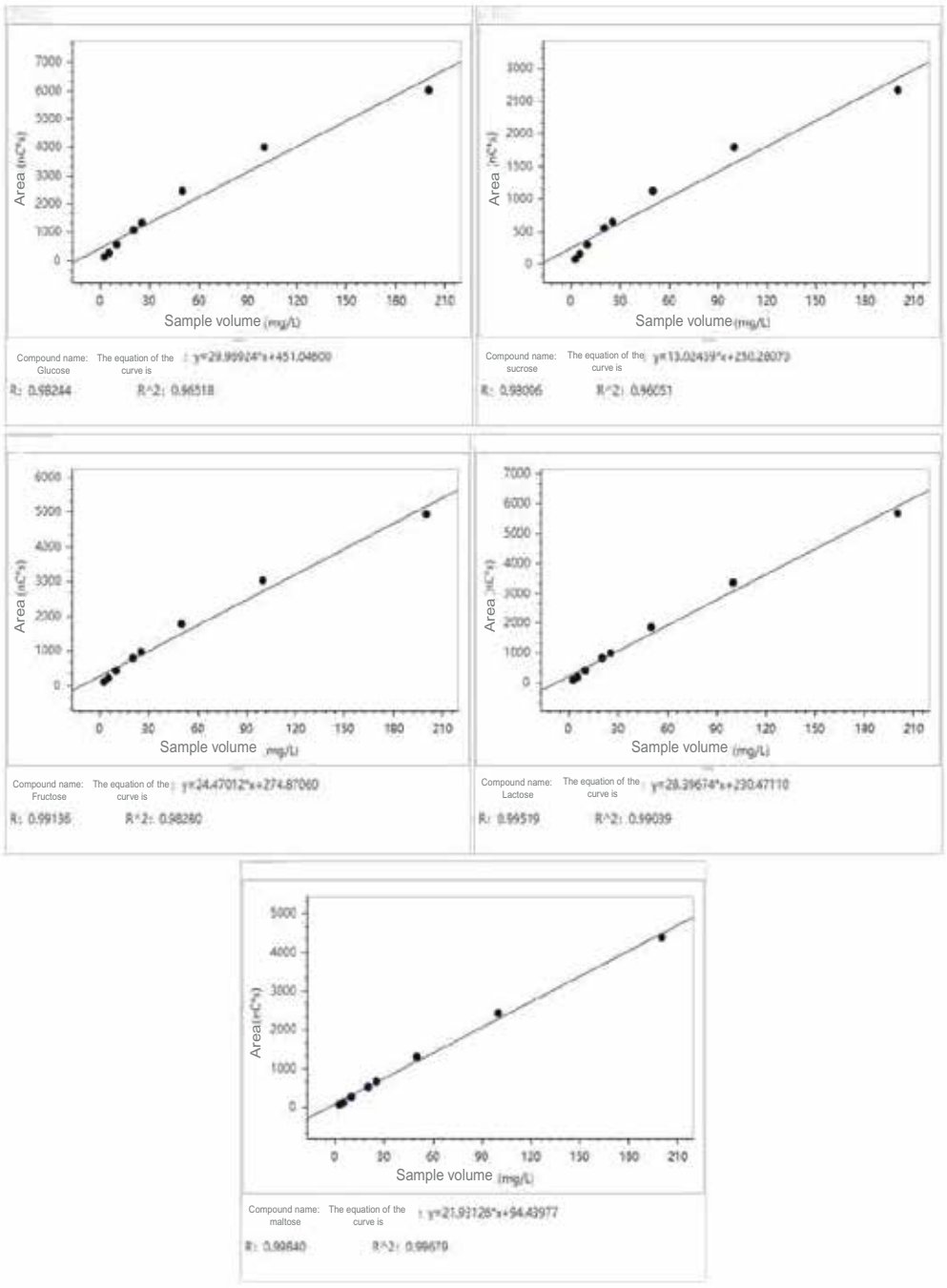
2.1 Chromatographic conditions

chromatographic column	SweetSep AEX200ÿ 4.0×20mm,5ÿm		
Flow rate	0.7mL/min		
mobile phase	NaOH gradient elution, see Table 1 below for details.		
Injection volume,	10ÿL		
column	30ÿ	Detector temperature	35ÿ
temperature,	Au electrode	reference electrode	Ag / AgCl
working electrode detector	ampere detector	model	Pulse Integral
Filtering time constant	5S	Manual gain configuration	1
Waveform selection	Gold electrodes, carbohydrates, four potentials, see Table 2 below for details.		

Table 1. NaOH Gradient Elution Table

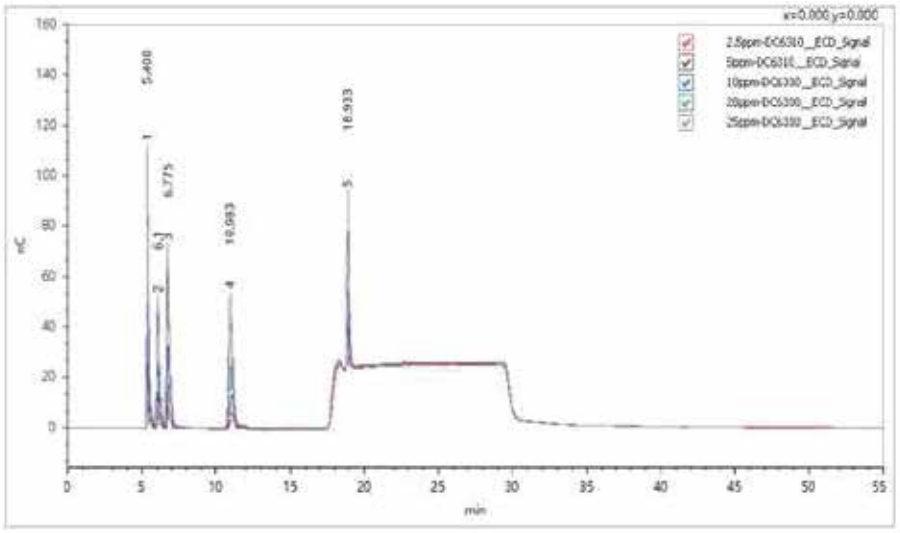
NaOH gradient elution table				A: Purewater	B:200mM NaOH
Serial Number	Time- linear flow rate	(mL/min) A%			B%
1	0	linear	0.7	94	6

Linear values of five sugars (2.5 mg/L~200 mg/L)

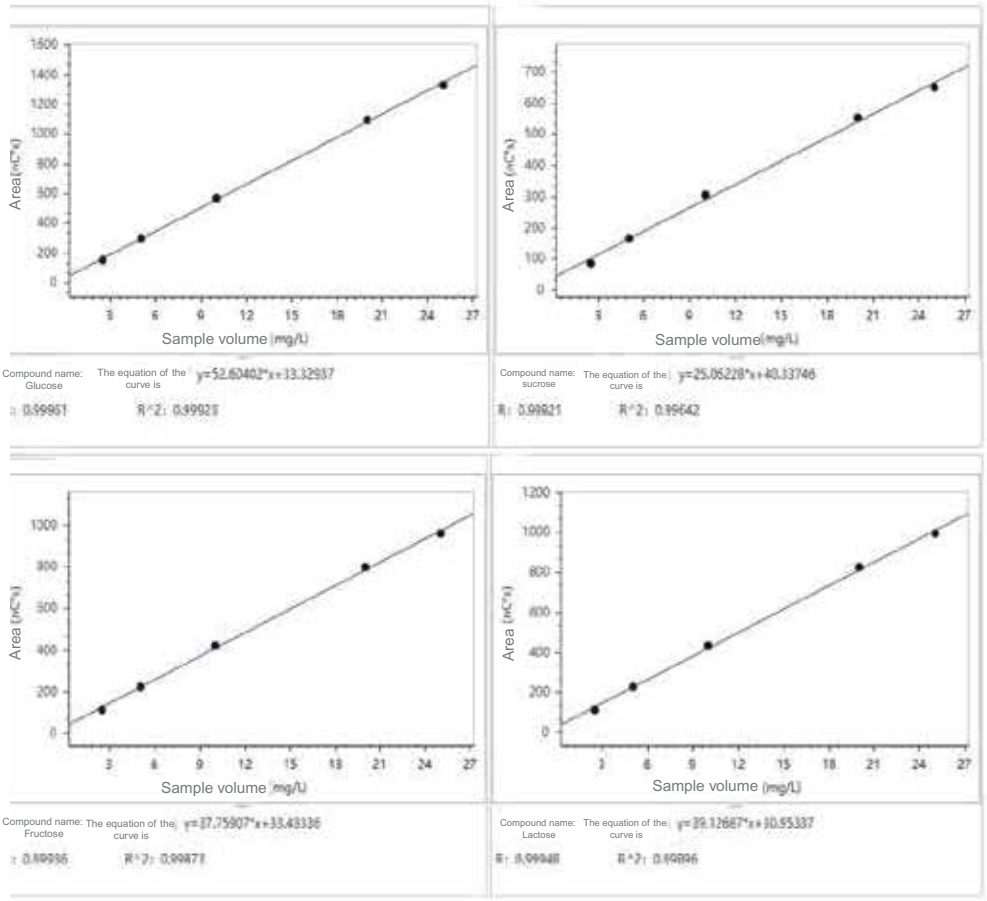


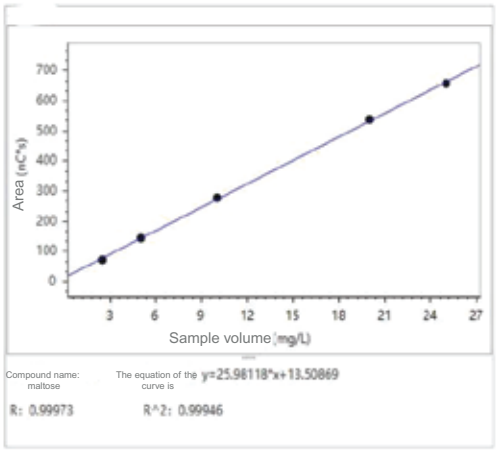
Note: The linear correlation coefficient of the HyperCHROMZ column for testing the concentrations of five sugars in the range of 2.5 mg/L to 200 mg/L is [missing information]. The value is 0.980~0.998, which does not meet the test requirement that the linear correlation coefficient R is greater than 0.999.

Overlapping graph of standard curve test results (2.5 mg/L~200 mg/L)



Linear values of five sugars (2.5 mg/L~25 mg/L)

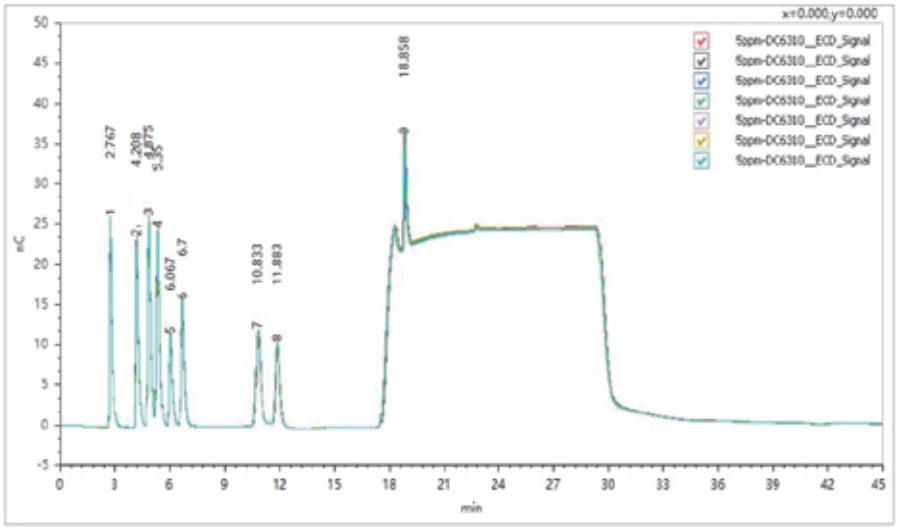




Note: The linear correlation coefficients for the five sugars tested using the HyperchromeZ column within the concentration range of 2.5 mg/L to 25 mg/L were all greater than [value missing].
0.999, which meets the testing requirements.

2.2.2 Nine-sugar mixed standard test (refer to HyperCHROMZ column instruction manual)

Spectrum of nine sugar mixed standard test



Testdata

Compound Name	Retention Time (min) Peak Area (nC*s)	Resolution	Tailing Factor	Theoretical	Plate Number
Fucose	2.767	251.023	6.004	2.179	2326
Arabic sugar	4.217	243.948	2.562	1.850	4424
Galactose	4.883	262.695	1.735	1.513	5359
glucose	5.358	260.66	2.444	1.632	5834
fructose	6.075	135.811	2.047	1.518	6308
sucrose	6.717	196.694	10.737	1.590	7001
lactose	10.858	207.576	2.282	1.338	9298
lactulose	11.917	186.305	20.02	1.331	9977

maltose	18.842	126.372	n.a.	2.007	123357

Note: When testing a mixed standard of 9 sugars using an HyperCHROMZ column, the resolution of the 9 sugars ranged from 1.735 to 20.02, indicating that most sugars were well separated.
The separation efficiency was around 2.0, indicating only average separation performance, not as good as described in the instructions; the tailing factor of the 9 sugars was...
Between 1.331 and 2.179, the trailing effect is quite noticeable.

Compoundname	Fucose		Storage time		Galactose	
Serial Number	Retention time (min)	peak area (nC*s)	ofarabinose (min)	Peak area (nC*s)	Retention time (min)	Peak area (nC*s)
1	2.767	251.023	4.217	243.948	4.883	262.695
2	2.767	250.815	4.208	243.817	4.875	259.984
3	2.767	250.859	4.208	243.061	4.883	260.138
4	2.767	250.542	4.208	242.313	4.883	259.129
5	2.767	250.160	4.208	241.398	4.875	258.974
6	2.767	250.476	4.217	241.188	4.883	257.539
7	2.767	250.230	4.208	240.391	4.875	257.957
average	2.767	250.586	4.211	242.302	4.880	259.488
RSD(%)	0.000	0.130	0.104	0.566	0.088	0.658

Compoundname	Glucose		Fructose		sucrose	
Serial Number	retention time (min)	Peak area (nC*s)	retention time (min)	Peak area (nC*s)	Retention time (min)	Peak area (nC*s)
1	5.358	260.660	6.717	196.694	6.075	135.811
2	5.350	260.285	6.700	196.601	6.058	135.436
3	5.350	259.981	6.708	195.544	6.067	134.269
4	5.350	259.435	6.708	195.422	6.067	133.787
5	5.350	258.940	6.700	194.321	6.067	132.641
6	5.358	258.966	6.708	194.323	6.067	132.431
7	5.350	258.432	6.700	193.405	6.067	131.220
Average	5.352	259.528	6.706	195.187	6.067	133.656
RSD(%)	0.073	0.312	0.094	0.632	0.081	1.247

Compoundname	Lactose		Lactulose		maltose	
Serial Number	retention time (min)	Peak area (nC*s)	retention time (min)	Peak area (nC*s)	Retention time (min)	Peak area (nC*s)
1	10.858	207.576	11.917	186.305	18.842	126.372
2	10.833	207.761	11.883	186.658	18.900	127.273
3	10.842	207.248	11.892	186.042	18.900	126.051
4	10.842	206.417	11.892	185.204	18.892	125.883
5	10.833	205.579	11.892	184.336	18.875	124.433

6	10.842	205.999	11.892	184.106	18.867	125.314
7	10.833	205.021	11.883	183.210	18.858	124.715
Average	10.840	206.514	11.893	185.123	18.876	125.720
RSD(%)	0.083	0.508	0.096	0.694	0.118	0.782

Note: The RSD values for retention time are between 0% and 0.119%, and the RSD values for peak area are betwee 0.130% and 1.247%.

3.1Chromatographic conditions

chromatographic column	PA1ÿ 4.0×250mm		
Flow rate	1.0mL/ min		
Mobile	NaOH gradient elution, see Table 1 below for details.		
injection volume	10ÿL		
column temperature	30ÿ	Detector temperature	35ÿ
working electrode	Auelectrode	Reference electrode	Ag/ AgCl
Detector	Amperedetector	Manual	Pulse Integral
filter time constant	5S	gain configuration for gold	1
waveform selection	electrodes,carbohydrates,and four potentials is detailed in Table 2 below.		

Table 1. NaOH Gradient Elution Table

NaOH gradient elution table				A:Pure water	B:200mM NaOH
Serial Number	time	Linear flow rate	(mL/ min) A%		B%
1	0	linear	1	88	12
2	21	linear	1	88	12
3	21.1	linear	1	0	100
4	33	linear	1	0	100
5	33.1	linear	1	88	12
6	55	linear	1	88	12

Table 2 Waveform Selection

Serial Number	Time	Cell voltage (V)	Start- end integration interval
1	0	0.1	/
2	200	0.1	ÿ
3	400	0.1	ÿ
4	410	-2	/
5	420	-2	/
6	430	0.6	/

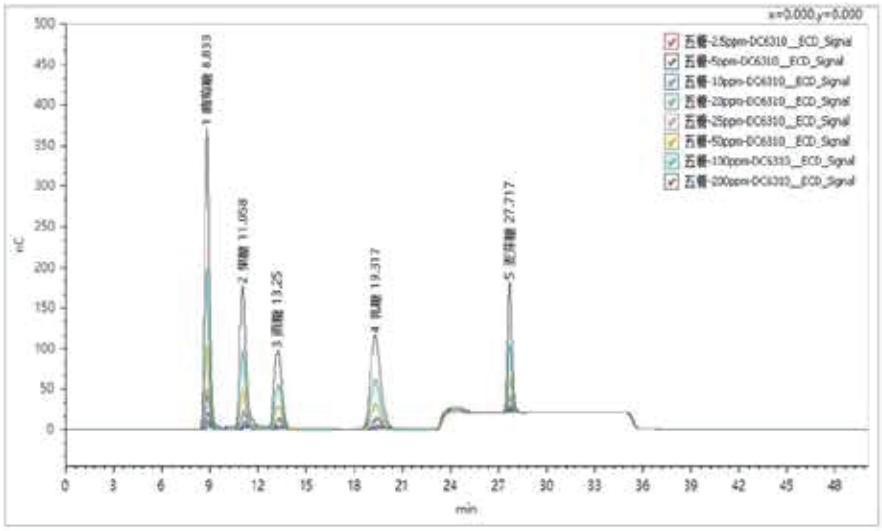
7	440	-0.1	/
8	500	-0.1	/

3.2 Test Spectra and Results

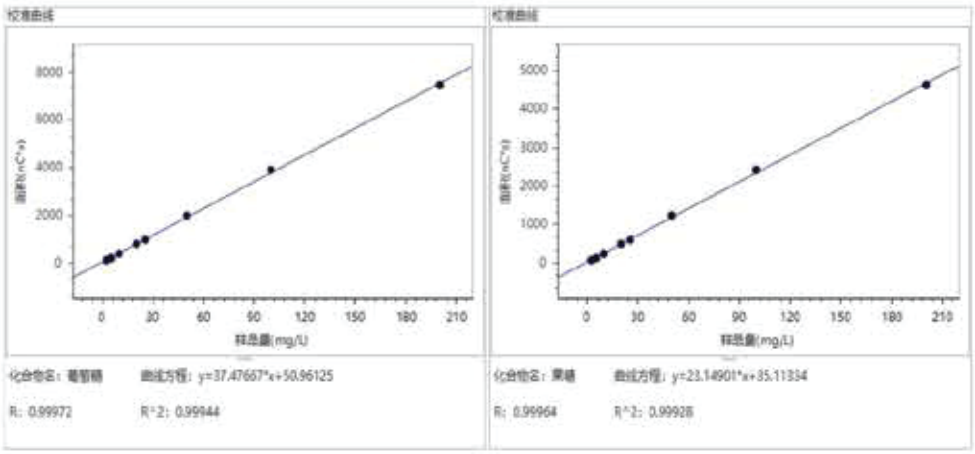
3.2.1 Linearity Test

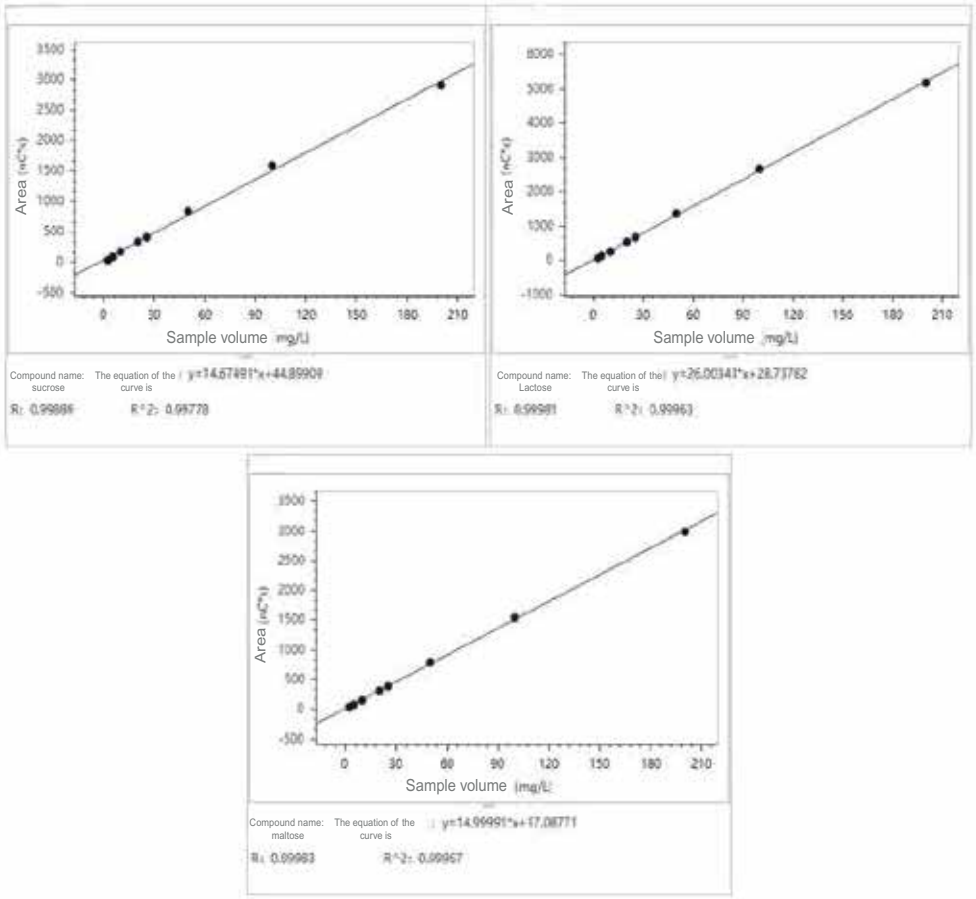
Standard curve series concentration gradient table (mg/L)								
Compound Name	Track1	Track 2	Track 3	Track 4	Track 5	Track 6	Track7	Track 8
Glucose 2.5		5	10	20	25	50	100	200
Sucrose 2.5		5	10	20	25	50	100	200
Fructose	2.5	5	10	20	25	50	100	200
and lactose	2.5	5	10	20	25	50	100	200
Maltose 2.5		5	10	20	25	50	100	200

Standard curve test spectrum overlap plot



Five sugars linear





Note: The linear correlation coefficients of the five sugars within the concentration range of 2.5 mg/L to 200 mg/L were all greater than 0.999, meeting the test requirements.

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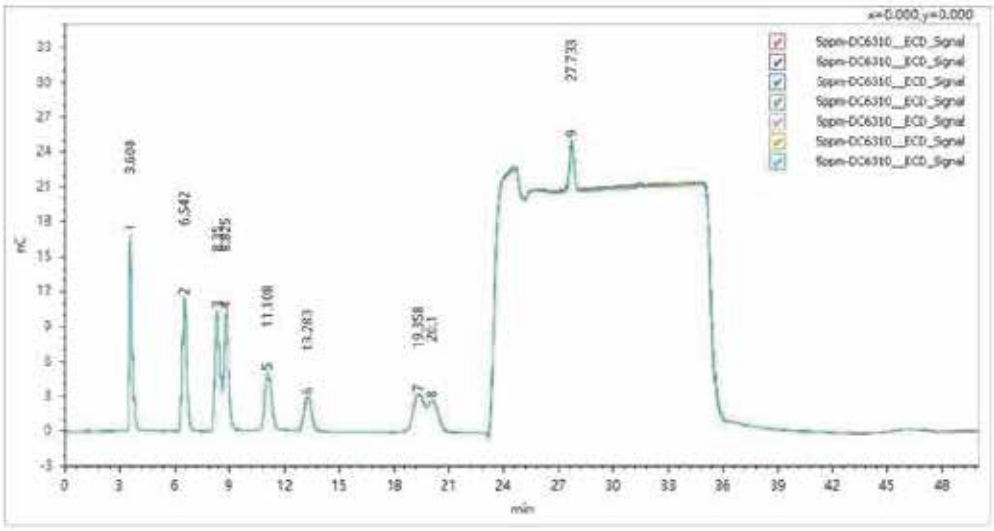
1.2.2 Nine-sugar mixed standard test (refer to HyperCHROMZ column instruction manual)

Spectrum of nine sugar mixed standard test



Test data

Compound Name	Retention Time (min) Peak Area (nC*s)	Resolution	Tailing Factor	Theoretical	Plate Number
Fucose	3.608	183.46	8.947	1.598	2964
Arabic sugar	6.542	174.862	4.464	1.223	4487
Galactose	8.350	122.687	1.1	0.911	6337
glucose	8.825	128.471	4.276	1.21	6308
fructose	11.108	120.787	3.15	1.148	5079
sucrose	13.283	82.765	7.531	1.032	4919
lactose	19.358	56.916	0.866	0.859	8187
lactulose	20.100	36.084	11.443	1.419	8799
maltose	27.733	77.059	n.a.	1.114	54090

Note: PA1 column was used to test a mixed standard of 9 sugars. The resolution between galactose and glucose was 1.1, while the resolution between lactose and lactulose was...
The separation degree was 0.866, and the separation degree of other sugars was greater than 3; the tailing factor of the nine sugars, except for lactose and lactulose, ranged from 0.859 to 1.598.
The abnormal tailing factor of lactose may be due to the poor separation between the two sugars.

Compound name	Fucose		Storage time		Galactose	
Serial Number	Retention time (min)	peak area (nC*s)	ofarabinose (min)	Peak area (nC*s)	Retention time (min)	Peak area (nC*s)
1	3.608	183.460	6.542	174.862	8.350	122.687
2	3.617	183.022	6.542	174.307	8.350	122.965
3	3.617	183.440	6.550	174.772	8.358	123.112
4	3.617	182.725	6.542	174.015	8.350	122.270
5	3.608	183.158	6.542	173.501	8.350	122.348
6	3.608	183.136	6.542	173.412	8.350	122.421
7	3.617	182.727	6.550	173.116	8.367	122.895
average	3.613	183.095	6.544	173.998	8.354	122.671
RSD(%)	0.133	0.163	0.060	0.393	0.079	0.270

Compoundname	Glucose		Fructose		sucrose	
Serial Number	retention time (min)	Peak area (nC*s)	retention time (min)	Peak area (nC*s)	Retention time (min)	Peak area (nC*s)
1	8.825	128.471	11.108	120.787	13.283	82.765
2	8.833	128.141	11.108	120.905	13.300	81.903
3	8.842	128.068	11.117	120.916	13.308	81.812
4	8.833	127.905	11.108	119.963	13.308	81.703
5	8.833	127.697	11.108	120.473	13.308	81.730

6	8.825	127.319	11.108	120.323	13.300	81.417
7	8.842	127.072	11.125	120.529	13.317	81.608
average	8.833	127.810	11.112	120.557	13.303	81.848
RSD(%)	0.079	0.381	0.061	0.287	0.080	0.529

Compound name	Lactose		Lactulose		maltose	
Serial Number	retentiontime (min)	Peak area (nC*s)	retention time (min)	Peak area (nC*s)	Retentiontime (min)	Peakarea (nC*s)
1	19.358	56.916	20.100	36.084	27.733	77.059
2	19.367	57.014	20.108	35.767	27.725	77.260
3	19.375	57.240	20.117	35.554	27.750	77.099
4	19.367	56.611	20.092	35.747	27.725	77.086
5	19.375	56.574	20.108	35.325	27.742	76.497
6	19.358	56.017	20.100	35.823	27.758	76.563
7	19.400	57.577	20.125	34.858	27.742	76.509
Average	19.371	56.850	20.107	35.594	27.739	76.868
RSD(%)	0.074	0.891	0.056	1.125	0.045	0.428

Note: The RSD values for retention time are between 0.045% and 0.133%, and the RSD values for peak area are between 0.163% and 1.125%.

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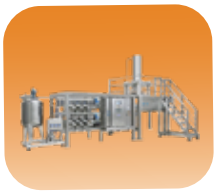

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Corporate & Regd. Office:
Analytical House, # E67 & E68,
Ravi Park, Vasna Road, Baroda,
Gujarat 390 015. INDIA

T: +91 265 2253620
+91 265 2252839
+91 265 2252370
F: +91 265 2254395

E :- info@analyticalgroup.net
info@hplctechnologies.com
info@multiplelabs.com

W:- www.analyticalgroup.net
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