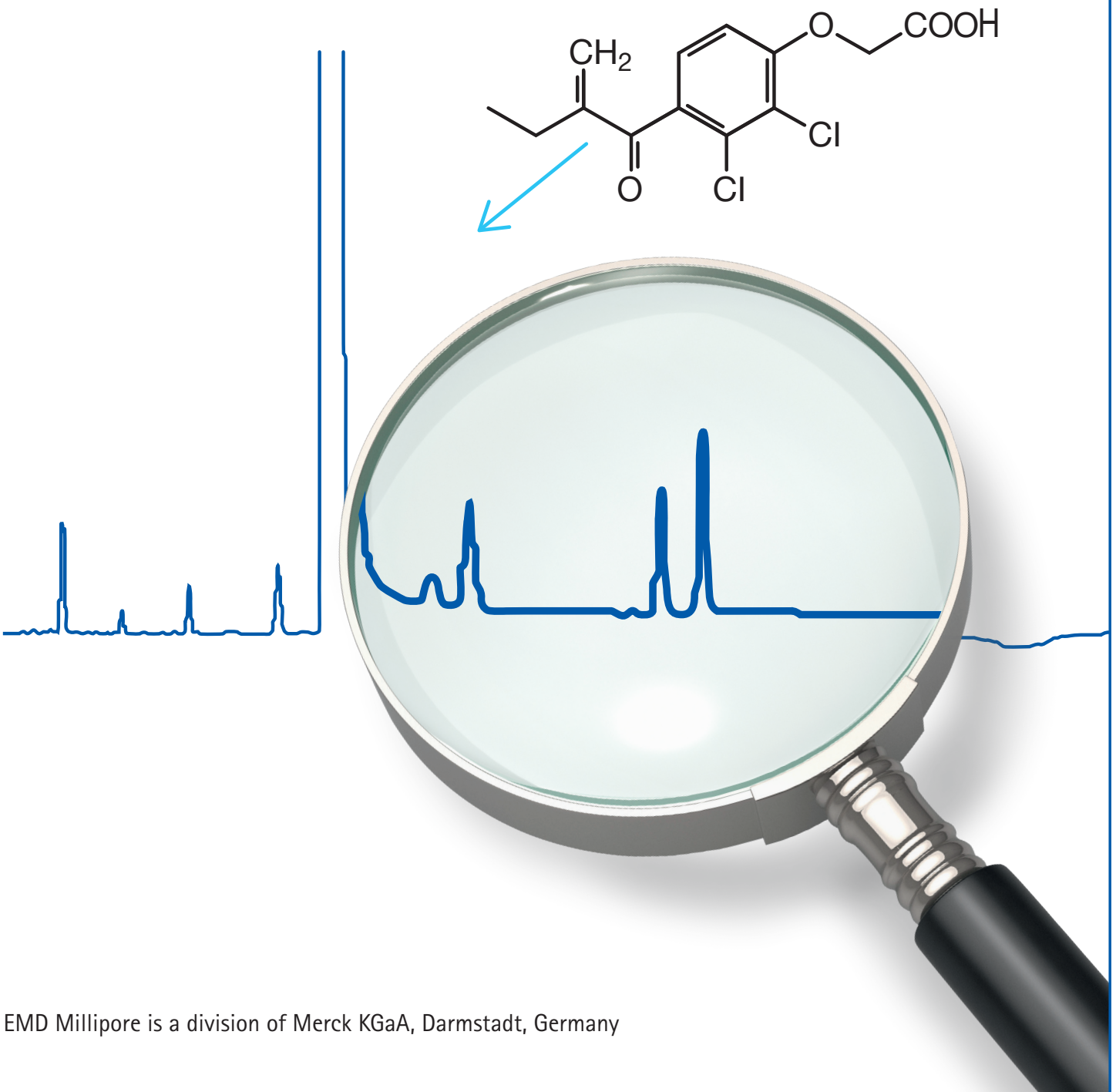
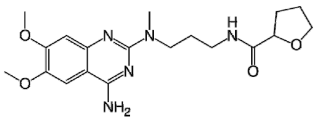
Pharmaceutical impurity profiling
Application guide



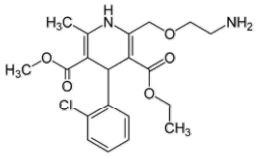
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

Content

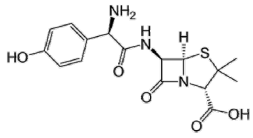
Molecular Structures



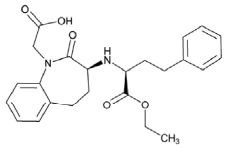
Alfuzosin



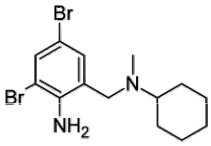
Amlodipine



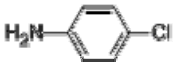
Amoxicillin



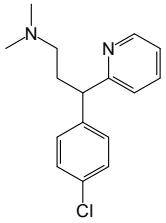
Benazepril



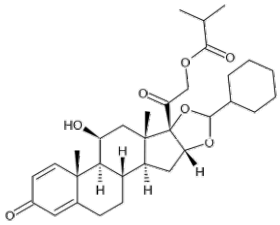
Bromhexine



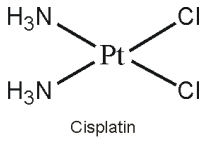
4-Chloroaniline



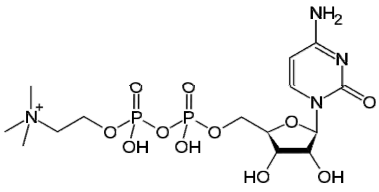
Chlorpheniramine



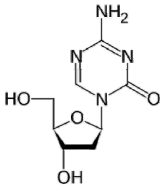
Ciclesonide



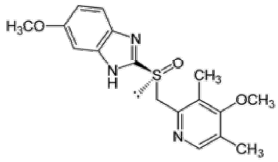
Cisplatin



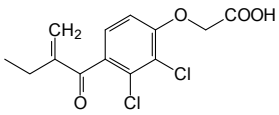
Citicoline



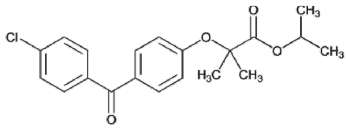
Decitabine



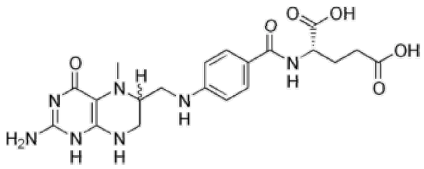
Esomeprazole



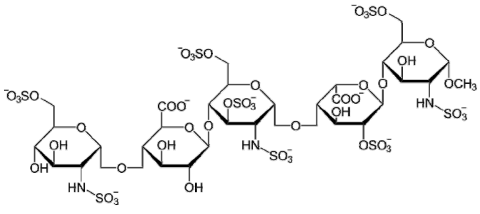
Ethacrynic acid



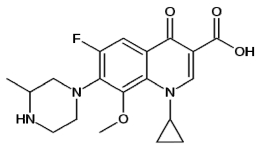
Fenofibrate



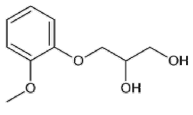
L-methyl-folate



Fondaparinux

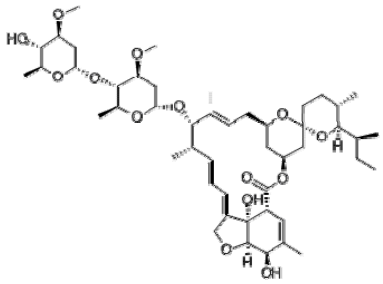


Gatifloxacin

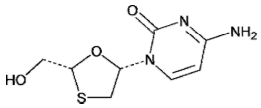


Guaifenesin

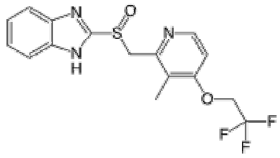
Molecular Structures



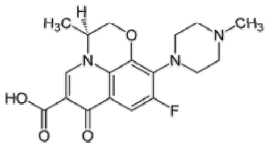
Ivermectin



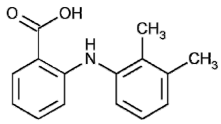
Lamivudine



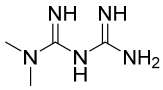
Lansoprazole



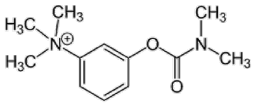
Levofloxacin



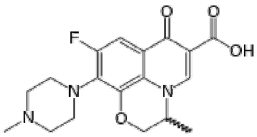
Mefenamic acid



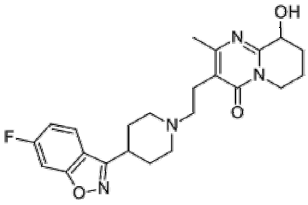
Metformin



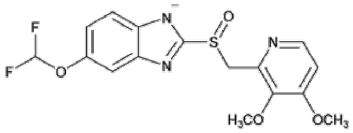
Neostigmine



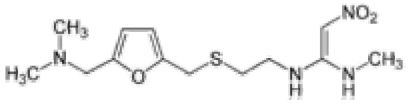
Ofloxacin



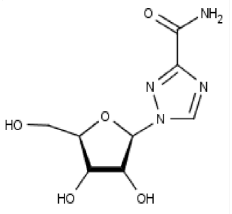
Paliperidone



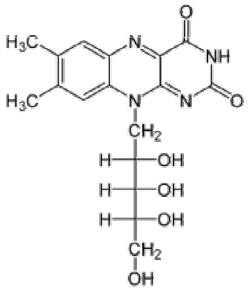
Pantoprazole



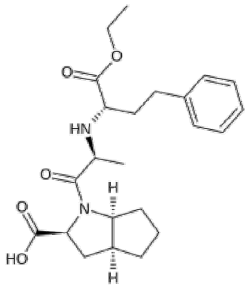
Ranitidine



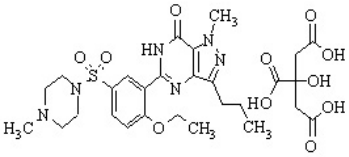
Ribavirin



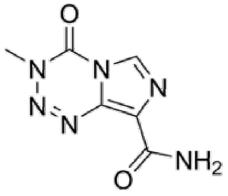
Riboflavin



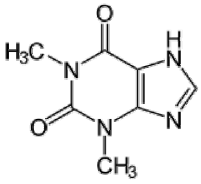
Ramipril



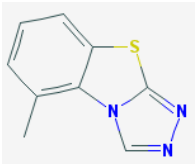
Sildenafil



Temozolomide



Theophylline



Tricyclazole

Application Index

Application Index

For more information about column specifications please contact your sales representative or visit our analytical chromatography webpage; see below. For technical assistance or application support please send an email to the EMD Millipore analytical chromatography support team, see email address below..

Introduction

Various regulatory authorities like International conference of harmonization (ICH), European Directorate for the Quality of Medicines and Healthcare (EDQM), United States Food and Drug Administration (USFDA), and Canadian Drug and Health Agency are emphasizing on the purity requirements and the identification of impurities in Active Pharmaceutical Ingredient's (API's).

Qualification of the impurities is the process of acquiring and evaluating data that establishes biological safety of an individual impurity; thus, revealing the need and scope of impurity profiling of drugs in pharmaceutical research and manufacturing. Identification of impurities is carried out with various analytical techniques, and where different chromatographic and spectroscopic techniques are common. Either alone or in combination with other techniques.

There are different methods for detecting and characterizing impurities with thin layer chromatography (TLC), high performance thin layer chromatography HPTLC, gas chromatography (GC), high performance liquid chromatography (HPLC), and atomic absorption spectroscopy (AAS) besides more classical tests based on titration.

Especially HPLC has been widely exploited for impurity profiling methods, and reasons for this is the wide range of detectors available that connect easily with HPLC along with the variety of column chemistries (stationary phases) commercially available. In simple words, with HPLC it is possible to develop robust and reliable methods having necessary sensitivity and linearity, that meet requirement in selectivity and provide cost effectiveness to the laboratory.

This guide focus on impurity profile analysis. The included methods show separations of different type of molecules; from hydrophobic to very hydrophilic drugs and their impurities. Thus, both reversed phase columns with RP-8 endcapped, RP-18 endcapped, and Phenyl stationary phases on either particulate silica particles (Purospher® STAR) or monolithic backbones (Chromolith® HighResolution) as well as hydrophilic interaction liquid chromatography (HILIC) columns (SeQuant® ZIC®-cHILIC and SeQuant® ZIC®-HILIC) have been used.

All methods are shown with complete experimental details to ease the implementation in your laboratory. EMD Millipore can offer virtually everything, beside the instrument, for your needs.

Changing a Regulated Method

What changes are allowed in a monograph method?

- Can we change the column material?
- Are we allowed to use a different column dimension?
- Is it allowed to scale down to smaller ID columns to save solvent?
- Is there a possibility to speed up separation?

The answer is YES to all these questions...but how?

	USP	EP
Column length	± 70%	± 70%
Inner diameter	Can be adjusted if linear velocity is kept constant	± 25%
Particle size	Reduction of 50%, no increase	Reduction of 50%, no increase
Flow rate	± 50% or more as long as linear velocity is kept constant	± 50%
Column temperature	± 10° C	± 10° C (max 60° C)
Injection volume	May be decreased (if LOD and repeatability is ok)	May be decreased (if LOD and repeatability is ok)
pH	± 0.2 units	± 0.2 units (± 1.0% for neutral substances)
UV wavelength	No adjustment permitted	No adjustment permitted
Buffer salts concentration	± 10%	± 10%
Mobile phase composition	± 30% relative or ± 10% absolute whichever is smaller	± 30% relative or ± 2% absolute whichever is larger

As long as the changes of a monograph method are within these limits it is possible to carry out only a partial revalidation followed by internal documentation of the updated method.
If changes are beyond these limits, a complete revalidation and documentation is required followed by discussion with auditor and regulating authorities for approval. It is (of course) also possible to submit completely new monograph methods to authorities. For more information, please read the EMD Millipore compilation about *monograph modernization* from 2013.

Amlodipine Besylate and Related Substances

From Particulate to Monolithic Column

The current impurity profiling method in USP36-NF31 for amlodipine besylate is based on TLC (test 1) and HPLC (test 2) where the liquid chromatograph is equipped with a 237 nm detector and a 150x3.9 mm column that contains packing L1. The flow rate is about 1.0 mL per minute.

Performance criteria to be met:
-For the purpose of identification, the relative retention times are about 0.2 for benzene sulfonate, 0.5 for amlodipine impurity A, and 1.0 for amlodipine. Amlodipine impurity A is 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate

The monograph was followed using a 150x4.6 mm Purospher® STAR RP-18 endcapped with 5 µm particle size, but not adjusting the flow-rate for the slightly larger inner diameter, page 10.
The monograph method was thereafter transferred using another column, see page 11.
No specific particle size is mentioned wherefore any type of column backbone can be used, and thus to comply with pharmacopoeial changes and perform only partial revalidation, the method can be changed by:

- Reduction of particle size to maximum 2.5 µm (50% since the first method uses a 5 µm particle in the written standard operating procedure - SOP) or use a monolithic column
- Shortening the column to a length of 45 mm (70%)
- Reduction of inner diameter if linear velocity is kept constant
- Reduction of injection volume as long as limit of detection (LOD) and linearity is OK.

A Chromolith® HighResolution RP-18 endcapped 100x4.6 mm column was chosen as the alternative for amlodipine besylate and its related impurities, page 11.

The alternative column met the performance criteria so why change to this alternative?

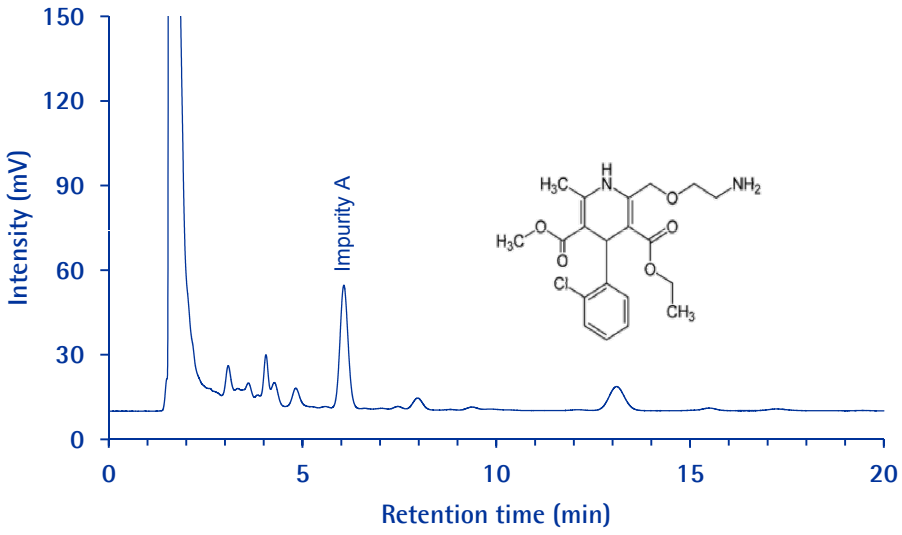
1. **The method will run faster** (Time-saving: 10 minutes per sample)
   (the column length is 33% shorter but total chromatographic analysis time is shortened by 50%).
2. **Higher chromatographic resolution**
   (Chromolith® HighResolution provide performance corresponding to sub-3 µm particle packed columns)
3. **Sensitivity enhancement**
   (Eluting peaks will have narrower width on a more efficient and shorter column and thus higher peak amplitude is attained)

Amlodipine Besylate and Related Substances

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 150x4.6 mm	1.51455.0001
Injection:	10 µL	
Detection:	UV 237 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Acetonitrile:Methanol:Buffer 15:35:50 (v/v)	
Buffer:	Add 7.0 ml of triethylamine in 1000 mL Milli-Q water, mix and adjust pH to 3.0 with orthophosphoric acid. Sonicate.	
Temperature:	25 °C	
Diluent	Mobile phase	
Standard:	Weigh 10 mg of Amlodipine Besylate in 20 mL volumetric flask. Add about 5.0 mL diluent and sonicate it. Dilute it up to the mark with the same. Further dilute 1.0 ml to 100 ml with diluent.	
Resolution Solution:	Weigh 5.0 mg of Amlodipine Besylate in 5.0 mL volumetric flask. Add 5.0 ml hydrogen peroxide. Heat it at 70°C for 45 min in water bath.	
Sample:	Crush 20 tablets. Weigh 50mg equivalent powder in 50 ml volumetric flask. Add about 30 ml diluent and sonicate it for 20 min. Dilute it up to the mark with the same.	



'does not need full revalidation'

Chromatographic Data :

No.	Compound	Retention Time (min)	RRT	Resolution	Theoretical plates
1	Amlodipine Impurity A	6.0	0.5	-	3966
2	Amlodipine Besylate	13.1	1.0	12.5	5026

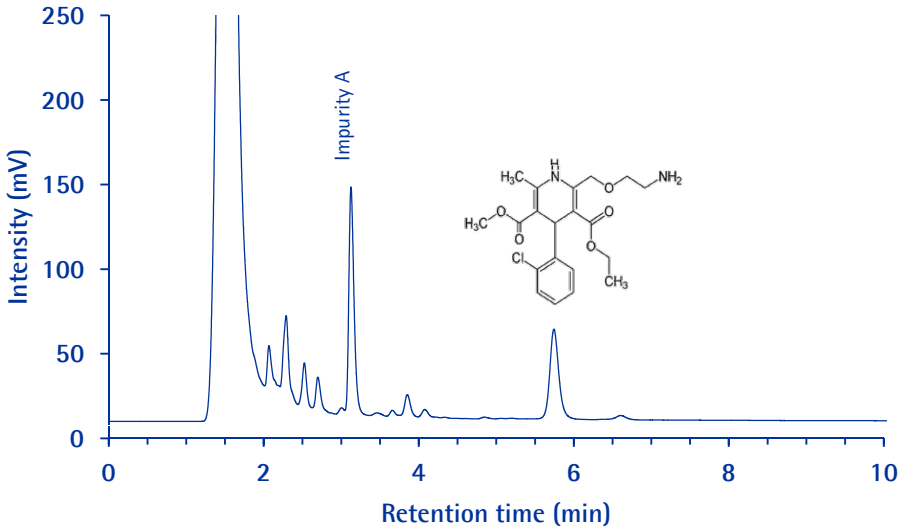
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Amlodipine Besylate and Related Substances

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® High Resolution RP-18 endcapped 100x4.6mm	1.52022.0001
Injection:	10 µL	
Detection:	UV 237 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Acetonitrile:Methanol:Buffer (15:35:50) (v/v)	
Buffer:	Add 7.0 mL of triethylamine in 1000 mL Milli-Q water. Mix and adjust pH to 3.0 with orthophosphoric acid. Sonicate.	
Temperature:	25 °C	
Diluent	Mobile phase	
Standard:	Weigh 10 mg of Amlodipine Besylate in 20 mL volumetric flask. Add about 5.0 mL diluent and sonicate it. Dilute it up to the mark with the same. Further dilute 1.0 ml to 100 ml with diluent.	
Resolution Solution:	Weigh 5.0 mg of Amlodipine Besylate in 5.0 mL volumetric flask. Add 5.0 mL hydrogen peroxide. Heat it at 70°C for 45 min in water bath.	
Sample:	Crush 20 tablets. Weigh 50mg equivalent powder in 50 ml volumetric flask. Add about 30 mL diluent and sonicate it for 20 min. Dilute up to the mark with diluent.	
Pressure Drop:	65-68 Bar (943-986 psi)	



'does not need full revalidation'

Chromatographic Data :

No.	Compound	Retention Time (min)	RRT	Resolution	Theoretical plates
1	Amlodipine Impurity A	3.1	0.5	-	11163
2	Amlodipine Besylate	5.7	1.0	16.1	12438

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Ethacrynic Acid and Related Compounds

From Particulate to Monolithic Column

Currently there is no impurity profiling method in USP for ethacrynic acid, **thus a new impurity profiling method would require method submission to authorities**. The assay method uses a 300x3.9 mm column that contains packing L1 (RP-18). Flow rate is about 1 mL per minute, and column efficiency determined from the analyte peak is not less than 1200 theoretical plates. The tailing factor for the analyte peak is not more than 2; the capacity (retention) factor, is not less than 0.8. Using these performance criteria, we developed an in-house method for ethacrynic acid and its seven impurities at one of our application laboratories. This new method was thereafter transferred within the scope of allowed monograph method changes, see page 8.
In the original impurity profiling method, see page 13, the liquid chromatograph should be equipped with 280 nm detector and a 250x4.6 mm column that contains packing L1 (RP-18). No specific particle size is mentioned, thus any type of column backbone can be used.

To comply with pharmacopoeial changes and perform only partial revalidation this method can be changed by:

- Reduction of particle size to maximum 2.5 µm (50% since method uses a 5 µm particle in written standard operating procedure - SOP) or use a monolithic column
- Shortening the column to 75 mm (70%)
- Reduction of inner diameter if linear velocity is kept constant
- Reduction of injection volume as long as limit of detection (LOD) and linearity is OK.

A monolithic Chromolith® HighResolution RP-18 endcapped 100x4.6 mm column was chosen as the alternative for ethacrynic acid and its related impurities, see page 14.

The alternative column met the performance criteria so why should you change?

1. **The method will run faster** (Time-saving: 30 minutes per sample)
   (yes...the column length is 60% shorter which is also the percentage of shortening of column).

2. **Better overall peak symmetry for Ethacrynic acid**
   (indicate a better loadability for ethacrynic acid on the monolithic over the particle packed column)

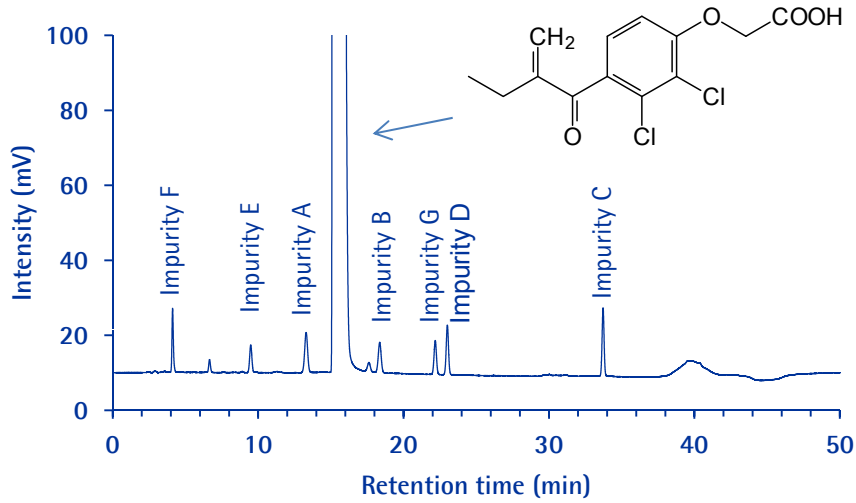
3. **Better overall resolution for more hydrophobic impurities**
   (whereas better overall resolution is attained for the less hydrophobic impurities on the particulate column)

Ethacrynic Acid and Related Impurities

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm	1.51456.0001
Injection:	20 µL	
Detection:	UV 280 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	A: Weigh 10 g of acetic acid and dilute to 1L with water. Adjust pH to 4.5 with dilute ammonia B: Methanol	
Gradient:	See Table	
Temperature:	50 °C	
Diluent:	Water and acetonitrile 65:45 (v/v)	
Sample:	Weigh 100 mg of ethacrynic acid in 100 mL volumetric flask. This gives a concentration of 1000 ppm. Add each impurity standard to get a 1 ppm level of impurities. Dilute with diluent.	
Pressure Drop:	83 Bar (1203 psi)	



Time (Min)	% A	% B
0.0	50	50
10.0	50	50
35.0	20	80
40.0	20	80
42.0	50	50
50.0	50	50

Chromatographic Data

No.	Compound	Time (min)	T_{USP}	Theoretical Plates*
1	Impurity F	4.1	1.3	8745
2	Impurity E	9.5	1.0	17736
3	Impurity A	13.3	1.0	19539
4	Ethacrynic acid	15.2	4.3	16100
5	Impurity B	18.4	1.0	39284
6	Impurity G	22.2	1.0	81421
7	Impurity D	23.0	1.0	96857
8	Impurity C	33.7	1.0	275157

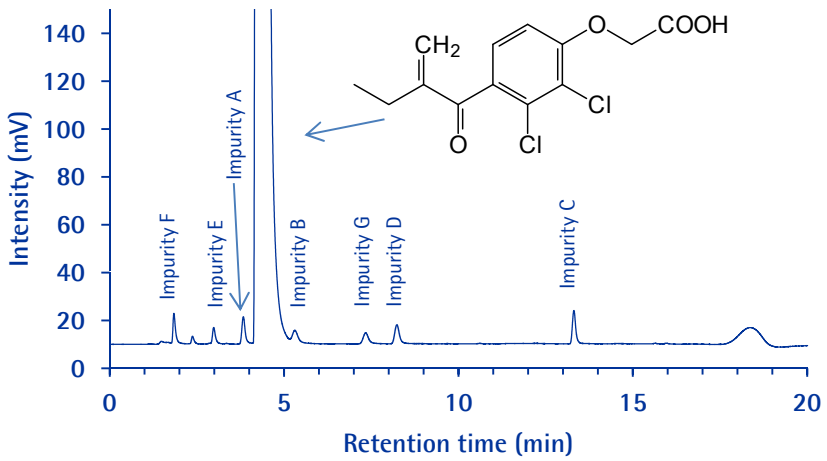
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Ethacrynic Acid and Related Impurities

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	10 µL	
Detection:	UV 280 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	A: Weigh 10 g of acetic acid and dilute to 1L with water. Adjust pH to 4.5 with dilute ammonia. B: Methanol	
Gradient:	See Table	
Temperature:	45 °C	
Diluent:	Water and acetonitrile 65:45 (v/v)	
Sample:	Weigh 100 mg of ethacrynic acid in 100 mL volumetric flask. This gives a concentration of 1000 ppm. Add each impurity standard to get a 1 ppm level of impurities. Dilute with diluent.	
Pressure Drop:	71 to 57 Bar (1029 to 826 psi)	



Time (Min)	% A	% B
0.0	50	50
4.0	50	50
14.0	20	80
16.0	20	80
16.8	50	50
20.0	50	50

Chromatographic Data

No.	Compound	Time (min)	T_{USP}	Theoretical Plates*
1	Impurity F	1.8	1.6	3482
2	Impurity E	2.9	1.6	6983
3	Impurity A	3.8	1.3	8653
4	Ethacrynic acid	4.3	2.4	1380
5	Impurity B	5.3	1.2	6814
6	Impurity G	7.3	1.3	11073
7	Impurity D	8.2	1.3	19091
8	Impurity C	13.3	1.3	99494

Ivermectin Injection Solution

From Particulate to Monolithic Column

As per the USP36 –NF31 monograph method for Ivermectin solution, the liquid chromatograph should be equipped with 245 nm detector and a 250x4.6 mm column that contains 5 µm packing L1 (RP-18). The Performance criteria to be met are:

- A relative retention time of about 1.3 to 1.5 to that of the principal peak is found
- The resolution between the first peak (H2B1b) and the second peak (H2B1a) is not less than 3.0

Within the scope of allowed monograph method changes, see page 8, and only to perform partial revalidation, the method can be changed by:

- Reduction of particle size to maximum 2.5 µm (50%)
- Shortening the column to a length of 75 mm (70%)
- Reduction of inner diameter if linear velocity is kept constant
- Reduction of injection volume as long as limit of detection (LOD) and linearity is OK.

Two different columns for the Ivermectin solution monograph method were used.
a) Purospher® STAR RP-18 endcapped (5µm) Hibar® 250x4.6 mm
b)Chromolith® HighResolution RP-18 endcapped 100x4.6 mm

The column alternative a) has the exact specification of the monograph method procedure and would only require a partial revalidation to prove that LOD, linearity, and performance criteria are met. Alternative b) the Chromolith® HighResolution RP-18 endcapped 100x4.6 mm column is a monolithic column (having no particle size), and thus would require a complete method revalidation and discussion/submission to auditor for acceptance.

Three reasons why we recommend changing to column alternative b) despite complete revalidation is required:

1. **The method will run three times faster** (Time-saving: 29 minutes per sample)
   (yes...the column length is 60% shorter, thus one reason for the method run-time reduction, but the shorter diffusion distances in a monolithic column gives advantages of particles).
2. **Higher chromatographic resolution between the two target molecules**
   (Chromolith® HighResolution provide performance corresponding to sub-3 µm particle packed columns)
3. **The method will run at 50% lower column backpressure**
   (No need to change instrument and still have high efficiency separation, and with low backpressure you also, as an added value get instrument safety at no extra cost. Less maintenance, less wear on pumps etc)

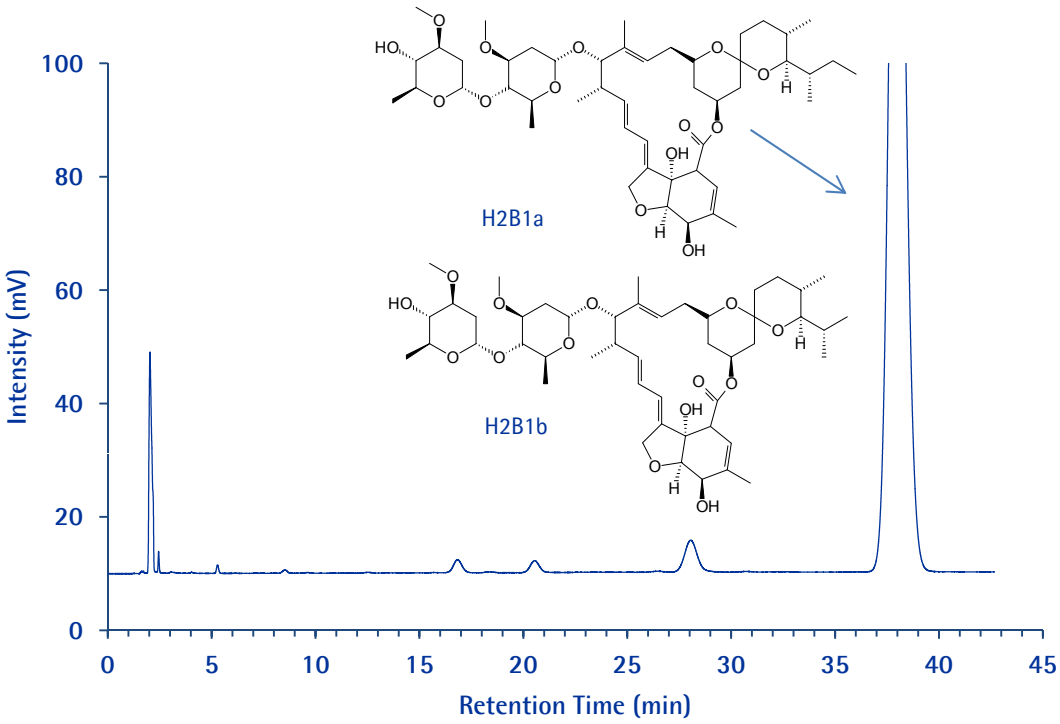
Ivermectin Injection Solution (USP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm	1.51456.0001
Injection:	20 µL	
Detection:	UV 245 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase :	Mixture of acetonitrile, methanol and water; 106:55:39 (v/v)	
Temperature:	Ambient	
Diluent:	Methanol	
Sample:	0.4 mg/mL (400 ppm) of each component in diluent	
Pressure Drop:	118 Bar (1711 psi)	

'does not need full revalidation'



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	T_{USP}	Resolution
1	H2B1b	28.0	1.0	1.1	–
2	H2B1a	38.0	1.36	1.1	7.9

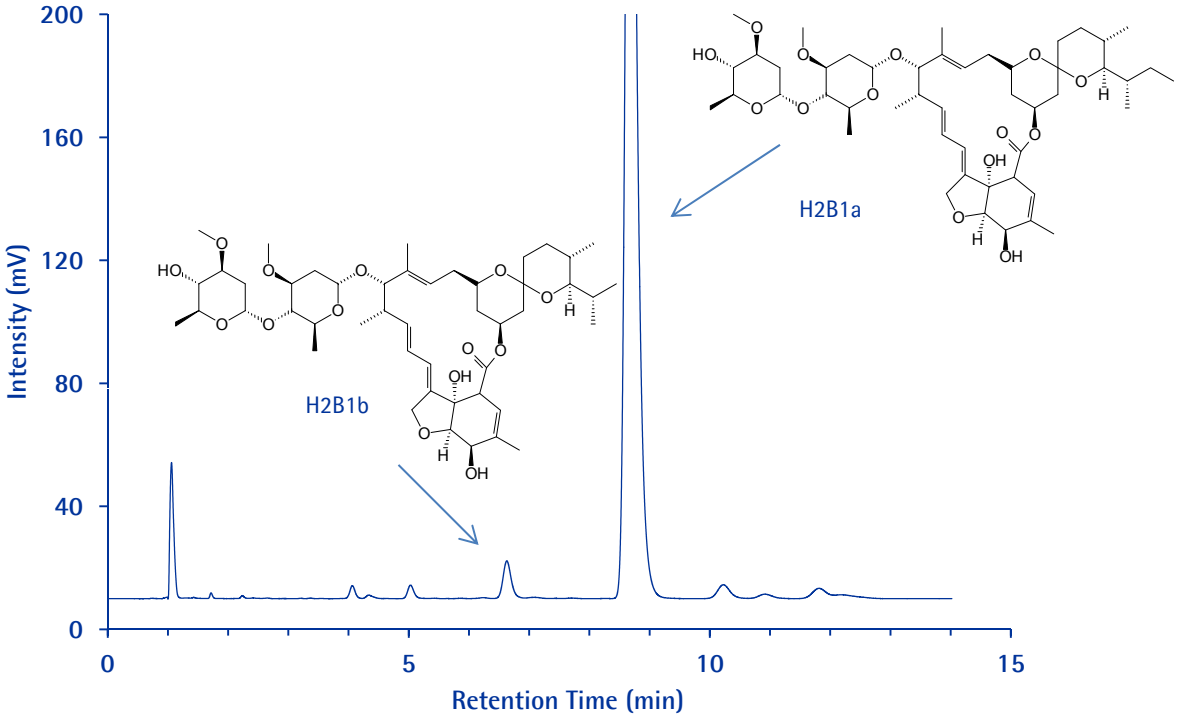
Ivermectin Injection Solution

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	10 µL	
Detection:	UV 245 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Mixture of acetonitrile, methanol and water; 106:55:39 (v/v)	
Temperature:	Ambient	
Diluent:	Methanol	
Sample:	0.4 mg/mL (400 ppm) of each component in diluent	
Pressure Drop:	50 Bar (725 psi)	

"needs full validation'



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	T_{USP}	Resolution
1	H2B1b	6.6	1.0	1.2	–
2	H2B1a	8.7	1.3	1.4	7.9

From HPLC to UHPLC

A transfer of HPLC methods to UHPLC requires scaling down from bigger to smaller inner diameter (e.g. 4.6 →2.1 mm i.d.) and from long to short columns (e.g. 250/150 to 100 or 50 mm length) in addition to the reduction of particle sizes (from e.g. 5 µm to 2 µm). To ensure equivalent chromatographic separation, it is also necessary to scale the flow rate, injection volume and the gradient parameters.

Adjusting the column length

The first step is to determine the appropriate column length in order to maintain the same separation. Keeping the same column length while decreasing the particle size will increase the number of theoretical plates as well as the backpressure. Therefore, when decreasing particle size, column length can be shortened without losing resolution.

$$\text{Column length } L_2 = L_1 \times dp_2 / dp_1$$

L_1 - HPLC column length
L_2 - UHPLC column length
dp_1 - HPLC particle size
dp_2 - UHPLC particle size

Scaling the flow rate

Decreasing the internal diameter of the column (e.g. from 4.6 to 2.1 mm) requires recalculating column flow rate in order to maintain same linear velocity. Linear velocity is defined as the distance which mobile phase travels over time (cm/min), whereas flow rate is the volume of mobile phase that travels over time (mL/min). To maintain the same linear velocity through a column with a smaller internal diameter, the flow rate must be decreased proportionally to the column internal diameter according to the equation below.

$$\text{Flow rate } f_2 = f_1 \times (d_2)^2 / (d_1)^2$$

f_1 - HPLC flow rate
f_2 - UHPLC flow rate (mL/min)
d_1 - HPLC column ID
d_2 - UHPLC column ID (mm)

Scaling the injection volume

Decreasing the column internal diameter and length, decreases the overall column volume and sample capacity. Therefore, we must alter the injection volume. Please note that since overall column volume has decreased, it is more important to match the sample solvent to the starting mobile phase composition. Mismatched sample solvents can cause irreproducible retention times, efficiencies, and even changes in selectivity. If using a larger injection volume than calculated, check for peak abnormalities and irreproducibility that could result from phase overload.

$$\text{Injection volume } V_2 = V_1 \times (d_2^2/d_1^2) \times (L_2 / L_1)$$

V_1 - HPLC Injection volume
V_2 - UHPLC Injection volume
d_1 - HPLC column ID
d_2 - UHPLC column ID (mm)
L_1 - HPLC column length
L_2 - UHPLC column length

Adjusting gradient time

When an analytical method is scaled down, the time program of the gradient also needs to be scaled down to keep the gradient volume the same.

$$\text{Time: } t_2 = t_1 \times (f_1/f_2) \times (d_2^2/d_1^2) \times (L_2 / L_1)$$

t_1 - HPLC time
t_2 - UHPLC time
f_1 - HPLC flow rate
f_2 - UHPLC flow rate (mL/min)
L_1 - HPLC column length
L_2 - UHPLC column length

Ramipril and Related Substances

From HPLC to UHPLC

The benefit of scaling from HPLC to UHPLC is illustrated with the USP36 –NF31 monograph method for ramipril related compounds, where the liquid chromatograph should be equipped with 210 nm detector and a 250x4.0 mm column that contains 3 µm packing L1 (RP-18) and is maintained at a temperature of 65°C.

Within the scope of allowed monograph method changes, and only to perform partial revalidation, this method can be changed by:

- Reduction of particle size to maximum 1.5 µm (50%)
- Shortening the column to a length of 75 mm (70%)
- Reduction of inner diameter if linear velocity is kept constant
- Reduction of injection volume as long as limit of detection (LOD) and linearity is OK.

Using the same mobile phases and gradient program as per monograph, this method was first finalized on a 250x4.6 mm Purospher® STAR RP-18 endcapped column with 5 µm packing, page 20, and thereafter scaled to a 100x2.1 mm Purospher® STAR RP-18 endcapped column with 2µm packing, see page 21. The UHPLC application is an allowed monograph modification per USP guidelines but the application using the larger HPLC column is not allowed. It is possible to reduce particle size, by maximum 50 %, but no increase.

Performance criteria:
Chromatograph the Resolution solution, and record the peak responses as directed for Procedure: the **resolution, R, between ramipril related compound A and ramipril is not less than 3.0.** Similarly chromatograph the Test solution, and record the peak responses as directed for procedure: **the retention time for ramipril is between 16 and 19 minutes; and the tailing factor for the ramipril peak is between 0.8 and 2.0.** Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 5.0%. [**The relative retention times are about 0.8 for ramipril related compound A, 1.0 for ramipril, 1.3 for ramipril related compound B,** 1.5 for ramipril related compound C, and 1.6 for ramipril related compound D.]

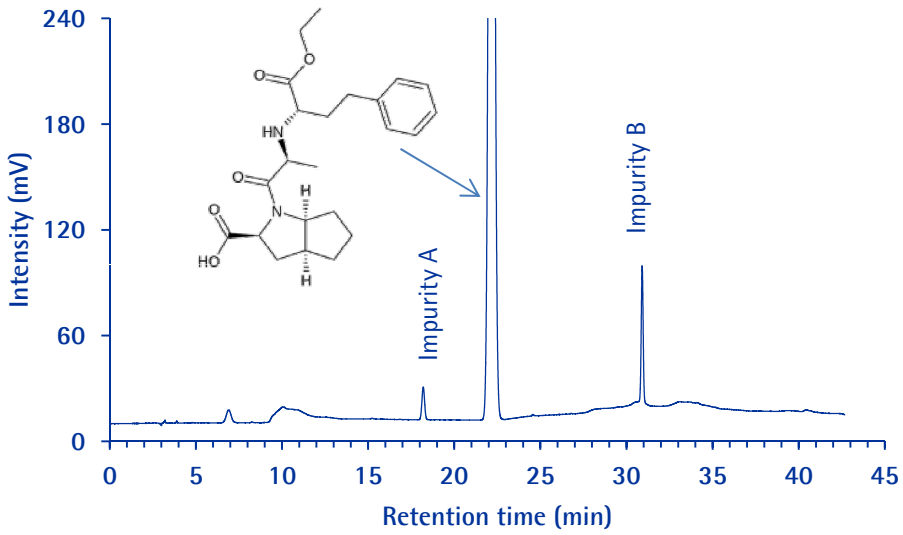
NOTE: no Ramipril related compound C and D were available at time of developing this application.
Thus reason why it is not marked as an USP method, despite it follow the monograph experimental conditions.

Ramipril and Related Substances

Purospher® STAR RP-18 endcapped (HPLC)

Chromatographic Conditions

Column: Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm 1.51456.0001
Injection: 10 µL
Detection: UV 210 nm
Cell: 10 µL
Flow Rate: 1.0 mL/min
Mobile Phase: A: Dissolve 2.0 g of sodium perchlorate in a mixture of 800 mL of Milli-Q water and 0.5 ml of triethylamine. Adjust pH to 3.6 with phosphoric acid. Add 200 mL and acetonitrile and mix.
B: Dissolve 2.0 g of sodium perchlorate in a mixture of 300 mL of Milli-Q water and 0.5 ml of triethylamine. Adjust pH to 2.6 with phosphoric acid. Add 700 mL acetonitrile and mix.
Gradient: See table
Temperature: 65 °C
Diluent Solution A
Sample: Dissolve 25 mg of sample in diluent and dilute to 25 ml with same solvent.
Pressure Drop: 61 to 74 Bar (884 to 1073 psi)



Time (min)	% A	% B
0.0	90	10
6.0	90	10
7.0	75	25
20.0	65	35
30.0	25	75
40.0	25	75
45.0	90	10
55.0	90	10

Chromatographic Data

No.	Compound	Retention Time (min)	RRT	Asymmetry
1	Ramipril RS A	18.2	0.82	1.0
2	Ramipril	22.2	1.00	1.0
3	Ramipril RS B	30.9	1.39	1.0

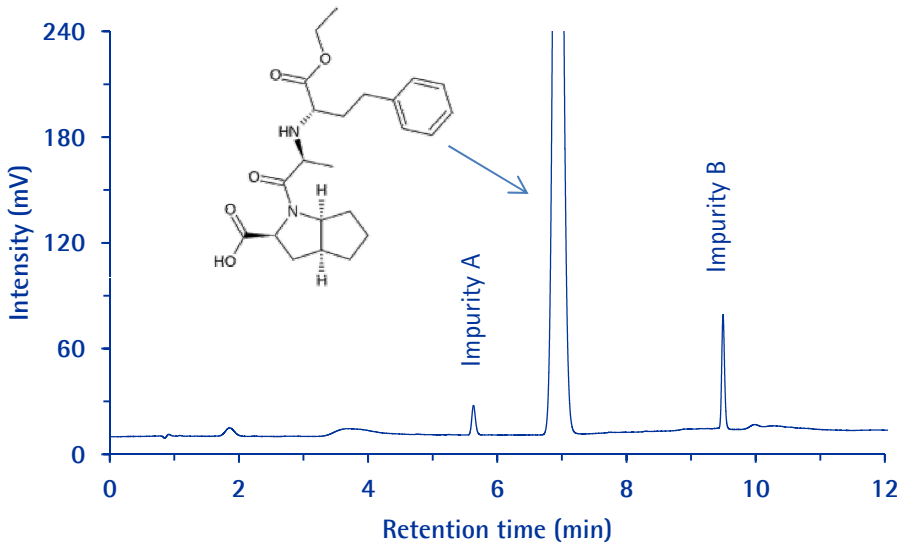
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Ramipril and Related Substances

Purospher® STAR RP-18 endcapped (UHPLC)

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (2µm) Hibar® HR 100x2.1 mm	1.50648.0001
Injection:	2 µL	
Detection:	UV 210 nm	
Cell:	2.5 µL (Use 0.1 mm tubing)	
Flow Rate:	0.3 mL/min	
Mobile Phase:	A: Dissolve 2.0 g of sodium perchlorate in a mixture of 800 mL of Milli-Q water and 0.5 ml of triethyl-amine. Adjust pH to 3.6 with phosphoric acid. Add 200 mL and acetonitrile and mix. B: Dissolve 2.0 g of sodium perchlorate in a mixture of 300 mL of Milli-Q water and 0.5 ml of triethyl-amine. Adjust pH to 2.6 with phosphoric acid. Add 700 mL acetonitrile and mix	
Gradient:	See table	
Temperature:	65 °C	
Diluent	Solution A	
Sample:	Dissolve 25 mg of sample in diluent and dilute to 25 ml with same solvent.	
Pressure Drop:	196 to 164 Bar (2827 to 2378 psi)	



Time (min)	% A	% B
0.0	90	10
1.66	90	10
1.93	75	25
5.54	65	35
8.31	25	75
11.08	25	75
12.46	90	10
15.23	90	10

'does not need full revalidation'

Chromatographic Data

No.	Compound	Retention Time (min)	RRT	Asymmetry
1	Ramipril RS A	5.6	0.81	1.1
2	Ramipril	6.9	1.00	1.1
3	Ramipril RS B	9.5	1.38	1.1

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Ramipril and Related Substances

From HPLC to UHPLC

As can be seen on page 20 and 21, both columns meet the performance criteria in terms of:

a) The resolution, R, between ramipril related compound A and ramipril (not less than 3.0)
b) The relative retention time between ramipril related compound A (ramipril RS A), ramipril and ramipril related compound B (ramipril RS B)
c) The tailing factor for the ramipril peak (between 0.8 and 2.0).

d) The application using HPLC conditions also meet the retention time requirement for ramipril

The UHPLC column - Purospher® STAR RP-18 endcapped (2µm) 100x2.1 mm thus seem to meet monograph and the customer would benefit from:

1. **Faster method** (Time-saving: 40 minutes per sample or 360%)
   (yes...the column length is 60% shorter and this provide 60% time saving but the real gain is to scale the method to a column with smaller particle size and not having to keep same linear velocity).

2. **Higher chromatographic resolution and efficiency**

....but this is not true. The retention time requirement for ramipril is NOT between 16 and 19 minutes. In addition, the flow rate has not been scaled to maintain same linear velocity. Monograph method is documented at 1.0 mL/min on 4.6 mm column and thus the flow rate should be reduced by a factor or 4.8 for the 2.1 mm i.d. UHPLC column, calculations see page 18. A flow rate of 0.2 mL/min should have been used instead of 0.3 mL/min. With the current experimental conditions, this would give comments from an auditor and very likely a request for method change.

The larger Purospher® STAR RP-18 endcapped (5µm) 250x4.6 mm column can definitely not be used. The particle size is larger than monograph method and would require complete revalidation and discussion with auditor and authorities. Most likely it would not be an accepted method.

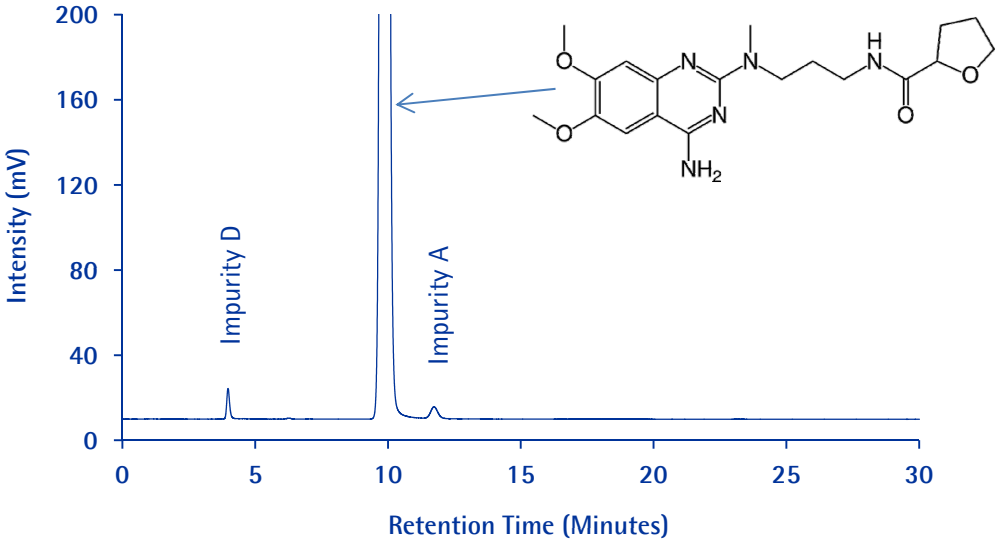
More information about EMD Millipore UHPLC columns and how to appropriately scale methods can be found at www.EMDmillipore.com/chromatography; in Chrombook and the 2013 application guide – UHPLC[2].

Alfuzosin and Related Substances (USP)

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 150x4.6 mm	1.51455.0001
Injection:	10 µL	
Detection:	UV 254 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase (v/v):	5 ml of perchloric acid in 900 mL Milli-Q water. Adjust pH to 3.5 with 2M NaOH. Diluter to 1000 mL with water. Mix buffer, acetonitrile & THF 80:20:1 (v/v)	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	400 ppm (0.4 mg/mL) Alfuzozin RS in mobile phase	
Pressure Drop:	150 Bar (2175 psi)	



Chromatographic Data

No	Compound	Time (min)	Resolution	Asymmetry (T_{USP})	RRT
1	Impurity D	4.0	-	1.2	0.4
2	Alfuzosin	9.9	-	1.1	1.0
3	Impurity A	11.7	4.1	1.1	1.2

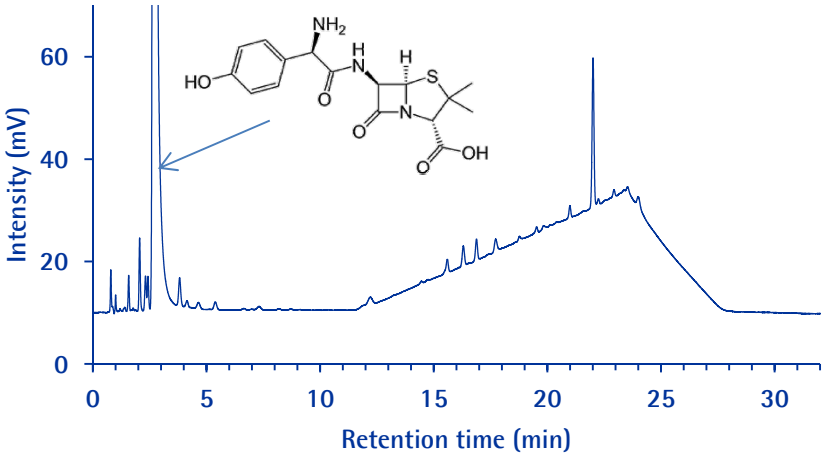
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Amoxicillin and Related Substances

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (3µm) Hibar® RT 100x4.6 mm	1.50469.0001
Injection:	10 µL	
Detection:	UV 210 nm	
Flow Rate:	1.5 mL/min	
Mobile Phase(v/v):	Solution A: 2.72 g/L of monobasic potassium phosphate. Adjust pH to 5.0 with 1M potassium hydroxide solution.. Solution B: Methanol	
Gradient:	See table	
Temperature:	40 °C (column) and 4 °C (autosampler)	
Standard:	Dissolve 1.25 mg of amoxicillin standard in Solution A and dilute to 100 mL with same solvent.	
Sample:	Weigh 125 mg of amoxicillin sample and dissolve in solution A. Dilute to 100 mL with same solvent. Use solution within four hours of preparation.	
Pressure Drop:	160 Bar to 215 Bar (2320 psi to 3118 psi)	



Time	% A	% B
0.01	97	3
10.0	97	3
22.0	75	25
26.0	97	3
32.0	97	3

Chromatographic Data

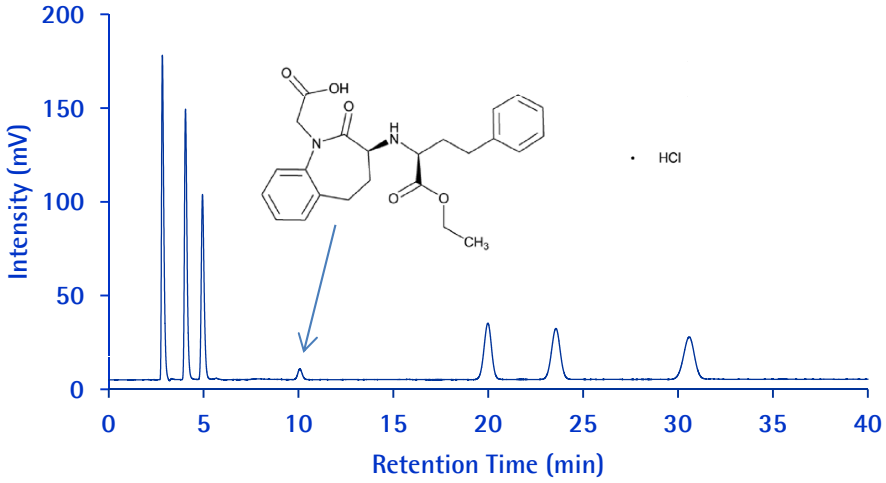
No.	Compound	Retention Time (min)	RRT	Tailing Factor
1	Amoxicillin related compound I	1.00	0.36	1.0
2	Amoxicillin related compound D	1.58	0.57	1.2
3	Amoxicillin related compound A	2.06	0.75	1.0
4	Amoxicillin related compound B	2.32	0.84	1.2
5	**Amoxicillin**	**2.75**	**1.00**	
6	Amoxicillin related compound E	12.21	4.44	1.1
7	Amoxicillin related compound M	16.30	5.93	1.0
8	Amoxicillin related compound F	16.88	6.12	0.9
9	Amoxicillin related compound C	17.73	6.45	1.0
10	Amoxicillin related compound J	24.00	8.73	1.0

Benazepril HCl and Related Substances (USP)

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5µm) 250x4.0 mm	1.50037.0001
Injection:	25 µL	
Detection:	UV 240 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Buffer: 0.81 gram of tetrabutyl ammonium bromide in 360 mL water containing 0.2 mL acetic acid. Mix. buffer and Methanol 36:64 (v/v).	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	Benazepril (1 ppm) + imp B, C, D, E, F and G (10 ppm each)	
Pressure Drop:	200 Bar (2900 psi)	



Chromatographic Data

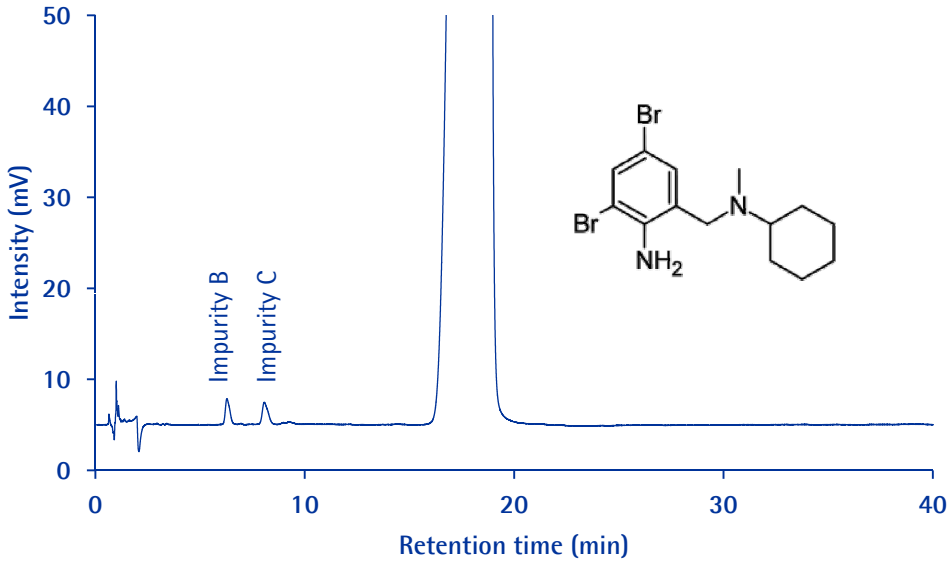
No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Impurity E	2.8	0.3	0.0	1.6
2	Impurity F	4.0	0.4	5.3	1.4
3	Impurity C	4.9	0.5	3.5	1.3
4	**Benazepril**	10.1	1.0	14.7	1.1
5	Impurity B	20.0	2.0	17.1	1.0
6	Impurity D	23.6	2.3	4.5	1.0
7	Impurity G	30.6	3.0	7.3	1.0

Bromhexine and Related Substances (BP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (3µm) Hibar® RT 100x4.6 mm	1.50469.0001
Injection:	10 µL	
Detection:	UV 248 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Add 0.5 mL orthophosphoric acid in 950 mL Milli-Q water. Adjust pH to 7.0 with triethylamine. Dilute solution to 1000 mL with Milli-Q water. Mix buffer and acetonitrile 20:80 (v/v)	
Temperature:	25 °C	
Diluent	Methanol	
Sample:	50 mg of sample in 10 mL of diluent (5000 ppm).	
Pressure Drop:	75 Bar (1087 psi)	



Chromatographic Data

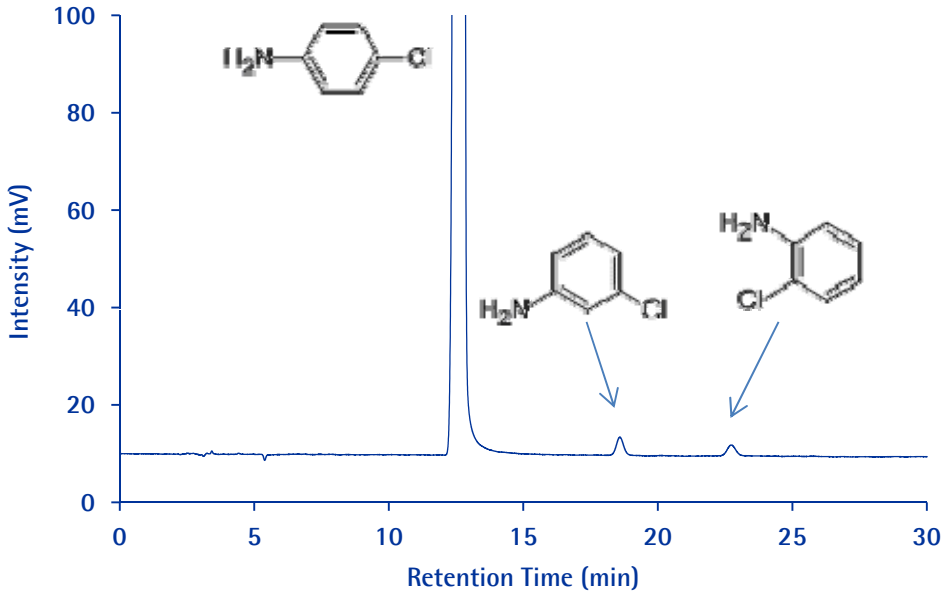
No.	Compound	Retention Time (min)	Resolution	Relative Retention Time
1	Impurity B	6.3	0.0	0.34
2	Impurity C	8.1	4.0	0.44
3	Bromhexine	18.5	14.8	1.00

4-Chloroaniline and Related Substances

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	UV 220 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	2 mL triethylamine in 1000 mL Milli-Q water. Adjust pH to 3.0 ± 0.1 with orthophosphoric acid. Mix buffer and acetonitrile 70:30 (v/v)	
Temperature:	25 °C	
Diluent	Mobile phase	
Sample:	500 ppm of 4-Chloroaniline, 1 ppm of each 3-Chloroaniline and 2-Chloroaniline in diluent	
Pressure Drop:	150 Bar (2175 psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	Resolution	Theoretical plates*
1	4-Chloroaniline	12.7	-	12490
2	3-Chloroaniline	18.6	12.3	22606
3	2-Chloroaniline	22.7	7.7	24143

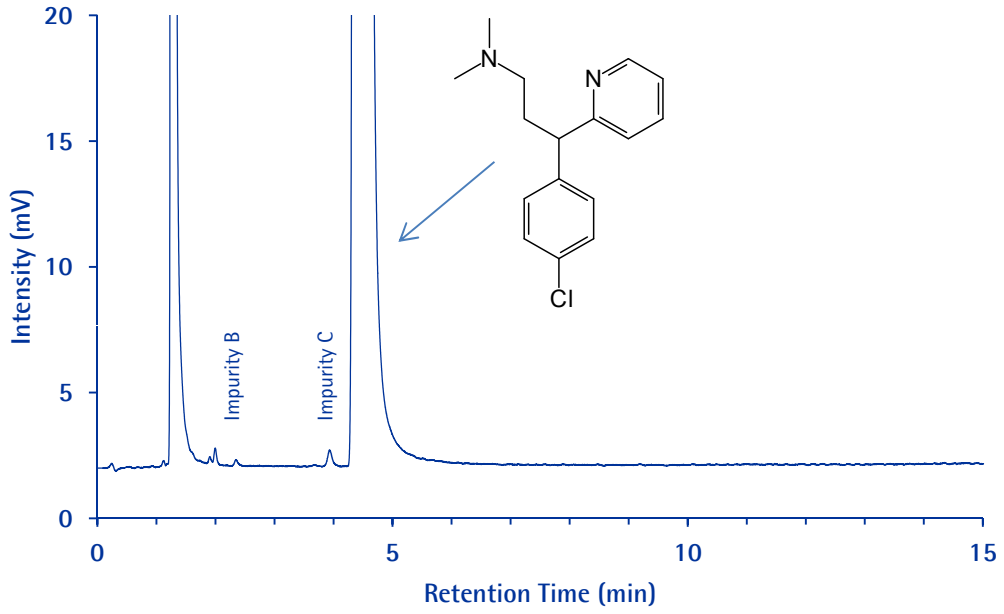
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Chloropheniramine Maleate Related Substances

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	5 µL	
Detection:	UV 225 nm	
Cell:	10 µL	
Flow Rate:	1.2 mL/min	
Mobile Phase:	8.57 g ammonium di-hydrogen phosphate in 1L water. pH adjust to 3.0 with ortophosphoric acid. Mix buffer and acetonitrile 80:20 (v/v).	
Temperature:	25 °C	
Diluent:	Mobile phase	
Sample:	100 mg of each substance in 100 ml mobile phase.	
Pressure Drop:	65 Bar (943 psi)	



Chromatographic Data

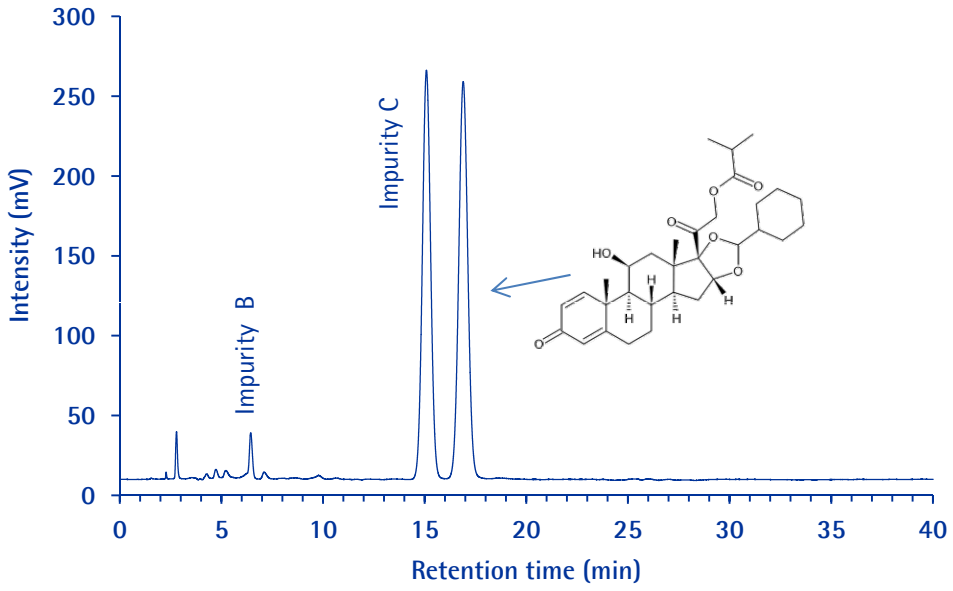
No.	Compound	Time (min)	Resolution	Relative Retention Time (RRT)
1	Maleic Acid	1.3	–	0.23
2	Impurity B	2.0	10.4	0.45
3	Impurity C	3.9	13.9	0.90
4	Chlorpheniramine	4.4	2.2	1.00

Ciclesonide and Related Substances

Purospher® STAR Phenyl

Chromatographic Conditions

Column:	Purospher® STAR Phenyl (5µm) Hibar® RT 250x4.6 mm	1.51918.0001
Injection:	20 µL	
Detection:	UV 243 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Mix water and anhydrous ethanol 38:62 (v/v)	
Temperature:	60 °C	
Standard:	Dissolve 3 mg of impurity B, 3 mg of impurity C, 5 mg impurity A in anhydrous ethanol and dilute to 10.0 mL with same solvent.	
Sample:	Dissolve 50 mg of substance to be examined in anhydrous ethanol dilute to 50 mL with ethanol.	
Pressure Drop:	140 Bar (1988 psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	RRT	Resolution
1	Impurity B	6.4	0.38	
2	Impurity C	15.1	0.89	
3	Ciclesonide	16.9	1.00	2.30

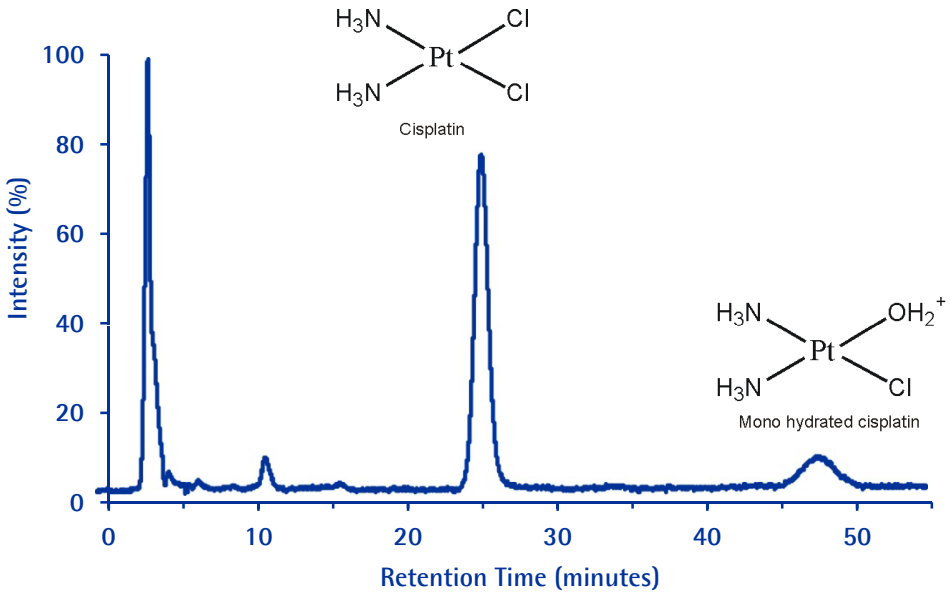
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Cisplatin and Related Substances

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5µm, 200Å) PEEK 150x2.1 mm,	1.50454.0001
Injection:	1 µl	
Detection:	UV 305nm	
Flow Rate:	0.1 mL/min	
Mobile Phase :	Buffer: Ammonium formate 25mM pH 6.5. Mix 1,4-dioxane and Buffer 80:20 (v/v) (Total ionic strength: 5mM).	
Temperature:	Ambient	
Diluent	Mobile phase without buffer	
Sample:	Cisplatin	



Chromatographic Data

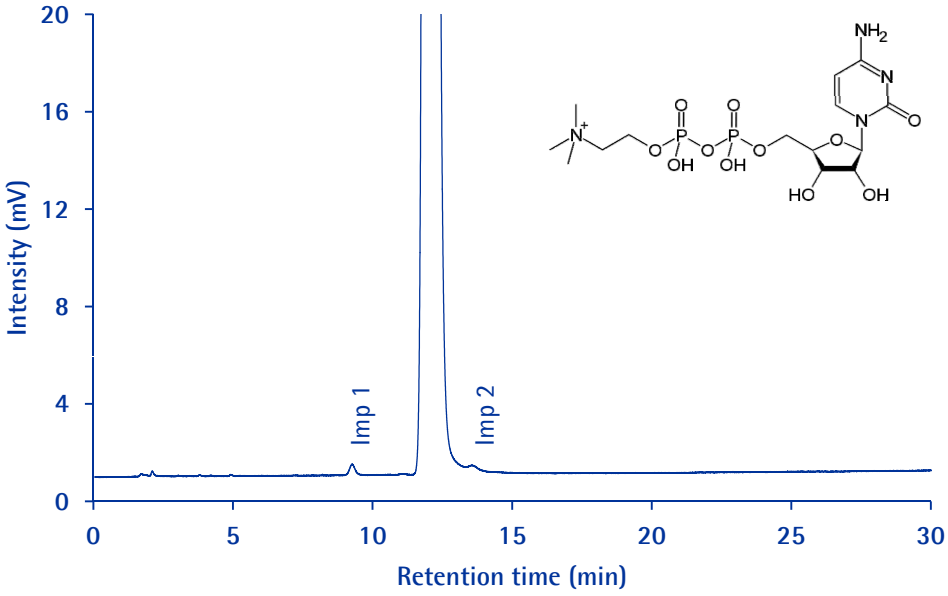
No.	Compound	Retention Time (min)	Resolution	Asymmetry
1	Cisplatin	25.6	-	-
2	Monohydrated cisplatin	47.8	-	-

Citicoline and Related Impurities

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC (3 µm, 100 Å) PEEK 150×4.6 mm	1.50661.0001
Injection:	20 µL	
Detection:	UV 276 nm	
Flow Rate:	0.75 mL/min	
Mobile Phase (v/v):	Buffer: Weigh 7.7 g ammonium acetate and dissolve in in 1L MilliQ water. Mix acetonitrile and buffer 70:30 (v/v). Total ionic strength:30 mM	
Temperature:	30 °C	
Diluent	Mobile phase	
Sample:	Weigh 25 mg citicoline in 50 ml volumetric flask. Dissolve in diluent.	
Pressure:	59 Bar (856 psi)	



Chromatographic Data

No.	Compound	Time	T_{USP}	Resolution*
1	Impurity 1	9.3	1.1	-
2	Citicoline	12.0	1.1	7.1
3	Impurity 2	13.6	1.1	3.9

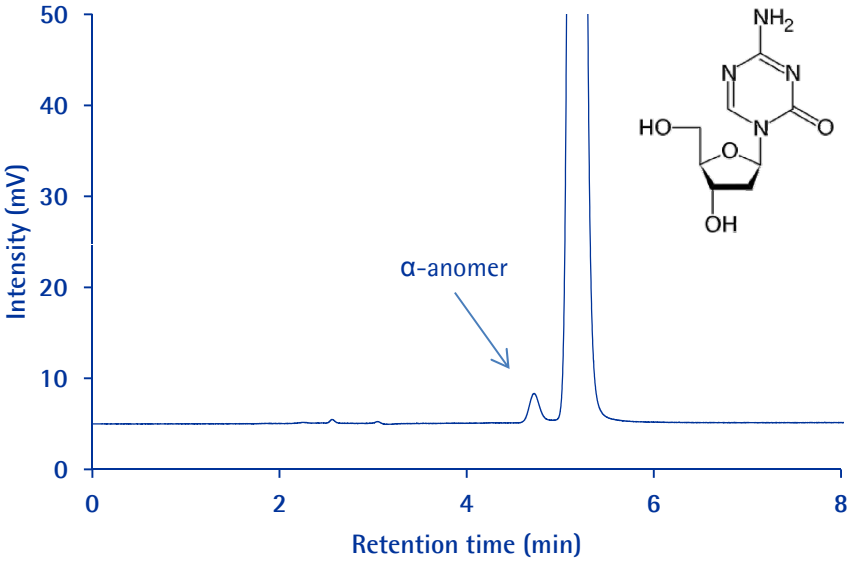
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Decitabine and Related Impurities

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5µm,200Å) PEEK 150x4.6 mm	1.50455.0001
Injection:	10 µL	
Detection:	UV 254 nm	
Cell:	10 µL	
Flow Rate:	0.75 mL/min	
Mobile Phase:	Dissolve 3.84 g of ammonium acetate in 1L water (50 mM). Mix buffer & acetonitrile 15:85 (v/v)	
Temperature:	Oven: 25 °C	
Diluent:	Mobile phase	
Sample:	Weigh 100 mg of sample in 100 mL volumetric flask. Dilute up to the mark with mobile phase. Pipette out 10 mL of the above solution and dilute to 50 ml with mobile phase.	
Pressure Drop:	30 Bar (435 psi)	



Chromatographic Data :

No.	Compound	Time (min)	Tailing Factor	Resolution
1	α-anomer	4.7	1.1	
2	Decitabine	5.2	1.1	2.2

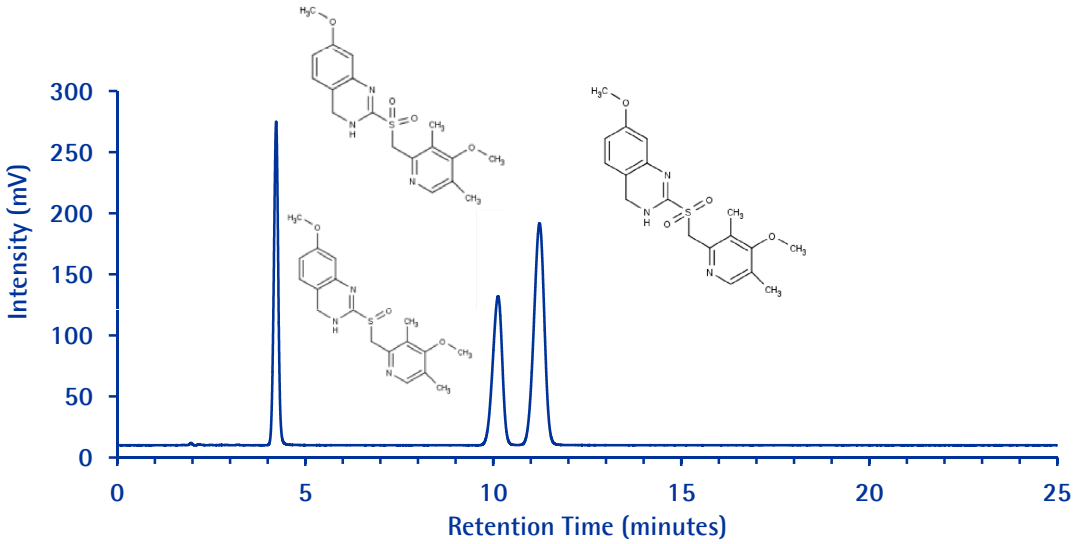
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Esomeprazole Magnesium – Impurities (USP)

Purospher® STAR RP-8 endcapped

Chromatographic Conditions

Column:	Purospher STAR RP-8 endcapped (5µm) Hibar® RT 150x4.6 mm	1.51453.0001
Injection:	50 µL	
Detection:	UV 280 nm	
Cell:	13 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Buffer: 0.725 g of monobasic sodium phosphate and 4.472g of anhydrous dibasic sodium phosphate in 300 mL of water, and diluting with water to 1000 mL. Dilute 250 mL of this solution with water to 1000 mL. Adjust pH to 7.6 with phosphoric acid if necessary. Mix acetonitrile and buffer 25:75 (v/v)	
Temperature:	25 °C	
Diluent	Mobile phase	
Sample:	System suitability solution:1 mg of USP Omeprazole RS and 1 mg of USP Omeprazole Related Compound A RS in 25 ml of diluent.	
Pressure Drop:	103 Bar (1493.5 psi)	



Chromatographic Data

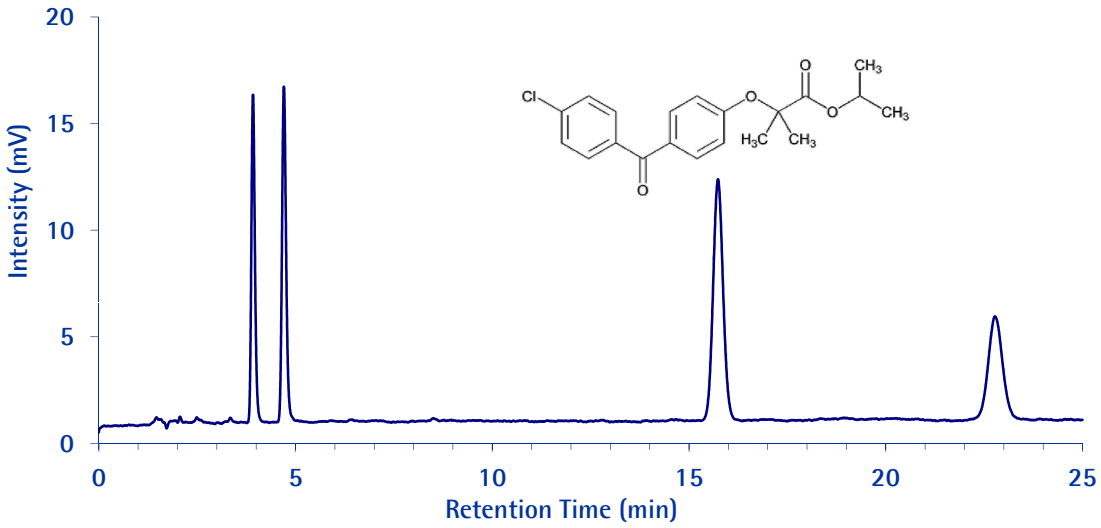
No.	Compound	Retention Time (min)	RRT	Resolution
1	t0	1.95	-	-
2	Omeprazole Related Compound I	4.23	0.38	-
3	Omeprazole Related Compound A	10.13	0.9	17.0
4	Omeprazole RS	11.23	1.0	3.0

Fenofibrate and Related Substances (USP)

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5µm) 250x4.0 mm	1.50037.0001
Injection:	20 µL	
Detection:	UV 286 nm	
Cell:	13 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Acetonitrile and water acidified with phosphoric acid to a pH of 2.5. Mix water and acetonitrile 30:70.	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	1 ppm of Fenofibrate, Fenofibrate RS A and RS B, and 2ppm Fenofibrate RS C	
Pressure Drop:	225 Bar (3263 psi)	



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Plates (N)	Resolution	Asymmetry (T_{USP})
1	Fenofibrate RS A	3.9	0.25	8919	-	1.2
2	Fenofibrate RS B	4.7	0.30	9719	4.4	1.2
3	**Fenofibrate**	15.7	1.00	17459	33.1	1.1
4	Fenofibrate RS C	22.7	1.45	17947	12.2	1.1

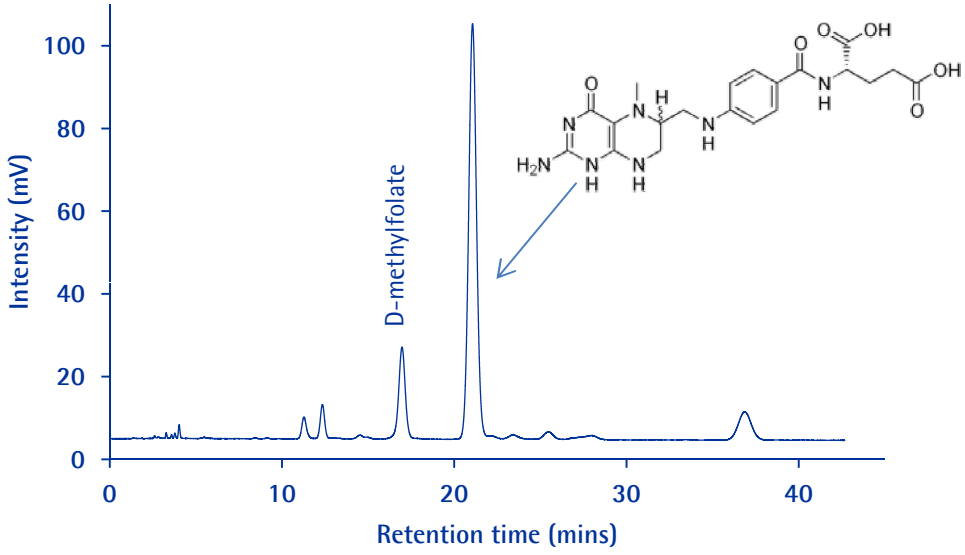
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

L-methyl folate and D-methyl folate

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC (3µm, 100Å) PEEK 150x4.6 mm	1.50661.0001
Injection:	10 µL	
Detection:	UV 280 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Dissolve 3.54 g of ammonium acetate in 1000mL Milli-Q water. Mix buffer and acetonitrile 23:77 (v/v)	
Temperature:	30 °C	
Standard:	Take 25 mg of L-methyl folate standard in 100 mL volumetric flask and dissolve in 30 mL of buffer. Sonicate solution and make up to final volume with acetonitrile.	
Sample:	Take 25 mg of sample in 100 mL volumetric flask and dissolve in 30 ml of buffer. Sonicate solution and make up to final volume with acetonitrile.	
Pressure Drop:	70 Bar(1015 psi)	



Chromatographic Data :

No.	Compound	Retention Time (min)	Theoretical Plate	Resolution
1	D-methyl folate	16.0	9811	0
2	L-methyl folate	21.1	10054	5.4

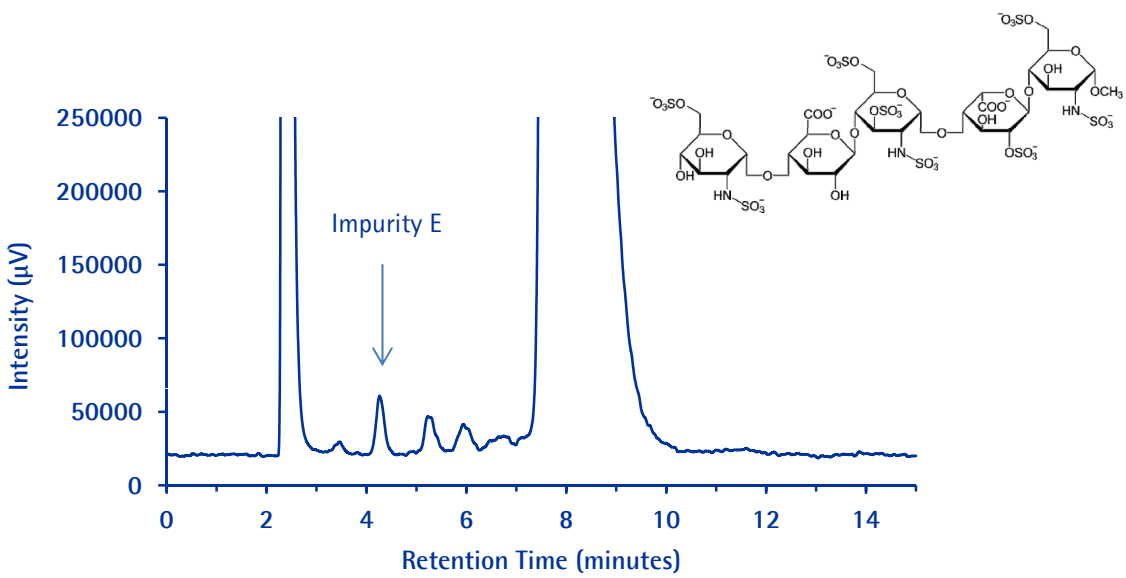
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Fondaparinux and Related Impurities

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC (3µm, 100Å) PEEK 150×4.6 mm	1.50661.0001
Injection:	20 µL	
Detection:	ELSD	
Cell:	Standard HPLC nebulizer	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Buffer: 160mM Ammonium acetate pH5.0. Mix Methanol and Buffer 45:55 (v/v)	
Temperature:	40 °C	
Diluent	Methanol/water 50/50	
Sample:	9000 ppm (9mg/ml Fondaparinux, 90 ppm Impurity E (1%)	
Pressure Drop:	140 bar (2000 psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	Resolution	Tailing Factor
1	t'0	2.4		
2	Impurity E	4.2	6.0	1.2
3	Fondaparinux	8.2	4.3	1.2

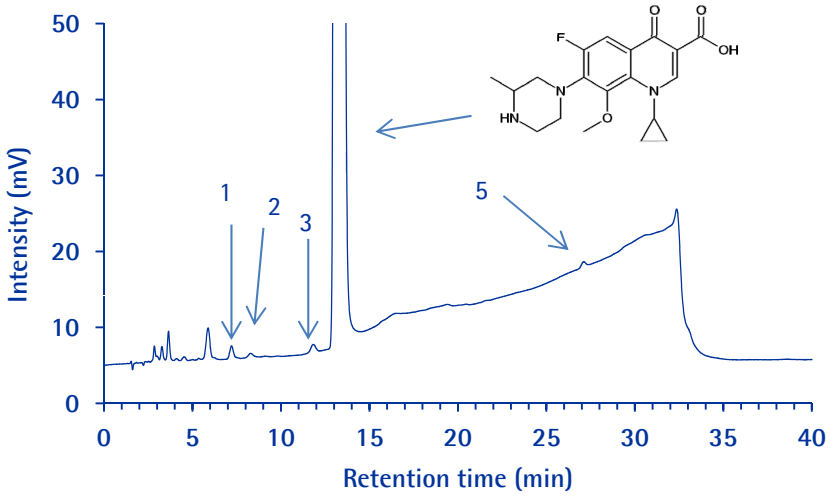
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Gatifloxacin and Related Substances (Eye Drops)

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column: Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm 1.51456.0001
Injection: 20 µL
Detection: UV 240 nm & 285 nm
Flow Rate: 1.5 mL/min
Mobile Phase: Dissolve 6.6 mL of 40 % Tetrabutylammonium hydroxide solution and 6.6 g of di-ammonium hydrogen phosphate in 1000 mL water. Adjust pH to 9.5 ± 0.05 with ammonia solution (25%).
Filter through 0.45 µm nylon membrane filter, 47mm.
Solution A: Buffer and acetonitrile 84:16 (v/v)
Solution B: Buffer, acetonitrile and methanol 65:25:10 (v/v/v)
Gradient: See table
Temperature: 40 °C
Diluent: Water and acetonitrile 90:10 (v/v)
Standard: Dissolve 10 mg of Gatifloxacin in 100 mL of diluent. Dilute the stock solution 100 times with diluent.
Sample: Weigh 4 gm of eye drops and dilute to 20 ml with diluent.



Time	%A	%B
0.0	100	0
8.0	100	0
30.0	0	100
30.1	100	0
40.0	100	0

Chromatographic Data

No.	Compound	Retention Time (min)	RRT	Asymmetry
1	Desmethyl Gatifloxacin	7.2	0.55	1.1
2	8-Hydroxy Gatifloxacin	8.3	0.63	1.4
3	Isogatifloxacin	11.8	0.90	0.9
4	Gatifloxacin	13.2	1.00	1.3
5	Difluoromethoxy Gatifloxacin	27.1	2.05	1.1

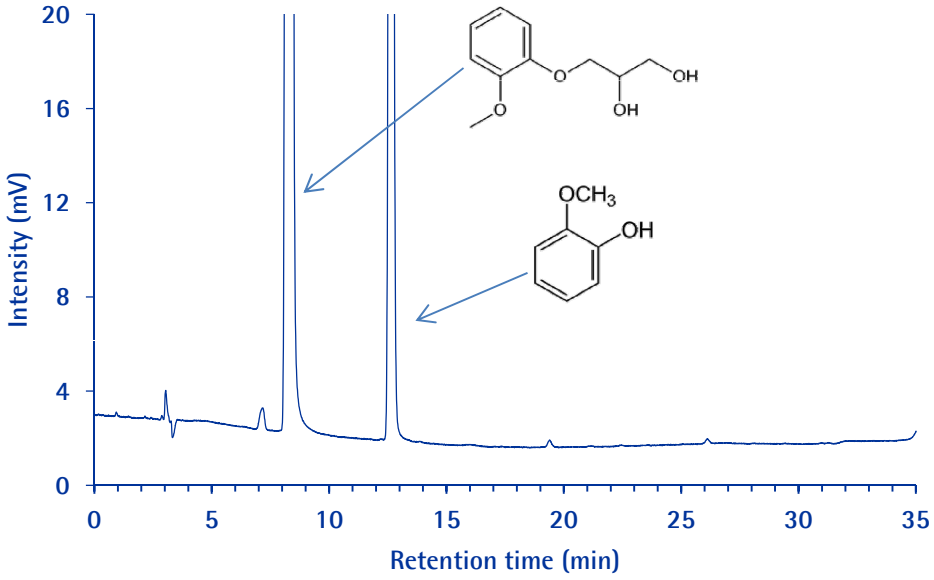
Guaifenisin and Related Substances (USP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	UV 276 nm	
Cell:	8 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase	Solution A: Glacial acetic acid and water (10:990) Solution B: Acetonitrile	
Gradient:	See Table:	
Temperature:	30° C	
Diluent	mobile phase	
Sample:	500 ppm (0.5 mg/mL) Guaiphenesin and 20 ppm (0.02 mg/mL) Guaiacol	
Pressure Drop:	142 Bar (2059 psi)	

Time (min)	A (%)	B (%)
0-32	80→50	20→50
32-35	50→80	50→20



Chromatographic Data

No.	Compound	Time (min)	(T_{USP})	Relative Retention Time (RRT)	Resolution
1	Guaifenesin beta isomer	7.2	1.0	0.9	
2	Guaifenesin	8.3	1.0	1.0	
3	Guaiacol	12.6	1.1	1.5	4.8

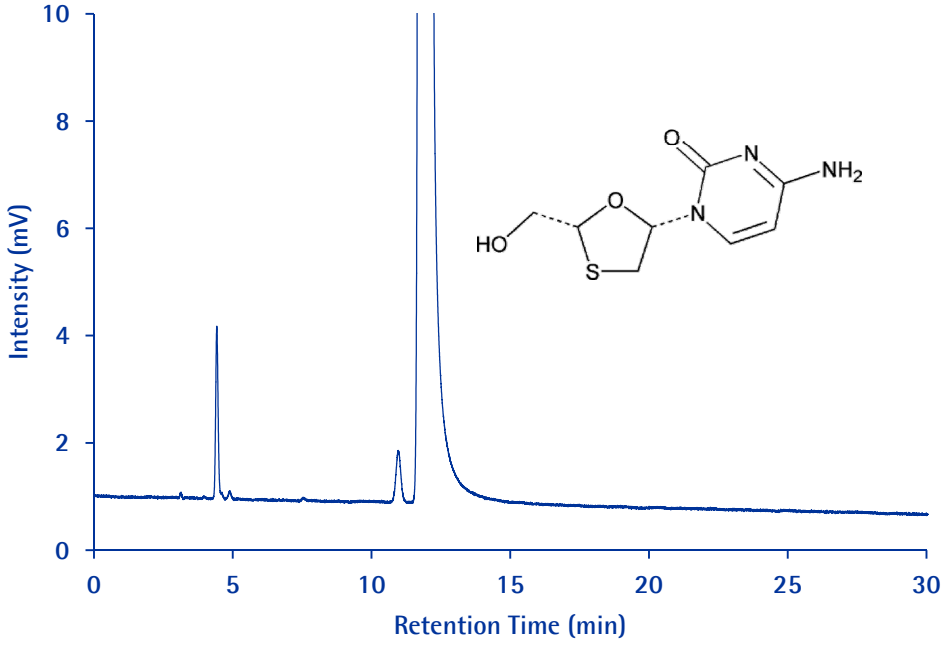
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Lamivudine and Related Substances (USP)

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	UV 277 nm	
Cell:	8 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Buffer: 0.025 M Ammonium acetate solution, with pH adjusted to 3.8 ± 0.2 with acetic acid Mix buffer and methanol 95:5 (v/v).	
Temperature:	35° Celsius	
Diluent	mobile phase	
Sample:	250 ppm (0.25 mg/mL) Lamivudine and traces of lamivudine diastereomer	
Pressure Drop:	134 Bar (1943 psi)	



Chromatographic Data

No.	Compound	Time (min)	Tailing Factor (TUSP)	Relative Retention Time (RRT)	Resolution (Rs)
1	Specified Impurity 1	4.4	1.1	0.4	
2	Specified Impurity 2	11.0	1.0	0.9	
3	Lamivudine	11.9	1.0	1.0	2.9

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

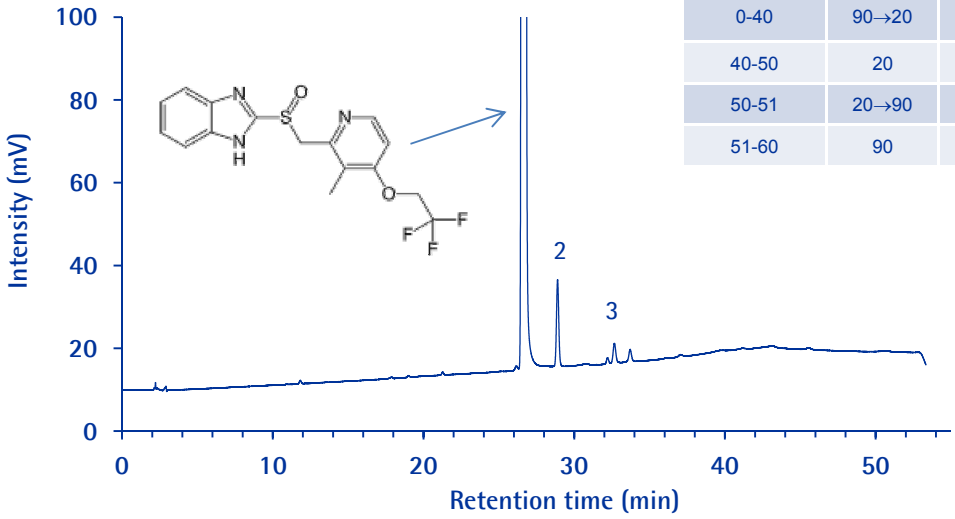
Lansoprazole and Related Substances (USP)

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5µm) Hibar® RT 150x4.6 mm	1.51455.0001
Injection:	40 µL	
Detection:	UV 285 nm	
Cell:	8 µL	
Flow Rate:	0.8 mL/min	
Mobile Phase:	Solution A: 100% Water Solution B: Acetonitrile, water, and triethylamine; 160:40:1 (v/v)with a pH of 7.0	
Gradient	See Table	
Temperature:	Ambient	
Diluent	mixture of 0.1 N sodium hydroxide solution and methanol; 75:25 (v/v)	
Sample:	250 ppm of Lansoprazole	

Time (min)	A (%)	B (%)	Elution
0-40	90→20	10→80	Linear
40-50	20	80	Isocratic
50-51	20→90	80→10	Linear
51-60	90	10	Isocratic



Chromatographic Data

No.	Compound	Time (min)	T_{USP}	Relative Retention Time (RRT)	Resolution
1	Lansoprazole	26.6	1.0	1.0	
2	Lansoprazole RS A	28.9	1.1	1.1	8.0
3	Lansoprazole RS B	32.7	1.1	1.2	

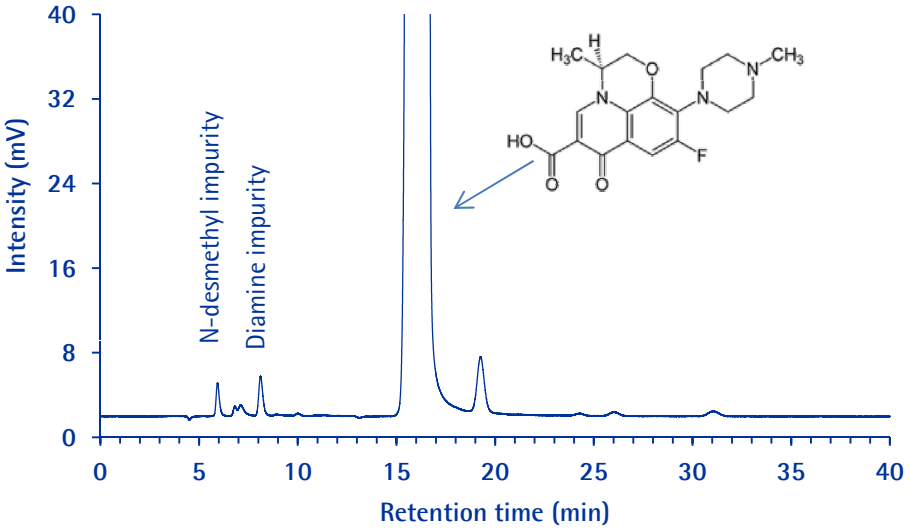
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Levofloxacin and Related Substances

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm	1.51456.0001
Injection:	25 µL	
Detection:	UV 360 nm	
Cell:	10 µL	
Flow Rate:	0.8 mL/min	
Mobile Phase:	8.5 g ammonium acetate, 1.25 g cupric sulphate pentahydrate, .3 g of L-isoleucine in 1000 mL water. Mix buffer and Methanol 7:3 (v/v)	
Temperature:	45° C	
Sample:	Weigh 10 mg substance and dissolve in mobile phase. Dilute the same to 10 ml with same solvent.	
Pressure Drop:	112 Bar(1624 psi)	



Chromatographic Data :

No.	Compound	Retention Time (min)	Asymmetry	Relative Retention Time (RRT)
1	N-desmethyl impurity	5.9	1.4	0.4
2	Diamine impurity	8.1	1.3	0.5
3	Levofloxacin	16.5	0.6	1.0
4	D-isomer	19.3	1.1	1.2

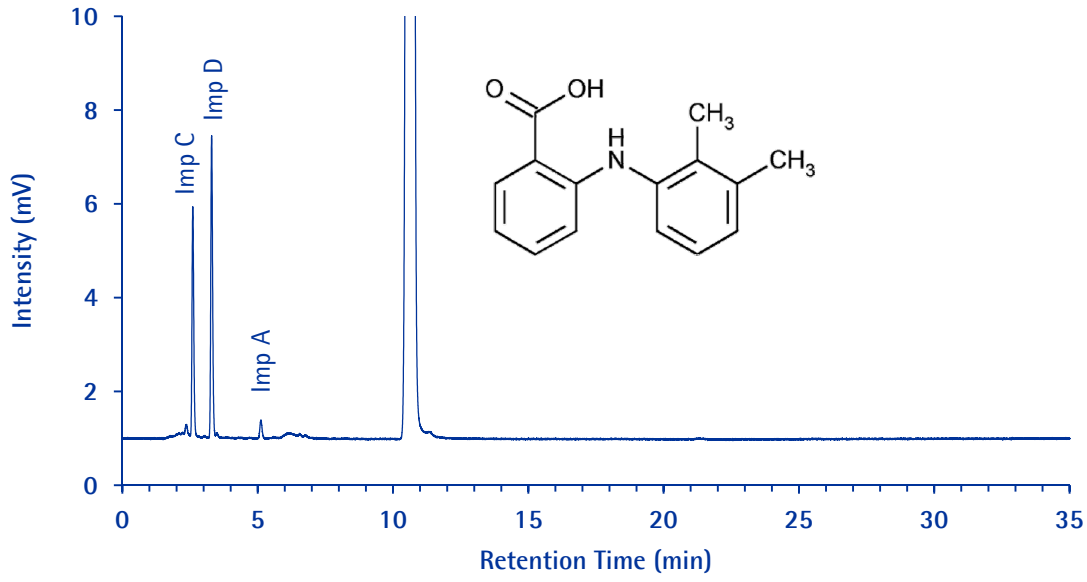
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Mefenamic Acid and Related Substances (USP)

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) Hibar® RT 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	UV 254 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Buffer: 50 mM of monobasic ammonium phosphate, adjusted with 3 M ammonium hydroxide to a pH of 5.0. Mix tetrahydrofuran, buffer and acetonitrile 14:40:46 (v/v)	
Temperature:	25°C	
Diluent	Mobile phase	
Sample:	5 ppm of Impurity C and D, 0.1 ppm of Impurity A and 100 ppm Mefenamic Acid	
Pressure Drop:	140 Bar (2030 psi)	



Chromatographic Data

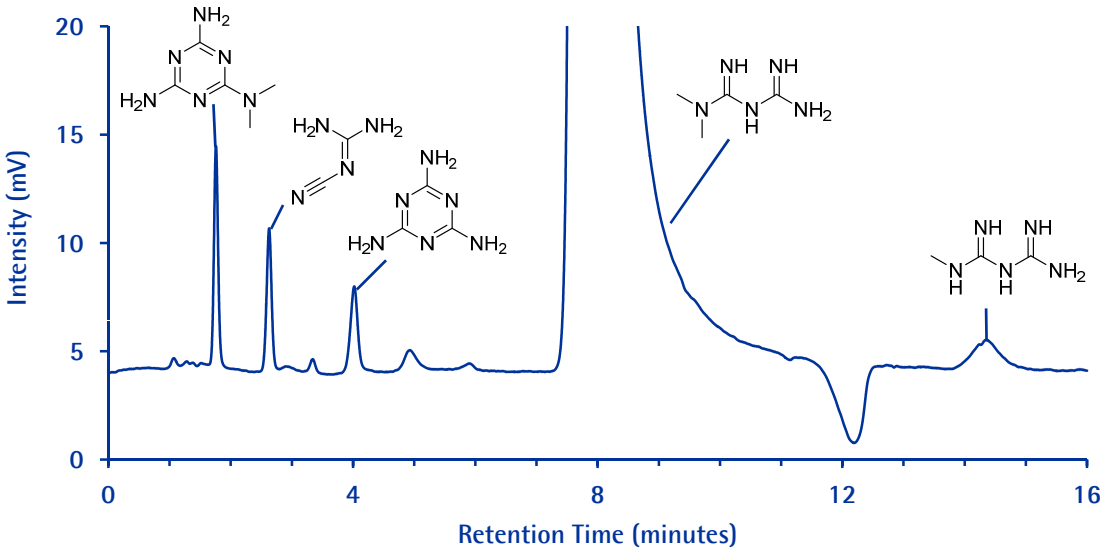
No	Compound	Time (min)	Relative Retention Time (RRT)	Asymmetry	Plates
1	Impurity C	2.6	0.24	1.2	7697
2	Impurity D	3.3	0.31	1.2	11439
3	Impurity A	5.1	0.48	1.1	19510
4	Mefenamic acid	10.7	1.00	0.9	18758

Metformin and Related Impurities

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC (3µm, 100Å) PEEK 150x4.6 mm	1.50661.0001
Injection:	5 µL	
Detection:	UV 218 nm	
Cell:	8 µl	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Buffer: Dissolve 4.62 g of ammonium acetate in 1000 ml water (60 mM). Adjust buffer to pH 5 using glacial acetic acid. Mix Acetonitrile and Buffer 90:10 (v/v)	
Temperature:	30 °C	
Diluent	Mobile phase	
Sample:	5000 ppm metformin & 1 ppm of each impurity: A, C and melamine & 5 ppm of impurity B in mobile phase	
Pressure Drop:	105 bar (1522 psi)	



Chromatographic Data

No.	Compounds	Retention Time (min)	k'	Resolution	Area (%)	Theoretical Plates	Tailing Factor
1	C: Dimethylmelamine	1.8	0.6	-	0.1	3100	1.1
2	A: Cyanoguanidine	2.6	1.4	6.3	0.1	4900	1.0
3	Melamine	4.0	2.7	7.4	0.1	5000	1.1
4	Metformin	8.0	6.5	10.0	99.6	3100	0.8
5	B: Methylbiguanide	14.4	12.3	9.1	0.1	4900	1.2

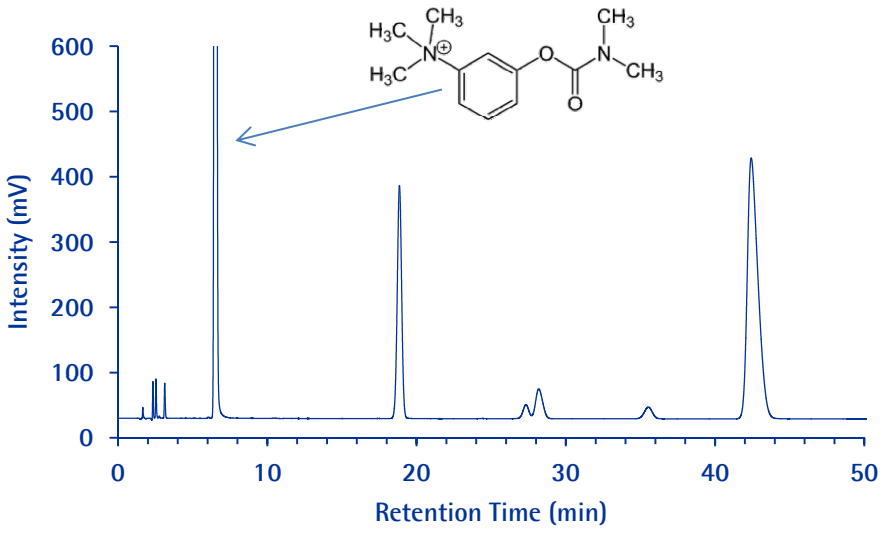
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Neostigmine Sulfate and Related Impurities

Purospher® STAR RP-8 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-8 endcapped (5µm) Hibar® RT 250x4.6 mm	1.51454.0001
Injection:	20 µL	
Detection:	UV 220 nm	
Cell:	10 µL	
Flow Rate:	1.2 mL/min	
Mobile Phase:	Dissolve 4.14 g of sodium dihydrogenphosphate in 1000 mL Milli-Q water. 300 ml of Acetonitrile & 4.33 gm of Sodium dodecyl sulfate & filter.	
Temperature:	30 °C	
Standard:	Take 50 mg of Neostigmine methylsulfate in 50 ml volumetric flask and dissolve in mobile phase.	
Sample:	Take 50 mg of sample in 50 ml volumetric flask and dissolve in mobile phase.	
Pressure Drop:	140 Bar(2030 psi)	



Chromatographic Data :

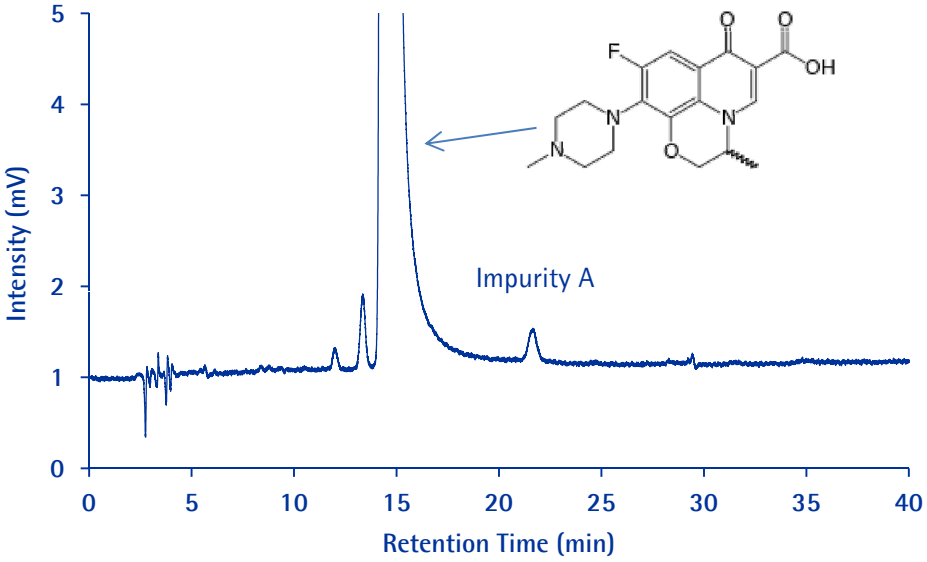
No.	Compound	Time (min)	Asymmetry	Theoretical Plate
1	3-Dimethylphenol	2.3	1.3	11233
2	3-Trimethylammoniumphenolmethylsulfate	2.5	1.2	10152
3	3-Dimethyl-Carbamoyl-N,N-dimethylaniline	3.1	1.2	12226
4	Neostigmine methylsulfate	6.5	1.3	13936
5	Methylneostigmine	18.9	1.0	19297
6	Ethylneostigmine	42.4	1.4	15790

Ofloxacin and Related Substances (USP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5µm) Hibar® RT 150x4.6 mm	1.51455.0001
Injection:	10 µL	
Detection:	UV 295 nm	
Cell:	10 µL	
Flow Rate:	0.5 mL/min	
Mobile Phase:	4 gm of ammonium acetate& 7.0 gm of sodium perchlorate in 1300 ml water. Adjust pH to 2.2 with orthophosphoric acid. Mix Buffer & Acetonitrile 130:24 (v/v)	
Temperature:	45 °C	
Diluent	Mobile phase	
Sample:	200 ppm of Ofloxacin in mixture of water and acetonitrile 6:1 (v/v)	
Pressure Drop:	50 Bar (725 psi)	



Chromatographic Data :

No.	Compound	Retention Time (min)	Resolution	RRT
1	Impurity A	13.4	-	0.94
2	Ofloxacin	14.2	2.5	1.00

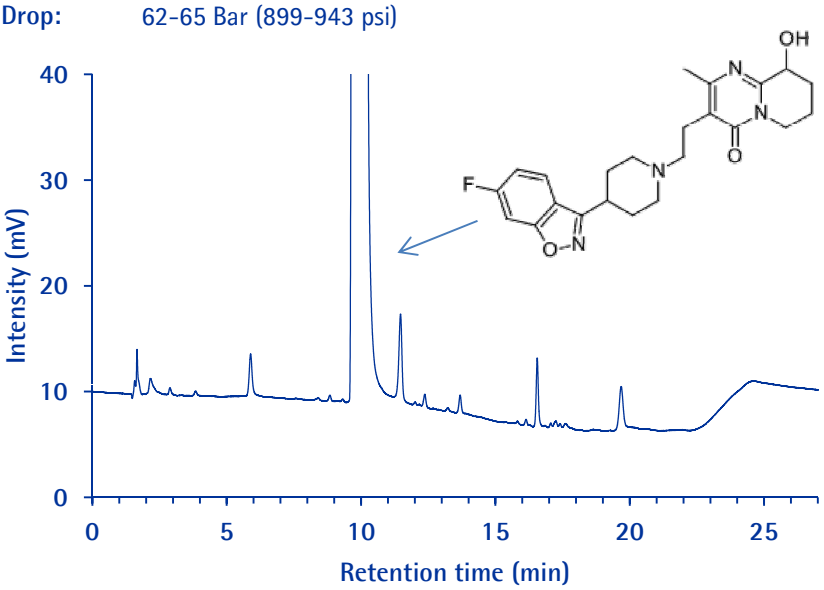
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Paliperidone and Related Substances

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 100x4.6mm	1.52022.0001
Injection:	10 µL	
Detection:	UV 238 nm	
Flow Rate:	1.0 mL/min	
Buffer:	Dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL with Milli-Q water. Adjust pH to 2.0 with orthophosphoric acid. Mobile phase A: buffer and mobile phase B: acetontrile	
Gradient	See table	
Temperature:	25 °C	
Diluent	Buffer and acetonitrile 80:20 (v/v)	
Reference solution:	1ppm solution of hydroxyimpurity in 1000 ppm of Paliperidone standard in diluent.	
Sample:	Dissolve 10 mg of sample in 10 ml diluent.	
Pressure Drop:	62-65 Bar (899-943 psi)	



Time	A %	B %
0	90	10
2	90	10
15	70	30
20	70	30
22	90	10
27	90	10

Chromatographic Data

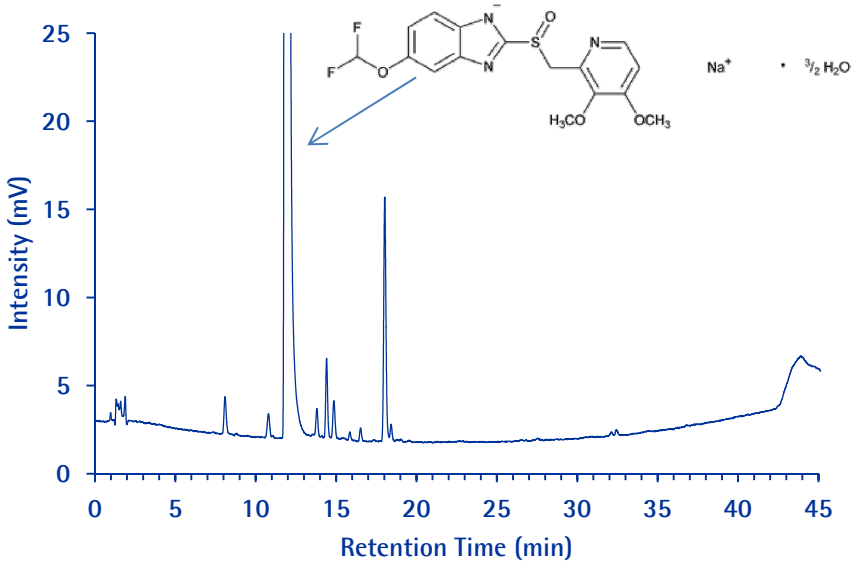
No.	Compound	Retention Time (min)	Resolution	Relative Retention Time
1	Hydroxy Impurity	5.9	0.0	0.60
2	Paliperidone	9.8	10.6	1.00
3	Impurity 1	11.5	4.6	1.17
4	Impurity 2	12.4	5.0	1.27
5	Impurity 3	13.7	8.4	1.40
6	Impurity 4	16.6	18.7	1.70

Pantoprazole Sodium Related Substances (USP)

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5µm) Hibar® RT 150x4.6 mm	1.51455.0001
Injection:	20 µL	
Detection:	UV 290 nm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Solution A: Prepare a solution of dibasic potassium phosphate (1.74 g/L) adjusted with a solution of phosphoric acid (330 g/L) to a pH of 7.00 ± 0.05. Solution B: acetonitrile	
Gradient:	See gradient table	
Temperature:	40°C	
Diluent	1:1 mixture of acetonitrile and 1 mM NaOH	
Sample:	460 ppm of Pantoprazole in diluent	
Pressure Drop:	101 to 56 Bar (1465 to 812 psi)	



Time	A (%)	B (%)
0-40	80→20	20→80
40-45	20→80	80→20
45-55	80	20

Chromatographic Data

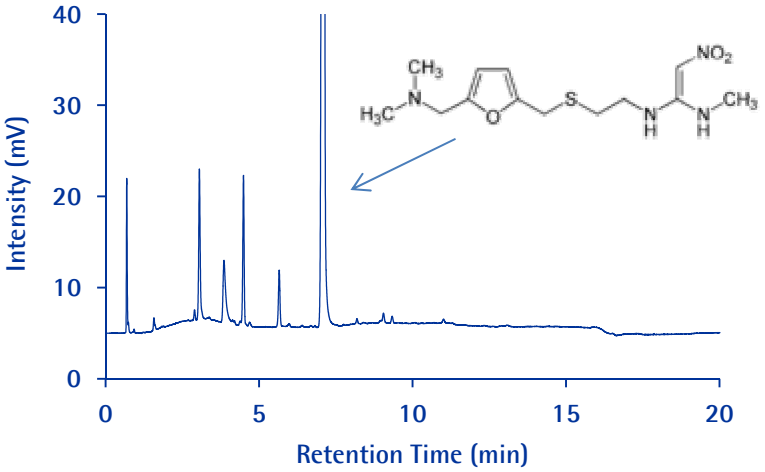
No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Pantoprazole RS C	8.1	0.7	-	1.1
2	Pantoprazole RS A	10.8	0.9	-	1.0
	Pantoprazole Na	11.9	1.0	-	1.1
3	Pantoprazole RS F	13.8	1.2	-	1.1
4	Pantoprazole RS D	14.4	1.2	2.9	1.1
5	Pantoprazole RS E	14.7	1.2	2.0	1.1
6	Pantoprazole RS B	18.0	1.5	-	1.2

Ranitidine HCl and Related Substances

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (3µm) Hibar® RT 100x4.6 mm	1.50469.0001
Injection:	10 µL	
Detection:	UV 230 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Buffer: Place 1900 mL of Milli-Q water in 2L volumetric flask. Add 6.8 mL of phosphoric acid and mix. Add 8.6 mL of 50% sodium hydroxide solution and dilute to final volume. If necessary adjust the pH to 7.1 with 50% sodium hydroxide solution. Solution A: buffer and acetonitrile 98:2 (v/v): Solution B: buffer and acetonitrile 78:22(v/v)	
Gradient:	See table	
Temperature:	35 °C	
Diluent	Solution A	
Sample:	Dissolve 1.3 mg of resolution mixture in diluent & dilute to 10 ml with same solvent.	
Pressure Drop:	154 to 175 Bar(2233 to 2537 psi)	



Time (min)	%A	%B
0.0	100	0
10.0	0	100
15.0	0	100
16.0	100	0
20.0	100	0

Chromatographic Data

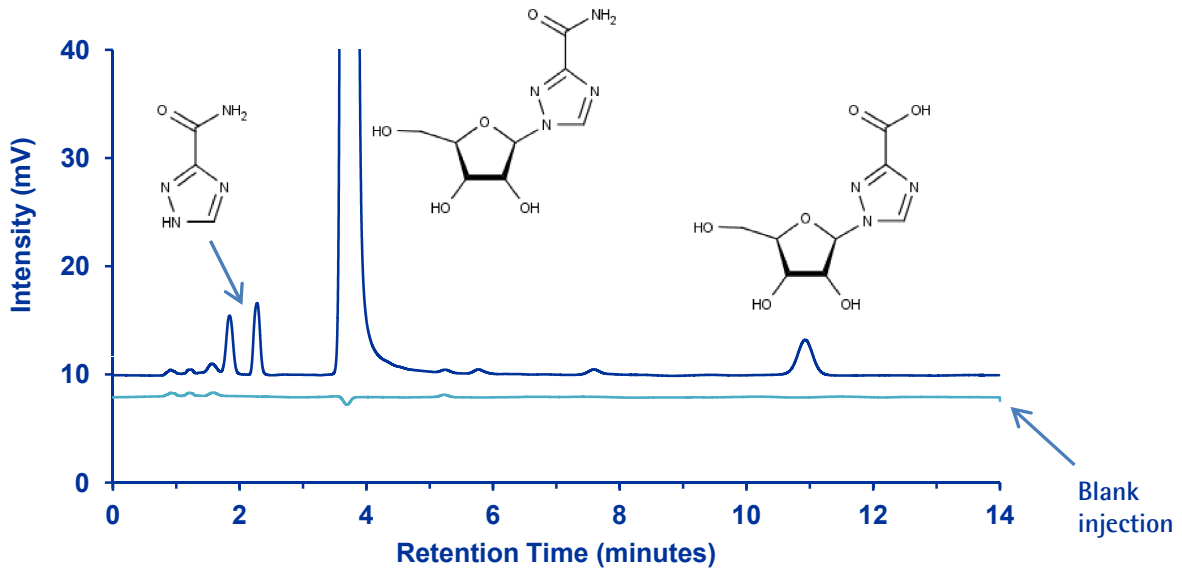
No.	Compound	Retention Time (min)	RRT	Asymmetry
1	Ranitidine Oxime	1.6	0.22	1.4
2	Amino alcohol	3.1	0.44	1.3
3	Ranitidine diamine	3.9	0.55	1.8
4	Ranitidine S-oxide	4.5	0.63	1.0
5	Complex nicotinamide	5.7	0.80	1.0
6	Ranitidine	7.1	1.00	1.0
7	Formaldehyde adduct	9.3	1.31	1.0

Ribavirin and Related Substances

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC (3µm, 100Å) PEEK 150x4.6 mm	1.50661.0001
Injection:	10 µL	
Detection:	UV 220 nm	
Cell:	13 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Buffer: Dissolve 0.385 g of ammonium acetate in 1000 ml water (5 mM). Mix Acetonitrile and Buffer 85:15 (v/v)	
Temperature:	25 °C	
Diluent	Mobile phase	
Sample:	Ribavirin CRS 1000 ppm, Ribavirin impurity A 5ppm and Ribavirin impurity D 0.5 ppm	
Pressure Drop:	92 Bar (1334 psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	Retention factor K°	Area %
1	Unknown impuitry	1.9	1.1	0.2
2	Ribavirin impurity D	2.2	1.5	0.2
3	Ribavirin CRS	3.7	3.1	99.4
4	Ribavirin impurity A	10.9	11.1	0.2

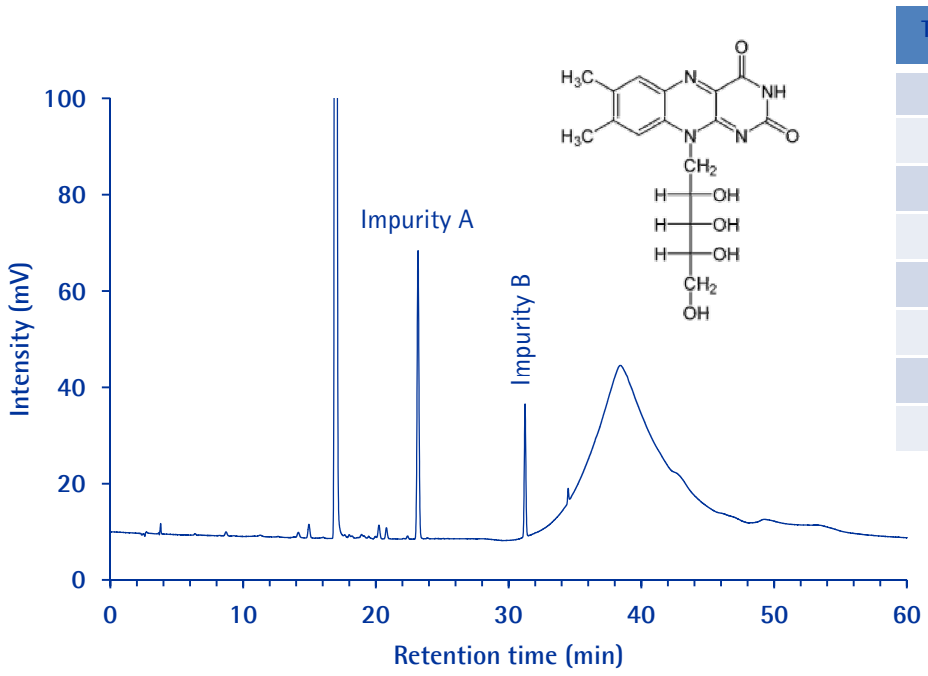
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Riboflavin and Related Substances

Purospher® STAR RP-18 endcapped (HPLC)

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	UV 267 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Mobile Phase A: Mix water & ortho-phosphoric acid 1000:1 (v/v) Mobile Phase B: Acetonitrile	
Gradient:	See table	
Temperature:	25 °C	
Sample:	In order to see impurity A & B, dissolve 10 mg substance in 1 mL 0.5 M NaOH solution. Expose to daylight for 1.5 hour. Add 0.5 mL of acetic acid and dilute to 100 mL with Milli-Qwater.	
Pressure Drop:	156 to 134 Bar(2262 to 1943 psi)	



Time (min)	%A	%B
0.0	90	10
5.0	90	10
20.0	80	20
25.0	80	20
35.0	50	50
45.0	50	50
50.0	90	10
60.0	90	10

Chromatographic Data

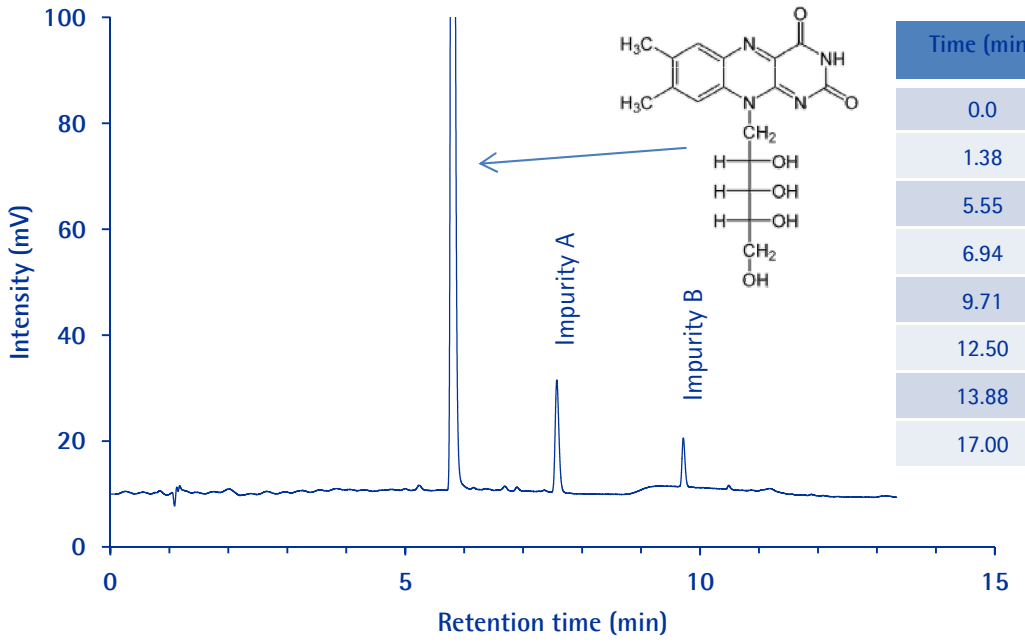
No.	Compound	Retention Time (min)	RRT	Resolution
1	Riboflavin	17.0	1.00	
2	Impurity A	23.2	1.36	
3	Impurity B	31.2	1.84	32.7

Riboflavin and Related Substances

Purospher® STAR RP-18 endcapped (UHPLC)

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (2µm) Hibar® HR 100x2.1 mm	1.50648.0001
Injection:	2 µL	
Detection:	UV 267 nm	
Cell:	2.5 µL (micro flowcell)	
Flow Rate:	0.3 mL/min	
Mobile Phase:	Mobile Phase A: Mix water & ortho-phosphoric acid 1000:1 (v/v) Mobile Phase B: Acetonitrile	
Gradient:	See table	
Temperature:	25 °C	
Sample:	In order to see impurity A & B, dissolve 10 mg substance in 1 mL 0.5 M NaOH solution. Expose to daylight for 1.5 hour. Add 0.5 mL of acetic acid and dilute to 100 mL with Milli-Qwater.	
Pressure Drop:	375 to 330 Bar(5438 to 4785 psi)	



Time (min)	%A	%B
0.0	90	10
1.38	90	10
5.55	80	20
6.94	80	20
9.71	50	50
12.50	50	50
13.88	90	10
17.00	90	10

Chromatographic Data

No.	Compound	Retention Time (min)	RRT	Resolution
1	Riboflavin	5.8	1.00	
2	Impurity A	7.8	1.34	
3	Impurity B	9.7	1.67	20.5

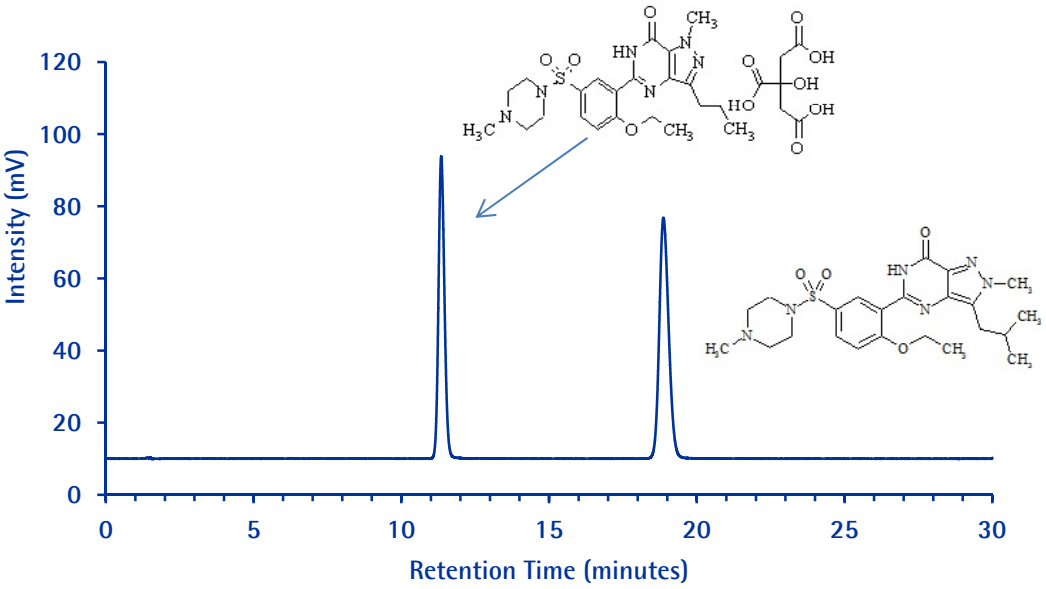
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Sildenafil Citrate and Related Substances (USP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (3µm) Hibar® RT 150x4.6 mm	1.50470.0001
Injection:	20 µL	
Detection:	UV 290 nm	
Cell:	13 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Buffer: 7 mL of triethylamine to total 1L water. Adjust with phosphoric acid to a pH=3.0±0.1 Mix the buffer, methanol and acetonitrile 58:25:17 (v/v)	
Temperature:	30°C	
Diluent	Mobile phase	
Sample:	Sildenafil Citrate RS 28ppm, Sildenafil Related compound A 28ppm in diluent	
Pressure Drop:	217 Bar (3146.5 psi)	



Chromatographic Data

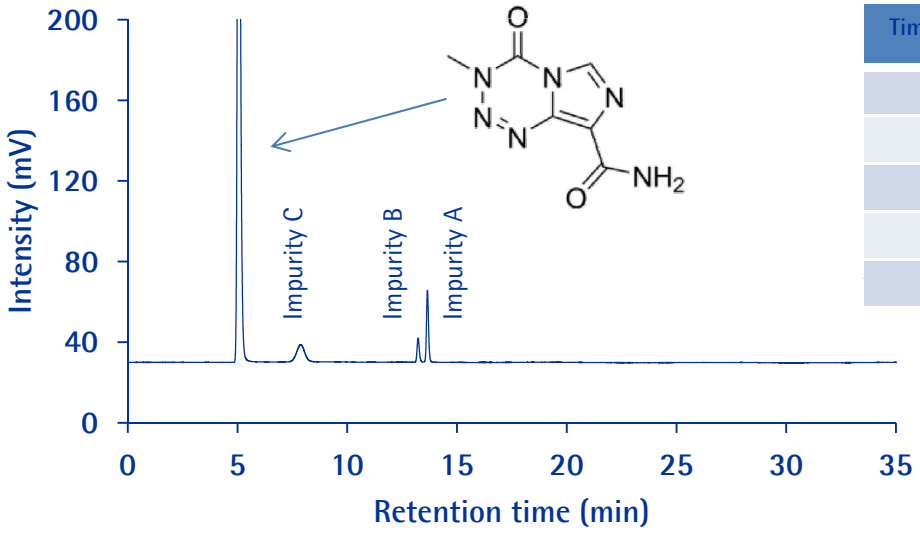
No.	Compound	Retention Time (min)	Retention factor K°	Asymmetry
1	T0	1.4	-	-
2	Sildenafil Citrate RS	11.3	7.1	1.1
3	Sildenafil Related compound A	18.8	12.4	1.1

Temozolomide and Related Impurities

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5.µm, 200Å) PEEK 250x4.6 mm	1.51458.0001
Injection:	10 µL	
Detection:	UV 254 nm	
Cell:	10 µL	
Flow Rate:	0.8 mL/min	
Mobile Phase:	A: 3.08 gm of ammonium acetate in 1000 ml water B: Acetonitrile	
Gradient:	See table	
Temperature:	30° C (sample cooler at 15° C)	
Diluent:	Acetonitrile	
Sample:	400 ppm of Temozolomide & 1 ppm of each imp A, B & C in acetonitrile. Keep the solution for 24 hrs in amber glassware before analysis for stabilization. Use amber coloured vial for analysis.	
Pressure Drop:	85 Bar(1232 psi)	



Time (min)	% A	% B
0.0	3	97
2.0	3	97
25.0	50	50
30.0	3	97
35.0	3	97

Chromatographic Data :

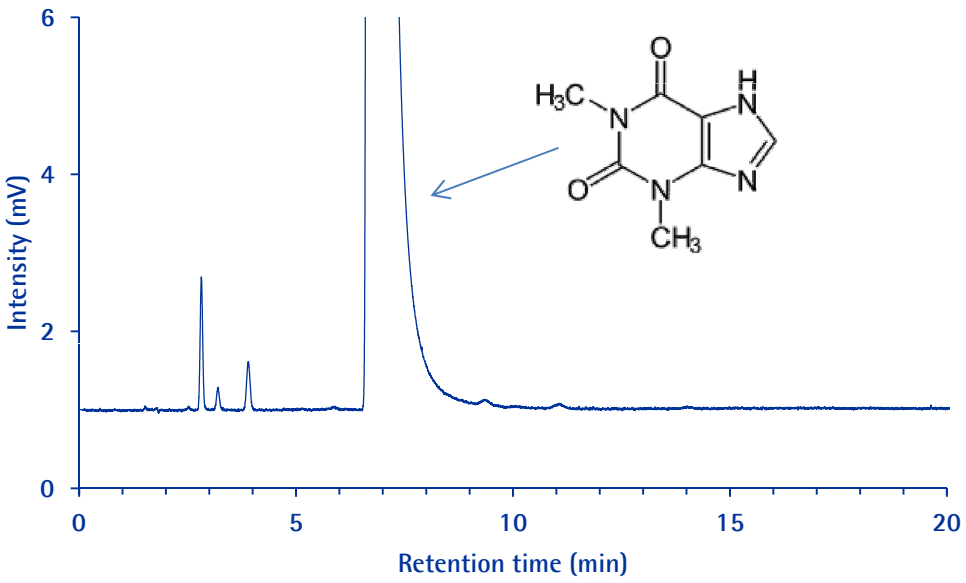
No.	Compound	Time (min)	Tailing Factor	Resolution
1	Temozolomide	5.1	1.3	
2	Impurity C	7.3	1.0	6.3
3	Impurity B	13.1	1.2	12.5
4	Impurity A	13.5	1.1	2.4

Theophylline and Related Impurities

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm	1.51456.0001
Injection:	20 µL	
Detection:	UV 272 nm	
Cell:	10 µL	
Flow Rate:	2.0 mL/min	
Mobile Phase:	Weigh 1.36 g of sodium acetate and dissolve in 1000mL of Milli-Q water containing 5 mL glacial acetic acid. Mix buffer and acetonitrile 93:7 (v/v)	
Temperature:	25 °C	
Diluent	Mobile phase	
Sample:	Dissolve 40 mg of substance in 20 mL mobile phase	
Pressure Drop:	257 Bar (3726 psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	Resolution	RRT
1	Impurity C	2.8	-	0.41
2	Impurity B	3.2	3.4	0.48
3	Impurity D	3.9	5.2	0.58
3	Theophylline	6.7	14.8	1.00

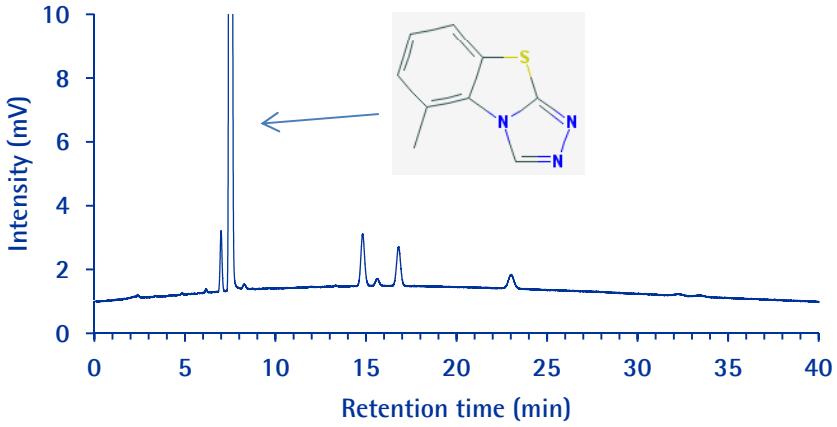
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.emdmillipore.com/chromatography
chromatography@merckgroup.com

Tricyclazole and Related Substances

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6mm	1.51456.0001
Injection:	20 µL	
Detection:	UV 254 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Dissolve 2.6 g of potassium dihydrogen phosphate in 1000 mL Milli-Q water. Filter through 0.45 µm filter paper. Mix buffer and acetonitrile 70:30 (v/v) and sonicate for 15 minutes.	
Temperature:	30 °C	
Diluent	Mobile phase	
Standard:	Weigh 10 mg Chlorotricyclazole in 100 mL volumetric flask. Dissolve in diluent and make up the volume with same solvent (Solution A). Weigh 10 mg Tricyclazole in another 100 ml volumetric flask. Add 5 ml of Solution A. Add diluent to dissolve and dilute to final volume with same solvent.	
Sample:	Weigh 10 mg of sample in 100 mL volumetric flask. Dissolve in diluent.	
Pressure Drop:	123 Bar (1784 psi)	



Chromatographic Data

No.	Compound	Time (min)	T_{USP}	Resolution
1	Impurity 1	7.0	1.1	0.0
2	Tricyclazole	7.5	1.1	2.6
3	Impurity 2	8.3	1.0	3.6
4	Impurity 3	14.8	1.1	21.7
5	Chlorotricyclazole	15.6	1.0	2.3
6	Impurity 4	16.8	1.0	3.3
7	Impurity 5	23.0	1.0	14.3

Solvents and Reagents

Product	P/N
Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.00063
Acetonitrile for Chromatography	1.14291
Acetonitrile Gradient Grade for Chromatography	1.00030
Ammonia solution 28-30% for analysis EMSURE® ACS,Reag. Ph Eur	1.05423
Ammonium acetate	1.01116
Ammonium dihydrogen phosphate for analysis EMSURE® ACS,Reag. Ph Eur	1.01126
1,4-Dioxane for liquid chromatography	1.03132
Copper(II) sulfate pentahydrate for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.02790
Heptane-1-sulfonic acid sodium salt for ion pair chromatography LiChropur®	1.18306
Hexane-1-sulfonic acid sodium salt for ion pair chromatography LiChropur®	1.18305
Methanol Gradient Grade for Chromatography	1.06007
Octane-1-sulfonic acid sodium salt for ion pair chromatography LiChropur®	1.18307
ortho-Phosphoric acid 85% for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.00573
Perchloric acid 70% for analysis (max. 0.0000005% Hg) EMSURE® ACS,ISO,Reag. Ph Eur	1.00514
Potassium dihydrogen phosphate	1.05108
di-Potassium hydrogen phosphate trihydrate buffer substance for chromatography	1.19754
Potassium hydroxide	1.05002
Sodium acetate anhydrous 99.99 Suprapur®	1.06264
Sodium dihydrogen phosphate dihydrate for analysis EMSURE® Reag. Ph Eur	1.06342
di-Sodium hydrogen phosphate dihydrate for analysis EMSURE®	1.06580
Sodium perchlorate monohydrate EMSURE®	1.06564
Tetrahydrofuran LiChrosolv®	1.08101
Water for chromatography*	1.15333
* or use a Milli-Q Integral Water Purification System	