Chinese Pharmacopoeia 2015
Monograph methods

Solutions for regulated Pharmaceutical
Instrumental Analysis
2016-2

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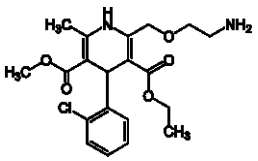
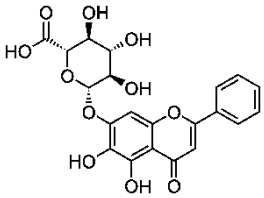
Molecular Structures



Amlodipine Besylate



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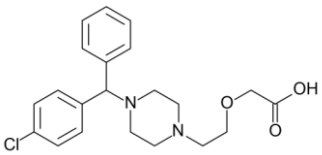
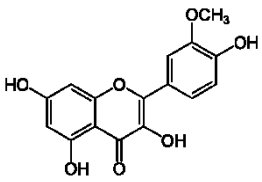
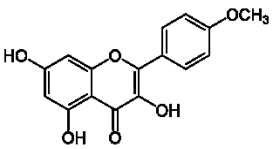


Cetirizine



Isorhamnetin
(3-methylquercetin)



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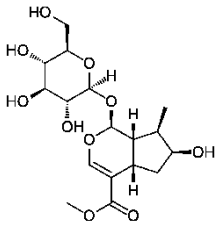

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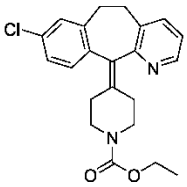
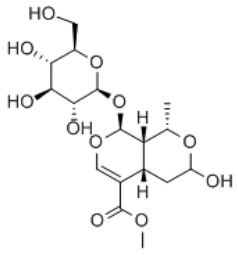


Loratidine



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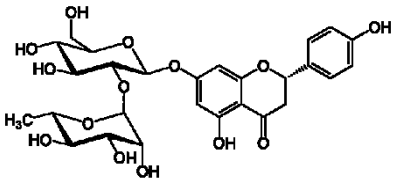

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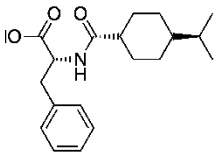

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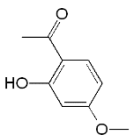

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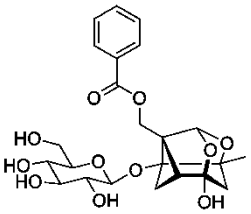
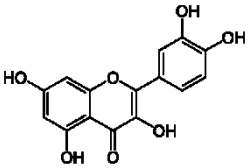
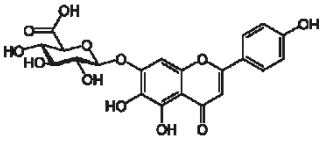


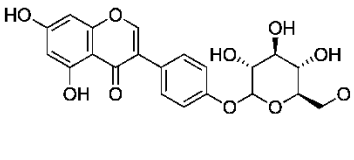
Paeoniflorin



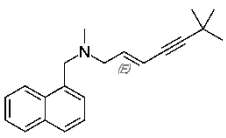
Quercetin



Scutellarin



Sophoricoside



Terbinafine

Application Index

Molecule Name	Products used	Page
Amlodipine Besylate Tablets Benhuangsuan Anludiping Pian 苯磺酸氨氯地平片	Purospher® Star STAR RP-18 endcapped (5µm) 250x4.6 mm Methanol, Acetonitrile, Triethylamine	
Breviscapine tablets Dengzhanhuasu Pian (灯盏花素片)	Purospher® Star STAR RP-18 endcapped (5µm) 150x4.6 mm Acetonitrile, Acetic acid	
Cetirizine Hydrochloride Tablets Yansuan Xitiliqin Pian (盐酸西替利嗪片)	Purospher® Star STAR RP-18 endcapped (5µm) 150x4.6 mm Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm Acetonitrile, Disodiumhydrogen phosphate, Phosphoric acid	
Gingko Leaves Tablets Yinxingye Pian (银杏叶片)	Purospher® Star STAR RP-18 endcapped (5µm) 150x4.6 mm Acetonitrile, Phosphoric acid	
Loratadine Tablets (ChP) Luleitading Pian (氯雷他定片)	Purospher® Star STAR RP-18 endcapped (5µm) 150x4.6 mm Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm Methanol, Disodiumhydrogen phosphate, Phosphoric acid	
Liuwei Dihuang Wan (ChP) Liuwei Dihuang pills 六味地黄丸	Purospher® Star STAR RP-18 endcapped (5µm) 250x4.6 mm Acetonitrile, Phosphoric acid	
Nateglinide tablets (ChP) Nagelienai Pian (那格列奈片)	Purospher® Star STAR RP-18 endcapped (5µm) 150x4.6 mm Chromolith® HighResolution RP-18 endcapped 100x4.6 mm Acetonitrile, Trifluoroacetic acid	
Xiaoyao Wan (逍遥丸) Free and Easy Wanderer pills	Purospher® Star STAR RP-18 endcapped (5µm) 150x4.6 mm Chromolith® HighResolution RP-18 endcapped 100x4.6 mm Acetonitrile, Phosphoric acid	
Huaijiao Wan (槐角丸)	Purospher® Star STAR Phenyl (5µm) 150x4.6 mm Methanol, Acetonitrile, acetic acid	
Terbinafine Hydrochloride Cream Yansuan Tebinaifen Rugao 盐酸特比萘芬乳膏	Purospher® Star STAR RP-18 endcapped (5µm) 150x3.0 mm Methanol, Acetonitrile Isopropanol, triethylamine, acetic acid, hydrochloric acid	

Introduction

This application compilation focus on the new 2015 Chinese pharmacopeia (ChP 2015) edition that officially replaced the previous ChP 2010 on December 1, 2015.

The total number of monographs is 5608, of which 1082 are new, in the new edition.
The number of excipients monographs has increased significantly; from 133 to 270.
The overall structure has changed, from three (3) to four (4) volumes

1. Traditional Chinese Medicine (TCM)
2. Chemical Medicine
3. Biologics
4. General Chapters (appendices), guidances and exipients monographs with new structure.

Coding	Category
0100 Series	General Chapter for Drug Formulation
0200 Series	Other General Chapter
0300 Series	General Identification Test
0400 Series	Spectroscopic Methodology
0500 Series	**Chromatography**
0600 Series	Physical Constants Test Method
0700 Series	Other test Method
0800 Series	Limit Test
0900 Series	Physical Properties Test method
1000 Series	Molecular Biological Technique
1100 Series	Biological Test Method
1200 Series	Biological Activity Test Method
2000 Series	Traditional Chinese Medicine Related Test Method
3000 Series	Biological Product Related Test Method
8000 Series	Reagents and Standard Substance
9000 Series	Guidance

The China Food and Drug Administration
http://eng.sfda.gov.cn

This application book focus on the chromatographic aspects of testing, and monographs have been selected from both Chemical Medicines as well as Traditional Chinese Medicines (TCM's).

Chapter 512 deals with HPLC where you find the general requirement for instruments and experimental conditions. In separate sections you find:

- general introduction for Columns (RP, NP, ion exchange, Chiral etc)
- mobile phases
- Detectors

As in other pharmacopeia clear guidance are provided, pointing out allowed/not allowed changes within packing material/stationary phase, mobile phase components, detector type etc.

Under system suitability tests (SST's)

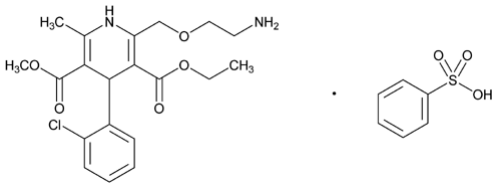
Repeatability

For internal standard method, inject five (5) injections of standard solution, RSD of peak area should be lower than 2.0%

For external standard method, inject three different concentration of standard solution twice, mean calibration factor should be lower than 2.0%.

On the following pages you will find a number of examples where we have used our HPLC columns combined with high purity solvents and reagents to comply with the set system suitability criteria for a number of monographs in the new ChP 2015.

Amlodipine Besylate Tablets
Benhuangsuan Anludiping Pian
苯磺酸氨氯地平片



Amlodipine Besylate

Original Manufacturer	Pfizer (Patent expired 2007)
Original Brand Name	Norvasc
Generic Names	Aforbes, Agen, Aken, Amlosun, Amcard, Amdepin, Amdipin, Amlodine, Amlodipine 5, Amlopin, Amlopine, Amlovasc, Asomex, ATECARD-AM, Camlodin, Dailyvasc, Istin, Lodopin, Lopin, Nopidin, Perivasc, Tenox...

Amlodipine (as besylate, mesylate or maleate) is a calcium channel blocker, and used as an anti-hypertensive and for treatment of angina. Amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance, thereby reducing blood pressure.

The experimental conditions in the new ChP for Amlodipine besylate tablets are similar to the corresponding USP monograph. In ChP it is written Phenomenex Luna C18, 250x4.6 mm, 5 µm, and in the USP the liquid chromatograph should use a 150x3.9 mm column that contains 5 µm C18 packing.

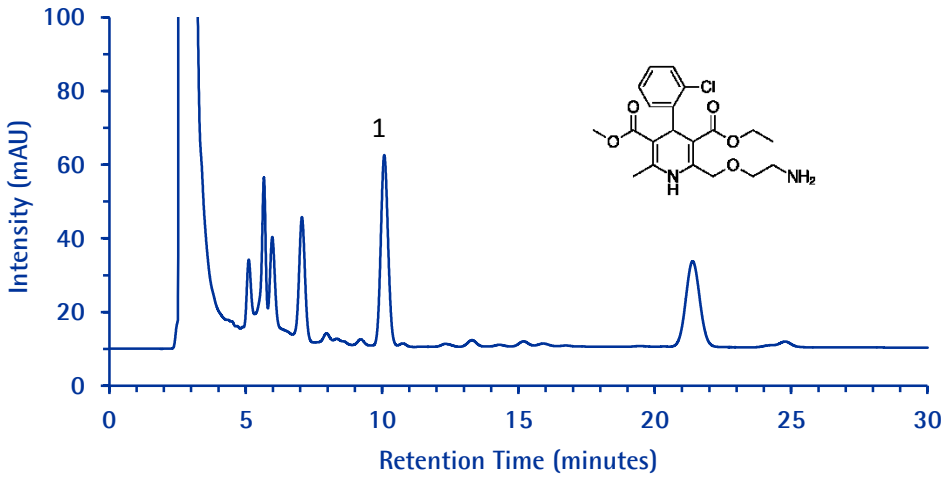
As shown on the following pages, a 250x4.6 mm Purospher® Star STAR RP-18 endcapped column with 5 µm particles can be excellent alternative.

Amlodipine Besylate Tablets (ChP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® Star STAR RP-18 endcapped (5µm) 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	UV @ 237 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Methanol:Acetonitrile:0.7% triethylamine aqueous solution 35:15:50 (v/v). Dissolve 7.0mL triethylamine in 1000mL water, adjust pH 3.0±0.1 with phosphate acid.	
Temperature:	30 °C	
Diluent	Mobile Phase	
Standard:	Dissolve appropriate amount of standard in mobile phase to obtain 50 µg/mL (50 ppm).	
Sample:	Take 10 tablets into a 50-mL volumetric flask, add into 5mL mobile phase, sonicate for 30 minutes. Cool down solution to room temperature, add mobile phase to volume. Shake well, filter, dilute with mobile phase to a concentration of 50 µg/mL (50 ppm).	
Pressure Drop:		

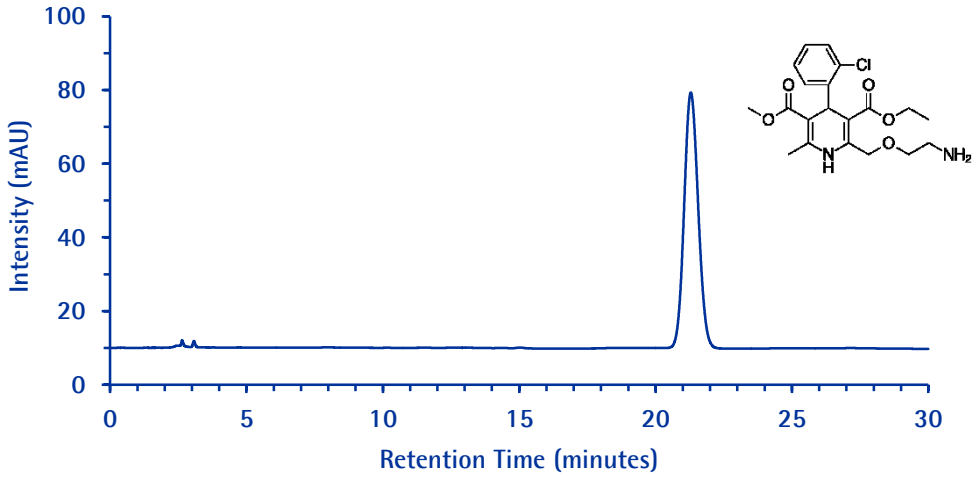


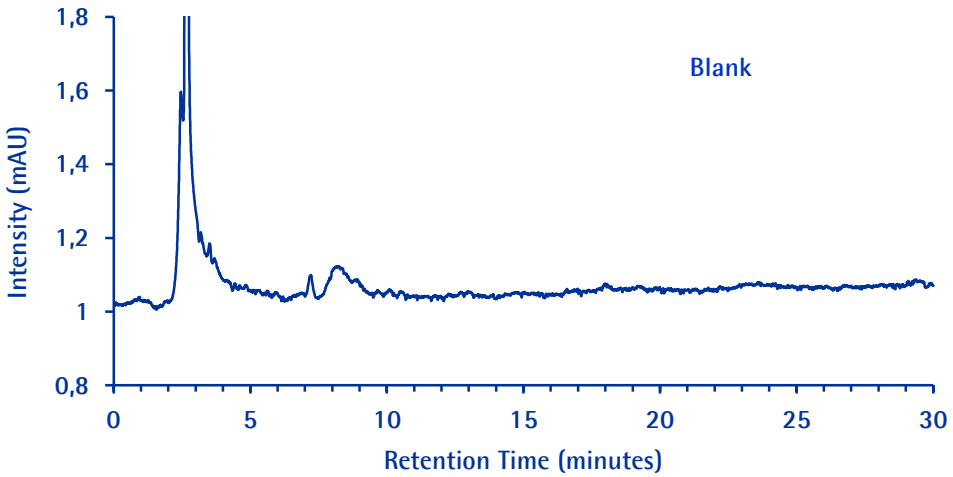
Chromatographic Data (System Suitability Solution)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Impurity	10.1	1.8	1.1	6695
2	Amlodipine	21.5	2.3	1.1	8081

Amlodipine Besylate Tablets (ChP)

Purospher® STAR RP-18 endcapped



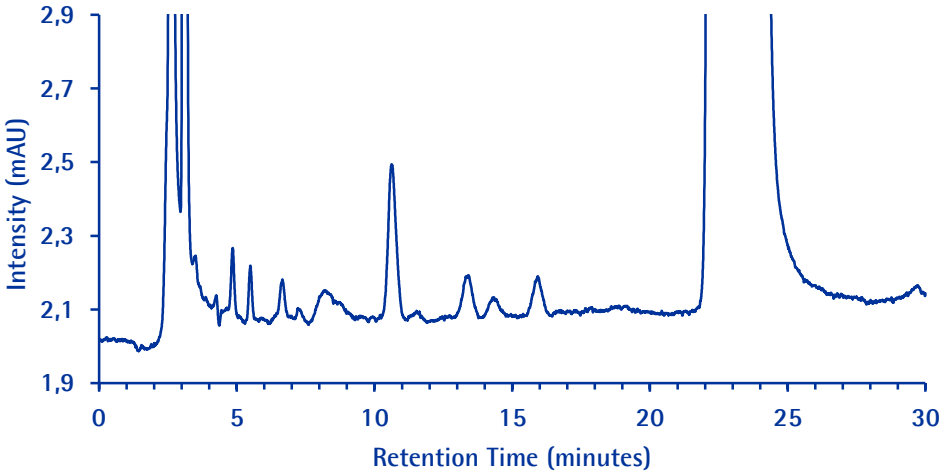


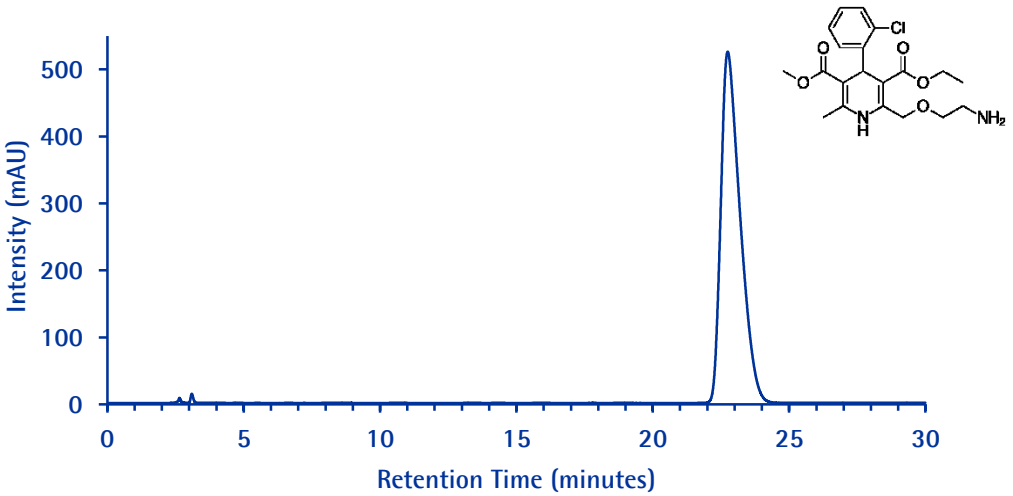
Chromatographic Data (Standard Solution 50 ppm)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
	Amlodipine	21.4	-	1.1	8065

Amlodipine Besylate Tablets (ChP)

Purospher® STAR RP-18 endcapped





Chromatographic Data (Tablet)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
	Amlodipine	22.8	7.0	1.9	4919

Amlodipine Besylate Tablets (ChP)

Validation and Verification Data

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time for Amlodipine and its peak purity.

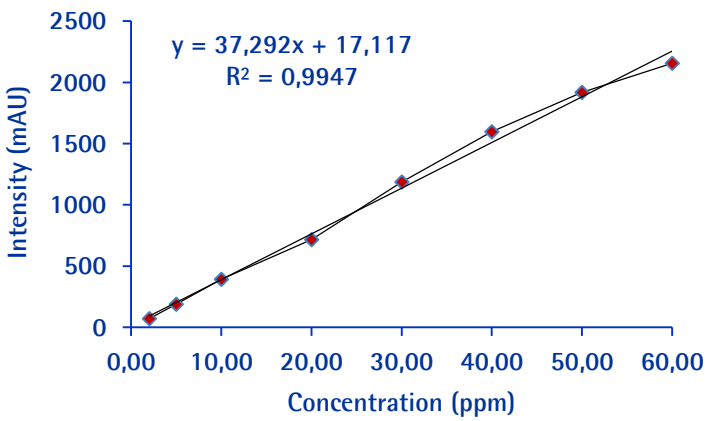
Compound	Retention Time (min)	RRT	Tailing factor	Peak Purity
Amlodipine	21.4	-	1.1	1.0000

2. **Repeatability.**
   Determined by injecting five (5) samples with a solution containing 10 ppm Amlodipine

	Amlodipine (Area units)
Standard 1	392.17209
Standard 2	391.29782
Standard 3	391.78897
Standard 4	391.95859
Standard 5	391.72211
Mean	391.788
Stdev	0.325
RSD (%)	0.1

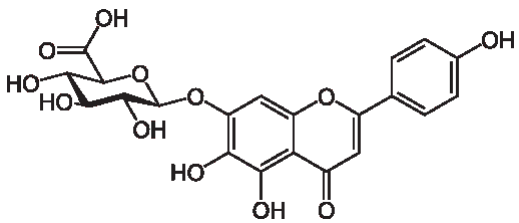
3. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting eight (8) concentration levels from 2-60 ppm of Amlodipine.

	Amlodipine (ppm)	Area
	2.0	71.44292
	5.0	190.08096
	10.0	392.17209
	20.0	716.32916
	30.0	1187.16626
	40.0	1597.69861
	50.0	1917.56018
	60.0	2156.8208
STEYX		63.453
SLOPE		35.710
LOD		5.9
LOQ		17.8



LOD (ppm)	LOQ (ppm)
6	18

Breviscapine tablets (ChP)
Dengzhanhuasu Pian
灯盏花素片



Scutellarin

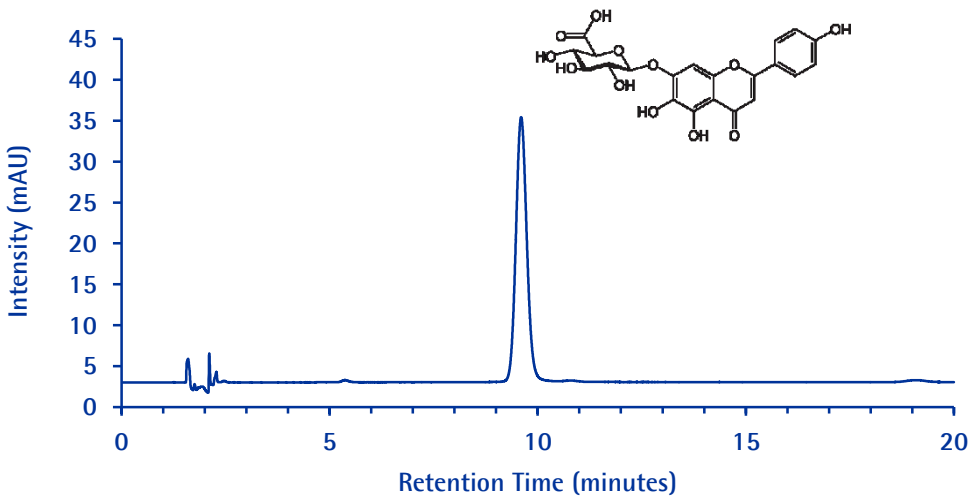
Breviscapine, a traditional Chinese medicine contains scutellarin (a flavone) and as shown on the following pages, a 150x4.6 mm Purospher® Star STAR RP-18 endcapped column with 5 µm particles can be an excellent choice for the analysis of Scutellarin in Breviscapine tablets (Dengzhanhuasu Pian) following the monograph in ChP 2015.

Breviscapine tablets (ChP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® Star RP-18 endcapped (5µm)150x4.6 mm	1.51455.0001
Injection:	10 µL	
Detection:	UV@ 335 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	1% glacial acetic acid solution and acetonitrile 83:17(v/v).	
Temperature:	30 °C	
Diluent	Methanol	
Standard:	Weigh Scutellarin, dissolve with methanol to obtain 30 µg/mL (30 ppm) of standard solution.	
Sample:	Take 10 tablets, weigh precisely, grind to powder. Weight appropriate amount (roughly contained 30mg Breviscapine) to a 50 mL volumetric flask, add methanol into, sonicate for 30 minutes (250W, 40kHZ). Cool down, add methanol to volume. Shake well, filtrate. Take 1 mL filtrate to a 25 mL volumetric flask, add methanol to volume.	
Pressure Drop:	110 Bar (1595 psi)	

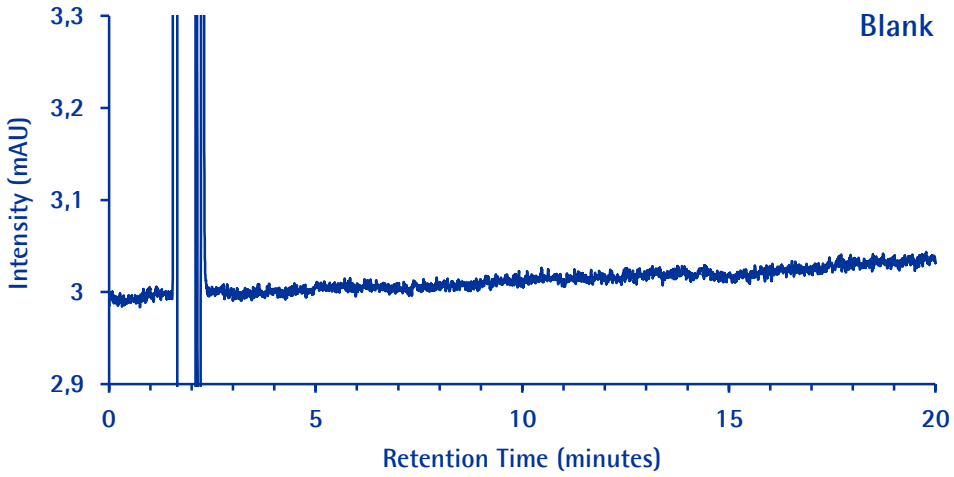


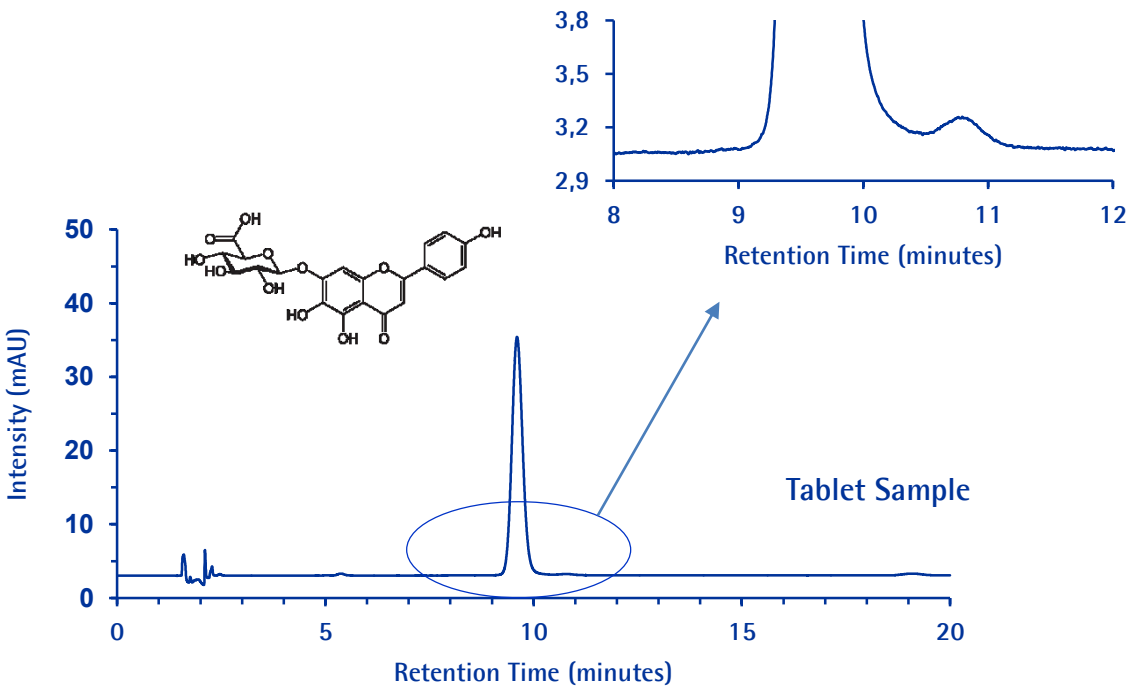
Chromatographic Data (Standard Solution 30 ppm)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
	Scutellarin	9.6	-	1.1	6519

Breviscapine tablets (ChP)

Purospher® STAR RP-18 endcapped





Chromatographic Data (Tablet sample)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
	Scutellarin	9.6	-	1.1	6433

Breviscapine tablets (ChP)

Validation and Verification Data

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time for Scutellarin and its peak purity.

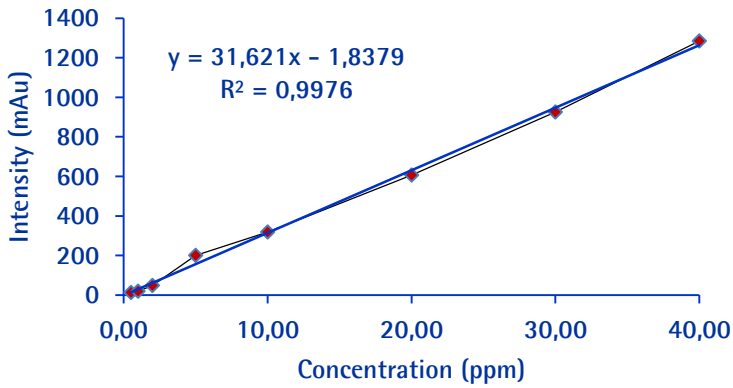
Compound	Retention Time (min)	Plates	Tailing factor	Peak Purity
Scutellarin	9.6	6519	1.1	1.0000

2. **Repeatability.**
   Determined by injecting five (5) samples with a solution containing 2 ppm Scutellarin.

	Scutellarin(Area units)
Standard 1	47.69291
Standard 2	47.51853
Standard 3	47.07962
Standard 4	47.35441
Standard 5	46.96062
Mean	47.32
Stdev	0.303
RSD (%)	0.6

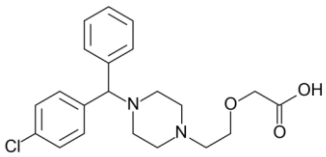
3. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting eight (8) concentration levels from 0.5-40 ppm of Scutellarin.

	Scutellarin (ppm)	Area
	0.50	12.78634
	1.00	18.86366
	2.00	47.69291
	5.00	200.2596
	10.00	318.97092
	20.00	607.05652
	30.00	925.39056
	40.00	1285.10791
STEYX		22.88
SLOPE		30.74
LOD		2.5
LOQ		7.5



LOD (ppm)	LOQ (ppm)
2.5	7.5

Cetirizine Hydrochloride Tablets (ChP)
Yansuan Xitiliqin Pian
盐酸西替利嗪片



Cetirizine

Cetirizine is a second-generation antihistamine.
Used in the treatment of hay fever, allergies, and angioedema
Cetirizine is a major metabolite of hydroxyzine, and a racemic selective H1 receptor antagonist.
Commercial brand names Zyrtec, Reactine...

Following the ChP 2015 monograph method, we first used a 150x4.6 mm Purospher® Star STAR RP-18 endcapped column with 5 µm particles for the analysis of Cetirizine in Cetirizine hydrochloride tablets (Yansuan Xitiliqin Pian).

The method was thereafter transferred to a shorter monolithic column, a 100x4.6 Chromolith® HighResolution RP-18 endcapped. 30% higher separation efficiency was achieved and it was possible to shorten the overall analysis time without loosing performance.

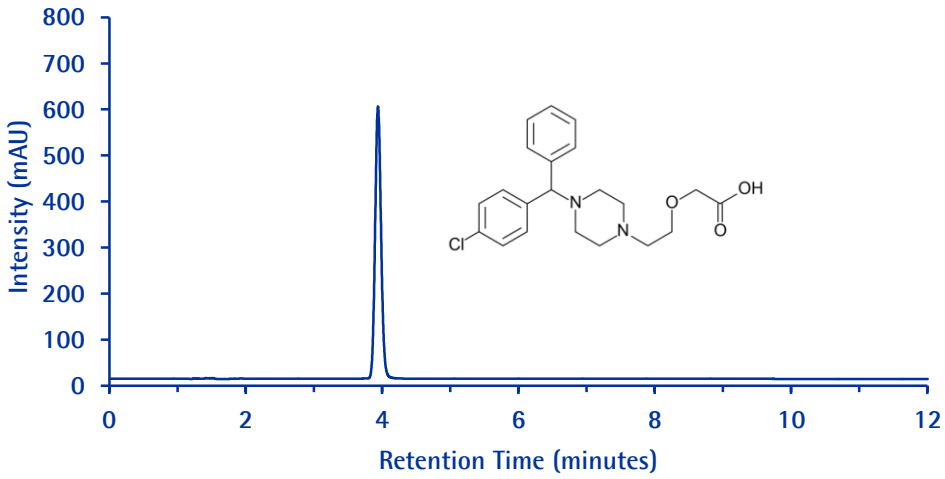
As a comparison, the current USP monograph for Cetirizine Hydrochloride Tablets uses an isocratic HPLC method but where a 250x4.6 mm column is prescribed with 5 µm L1 packing operating at 1.0 mL/min. Albeit possible to change to shorter column dimension/smaller particle size as it is a isocratic method.

Cetirizine Hydrochloride tablets (ChP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® Star RP-18 endcapped (5µm) 150x4.6 mm	1.51455.0001
Injection:	20 µL	
Detection:	UV@ 232 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Buffer: 100mM NaH2PO4 aqueous solution, adjusted to pH 3.7 with H3PO4 Buffer and acetonitrile 60:40 (v/v).	
Temperature:	30 °C	
Diluent	H2O	
Standard:	Weigh appropriate amount of Cetirizine, add diluent to obtain 100 µg/mL (100 ppm) concentration.	
Sample:	Take 10 Cetirizine Hydrochloride tablets, weigh precisely, grind to powder. Weight appropriate amount to a 100-mL volumetric flask, add appropriate amount of H2O. Sonicate, finally add H2O to volume. Filtrate.	
Pressure Drop:	101 Bar (1465 psi)	

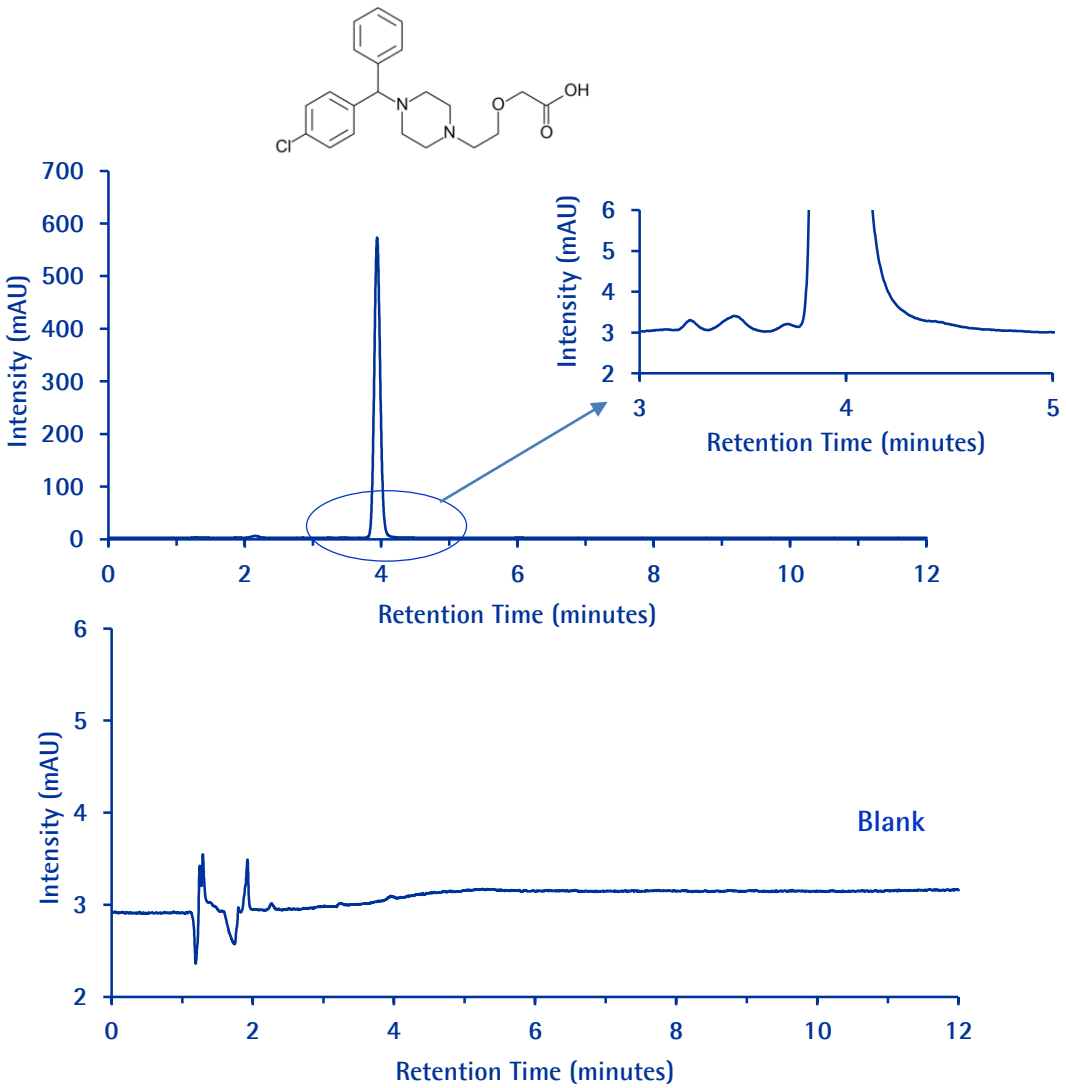


Chromatographic Data (100 ppm standard solution)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
	Cetirizine	3.9	-	1.1	9756

Cetirizine Hydrochloride tablets (ChP)

Purospher® STAR RP-18 endcapped



Chromatographic Data (Tablet sample)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
	Cetirizine	3.9	-	1.1	9709

Cetirizine Hydrochloride tablets (ChP)

Validation and Verification Data – particle packed column

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time for **Cetirizine** and its peak purity.

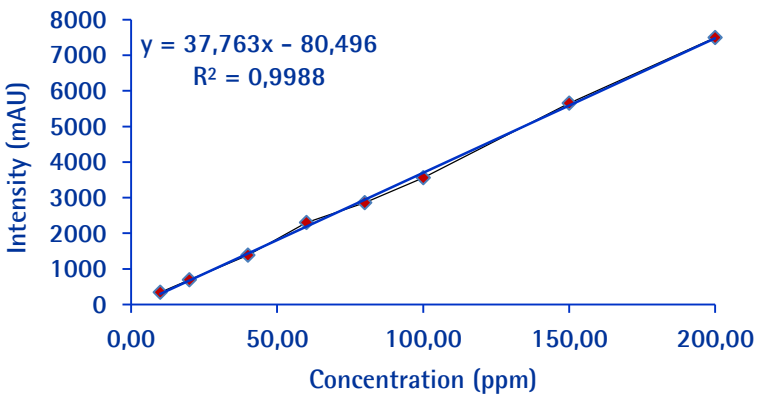
Compound	Retention Time (min)	Plates	Tailing factor	Peak Purity
Cetirizine	3.9	9756	1.1	1.0000

2. **Repeatability.**
   Determined by injecting five (5) samples with a solution containing 10 ppm Cetirizine.

	Cetirizine (Area units)
Standard 1	343.93933
Standard 2	344.14230
Standard 3	344.46487
Standard 4	344.43146
Standard 5	344.60220
Mean	344.3
Stdev	0.269
RSD (%)	0.1

3. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting eight (8) concentration levels from 10-200 ppm of Cetirizine.

	Cetirizine (ppm)	Area
	10.0	343.91907
	20.0	689.21588
	40.0	1385.32129
	60.0	2299.77612
	80.0	2858.01855
	100.0	3554.92944
	150.0	5655.96143
	200.0	7492.14648
STEYX		98.671
SLOPE		37.574
LOD		8.7
LOQ		26



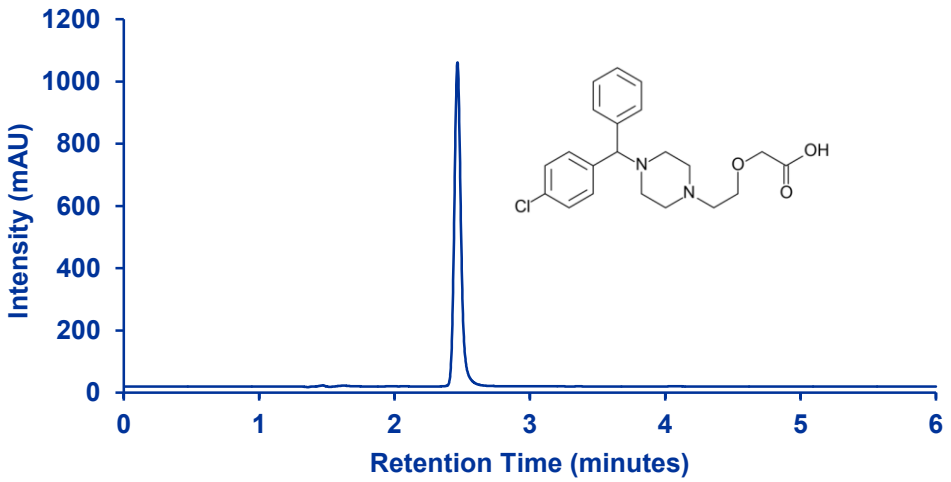
LOD (ppm)	LOQ (ppm)
8.7	26

Cetirizine Hydrochloride tablets (ChP)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	20 µL	
Detection:	UV@ 232 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Buffer: 100mM NaH_2PO_4 aqueous solution, adjusted to pH 3.7 with H_3PO_4 Buffer and acetonitrile 60:40 (v/v)	
Temperature:	30 °C	
Diluent	H_2O	
Standard:	Weigh appropriate amount of Cetirizine, dissolve with H_2O to obtain 100 µg/mL (100 ppm) solution.	
Sample:	Take 10 Cetirizine Hydrochloride tablets, weigh precisely, grind to powder. Weight appropriate amount to a 100-mL volumetric flask, add appropriate amount of H_2O. Sonicate, add H_2O to volume. Filtrate.	
Pressure Drop:	101 Bar (1465 psi)	

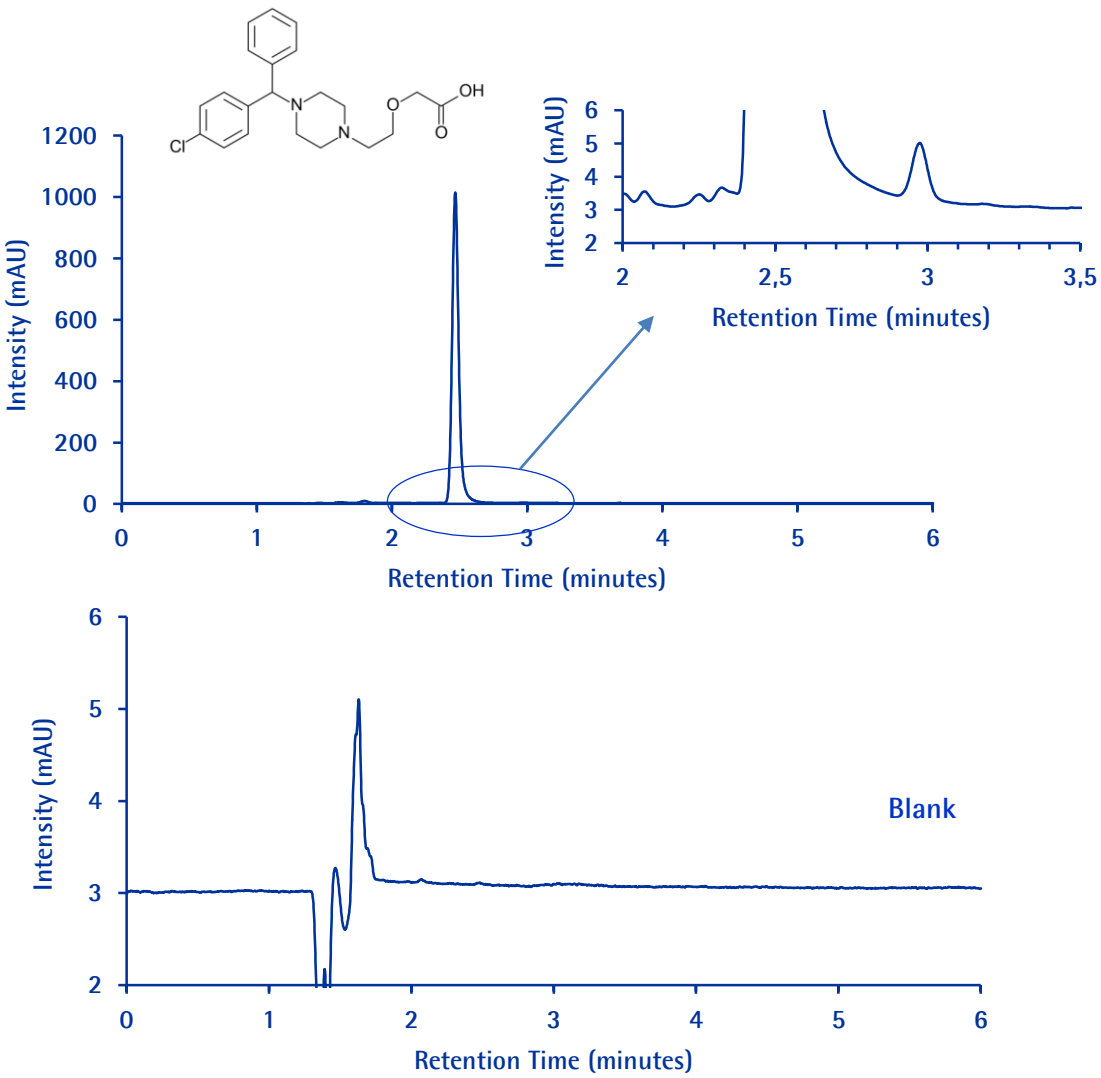


Chromatographic Data (100 ppm standard solution)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
	Cetirizine	2.5	-	1.2	13116

Cetirizine Hydrochloride tablets (ChP)

Chromolith® HighResolution RP-18 endcapped



Chromatographic Data (Tablet sample)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
	Cetirizine	2.5	-	1.2	13239

Cetirizine Hydrochloride tablets (ChP)

Validation and Verification Data – monolithic column

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time for **Cetirizine** and its peak purity.

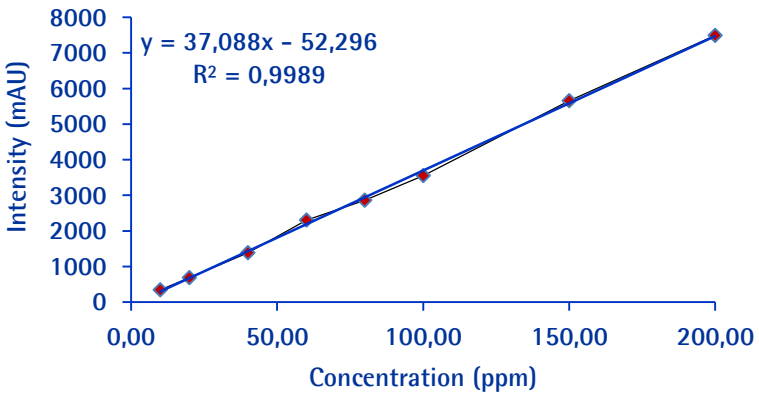
Compound	Retention Time (min)	Plates	Tailing factor	Peak Purity
Cetirizine	2.5	13116	1.2	1.0000

2. **Repeatability.**
   Determined by injecting five (5) samples with a solution containing 10 ppm Cetirizine.

	Cetirizine (Area units)
Standard 1	345.44650
Standard 2	345.69003
Standard 3	345.48767
Standard 4	345.42941
Standard 5	344.81512
Mean	345.37
Stdev	0.329
RSD (%)	0.1

3. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting eight (8) concentration levels from 10-200 ppm of Cetirizine.

	Cetirizine (ppm)	Area
	10.0	344.63641
	20.0	688.53162
	40.0	1384.75415
	60.0	2293.48999
	80.0	2851.61084
	100.0	3538.21655
	150.0	5614.33154
	200.0	7343.92432
STEYX		95,293
SLOPE		37,287
LOD		8,434
LOQ		25,557

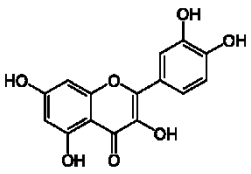


LOD (ppm)	LOQ (ppm)
8.4	25.6

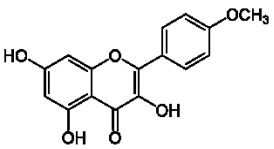
Gingko Leaves Tablets (ChP)

Yinxingye Pian

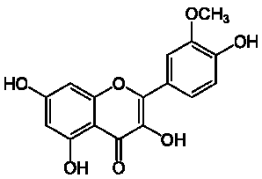
银杏叶片



Quercetin



Kaempferide



Isorhamnetin
(3-methylquercetin)

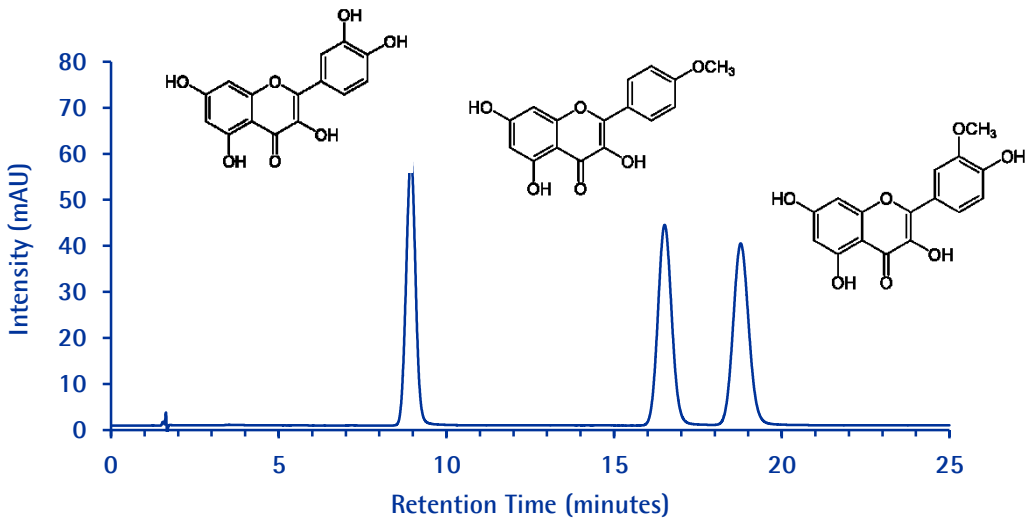
Gingko is commonly used in several traditional Chinese medicines.
Among others it contains Quercetin, Kaempferide and Isorhamnetin (flavones). Shown on the following pages, a 150x4.6 mm Purospher® Star STAR RP-18 endcapped column with 5 µm particles can be an excellent choice for the analysis of Quercetin, Kaempferide and Isorhamnetin in Gingko Leaves Form Tablets (Yinxingye Pian) following the monograph in ChP 2015.

Gingko Leaves Tablets (ChP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® Star RP-18 endcapped (5µm) 150x4.6 mm	1.51455.0001
Injection:	10 µL	
Detection:	UV@ 360 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Mix 0.4% H3PO4 aqueous solution and Methanol; 50:50 (v/v).	
Temperature:	30 °C	
Diluent	methanol	
Standard:	Dissolve appropriate amounte of Kaempferide, Isophamnetin and Quercetin in methanol to obtain a 30 µg/mL (30 ppm) of each in final solution.	
Sample:	Take 10 drug tablets, grind. Take propraite amount of drug powder (Contained 9.6 mg flavonol glycoside),add into 25mL methanol-25% HCL aqueous solution (4:1, v/v).Shake well, put into water bath to heat and reflux for 30 min. Cool rapidly to room temperature, transfer to 50-mL volumetric flask1,add methanol to volume. Shake well filtrate, inject the filtrate for HPLC analysis.	
Pressure Drop:	190 Bar (2755 psi)	

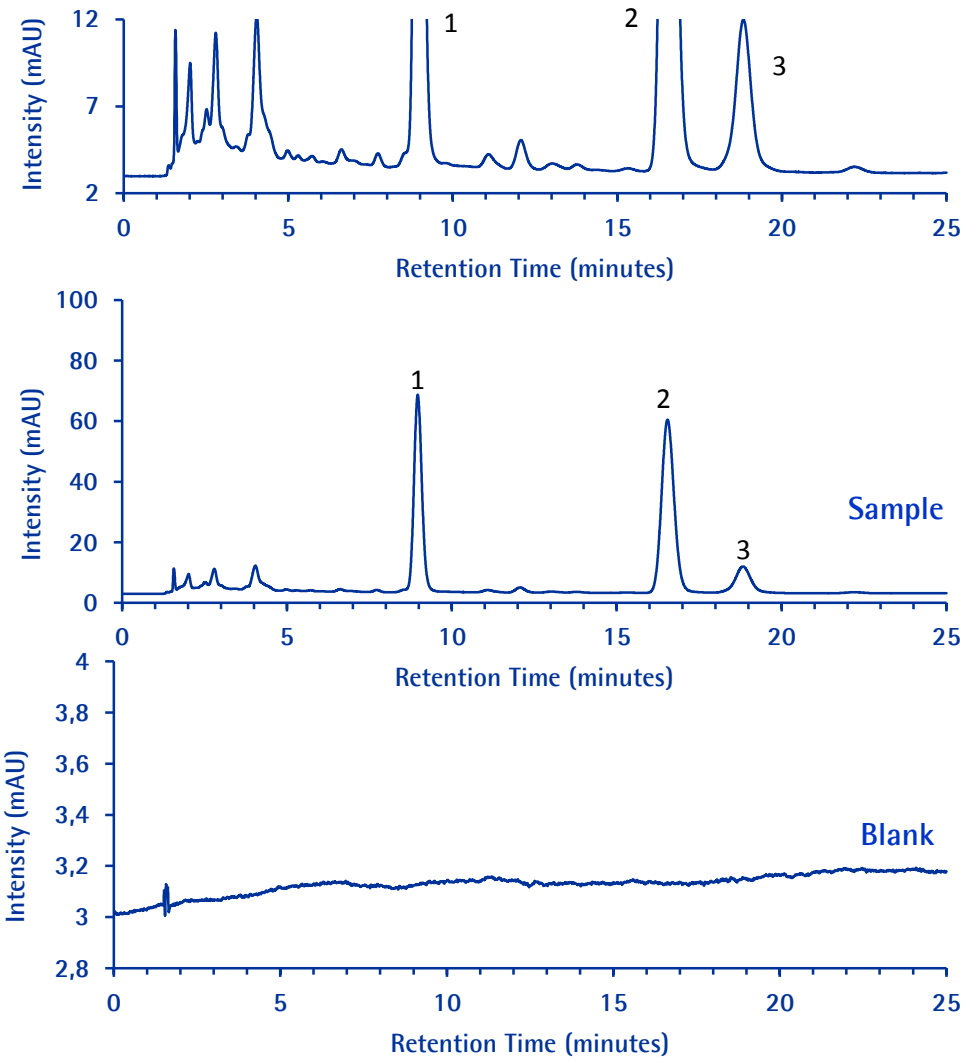


Chromatographic Data (Standard Solution 30 ppm)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Quercetin	8.9	-	1.0	4715
2	Kaempferide	16.5	-	1.1	7541
3	Isorhamnetin	18.8	-	1.1	7670

Gingko Leaves Tablets (ChP)

Purospher® STAR RP-18 endcapped



Chromatographic Data (Tablet sample)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Quercetin	8.9	3.1	1.1	4715
2	Kaempferide	16.5	1.7	1.2	8731
3	Isorhamnetin	18.8	2.9	1.0	7755

Gingko Leaves Tablets (ChP)

Validation and Verification Data

1. Specificity

Determined by injection of SST Solution and determination of the retention time for Quercetin, Kaempferide, and Isophamnetin, and their peak purity.

Compound	Retention Time (min)	Plates	Tailing factor	Peak Purity
Quercetin	8.9	4715	1.0	1.0000
Kaempferide	16.5	7541	1.1	1.0000
,Isophamnetin	18.8	7670	1.1	1.0000

2. Repeatability.

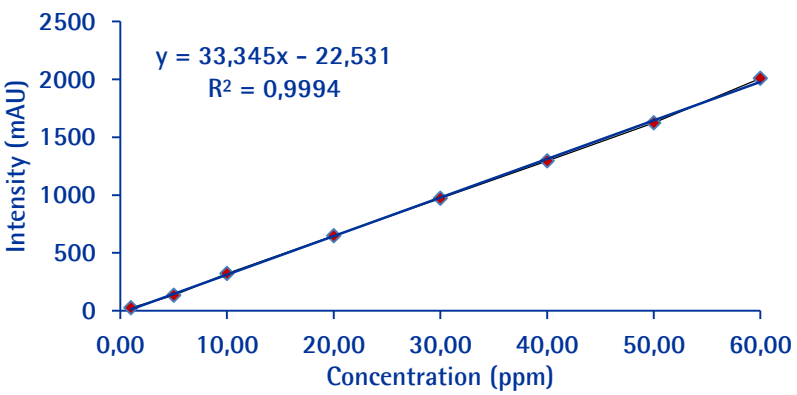
Determined by injecting five (5) samples with a solution containing 10 ppm of each Quercetin, Kaempferide, and Isophamnetin.

	Quercetin (Area units)	Kaempferide (Area units)	Isophamnetin (Area units)
Standard 1	320.61188	345.99832	302.67032
Standard 2	323.42340	348.07788	305.70883
Standard 3	323.76321	348.65967	306.75729
Standard 4	323.10254	349.91046	308.81110
Standard 5	322.20844	347.99069	307.03265
Mean	322.62	348.12740	306.20
Stdev	1.26	1.42	2,27
RSD (%)	0.4	0.4	0.7

3. Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Determined by injecting eight (8) concentration levels from 1-60 ppm of Quercetin.

	Quercetin (ppm)	Area
	1,0	25.46314
	5.0	131.23473
	10.0	320.61188
	20.0	647.20935
	30.0	970.4082
	40.0	1294.71252
	50.0	1624.1223
	60.0	2007.62427
STEYX		9.7668
SLOPE		32.802
LOD		0.98
LOQ		2.98



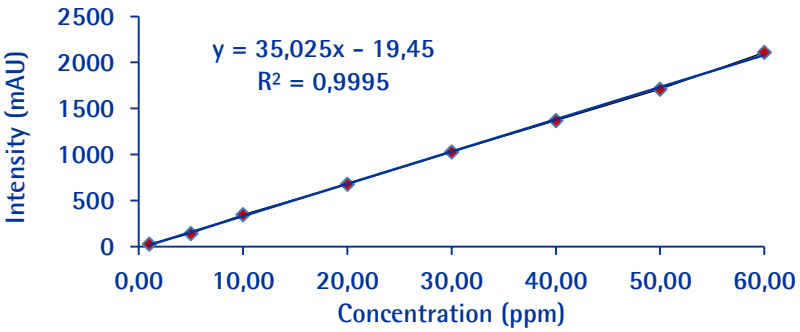
LOD (ppm)	LOQ (ppm)
1	3

Gingko Leaves Tablets (ChP)

Validation and Verification Data

4. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting eight (8) concentration levels from 1-60 ppm of Kaempferide.

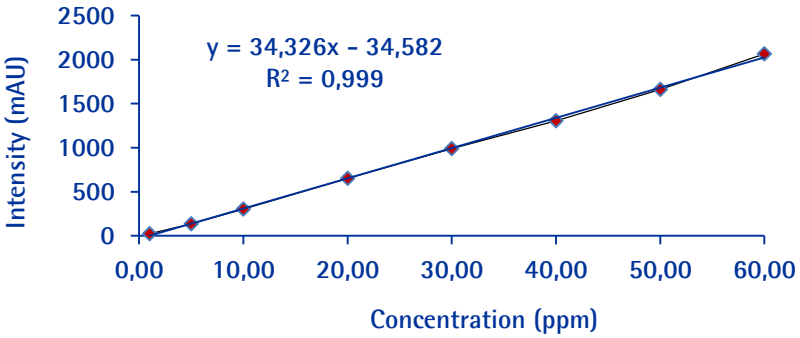
	Kaempferide (ppm)	Area
	1.0	27.47568
	5.0	140.68753
	10.0	345.99832
	20.0	677.09778
	30.0	1029.85388
	40.0	1370.50671
	50.0	1710.15723
	60.0	2107.96362
STEYX		11.329
SLOPE		34.547
LOD		1.1
LOQ		3.3



LOD (ppm)	LOQ (ppm)
1.1	3.3

5. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting eight (8) concentration levels from 1-60 ppm of Isophamnetin

	Isophamnetin (ppm)	Area
	1,0	23.58939
	5.0	135.67494
	10.0	302.67032
	20.0	652.36981
	30.0	988.96863
	40.0	1304.81311
	50.0	1663.04529
	60.0	2066.72437
STEYX		11.591
SLOPE		33.559
LOD		1.2
LOQ		3,5

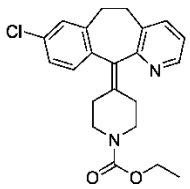


LOD (ppm)	LOQ (ppm)
1.2	3.5

Loratadine Tablets (ChP)

Luleitading Pian

氯雷他定片



Loratidine

Loratadine is a histamine H1-receptor blocker used to treat allergies, and came to market in 1993. Today it is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system. In chemical structure, it is closely related to tricyclic antidepressants, such as imipramine.

Following the ChP 2015 monograph method, we first used a 150x4.6 mm Purospher® Star STAR RP-18 endcapped column with 5 µm particles for the analysis of loratidine in loratidine tablets (Luleitading Pian).

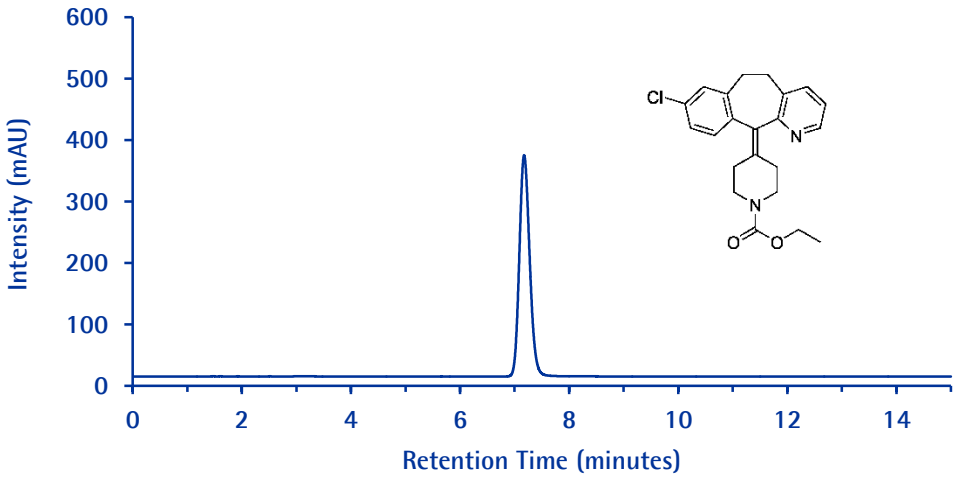
The method was also transferred to a 33% shorter monolithic column. The 100x4.6 Chromolith® HighResolution RP-18 endcapped provided more than 75% higher separation efficiency and 50% lower backpressure due to the superior mass transfer provided by the macropore/mesopore structure in monolithic columns. The loratidine peak eluted in less than half of the time and the overall method sensitivity was also improved.

Loratadine Tablets (ChP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5µm) 150x4.6 mm	1.51455.0001
Injection:	10 µL	
Detection:	UV@ 247 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Buffer: Dissolve 2.28 g K2HPO4 in 800mL H2O, adjust pH 6.0 with H3PO4, add H2O to 1000mL. Mix buffer solution and methanol 20:80 (v/v).	
Temperature:	30 °C	
Diluent	Mobile phase	
Standard:	Weigh appropriate amount of Loratadine to obtain 200 µg/mL (200 ppm). Dissolve with diluent.	
Sample:	Take 20 loratadine tablets, weigh precisely, grind to powder. Weight appropriate amount (roughly contained 10mg loratadine) to a 50-mL volumetric flask, add appropriate amount of mobile phase into,shake. add H2O to volume. Filtrate.	
Pressure Drop:	125 Bar (1813 psi)	

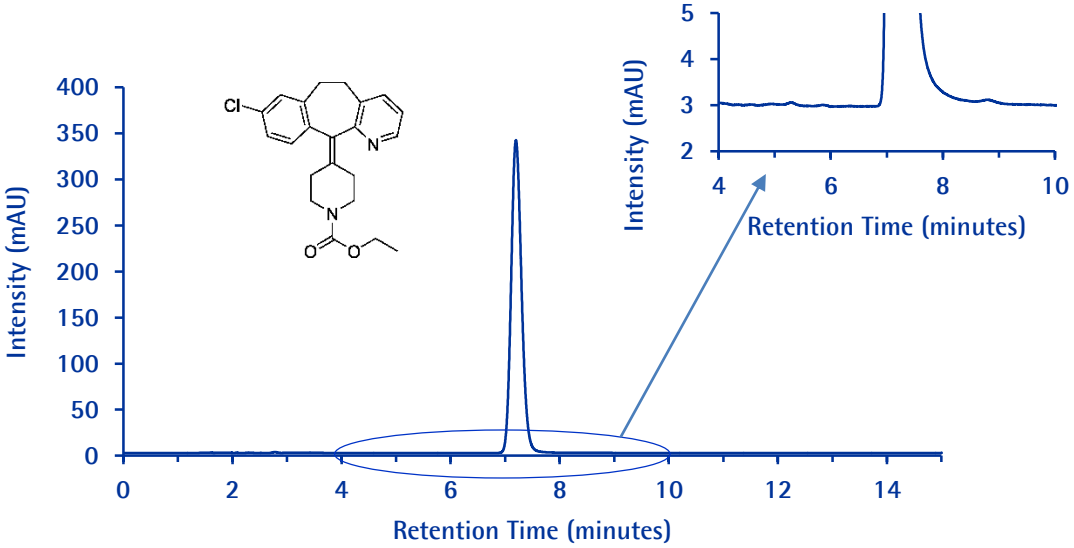


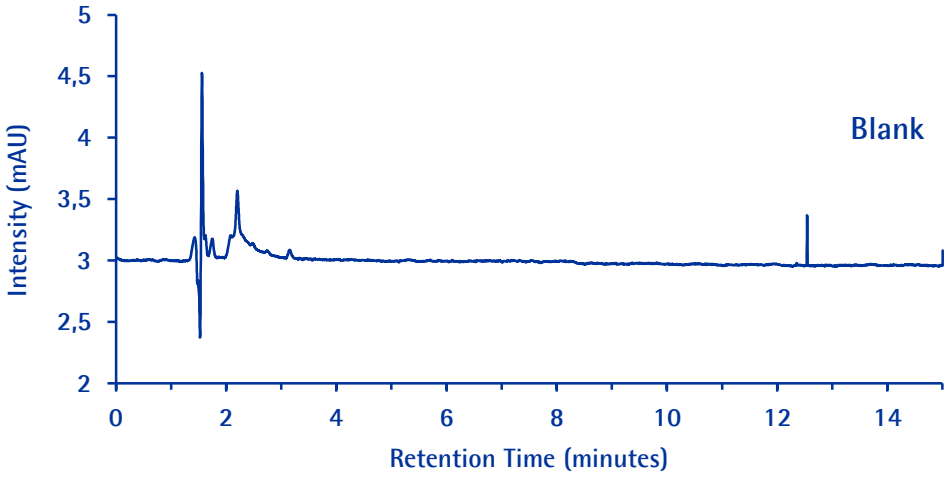
Chromatographic Data (standard solution 200 ppm)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
	Loratadine	7.2	-	1.2	6989

Loratadine Tablets (ChP)

Purospher® STAR RP-18 endcapped





Chromatographic Data (Tablet sample)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
	Loratadine	7.2	-	1.2	6921

Loratadine Tablets (ChP)

Validation and Verification Data – particle packed column

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time for Loratidine and its peak purity.

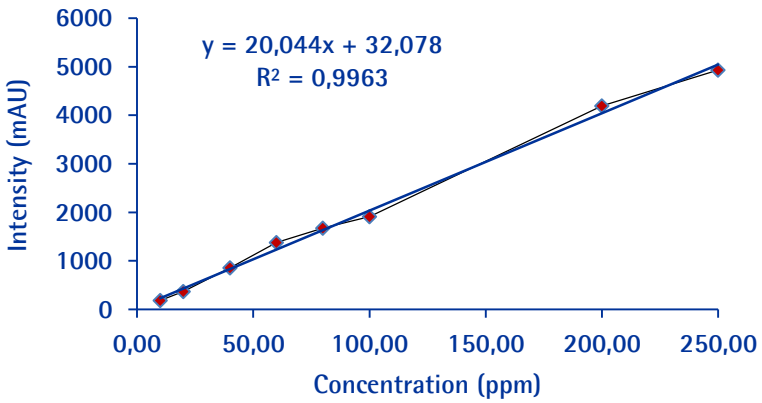
Compound	Retention Time (min)	Plates	Tailing factor	Peak Purity
Loratidine	7.2	6989	1.2	1.0000

2. **Repeatability.**
   Determined by injecting five (5) samples with a solution containing 10 ppm Loratidine.

	Loratidine (Area units)
Standard 1	183.37714
Standard 2	183.47730
Standard 3	183.27551
Standard 4	183.69872
Standard 5	184.10564
Mean	183.59
Stdev	0,3296
RSD (%)	0,2

3. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting seven (8) concentration levels from 10-250 ppm of Loratidine.

	Loratidine (ppm)	Area
	10.0	183.7714
	20.0	368.7944
	40.0	858.21564
	60.0	1374.34155
	80.0	1671.39624
	100.0	1912.24805
	200.0	4191.23535
	250.0	4930.85645
STEYX		98.280
SLOPE		20.842
LOD		15.6
LOQ		47



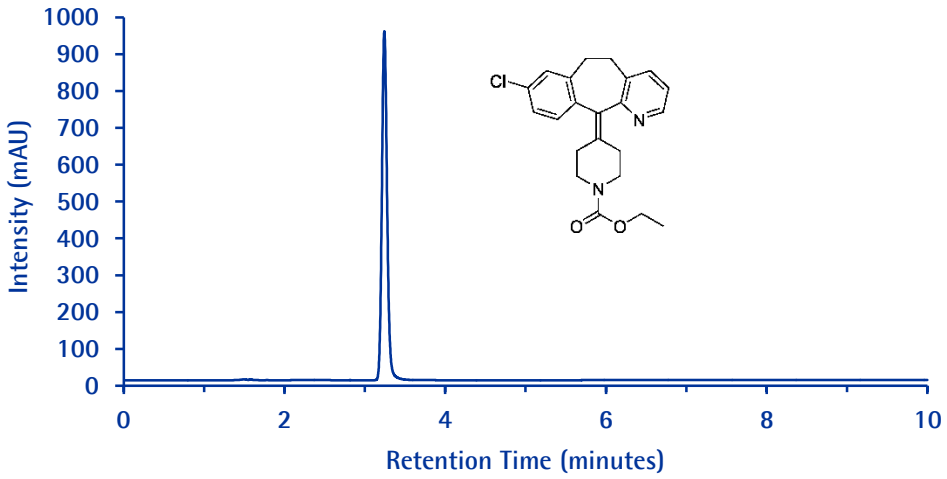
LOD (ppm)	LOQ (ppm)
15.6	47

Loratadine Tablets (ChP)

Chromolith® HighReslution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 100x4.6 mm	1.52022.0001
Injection:	10 µL	
Detection:	UV@ 247 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Buffer: Dissolve 2.28 g K2HPO4 in 800mL H2O, adjust pH 6.0 with H3PO4, add H2O to 1000mL. Mix buffer solution and methanol 20:80 (v/v).	
Temperature:	30 °C	
Diluent	Mobile phase	
Standard:	Weigh appropriate amount of Loratadine to obtain 200 µg/mL (200 ppm). Dissolve with diluent.	
Sample:	Take 20 loratadine tablets, weigh precisely, grind to powder. Weight appropriate amount (roughly contained 10mg loratadine) to a 50-mL volumetric flask, add appropriate amount of mobile phase into,shake. add H2O to volume. Filtrate.	
Pressure Drop:	62 Bar (899 psi)	

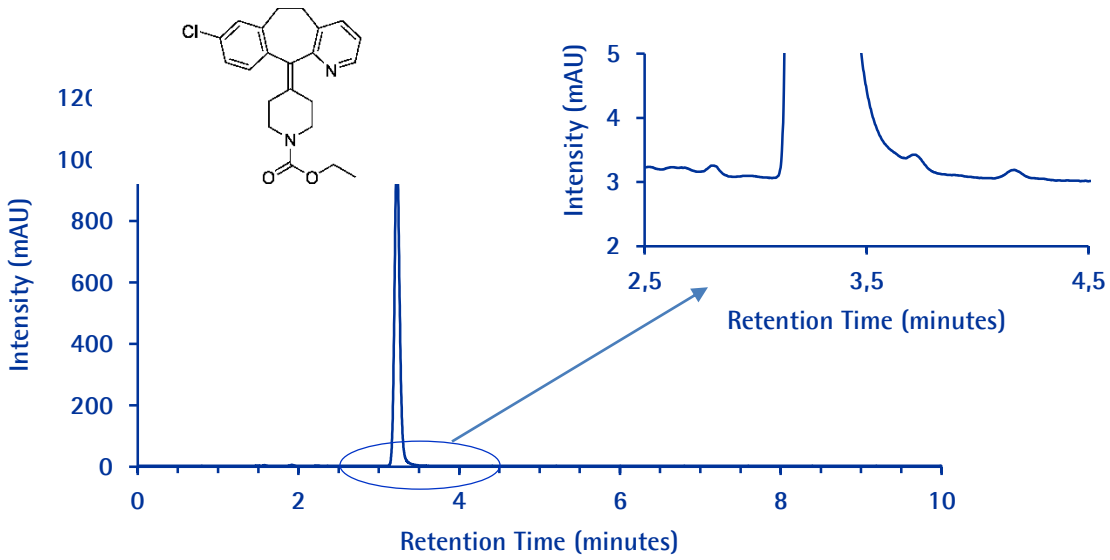


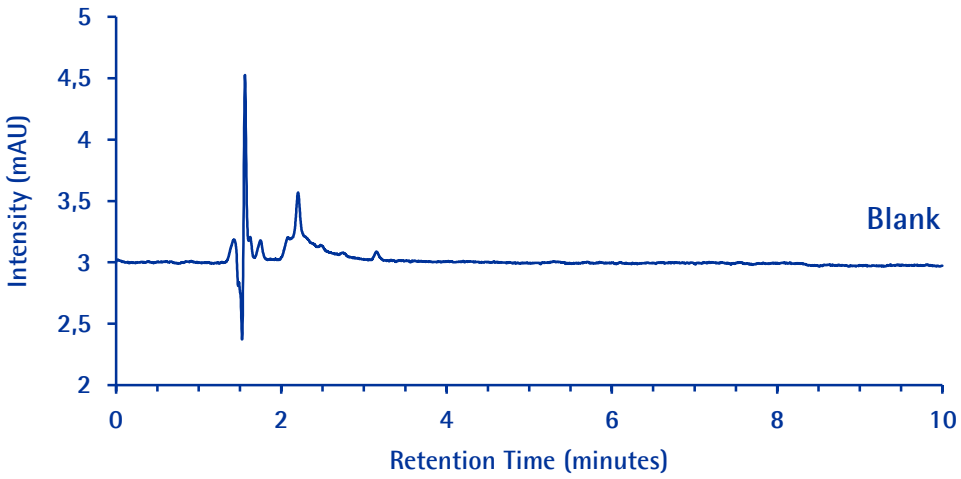
Chromatographic Data (standard solution 200 ppm)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
	Loratadine	3.2	-	1.2	12489

Loratadine Tablets (ChP)

Chromolith® HighReslution RP-18 endcapped





Chromatographic Data (Tablet sample)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
	Loratadine	3.2	-	1.2	12467

Loratadine Tablets (ChP)

Validation and Verification Data – monolithic column

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time for Loratadine and its peak purity.

Compound	Retention Time (min)	Plates	Tailing factor	Peak Purity
Loratadine	3.2	12489	1.2	1.0000

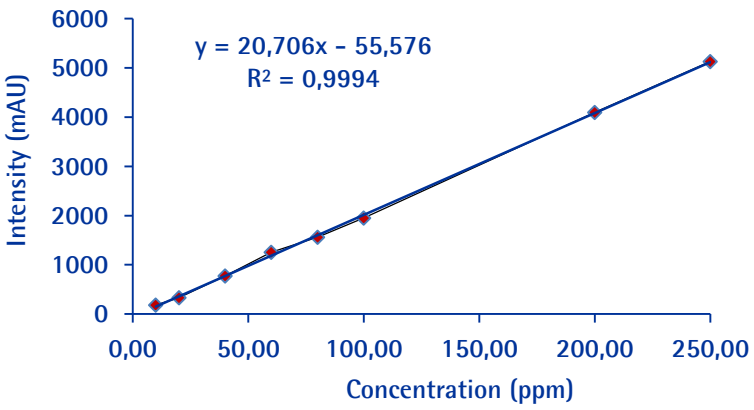
2. **Repeatability.**
   Determined by injecting five (5) samples with a solution containing 10 ppm Loratadine.

	Loratadine (Area units)
Standard 1	183.28259
Standard 2	183.33356
Standard 3	182.88661
Standard 4	182.68979
Standard 5	182.87520
Mean	183.01355
Stdev	0.281
RSD (%)	0.2

- Faster
- Less backpressure
- More sensitive

3. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting eightn (8) concentration levels from 10-250 ppm of Loratadine.

	Loratadine (ppm)	Area
	10.0	183.28259
	20.0	332.18817
	40.0	776.03052
	60.0	1256.86438
	80.0	1563.50977
	100.0	1949.31238
	200.0	4098.21631
	250.0	5132.63867
STEYX		49.624
SLOPE		20.623
LOD		7.9
LOQ		24.1

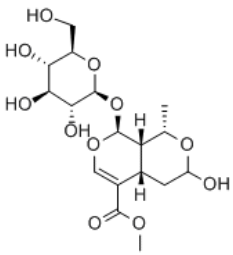


LOD (ppm)	LOQ (ppm)
8	24

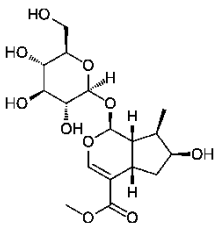
Liuwei Dihuang Wan (ChP)

Liuwei Dihuang pills

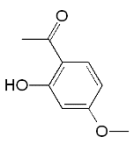
六味地黄丸



Morroniside



Loganin



Paeonol

The Liuwei Dihuang Wan is one of the most important Chinese patent medicines, and is widely used in eastern Asia. It is also known as also known as Liuwei Dihuang pills (六味地黄丸) or Six Flavor Rehmanni ((方剂 fāng jì). From its name one can easily understand that it is a mixture of six famous ingredients. These are Poria, China Root (Fu Ling); Tree Peony Root Cortex (Mu Dan Pi); Chinese Yam (Shan Yao); Asiatic Cornelian Cherry Fruit (Shan Zhu Yu); Rehmannia, Chinese Foxglove Root (Shu Di Huang) and Water Plantain Root (Ze Xie).

The ChP monograph specify Morroniside, Loganin and Paeonol as markers for the Liuwei Dihuang Wan. Shown on the following pages, a 150x4.6 mm Purospher® Star STAR RP-18 endcapped column with 5 µm particles can be an excellent choice for the analysis of these analytes in Liuwei Dihuang teapills.

Liuwei Dihuang Wan (ChP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column: Purospher® Star RP-18 endcapped (5µm) 250x4.6 mm 1.51456.0001

Injection: 10 µL

Detection: UV@ 240, 274 nm

Cell: 1ul/10mm

Flow Rate: 1.0 mL/min

Mobile Phase: Mobile Phase A: Acetonitrile
Mobile Phase B: 0.3% H3PO4 aqueous solution.

Time (min)	Solution A (%)	Solution B (%)
0	5	95
5	8	92
20	8	92
35	20	80
45	60	40
55	60	40

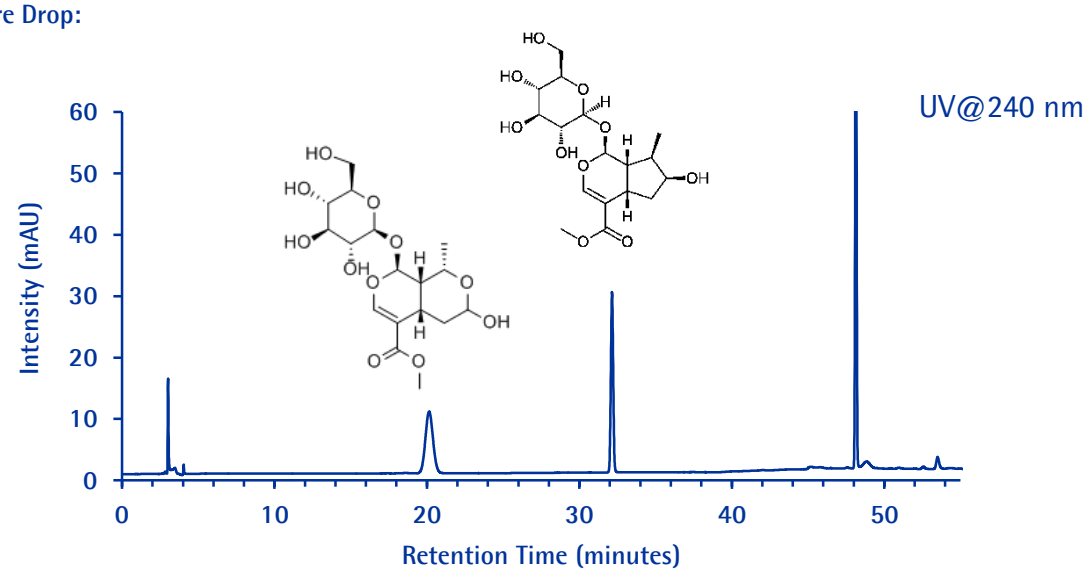
Temperature: 40 °C

Diluent 50% methanol/water solution

Standard: Dissolve appropriate amount of each analyte to give 20 µg/mL (20 ppm) of Morroniside and Loganin and 60 µg/mL (60 ppm) Paeonol in 50% methanol solution.

Sample: Take appropriate amount of drug pills, grind, weigh 1.0g small pills to a Erlenmeyer flask with cover, add into 25 mL 50% methanol aqueous solution, weigh the total weight, heat to reflux for 30minutes, cool down, weigh, make up weight with 50% methanol aqueous solution. Shake well and filter.

Pressure Drop:

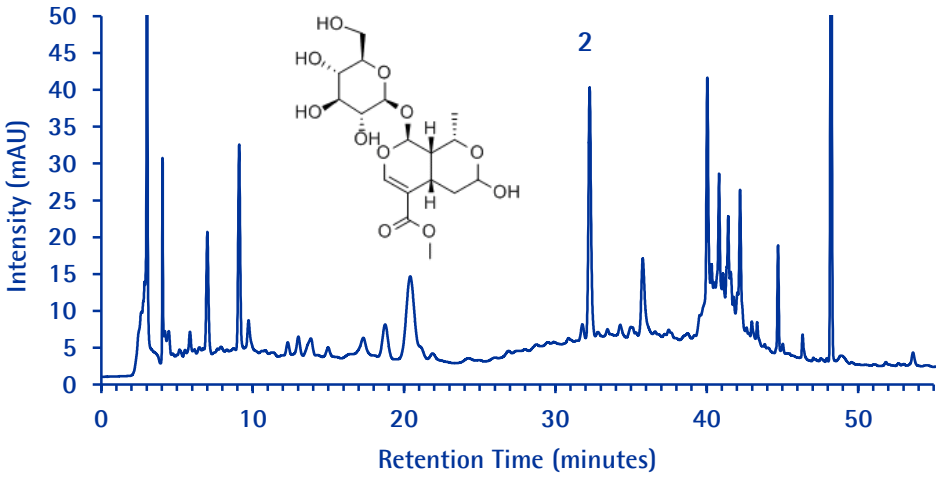


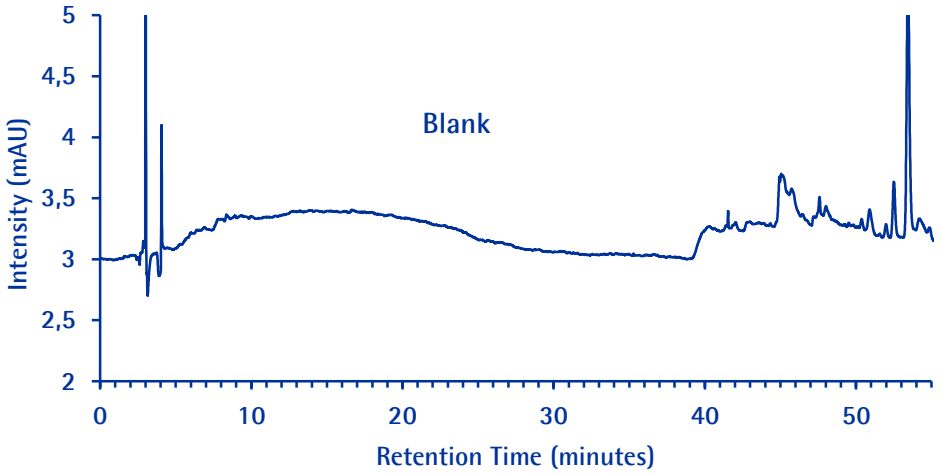
Chromatographic Data (Standard Solution)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Morroniside	20.1	19	1.1	7341
2	Loganin	32.1	65	1.0	201385

Liuwei Dihuang Wan (ChP)

Purospher® STAR RP-18 endcapped



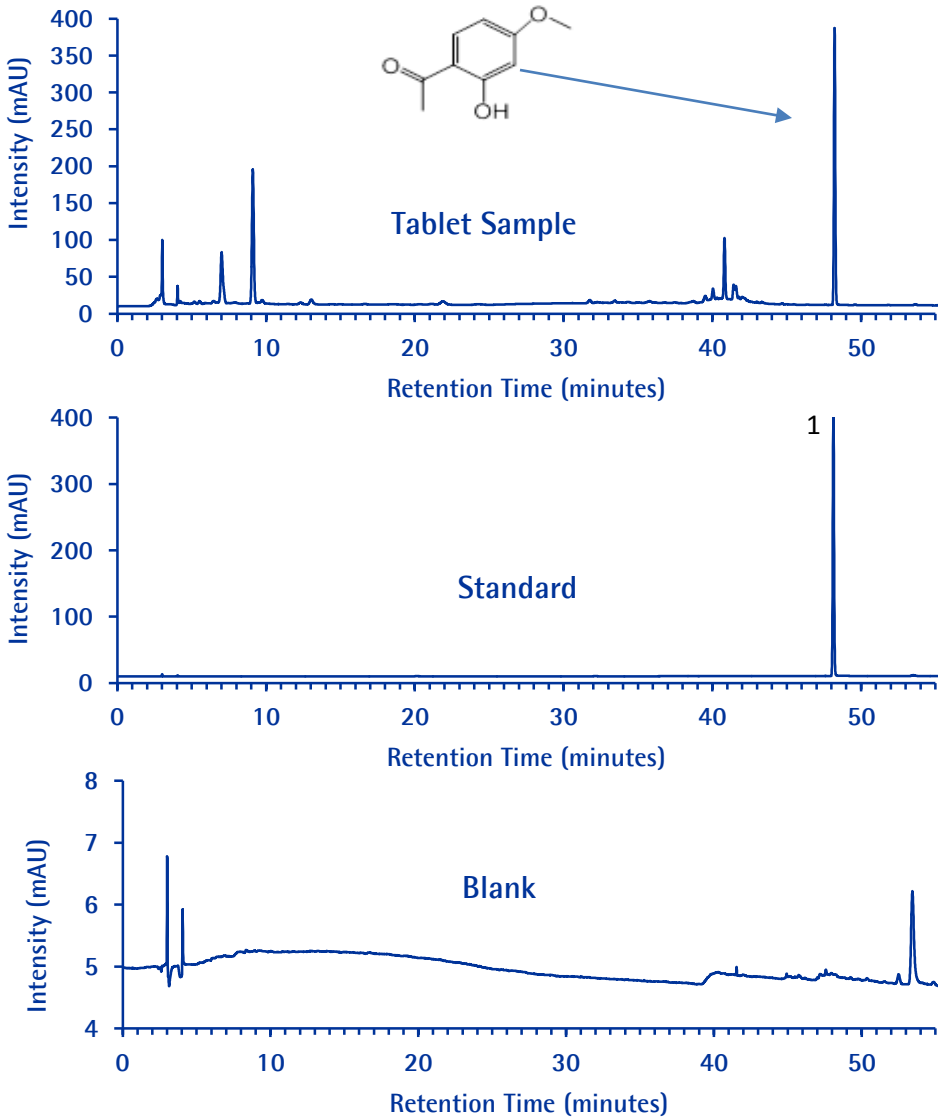


Chromatographic Data (Tablet sample)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Morroniside	20.4	2.0	1.2	6559
2	Loganin	32.3	1.6	1.2	174788

Liuwei Dihuang Wan (ChP)

Purospher® STAR RP-18 endcapped



Chromatographic Data (Tablet sample)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Paeonol	48.1	22.0	1.0	1298184

Liuwei Dihuang Wan (ChP)

Validation and Verification Data

1. Specificity

Determined by injection of SST Solution and determination of the retention time for Morroniside, Loganin and Paeonol, and their peak purity.

Compound	Retention Time (min)	Plates	Tailing factor	Peak Purity
Morroniside	20.1	7341	1.08	1.0000
Loganin	32.1	201385	1.04	1.0000
Paeonol	48.1	1341790	1.05	1.0000

2. Repeatability.

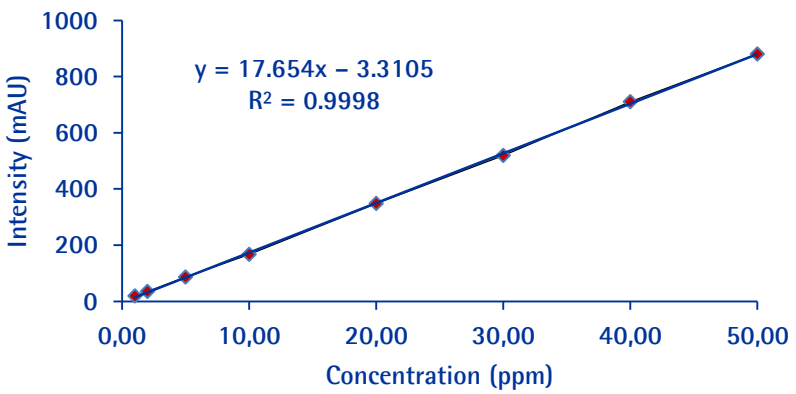
Determined by injecting five (5) samples with a solution containing 30 ppm of each Morroniside, Loganin and Paeonol.

	Morroniside(Area units)	Loganin (Area units)	Paeonol (Area units)
Standard 1	520.52740	478.52881	1611.39001
Standard 2	522.80353	480.26306	1612.67859
Standard 3	523.51221	480.26044	1613.54028
Standard 4	522.63538	479.61017	1613.24109
Standard 5	523.88458	479.80783	1613.69800
Mean	522.67	479.69	1612.91
Stdev	1.30	0.711	0.934
RSD (%)	0.2	0.1	0.1

3. Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Determined by injecting eight (8) concentration levels from 1-50 ppm of Morroniside.

	Morroniside (ppm)	Area
	1.0	18.50785
	2.0	34.48851
	5.0	86.3555
	10.0	167.45831
	20.0	347.55862
	30.0	518.33978
	40.0	709.97937
	50.0	880.23798
STEYX		5.458
SLOPE		17.634
LOD		1.0
LOQ		3.1



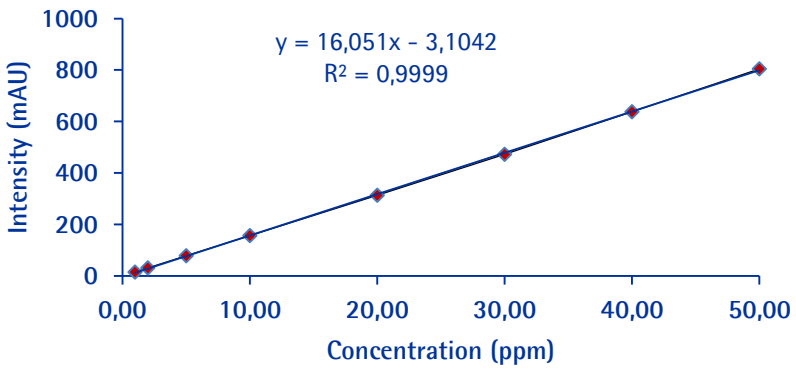
LOD (ppm)	LOQ (ppm)
1	3

Liuwei Dihuang Wan (ChP)

Validation and Verification Data

4. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting eight (8) concentration levels from 1-50 ppm of Loganin.

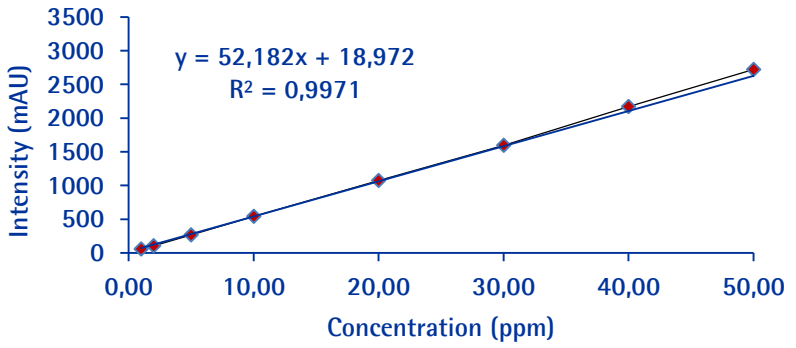
	Loganin (ppm)	Area
	1.0	15.67782
	2.0	30.99024
	5.0	78.47199
	10.0	156.32524
	20.0	313.44232
	30.0	473.07959
	40.0	638.4458
	50.0	804.76831
STEYX		5.1806
SLOPE		17.664
LOD		0.97
LOQ		2.9



LOD (ppm)	LOQ (ppm)
1	3

5. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting eight (8) concentration levels from 1-50 ppm of Paeonol

	Paeonol (ppm)	Area
	1.0	54.87699
	2.0	104.63998
	5.0	264.42062
	10.0	540.19263
	20.0	1072.12329
	30.0	1594.19653
	40.0	2171.53125
	50.0	2722.83228
STEYX		13.938
SLOPE		53.971
LOD		0.85
LOQ		2.6

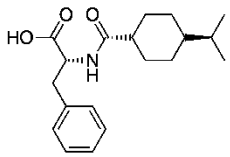


LOD (ppm)	LOQ (ppm)
0.85	2.6

Nateglinide tablets (ChP)

Nagelienai Pian

那格列奈片



Nateglinide

Nateglinide is a drug for the treatment of type 2 diabetes, and was developed by Ajinomoto (JP). It was introduced under the trade name Starlix by Novartis.

Following the ChP 2015 monograph method, we first used a 150x4.6 mm Purospher® Star STAR RP-18 endcapped column with 5 µm particles for the analysis of Nateglinide in Nateglinide tablets (Nagehenai Pian).

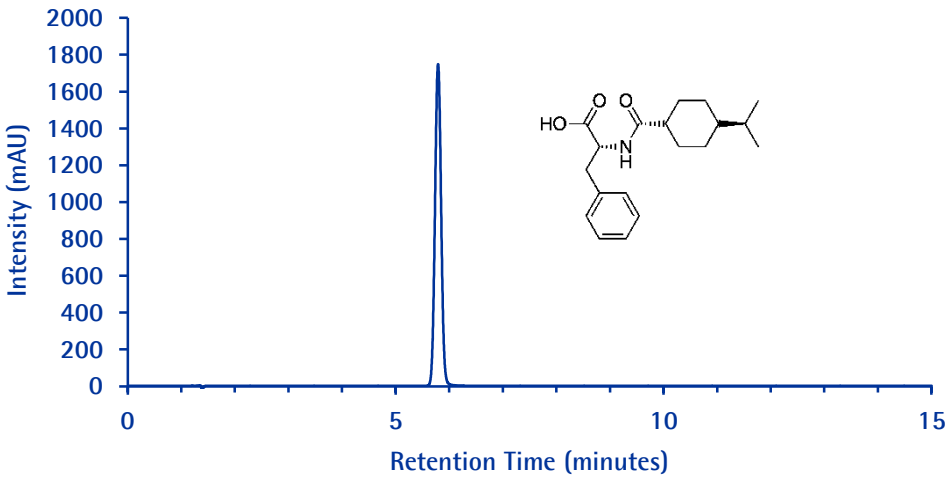
The method was thereafter transferred to a 33% shorter monolithic column. The 100x4.6 Chromolith® HighResolution RP-18 endcapped provided approximately same separation efficiency and thereby similar method sensitivity. The Nateglinide peak eluted in less than half of the time an at 50% lower backpressure, a benefit of the macropore/mesopore structure in monolithic columns.

Nateglinide tablets (ChP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® Star RP-18 endcapped (5µm) 150x4.6 mm	1.51455.0001
Injection:	10 µL	
Detection:	UV@ 210 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	0.05% trifluoroacetic acid solution and acetonitrile; 40:60 (v/v).	
Temperature:	30 °C	
Diluent	55% ACN in H2O	
Standard:	30 µg/mL (30 ppm) of Nateglinide in diluent to obtain the standard solution.	
Sample:	Take 10 tablets of Nagelienai Pian, weigh precisely, grind to powder. Weight appropriate amount (roughly contained 70mg Nateglinide) to a 100-mL volumetric flask, add appropriate amount of 55% ACN solution. Shake for 30 minutes. Add diluent to volume. Filtrate.	
Pressure Drop:	80 Bar (1160 psi)	

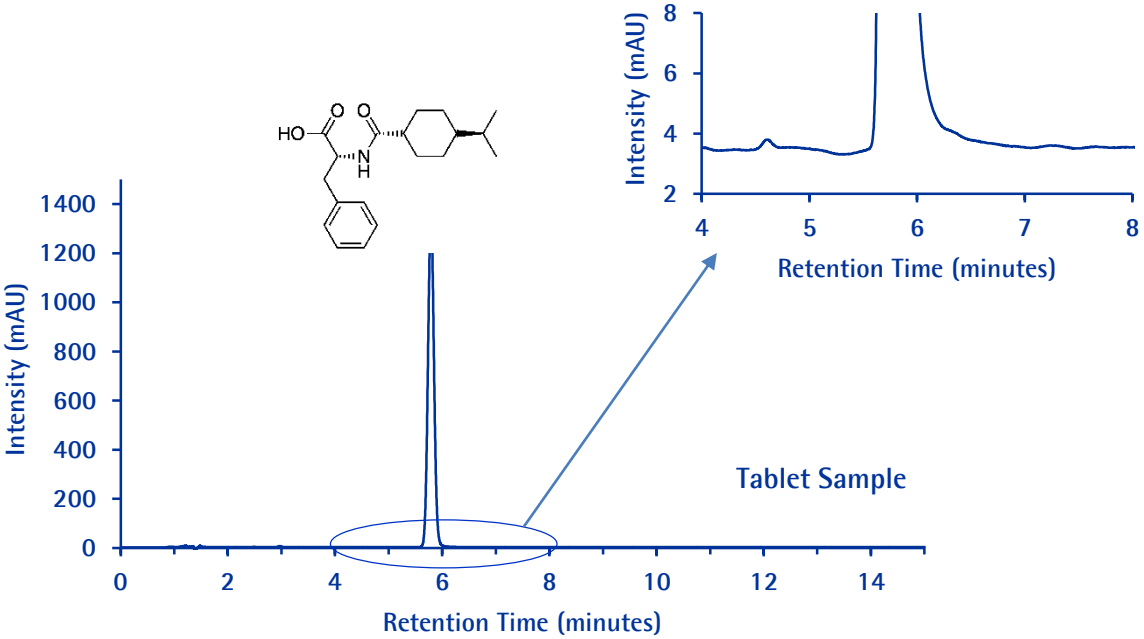


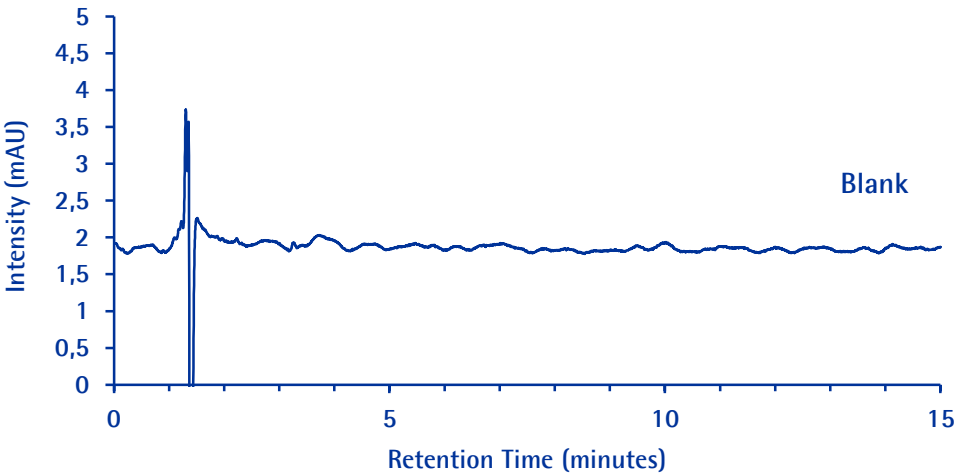
Chromatographic Data (standard solution 30 ppm)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Nateglinide	5.8	-	1.1	12001

Nateglinide tablets (ChP)

Purospher® STAR RP-18 endcapped





Chromatographic Data (Tablet sample)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Nateglinide	5.8	-	1.1	12001

Nateglinide tablets (ChP)

Validation and Verification Data – particle packed column

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time for Nateglinide and its peak purity.

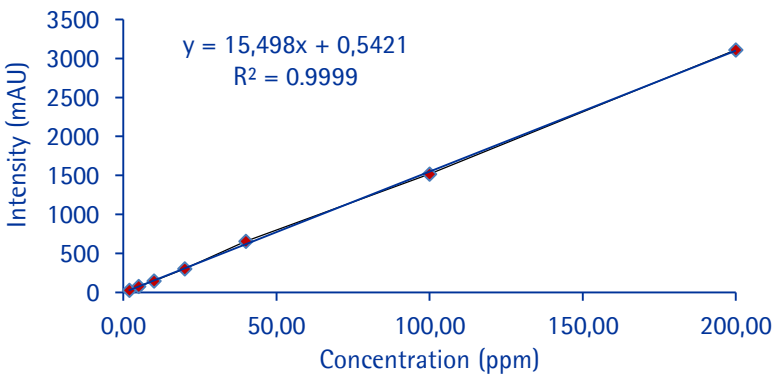
Compound	Retention Time (min)	Plates	Tailing factor	Peak Purity
Nateglinide	5.8	12001	1.1	1.0000

2. **Repeatability.**
   Determined by injecting five (5) samples with a solution containing 10 ppm Nateglidine.

	Nateglinide (Area units)
Standard 1	149.79939
Standard 2	151.85692
Standard 3	152.17433
Standard 4	150.22794
Standard 5	150.92566
Mean	150.997
Stdev	1.019
RSD (%)	0.7

3. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting seven (7) concentration levels from 2-200 ppm of Nateglinide.

	Nateglinide (ppm)	Area
	2.0	30.23459
	5.0	79.93151
	10.0	149.79939
	20.0	303.99387
	40.0	655.95764
	100.0	1518.79736
	200.0	3107.15625
STEYX		21.806
SLOPE		15.483
LOD		4.6
LOQ		14.1



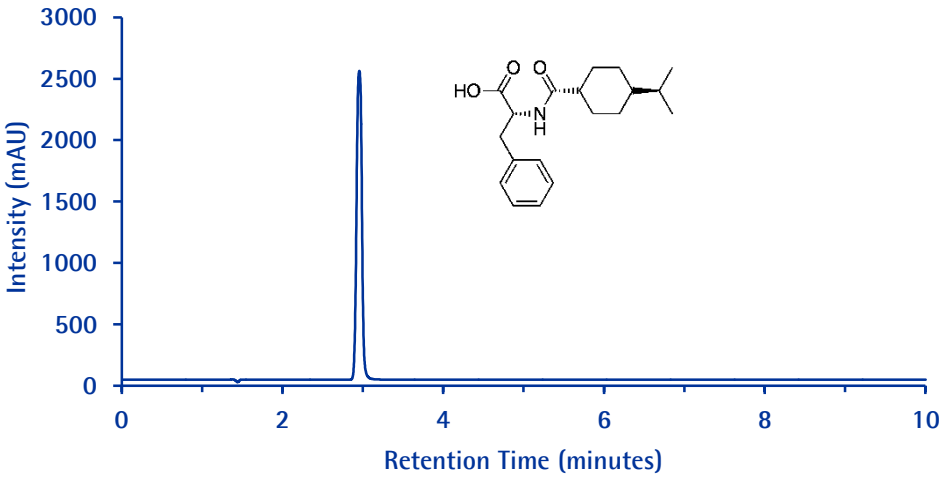
LOD (ppm)	LOQ (ppm)
4.6	14.1

Nateglinide tablets (ChP)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 100x4.6 mm	1.52022.0001
Injection:	10 µL	
Detection:	UV@ 210 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	0.05% Trifluoroacetic acid solution and acetonitrile 40:60 (v/v).	
Temperature:	30 °C	
Diluent	55% ACN in H2O	
Standard:	30 µg/mL (30 ppm) of Nateglinide in diluent to obtain the standard solution.	
Sample:	Take 10 tablets of Nagelienai Pian, weigh precisely, grind to powder. Weight appropriate amount (roughly contained 70mg Nateglinide) to a 100-mL volumetric flask, add appropriate amount of 55% ACN solution. Shake for 30 minutes. Add diluent to volume. Filtrate.	
Pressure Drop:	38 Bar (551 psi)	

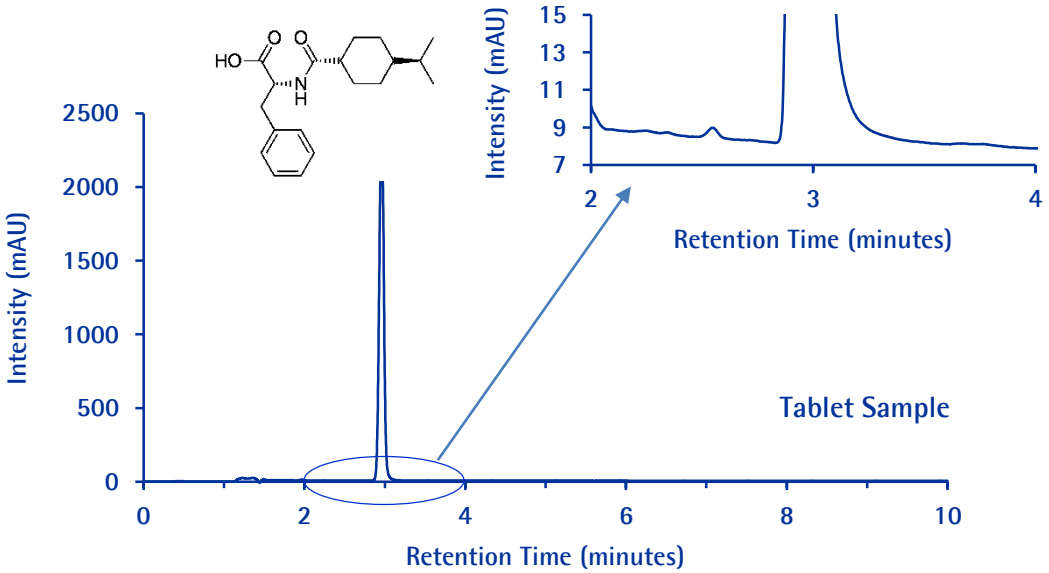


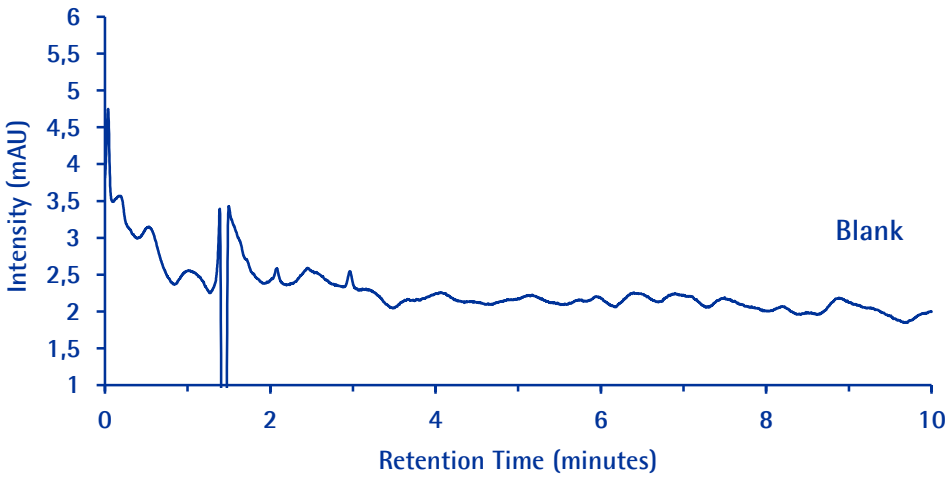
Chromatographic Data (standard solution 30 ppm)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Nateglinide	3.0	-	1.0	9370

Nateglinide tablets (ChP)

Chromolith® HighResolution RP-18 endcapped





Chromatographic Data (standard solution 30 ppm)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Nateglinide	3.0	10.6	1.0	11401

Nateglinide tablets (ChP)

Validation and Verification Data – monolithic column

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time for Nateglidine and its peak purity.

Compound	Retention Time (min)	Plates	Tailing factor	Peak Purity
Nateglidine	3.0	9370	1.0	1.0000

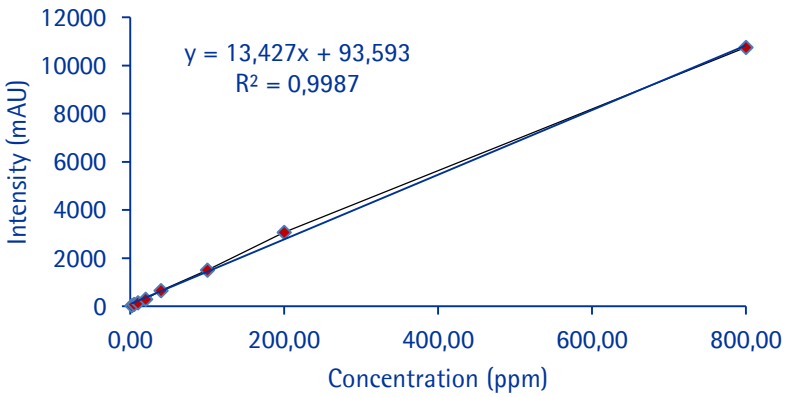
2. **Repeatability.**
   Determined by injecting five (5) samples with a solution containing 10 ppm Nateglidine.

	Nateglidine (Area units)
Standard 1	150.72525
Standard 2	149.98912
Standard 3	151.61790
Standard 4	152.37445
Standard 5	152.69785
Mean	151.48
Stdev	1.129
RSD (%)	0.7

- 50% faster
- Less backpressure
- Same sensitivity

3. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting seven (7) concentration levels from 2-200 ppm of Nateglidine.

	Nateglidine (ppm)	Area
	2.0	30.25955
	5.0	80.1163
	10.0	150.72525
	20.0	303.18668
	40.0	656.32147
	100.0	1503.63062
	200.0	3072.74316
STEYX		23.363
SLOPE		15.302
LOD		5.0
LOQ		15.3

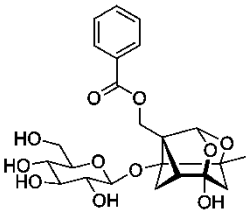


LOD (ppm)	LOQ (ppm)
5	15

Xiaoyao Wan (ChP)

Free and Easy Wanderer pills

逍遥丸



Paeoniflorin

The Xiaoyao Wan is a classical Chinese herbal formulation.
In English is known as free and easy wanderer pills.

The ChP monograph specify that Paeoniflorin should be checked in the Xiaoyao Wan.
A 150x4.6 mm Purospher® Star STAR RP-18 endcapped column with 5 µm particles can be an excellent choice for the analysis of these analytes in Xiaoyao Wan.

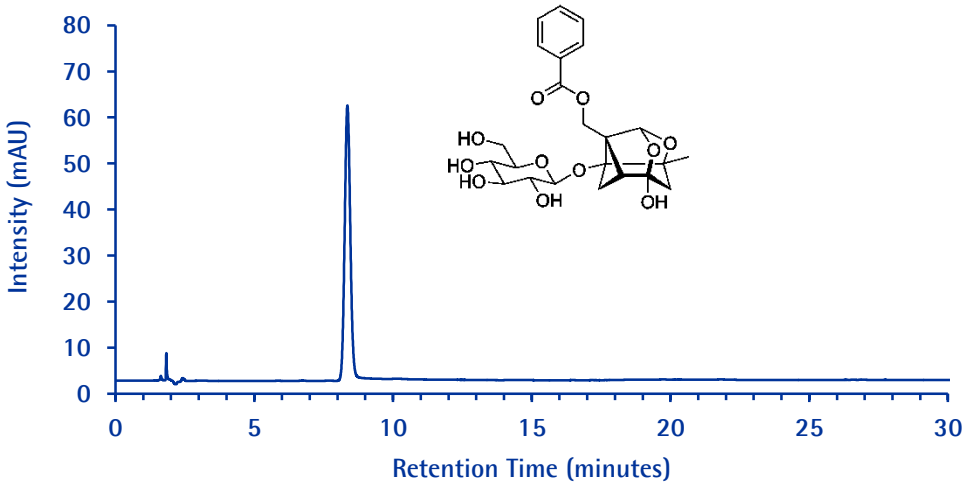
The method was also transferred to a 33% shorter monolithic column. The 100x4.6 Chromolith® HighResolution RP-18 endcapped provided similar method sensitivity, but where the Paeoniflorin peak eluted in less than half of the time at 50% lower backpressure, an benefit with the macropore/mesopore structure in monolithic columns.

Xiaoyao Wan (ChP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® Star RP-18 endcapped (5µm)150x4.6 mm	1.51455.0001
Injection:	10 µL	
Detection:	UV@ 230 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Mix 0.1% H3PO4 aqueous solution and acetonitrile 85:15 (v/v).	
Temperature:	30 °C	
Diluent	Dilute ethanol solution (dilute 529 mL ethanol with water to 1000 mL).	
Standard:	60 µg/mL (60 ppm) Paeoniflorin in diluent.	
Sample:	Take appropriate amount of drug pills, grind, weigh 0.4g powder a volumetric flask, add into 25 mL dilute ethanol aqueous solution, weigh the solution, sonicate (250w, 33kHZ) for 30minutes, cool, weigh, make up weight with diluent. Shake and filter.	
Pressure Drop:	106 Bar (1537 psi)	

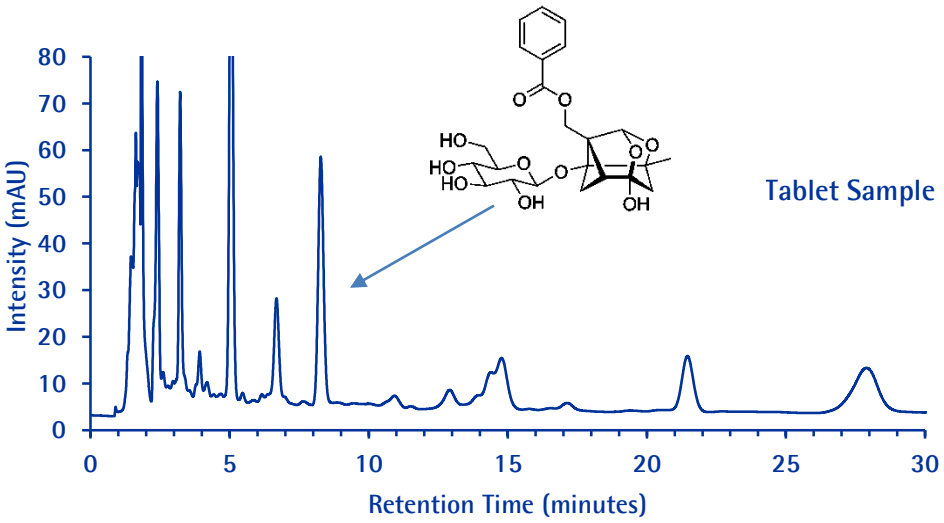


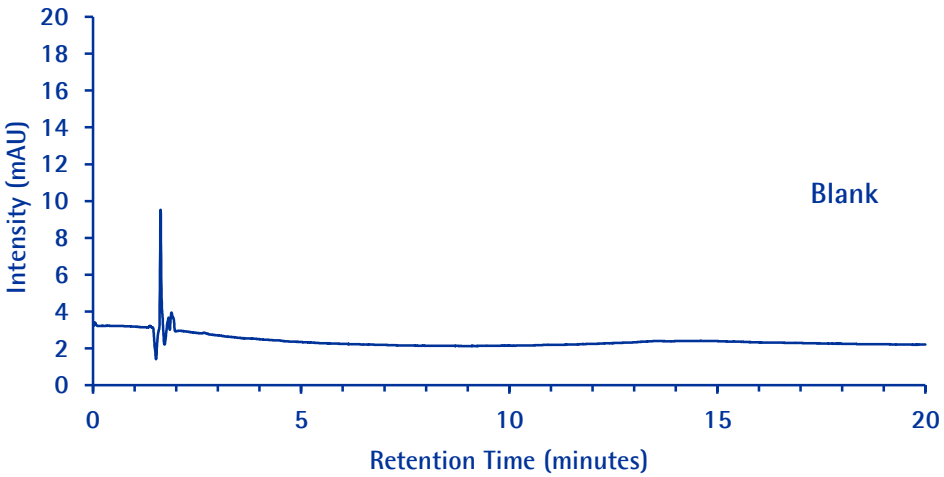
Chromatographic Data (standard solution 60 ppm)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Paeoniflorin	8.4	-	1.1	8164

Xiaoyao Wan (ChP)

Purospher® STAR RP-18 endcapped





Chromatographic Data (Tablet sample)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Paeoniflorin	8.3	1.6	1.1	8474

Xiaoyao Wan (ChP)

Validation and Verification Data – particle packed column

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time for Paeoniflorin and its peak purity.

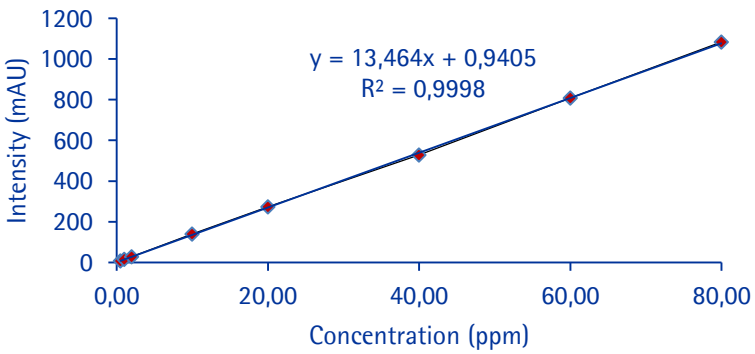
Compound	Retention Time (min)	Plates	Tailing factor	Peak Purity
Paeoniflorin	8.4	8164	1.1	1.0000

2. **Repeatability.**
   Determined by injecting five (5) samples with a solution containing 2 ppm Paeoniflorin.

	Nateglinide (Area units)
Standard 1	28.53910
Standard 2	28.16852
Standard 3	28.16190
Standard 4	28.10512
Standard 5	28.32438
Mean	28.260
Stdev	0.176
RSD (%)	0.6

3. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting seven (7) concentration levels from 0.5-80 ppm of Paeoniflorin.

	Paeoniflorin (ppm)	Area
	0.5	7.27269
	1.0	14.91638
	2.0	28.32438
	10.0	139.52818
	20.0	272.86389
	40.0	527.81866
	60.0	808.1062
STEYX		4.9969
SLOPE		13.3643
LOD		1.2
LOQ		3.7



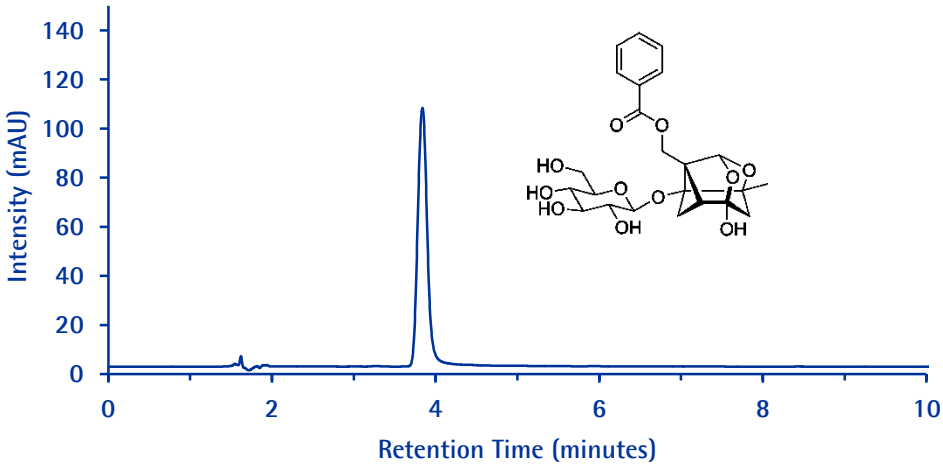
LOD (ppm)	LOQ (ppm)
1.2	3.7

Xiaoyao Wan (ChP)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 100x4.6 mm	1.52022.0001
Injection:	10 µL	
Detection:	UV@ 230 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Mix 0.1% H3PO4 aqueous solution and acetonitrile 85:15 (v/v).	
Temperature:	30 °C	
Diluent Standard:	Dilute ethanol solution (dilute 529 mL ethanol with water to 1000 mL). 60 µg/mL (60 ppm) Paeoniflorin in diluent.	
Sample:	Take appropriate amount of drug pills, grind, weigh 0.4g powder a volumetric flask, add into 25 mL dilute ethanol aqueous solution, weigh the solution, sonicate (250w, 33kHZ) for 30minutes, cool, weigh, make up weight with diluent. Shake and filter.	
Pressure Drop:	57 Bar (826 psi)	

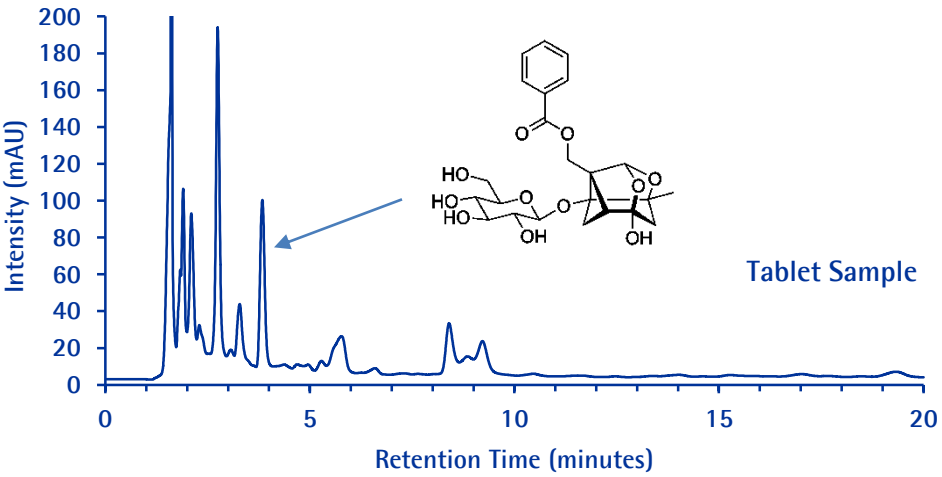


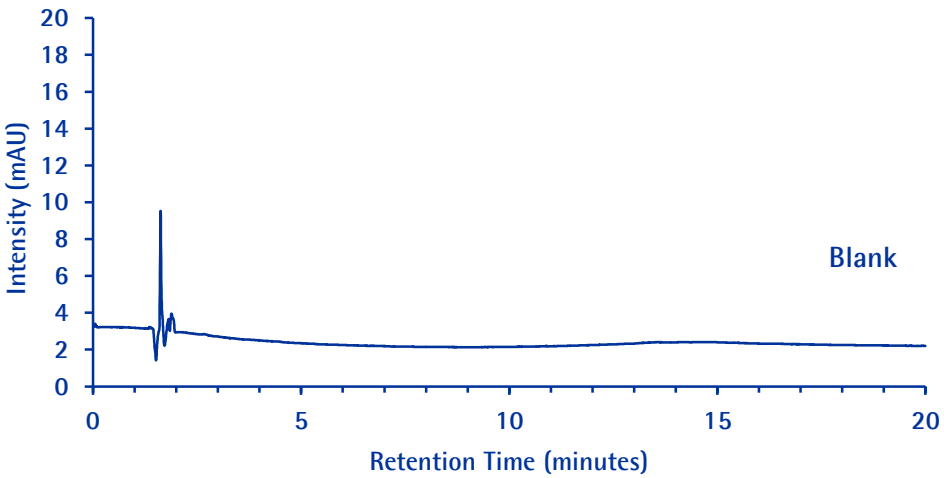
Chromatographic Data (standard solution 60 ppm)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Paeoniflorin	3.8	-	1.2	5314

Xiaoyao Wan (ChP)

Chromolith® HighResolution RP-18 endcapped





Chromatographic Data (Tablet sample)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Paeoniflorin	3.8	2.5	1.1	5272

Xiaoyao Wan (ChP)

Validation and Verification Data – monolithic column

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time for Paeoniflorin and its peak purity.

Compound	Retention Time (min)	Plates	Tailing factor	Peak Purity
Paeoniflorin	3.8	5314	1.2	1.0000

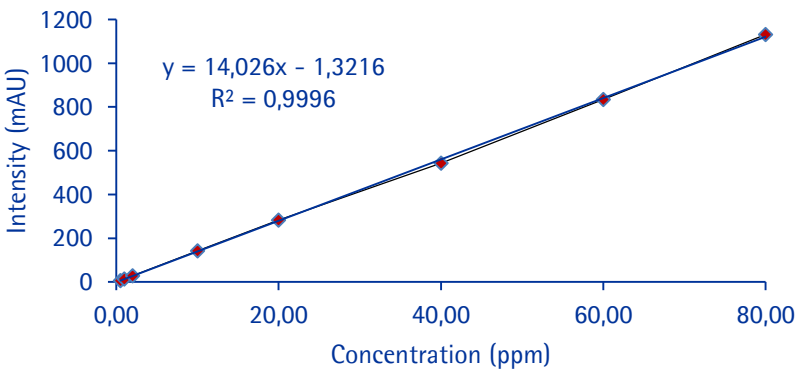
2. **Repeatability.**
   Determined by injecting five (5) samples with a solution containing 2 ppm Paeoniflorin.

	Paeoniflorin (Area units)
Standard 1	28.63140
Standard 2	28.46291
Standard 3	28.43642
Standard 4	28.24395
Standard 5	28.26835
Mean	28.409
Stdev	0.158
RSD (%)	0.6

- 50% faster
- Less backpressure
- Same sensitivity

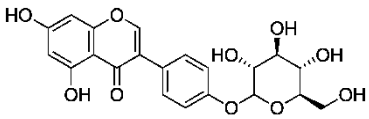
3. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting eight (8) concentration levels from 0.5-80 ppm of Paeoniflorin.

	Paeoniflorin (ppm)	Area
	0.50	6.8209
	1.0	13.77197
	2.0	28.6314
	10.0	142.7113
	20.0	282.02817
	40.0	543.86139
	60.0	834.25781
	80.0	1131.99866
STEYX		5.495
SLOPE		13.810
LOD		1.3
LOQ		4.0

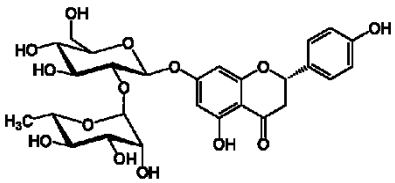


LOD (ppm)	LOQ (ppm)
1.3	4

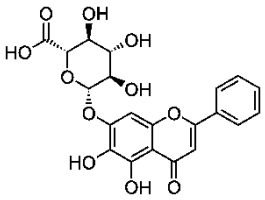
Huaijiao Wan (ChP)
槐角丸



Sophoricoside



Naringin



Baicalin

The Huaijiao Wan is a mixture of six ingredients; Fructus Sophorae, Radix Sanguisorbae, Radix Scutellariae, Fructus Aurantii, Radix Angelicae Sinensis and Radix Saposhnikoviae.

The ChP monograph specify Sophoricoside, Naringin and Baicalin as markers for the Huaijiao Wan. Shown on the following pages, a 150x4.6 mm Purospher® Star STAR Phenyl column with 5 µm particles can be an excellent choice for the analysis of Huaijiao Wan tablets.

Huaijiao Wan (ChP)

Purospher® Phenyl

Chromatographic Conditions

Column: Purospher® Star Phenyl (5µm) 150x4.6 mm 1.51919.0001

Injection: 10 µL

Detection: UV @ 260 and 280 nm

Cell: 1ul/10mm

Flow Rate: 1.0 mL/min

Mobile Phase: Mobile Phase A: 2% glacial acetic acid solution.
Mobile Phase B: Mix methanol and acetonitrile 2:1 (v/v).

Time (min)	A (%)	B (%)
0	89	11
23	89	11
50	70	30
51	10	90
57	10	90

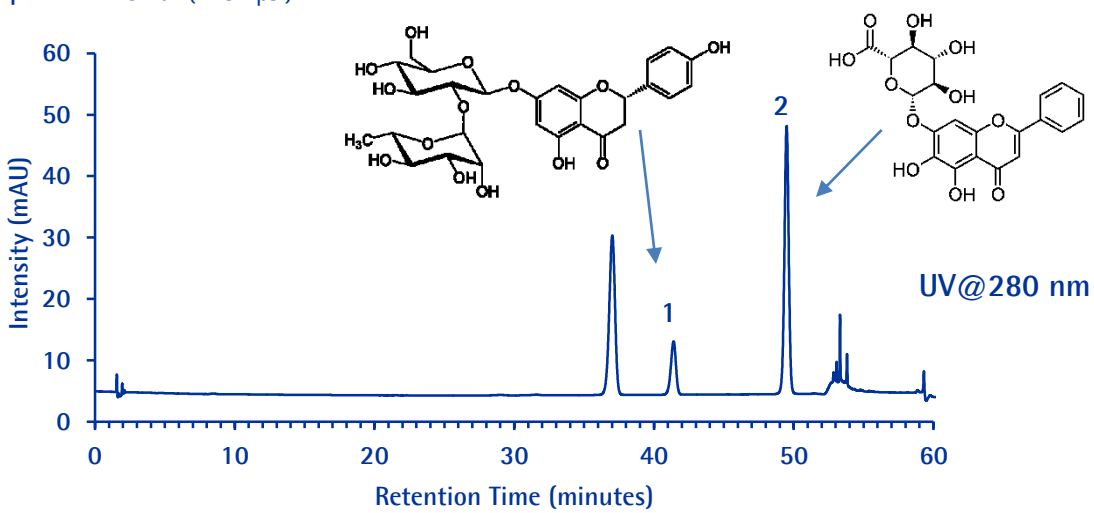
Temperature: 50 °C

Diluent 50% methanol solution

Standard: Dissolve appropriate amount of Sophoricoside, Naringin & Baicalin in methanol solution to obtain a 40, 20, 48 µg/mL (ppm) solution.

Sample: Take appropriate amount of drug pills, cut into pieces, weigh exactly 0.25g small pills to a mortar, grind four times with total 40 mL 50% methanol aqueous solution. Transfer it to a 50-mL volumetric flask, sonicate (250w, 33kHZ) for 30 minutes, cool down, weigh, make up weight with 50% methanol solution. Shake well and filter.

Pressure Drop: 78 Bar (1131 psi)

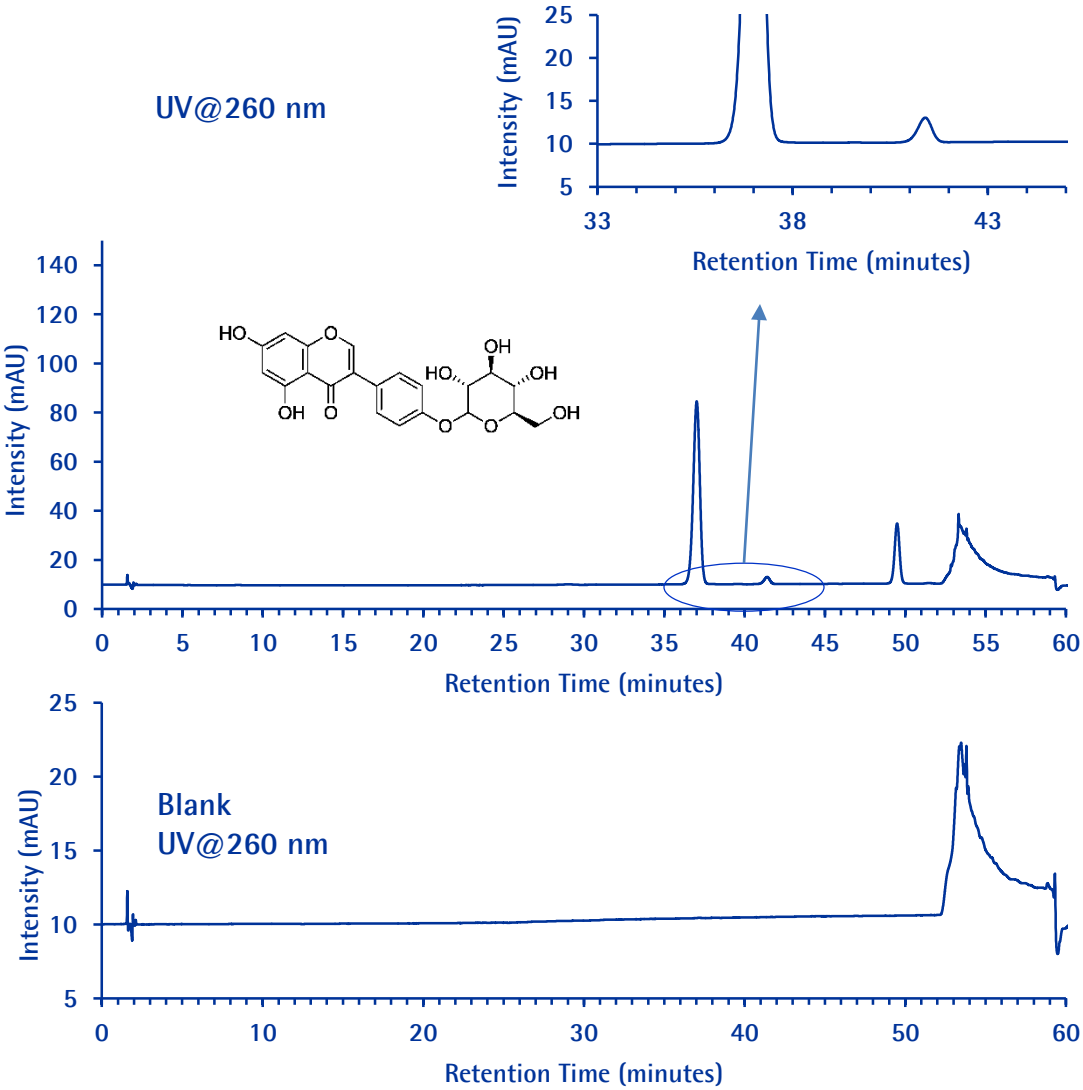


Chromatographic Data (standard solution)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Naringin	41.4	6.2	1.0	63521
2	Baicalin	49.5	13.3	1.0	126977

Huaijiao Wan (ChP)

Purospher® Phenyl

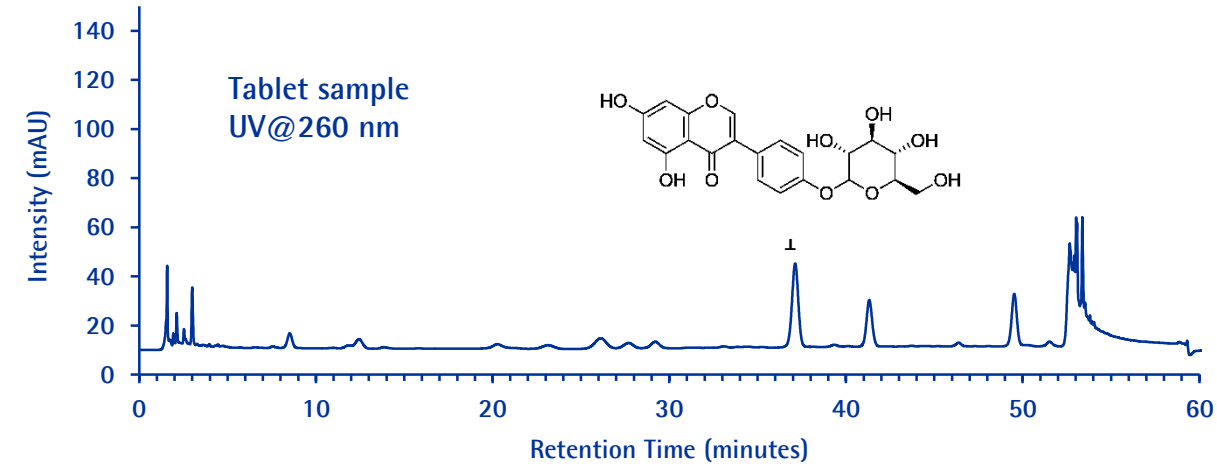


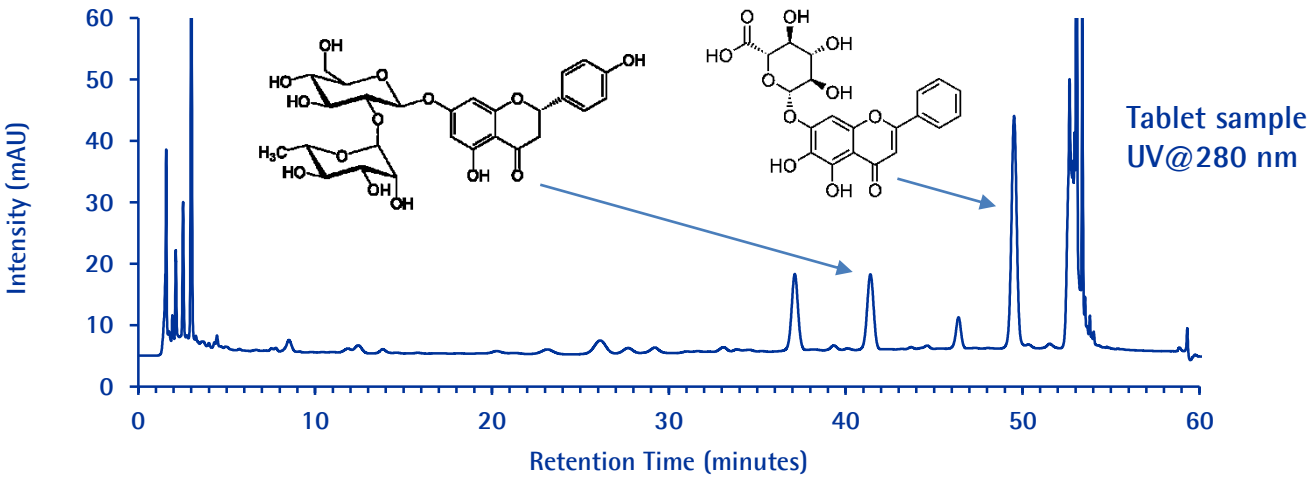
Chromatographic Data (standard solution)

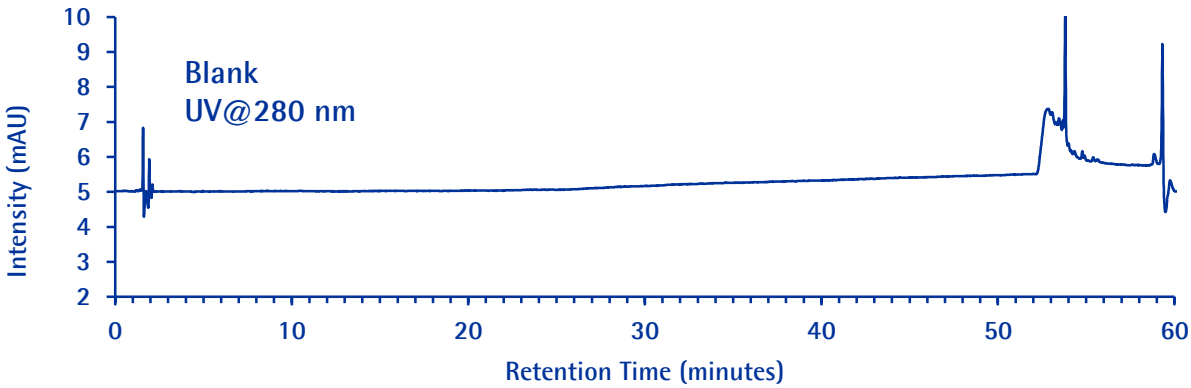
		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Sophoricoside	37.0	-	1.0	37753

Huaijiao Wan (ChP)

Purospher® Phenyl







Huaijiao Wan (ChP)

Validation and Verification Data

1. Specificity

Determined by injection of SST Solution and determination of the retention time for Sophoricoside, Naringin and Baicalin, and their peak purity.

Compound	Retention Time (min)	Plates	Tailing factor	Peak Purity
Sophoricoside	37.0	37753	0.9	1,0000
Naringin	41.4	63521	0,9	1,0000
Baicalin	49.5	126977	1,0	1,0000

2. Repeatability.

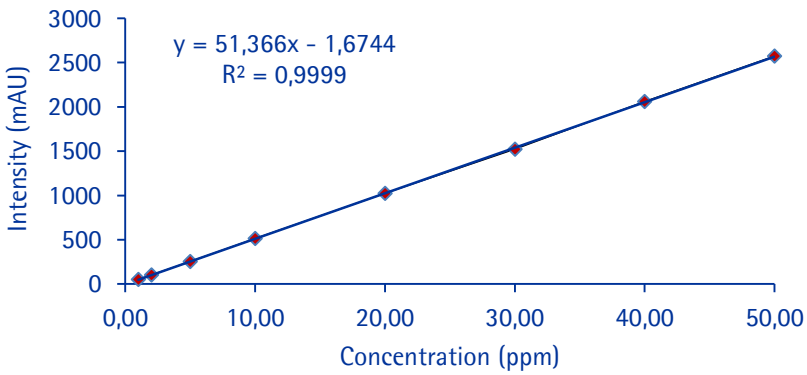
Determined by injecting five (5) samples with a solution containing 20 ppm of each Sophoricoside, Naringin and Baicalin.

	Sophoricoside (Area units)	Naringin (Area units)	Baicalin (Area units)
Standard 1	1025.13733	195.66806	354.13263
Standard 2	1021.60944	195.32964	353.57370
Standard 3	1024.02502	195.91623	354.21594
Standard 4	1025.08813	196.97253	352.65549
Standard 5	1025.51917	195.81633	350.83740
Mean	1024.28	195.94	353.08
Stdev	1.59	0.618	1.40
RSD (%)	0.2	0.3	0.4

3. Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Determined by injecting eight (8) concentration levels from 1-50 ppm of Sophoricoside.

	Sophoricoside (ppm)	Area
	1.0	52.29488
	2.0	102.45029
	5.0	254.01421
	10.0	516.11334
	20.0	1025.13733
	30.0	1521.93396
	40.0	2058.42822
	50.0	2572.06738
STEYX		8.047
SLOPE		51.228
LOD		0.5
LOQ		1.6



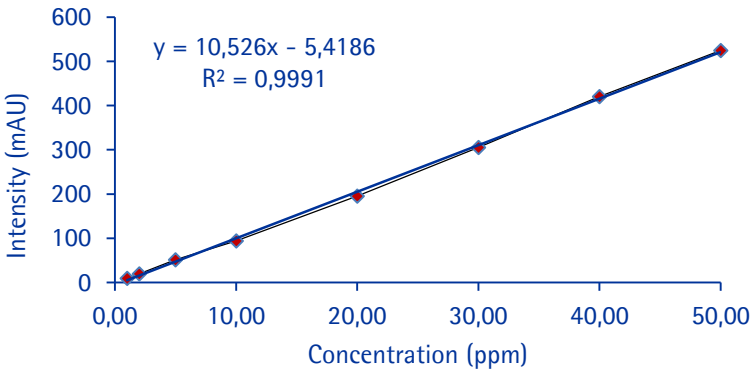
LOD (ppm)	LOQ (ppm)
0.5	1.6

Huaijiao Wan (ChP)

Validation and Verification Data

4. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting eight (8) concentration levels from 1-50 ppm of Naringin.

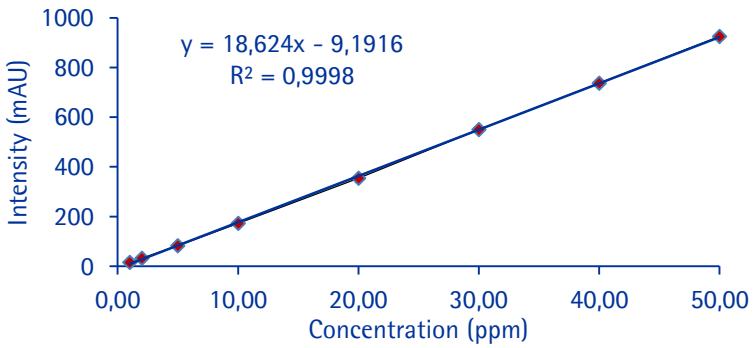
	Naringin (ppm)	Area
	1.0	9.44313
	2.0	19.18369
	5.0	51.61107
	10.0	93.79378
	20.0	195.66806
	30.0	305.18057
	40.0	420.37619
	50.0	524.49475
STEYX		6.309
SLOPE		10.434
LOD		2..0
LOQ		6.0



LOD (ppm)	LOQ (ppm)
2	6

5. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting eight (8) concentration levels from 1-50 ppm of Baicalin.

	Baicalin (ppm)	Area
	1.0	15.16125
	2.0	32.66272
	5.0	82.30071
	10.0	172.30835
	20.0	354.13263
	30.0	550.52325
	40.0	737.42834
	50.0	924.5946
STEYX		5.376
SLOPE		18.559
LOD		1.0
LOQ		3

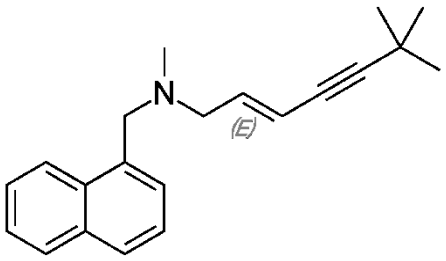


LOD (ppm)	LOQ (ppm)
1	3

Terbinafine Hydrochloride Cream (ChP)

Yansuan Tebinaifen Rugao

盐酸特比萘芬乳膏



Terbinafine

Terbinafine hydrochloride is an antifungal. It is highly hydrophobic molecule and tends to accumulate in hair, skin, nails, and fatty tissue. It was developed by Novartis and Omega Pharma, respectively, and is sold under various brand names, i.e. Lamisil, Terboderm, Corbinal, Terbisil, Undofen, Sebifin, Tinasil, Terbisil, Terbicor, and Tamsil.

It is listed on the WHO Model List of Essential Medicines.

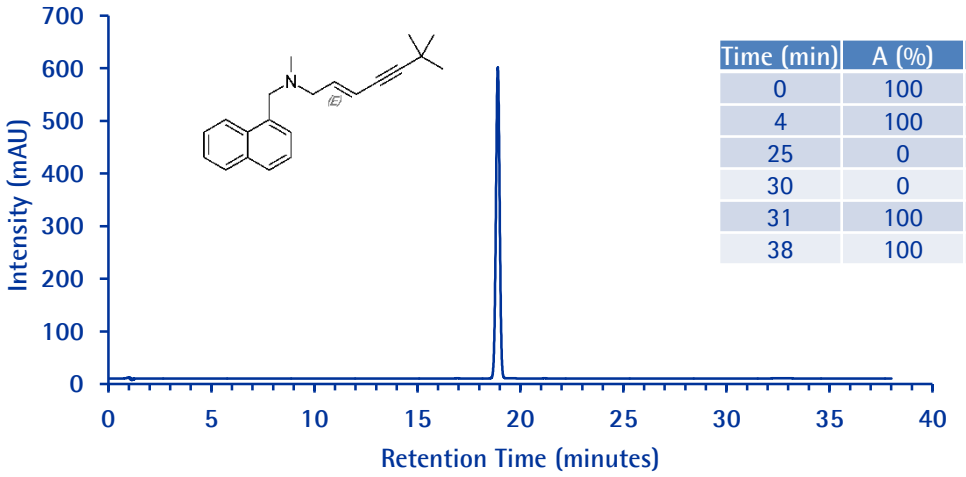
Following the ChP 2015 monograph method, we used a 150x3.0 mm Purospher® Star STAR RP-18 endcapped column with 5 µm particles for the analysis of Terbinafine in Terbinafine hydrochloride cream (Yansuan Tebinaifen Rugao).

Terbinafine HCl Cream (ChP) - Assay

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® Star RP-18 endcapped (5 µm) 150x3 mm	1.50626.0001
Injection:	20 µL	
Detection:	UV @ 280 nm	
Cell:	1ul/10mm	
Flow Rate:	0.8 mL/min	
Mobile Phase:	Buffer solution: 2.0 mL/L of triethylamine. Adjust pH 7.5 with glacial acetic acid. Mobile Phase A: Buffer:Methanol:Acetonitrile 30:42:28 (v/v). Mobile Phase B: Buffer:Methanol :Acetonitrile 5:57:38 (v/v).	
Temperature:	25 °C	
Diluent	50% ACN solution.	
Sample Preparation:	Sample: Dissolve appropriate amount of Terbinafine Hydrochloride Cream in isopropanol to obtain a 0.2 mg/mL (200 ppm) of solution, filter prior to analysis. Standard solution: Dilute appropriate amount of standard with isopropanol to obtain a 0.2 mg/mL (200 ppm) solution. Light degradation solution expose 1mg/mL of Terbinafine Hydrochloride in 50% ACN solution at 254 nm of UV light for 1h. Inject the solution to HPLC system, **3 major related impurity can be found with relative retention time (RRT) from 0.8 to 1.2 (Generally 0.87, 0.95,1.1)**.	
Pressure Drop:	166 Bar (2407 psi)	



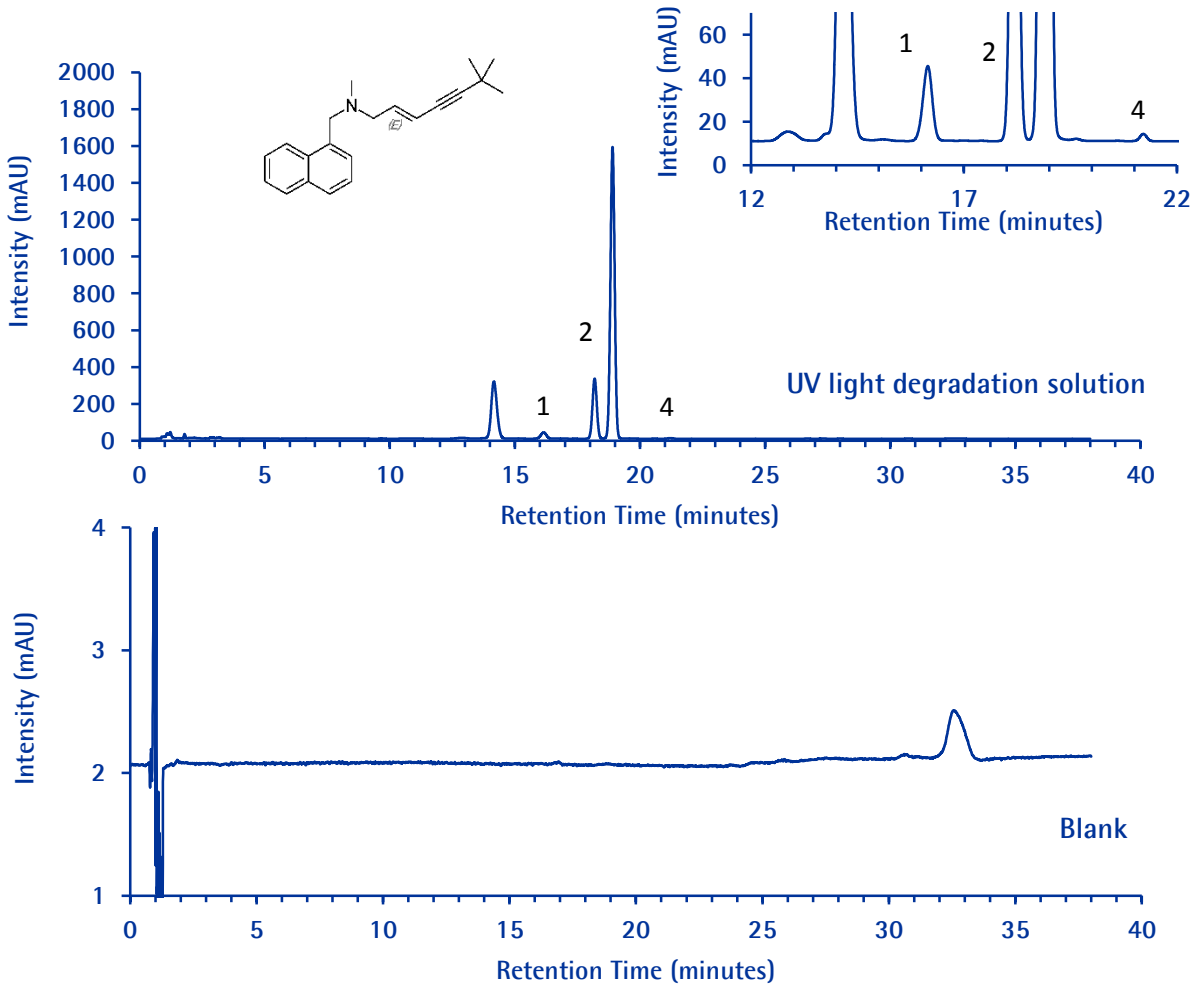
Time (min)	A (%)	B (%)
0	100	0
4	100	0
25	0	100
30	0	100
31	100	0
38	100	0

Chromatographic Data (200 ppm standard solution)

		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Terbinafine	18.9	-	1.0	50074

Terbinafine HCl Cream (ChP) - Assay

Purospher® STAR RP-18 endcapped



Chromatographic Data (standard solution)

		RT (min)	RRT	Tailing Factor	Theoretical Plates
1	Impurity 1	16.2	0.86	1.8	24073
2	Impurity 2	18.2	0.96	5.5	50095
3	Terbinafine Hydrochloride	18.9	1.0	2.2	56647
4	Impurity 3	21.2	1.1	4.7	75518

Terbinafine HCl Cream (ChP) - RS

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column: Purospher® Star RP-18 endcapped (5 µm) 150x3 mm 1.50626.0001

Injection: 20 µL

Detection: UV@ 280 nm

Cell: 1ul/10mm

Flow Rate: 0.8 mL/min

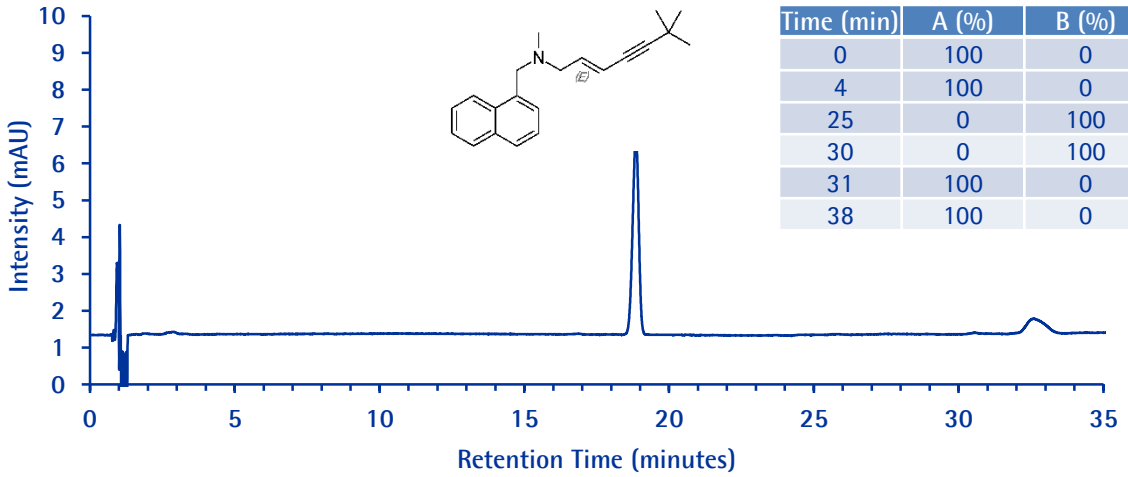
Mobile Phase: Buffer solution: 2.0 mL/L of triethylamine. Adjust pH 7.5 with glacial acetic acid.
Mobile Phase A: Buffer:Methanol:Acetonitrile 30:42:28 (v/v).
Mobile Phase B: Buffer:Methanol :Acetonitrile 5:57:38 (v/v).

Temperature: 25 °C

Diluent Isopropanol

Sample: Dissolve appropriate amount of Terbinafine Hydrochloride Cream in isopropanol to obtain a 0.5 mg/mL (500 ppm) of solution, filter prior to analysis. Dilute it to a 2.5ug/mL of solution with isopropanol as standard solution. Dilute the standard solution with isopropanol to 250ng/mL (250 ppb) of solution as sensitivity solution.

Pressure Drop: 166 Bar (2407 psi)



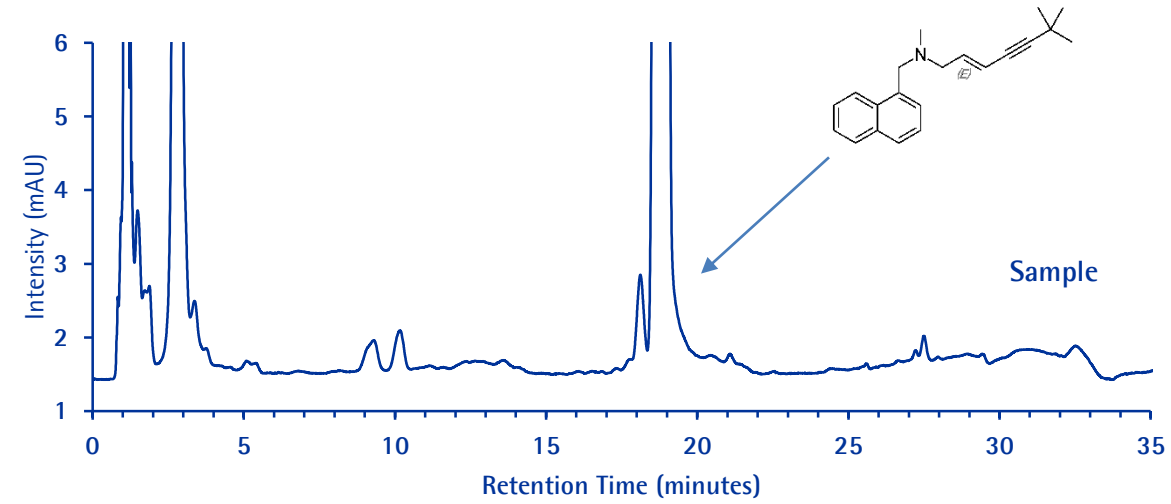
Time (min)	A (%)	B (%)
0	100	0
4	100	0
25	0	100
30	0	100
31	100	0
38	100	0

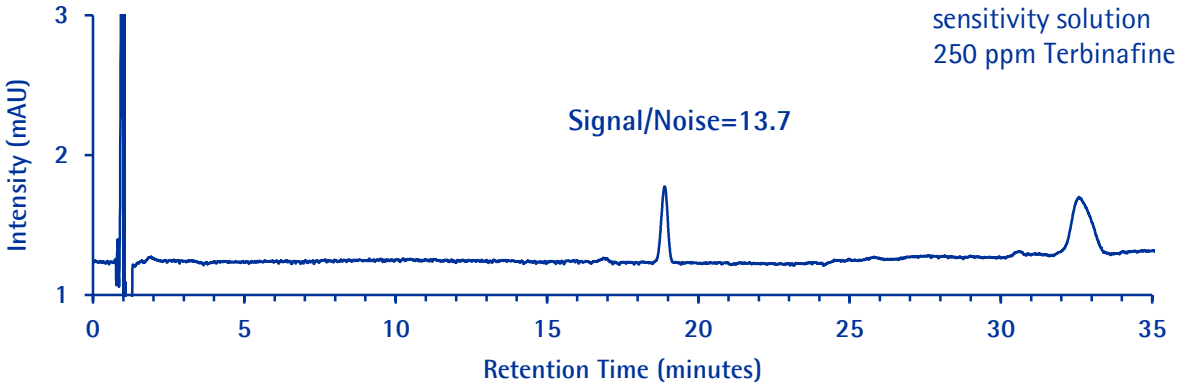
Chromatographic Data (standard solution)

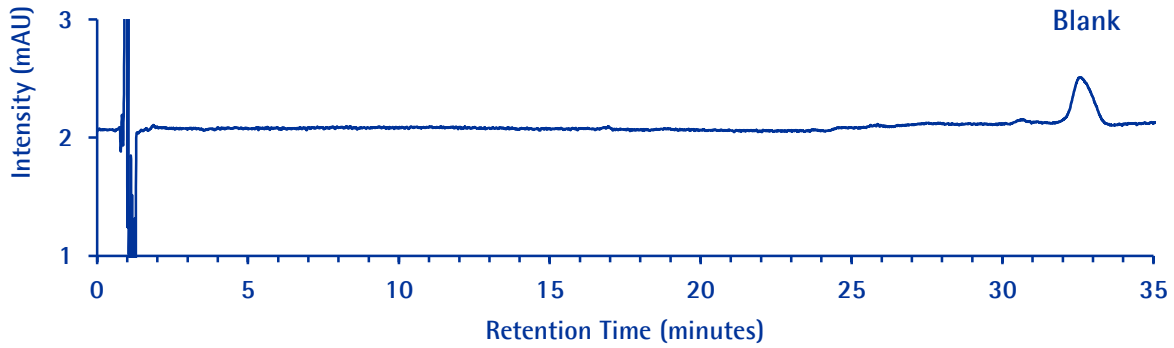
		RT (min)	Resolution	Tailing Factor	Theoretical Plates
1	Terbinafine	18.9	-	0.9	35668

Terbinafine HCl Cream (ChP) - RS

Purospher® STAR RP-18 endcapped







Terbinafine Hydrochloride Cream (ChP)

Validation and Verification Data

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time for Terbinafine and its peak purity.

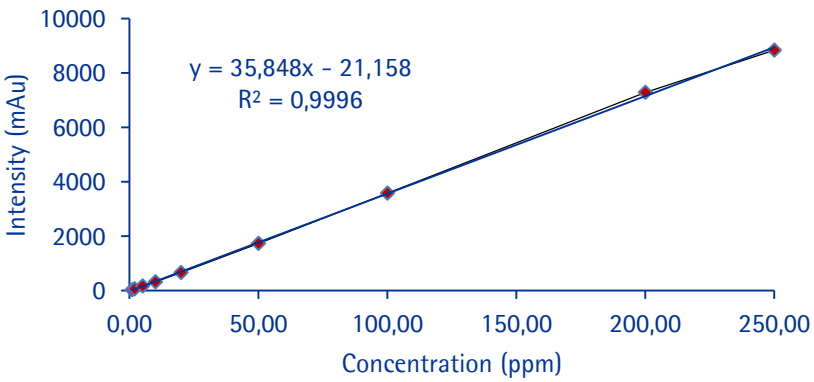
Compound	Retention Time (min)	Plates	Tailing factor	Peak Purity
Terbinafine	18.9	50074	1.0	1.0000

2. **Repeatability.**
   Determined by injecting five (5) samples with a solution containing 50 ppm Terbinafine.

	Terbinafine (Area units)
Standard 1	1732.44263
Standard 2	1730.52429
Standard 3	1731.75525
Standard 4	1731.38757
Standard 5	1734.54932
Mean	1732.13
Stdev	1.518
RSD (%)	0.1

3. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting eight (8) concentration levels from 1-250 ppm of Terbinafine

	Terbinafine (ppm)	Area
	1.0	31.54334
	2.0	63.9935
	5.0	168.31068
	10.0	315.39963
	20.0	666.85101
	50.0	1734.54932
	100.0	3578.61011
	200.0	7288.27686
STEYX		36.052
SLOPE		35.848
LOD		3.3
LOQ		10



LOD (ppm)	LOQ (ppm)
3.3	10