SeQuant® ZIC®-HILIC

for Pharmaceutical Analysis

Merck Millipore is a division of MERCK

Introduction

For hydrophobic substances, reliable and robust methods have successfully been developed on reversed phase columns over the past 30 years, but analyzing hydrophilic compounds in clean or complex samples have been a massive undertaking, involving derivatization and tedious sample preparation procedures. To retain polar and hydrophilic molecules on reversed phase columns ion-pairing reagents are required, or use of almost or complete aqueous mobile phases. Ionizable polar compounds can be retained on ion-exchange columns, but then only negative or positive analytes in each method, since ion exchange columns are either for cations or anions.

Technical development have provided us with new instrumentation and column chemistries, and thereby the possibility to develop comprehensive and sensitive methods. The challenge is to make them reliable and robust to work in diverse matrices and to combine the best detection technique with the most favorable separation mode for analytes of interest. Developing methods that are fast and accurate in a single matrix is straightforward, but in many cases a lot of information can be lost if the samples are analyzed with only one chromatographic selectivity, especially for impurity profiling or when sample composition is unknown. Screening methods, based on complementary chromatographic selectivities together with sensitive and specific detection techniques can therefore, all together, provide us with much more complete information.

The globalization process changes the demands and requirements literally as we speak. Currently official controlling organizations such as the United States Pharmacopeia (USP) are looking for new analytical alternatives to replace and modernize old regulated pharmaceutical control methods. Merck Millipore contributes in this context by offering analytical solutions and prompt technical support.

This compilation exemplify how hydrophilic interaction liquid chromatography (HILIC) in general, and the bonded zwitterionic SeQuant® ZIC®-HILIC stationary phase in particular is the ideal tool for analysis of polar compounds. Several application examples are presented, with experimental details, to demonstrate the usefulness of HILIC for pharmaceutical quality control. Merck Millipore offers virtually everything but the instrument to successfully implement these methods in your laboratory, and as you will see, HILIC definitely is useful for regulated pharmaceutical assay and impurity profiling methods.

What is HILIC and why ZIC®-HILIC?

Analysis of polar molecules in complex mixtures is problematic since the separation is difficult due to their inherently poor retention in traditional reversed-phase liquid chromatography (RP-LC). As a solution, Merck Millipore have developed the high-quality SeQuant® ZIC®-HILIC range of HPLC columns, designed to retain and separate all types of polar and hydrophilic compounds and for robust chromatography with high selectivity and reproducibility. These columns are used in Hydrophilic Interaction Liquid Chromatography (HILIC) mode, which means buffered aqueous eluents rich in organic solvents such as acetonitrile. With this mode of operation follows also a couple of characteristic advantages such as low column back-pressure allowing high-speed separations, enhanced sensitivity when interfaced with mass spectrometry (MS), and simplified sample preparation schemes. By employing ZIC®-HILIC columns, laboratories can be more efficient and deliver more secure analysis results for polar and hydrophilic analytes. And this is regardless if it is well-equipped with sophisticated instrumentation such as LC-MS/MS, or rely on more traditional HPLC with detection by UV light absorption, ELSD (evaporative light scattering) or refractive index (RI) detection.

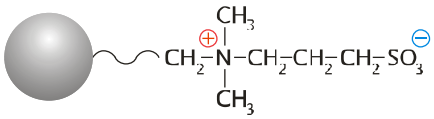
Separations are equally easy to develop on ZIC®-HILIC columns as on traditional RP HPLC columns since the eluents are similar, however, the difference is the effect of the water in the separation. In HILIC mode, water is the strongest solvent. To increase analyte retention, the organic portion of the mobile phase needs to be increased, and the water or buffer portions decreased. This will increase the hydrophilic partitioning into the water-enriched stationary phase, and thus increase the retention of the analyte.

The zwitterionic character of ZIC®-HILIC with a 1:1 balanced charge, gives further possibilities for selectivity by weak electrostatic interactions between the stationary phase and the molecules that are separated. This interaction can be tuned by changing buffer type and concentration, typically in the interval 5-50 mM. Buffer pH is also an important parameter to control retention, but also here the thinking is opposite to in RP mode; more ionized compounds will be more hydrophilic and thus have more retention on ZIC®-HILIC.

More technical details on how to develop methods with ZIC®-HILIC can be found in the booklet 'A Practical Guide to HILIC', which is available free of charge from Merck Millipore printed or in online format (www.sequant.com/hilicguide).

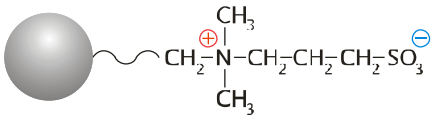
SeQuant® ZIC®-HILIC

Sorbent characteristics:	high-density zwitterionic sulfobetaine modification
Charge balance:	1:1
Particle material:	high-purity type B silica
Particle type:	spherical, fully porous
Particle size:	3.5 µm, 5 µm
Pore size:	100 Å , 200 Å
pH range:	pH 3 - 8
Max temperature:	70 °C
Max pressure:	350 bar (PEEK columns) or 400 bar
Column inner diameters:	0.075, 0.1, 0.3, 1.0, 2.1, 4.6, 7.5, 10, 21.2 mm
Column lengths:	20, 30, 50, 100, 150, 250 mm



SeQuant® ZIC®-*p*HILIC

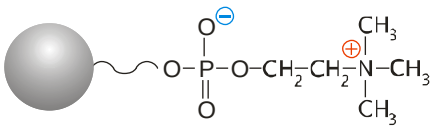
Sorbent characteristics:	high-density zwitterionic sulfobetaine modification
Charge balance:	1:1
Particle material:	high-purity polymer
Particle type:	spherical, fully porous
Particle size:	5 µm
pH range:	pH 2 - 12
Max temperature:	50 °C
Max pressure:	200 bar
Column inner diameters:	2.1, 4.6 mm
Column lengths:	50, 100, 150 mm



NEW PRODUCT

SeQuant® ZIC®-*c*HILIC

Sorbent characteristics:	high-density zwitterionic phosphorylcholine modification
Charge balance:	1:1
Particle material:	high-purity type B silica
Particle type:	spherical, fully porous
Particle size:	3 µm
Pore size:	100 Å
Column inner diameters:	0.1, 0.3, 1.0, 2.1, 4.6 mm
Column lengths:	50, 100, 150, 250 mm



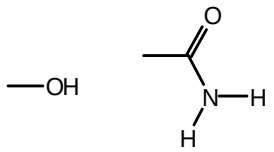
*For more information on specifications and launch of SeQuant® ZIC®-*c*HILIC, please contact your Merck Millipore sales representative or visit www.merckmillipore.com/chromatography or www.sequant.com/zicchilic.

How to use HILIC?

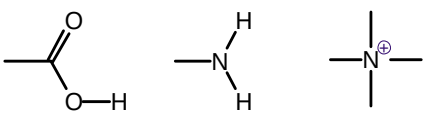
The bonded hydrophilic ZIC®-HILIC stationary phase consist of a highly polar, permanent zwitterion that ensures a stable environment for HILIC partitioning during analyte retention.

Separation selectivity is favoured by the 1:1 zwitterion charge balance, which makes the ZIC®-HILIC column overall neutral, with weak, but important, ionic interactions.
Tuning of the selectivity on the ZIC®-HILIC column during method development is facilitated by the pH-independent permanent zwitterion, ensuring that only the analytes (and not the column) is affected during eluent optimization.

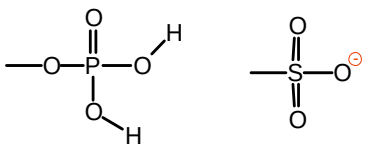
ZIC®-HILIC is compatible with a range of different buffers, organic solvents and temperatures, and hence makes it a straightforward task to develop robust isocratic and gradient methods for MS, ELSD, UV and other detection techniques.

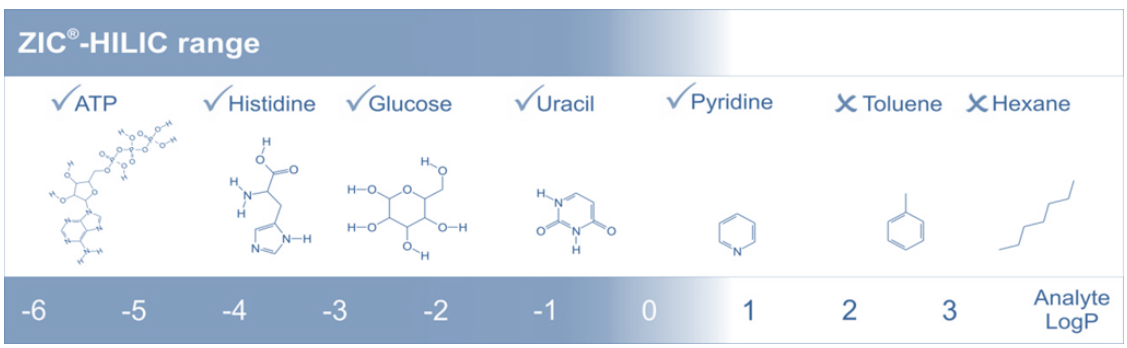


Typical HILIC compounds



1. Small or negative LogP value*
2. Hydrophilic, ionic or ionizable functional groups
3. Hydrophilic centre or difference in hydrophilic substitution





* octanol-water partition coefficient

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

Getting Started with ZIC®-HILIC

Sample Solvent and Solvent Strength

Sample solvents should consist of 60-100% organic solvent, or initial eluent composition.
Water should be minimized. Weak HILIC solvents such as acetonitrile are favoured.
It is recommended to have about 5% water in the autosampler wash solution.
The relative solvent strength for HILIC is:
Acetone < Acetonitrile < Isopropanol < Ethanol < Methanol < Water

Typical Elution Protocols

Isocratic elution: 80:20 (v/v) acetonitrile / NH4Ac, (5-20 mM) or other suitable buffer salt.
Gradient elution: 90% to 40% acetonitrile in 20 minutes (~2.5%/ min).

Mobile Phase Considerations

To obtain reproducible results, maintain at least 3% water in the mobile phase, in order to ensure sufficient hydration of the stationary phase particles.

Buffer Recommendations

Suitable buffer systems for HILIC separations are formate and acetate, due to their excellent solubility even in very high concentrations of organic solvent. Typically avoid phosphate, and other low solubility buffers, to prevent precipitation on the column bed. A buffer concentration in the range 5-20 mM is recommended for most analytes, with an upper limit of 200-300 mM, depending on the solubility in the eluent. TFA and other ion-pair reagents should be avoided, as they can interfere with the HILIC separation mechanism, and suppress MS signals.

Suitable pH range: 3-8 (ZIC®-HILIC) and 2-12 (ZIC®-*p*HILIC)
Temperature limit: 70 °C (ZIC®-HILIC) and 50 °C (ZIC®-*p*HILIC).

Column Regeneration and Storage

If the backpressure increases or a shift in selectivity is observed, use the following washing protocol to regenerate the column.
1. 30-60 column volumes of de-ionized water
2. 30 column volumes of 0.5 M NaCl
3. 30 column volumes of de-ionized water

Store columns as shipped: Acetonitrile / NH4Ac 25 mM, pH 6.8; 80:20 (v/v)

Getting Started with ZIC®-HILIC

Flow-rate, Backpressure and Injection Volume

The optimal flow rate on SeQuant® ZIC®-HILIC and expected backpressure at that flow-rate can be seen in the figure and table below. The recommended injection volume is also listed in the table as is the applicable flow-rate range. Never exceed the maximum backpressure.

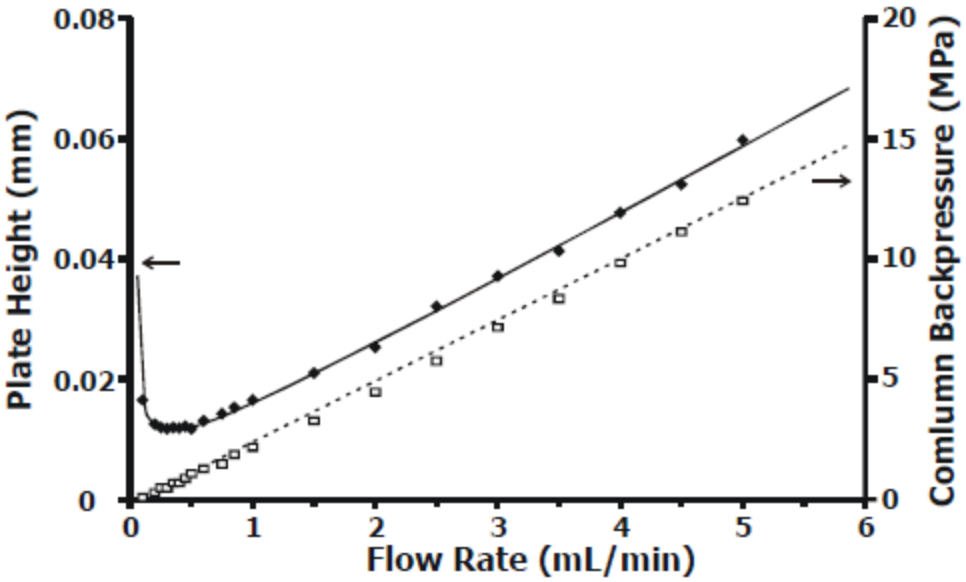
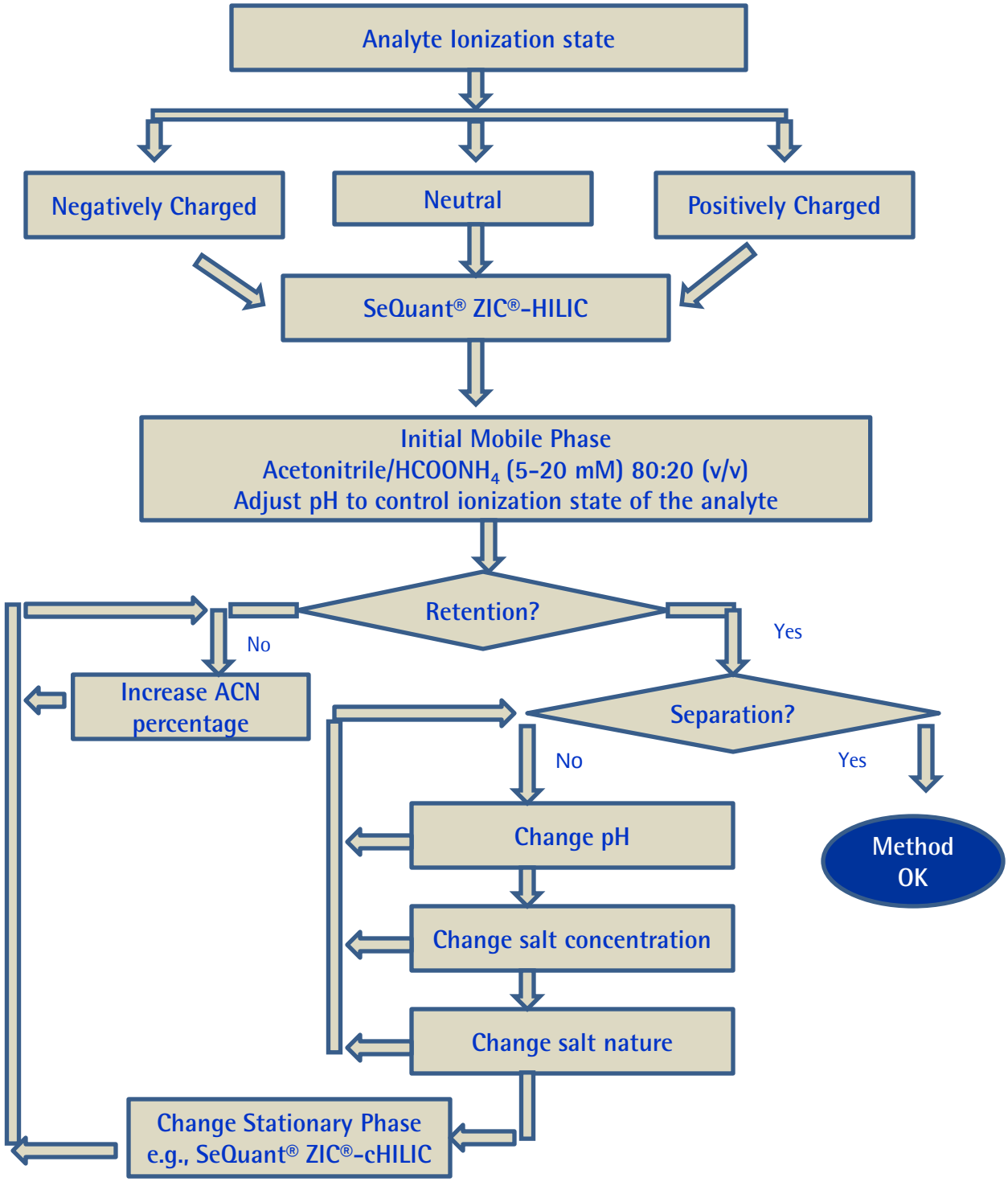


Figure: Column plate height (♦) and backpressure (□) vs. volumetric flow rate. Cytosine injected on a 50 x 4.6 mm ZIC®-HILIC column at k' 1.3 using a 80:20 acetonitrile/buffer eluent.

Column i.d. (mm)	Injection Volume (µL)	Flow-rate optimum (ml/min)	Flow-rate range (ml/min)	Backpressure (expected MPa)	Backpressure (Max MPa)
4.6	5 – 50	0.5	0.25 – 10*	1-5	35*
2.1	0.5 - 5	0.1	0.05 – 2*	1-5	35*

*Never exceed the maximum backpressure at any flow rate. Data in table valid for ZIC®-HILIC. Max backpressure for the polymer-based ZIC®-*p*HILIC is 20 MPa., which restricts the max flow rate.

HILIC Method Development Strategy



Tips and Tricks

Injection Solvent Effect in HILIC

Injection solvent with higher elution strength (i.e. more H_2O) than the mobile phase might cause problem in HILIC.

The effect of the injection solvent is a combination of solvent volume and elution strength
Therefore try to have the sample in mobile phase not water.
If high water content samples are injected – reduce injection volume

Be aware of the risk of sample precipitation with high concentration sample solutions dissolved in other solvents than the mobile phase.

Injection solvents with higher elution strength than the mobile phase might decrease the loading capacity.

Do's and Don'ts

Wash the system before and after use with water,
to ensure a clean system without buffers that risk to precipitate or contaminate.

Change auto sampler needle-wash solvent to something HILIC-compatible,
for example initial mobile phase composition without buffer salt or 90% acetonitrile in water.

Run slower gradients than in reversed phase,
typically do not change more than 3% B/minute to achieve reproducible separations.

HILIC is not just HILIC

Don't give up if the first HILIC column you try has retention but not enough selectivity
There are plenty columns commercially available, and it is not as with C18 columns. Different types of HILIC columns do not behave in same manner, but rather VERY differently.
The bonded zwitterionic SeQuant® HILIC columns from Merck Millipore can be used for all type of HILIC separations (differently to diol, plain silica and amino phases which are of more limited use). The weak electrostatic interactions provided by the stationary phase zwitterionic functional groups, overlayed with the hydrophilic partitioning, provide a very powerful tool for succesful operation in your laboratory. Separation of polar and hydrophilic compounds are not difficult to separate any more, but you need to pay more attention to the chemistry of the sample.

Application Index

Molecule Name	Column Used	Article No.	Page
Adrenaline & Noradrenaline	SeQuant® ZIC®-HILIC (3.5 µm, 100 Å) PEEK 100 x 2.1 mm	1.50441.0001	11
Allantoin	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 150 x 4.6 mm	1.50455.0001	12
Azacitidine & related compound	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 150 x 4.6 mm	1.50455.0001	13
Caffeine, Choline, Taurine, Inositol and Carnitine	SeQuant® ZIC®-HILIC (3.5 µm, 100 Å) PEEK 150 x 2.1 mm	1.50442.0001	14
2 Chloroethanol	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 150 x 4.6 mm	1.50455.0001	15
Decitabine	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 150 x 4.6 mm	1.50455.0001	16
5-Fluorouracil and 5-Fluorouracil Dimer	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 150 x 4.6 mm	1.50455.0001	17
Methanesulfonic Acid in Busulfan Injection	SeQuant® ZIC®-HILIC (3.5 µm, 100 Å) PEEK 150 x 4.6 mm	1.50444.0001	18
Metformin and Related Impurities	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 250 x 4.6 mm	1.50458.0001	19
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Risedronate sodium and Related impurities	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 100 x 4.6 mm	1.50453.0001	21
Temozolomide and Related Impurities	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 250 x 4.6 mm	1.50458.0001	22

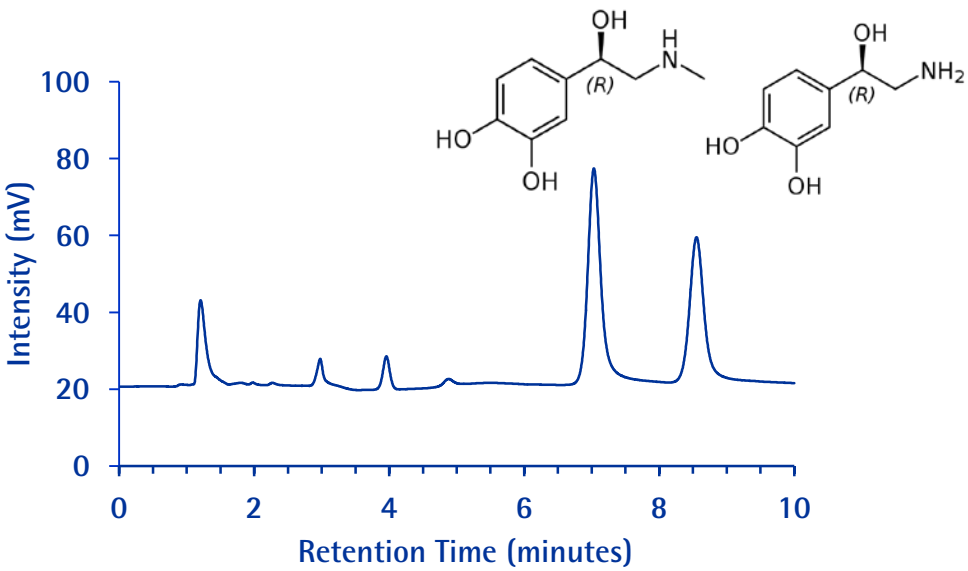
* For more information on specifications and launch of SeQuant® ZIC®-cHILIC, please contact your Merck Millipore sales representative or visit www.merckmillipore.com/chromatography or www.sequant.com/zicchilic.

Adrenaline and Noradrenaline

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (3.5 µm, 100 Å) PEEK 100 x 2.1 mm	1.50441.0001
Injection:	2 µL	
Detection:	UV @ 210 nm	
Cell:	Micro-flow cell (2.5 µl)	
Flow Rate:	0.20 mL/min	
Mobile Phase (v/v):	Buffer: 40 mM ammonium acetate in water, pH 6.8 Mix acetonitrile and buffer by volume 80:20.	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	25 µg/mL (25 ppm) Noradrenaline and 25 µg/mL (25 ppm) Adrenaline	
Pressure Drop:	56 Bar (806 psi)	



Chromatographic Data

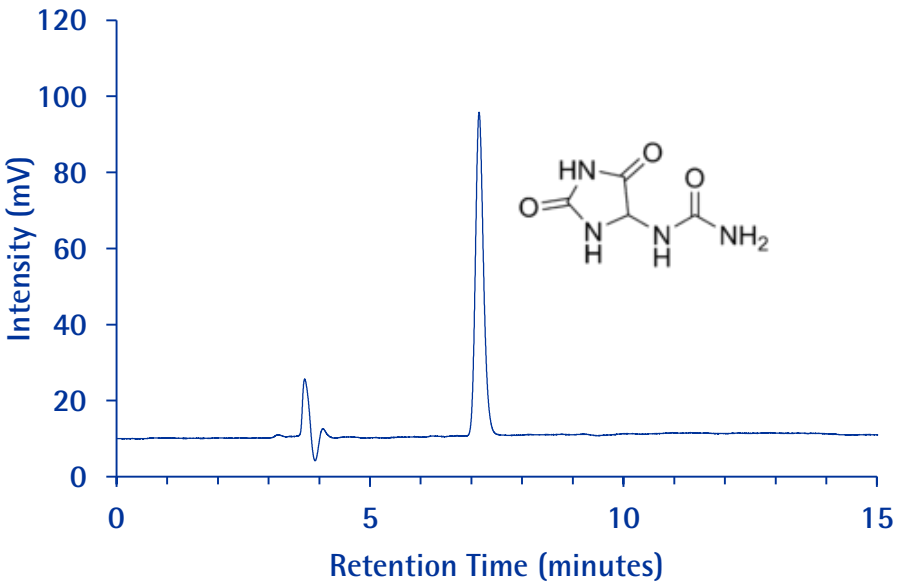
No.	Compound	Time	Tailing Factor	Retention Factor (k')	Resolution
1	void volume	1.0	-	-	-
2	Adrenaline (A)	7.0	1.2	6.0	-
3	Noradrenaline (NA)	8.6	1.2	7.6	4.2

Allantoin

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 150 x 4.6 mm	1.50455.0001
Injection:	20 µL	
Detection:	UV at 210 nm. Shimadzu Prominence	
Cell:	10 µL	
Flow Rate:	0.5 mL/min.	
Mobile Phase (v/v):	Add 1 ml of formic acid in 900 ml water. Mix well and dilute to 1L with water. Mix buffer and acetonitrile 25:75 (v/v)	
Temperature:	30 °C	
Diluent	Mobile phase	
Sample:	Weigh 30 mg of Allantoin in 100 ml volumetric flask. Dilute upto the mark with mobile phase. Pipette out 5 ml of the above solution and dilute to 50 ml with mobile phase.	
Pressure Drop:	16 bar (232 psi)	



Chromatographic Data

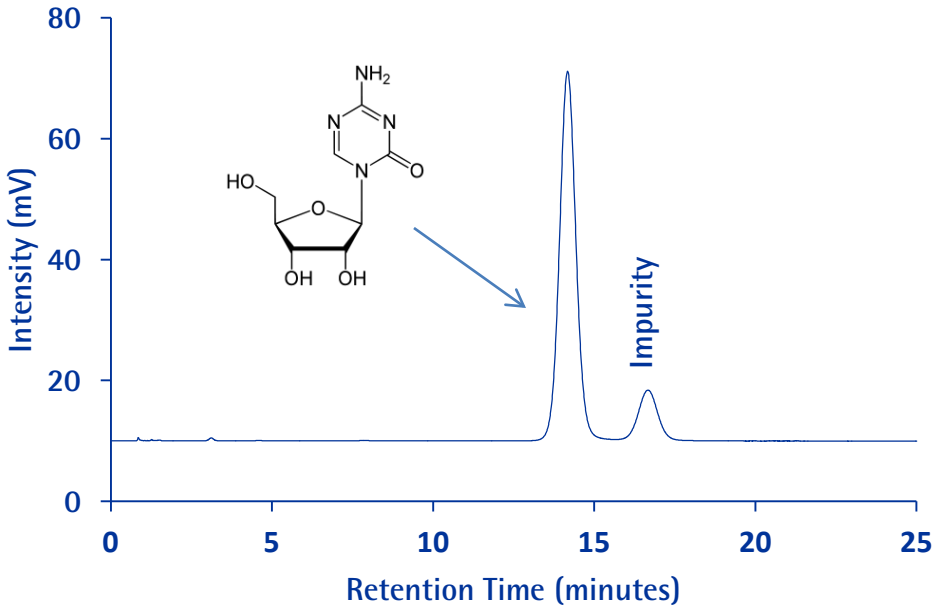
No.	Compound	Time (min)	Tailing Factor	Theoretical Plates
	Void volume (t0)	4.0	-	-
1	Allantoin	7.2	1.2	9907

Azacitidine and Related Impurities

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 150 x 4.6 mm	1.50455.0001
Injection:	10 µL	
Detection:	UV at 242 nm. Shimadzu Prominence	
Cell:	10 µL	
Flow Rate:	2.0 mL/min.	
Mobile Phase (v/v):	Dissolve 0.77 g of ammonium acetate in 1L water (10 mM). Mix buffer and acetonitrile 10:90 (v/v)	
Temperature:	25 °C	
Diluent	Mobile phase	
Sample:	Weigh 50 mg of sample in 100 ml volumetric flask. Dilute upto the mark with mobile phase.	
Pressure Drop:	57 bar (826 psi)	



Chromatographic Data

No.	Compound	Time (min)	Tailing Factor	Resolution
1	Azacitidine	14.2	1.1	-
2	Impurity	16.7	1.0	2.5

Caffeine, Choline, Taurine, Inositol and Carnitine

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column: SeQuant® ZIC®-HILIC (3.5 µm, 100 Å) PEEK 150 x 2.1 mm 1.50442.0001
Injection: 5 µl

Detection: ELSD (Sedere Sedex 85LT), 40°C, 3,5 bar pressurized air, Gain 6

Flow Rate: 0.4 mL/min

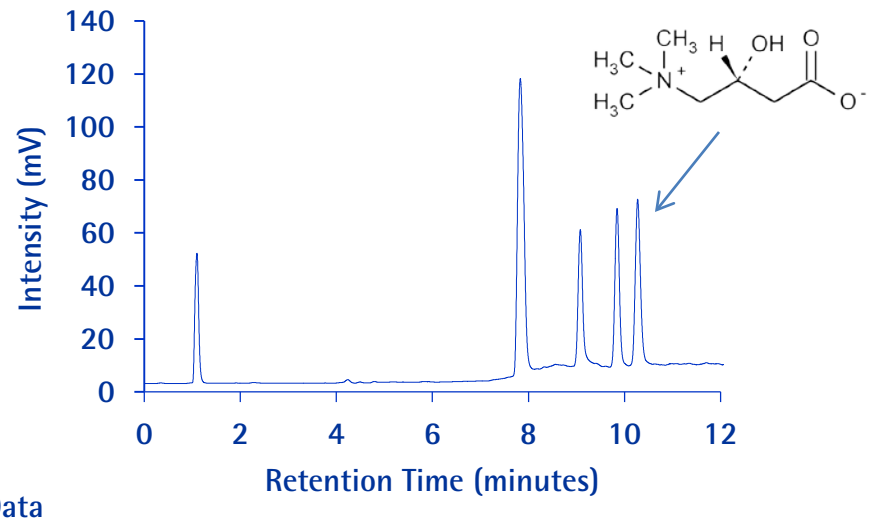
Mobile Phase (v/v): A: Acetonitrile and ammonium acetate 100 mM, pH 4.5. (90:10)
B: Ammonium acetate 100 mM, pH 4.5

Gradient: See Table

Time (min)	Solution A	Solution B	Elution
0.0-2.0	100	0	isocratic
2.01-12.0	100→60	0→40	gradient
12.01-17.0	100	0	equilibration

Temperature: 30°C
Diluent Initial mobile phase

Sample: 0.5 mg/mL of caffeine, taurine, inositol carnitine, and 0.75 mg/mL of Choline in diluent



Chromatographic Data

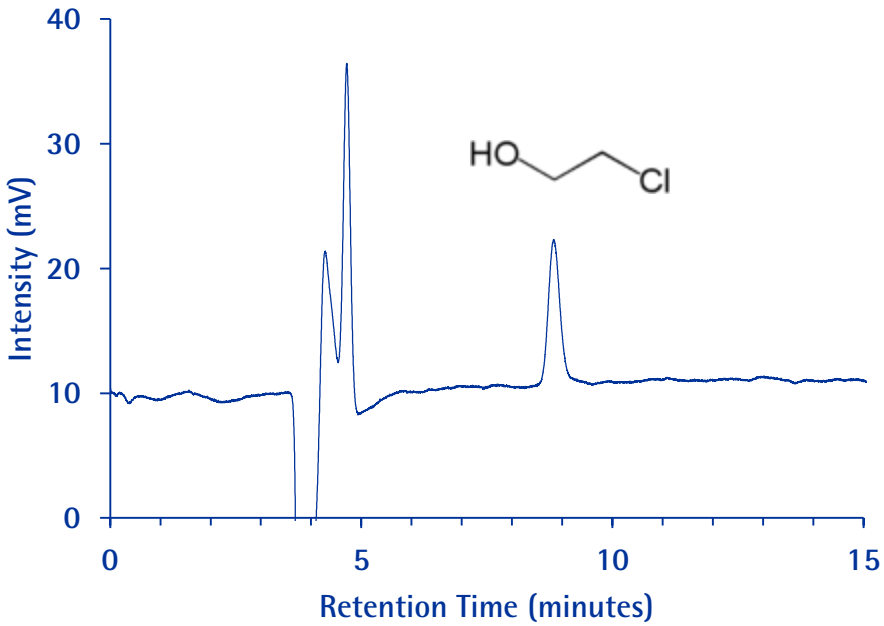
No.	Compound	Retention Time (min)	Resolution	Tailing Factor
1	Caffeine	1.1	-	1.1
2	Choline	7.8	35.6	1.3
3	Taurine	9.1	6.2	1.3
4	Inositol	9.8	4.6	1.1
5	Carnitine	10.3	2.3	1.2

2-Chloroethanol

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 150 x 4.6 mm	1.50455.0001
Injection:	25 µL	
Detection:	RI (Detection Range: 1, Cell temperature: 40°C) Shimadzu Prominence	
Cell:	9 µL	
Flow Rate:	0.5 mL/min.	
Mobile Phase (v/v):	Dissolve 3.08 g of ammonium acetate in 1L water (40 mM). Mix buffer and acetonitrile 25:75 (v/v)	
Temperature:	40 °C	
Diluent	Mobile phase	
Sample:	Weigh 100 mg of sample in 100 ml volumetric flask. Dilute upto the mark with mobile phase. Pipette out 10 ml of the above solution & dilute to 100 ml with mobile phase.	
Pressure Drop:	83 bar (1203 psi)	



Chromatographic Data

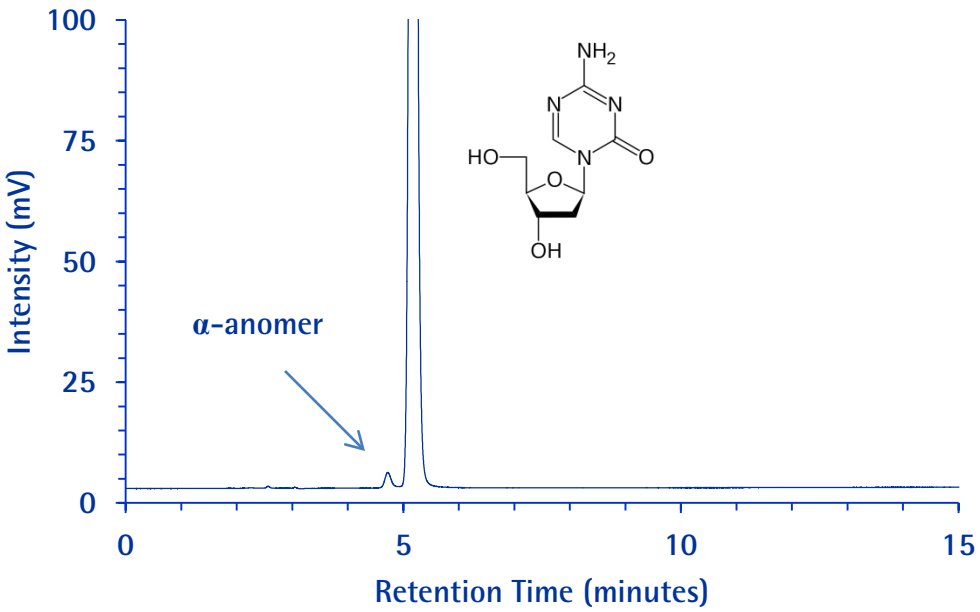
No.	Compound	Retention Time (min)	Tailing Factor	Theoretical Plate
1	2-Chloroethanol	8.8	1.2	7149

Decitabine

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 150 x 4.6 mm	1.50455.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence, UV@254 nm	
Cell:	10 µl	
Flow Rate:	0.75 mL/min	
Mobile Phase (v/v):	Buffer: 50 mM ammonium acetate in water, pH 6.8 Mix acetonitrile and buffer by volume 85:15.	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	200 ppm of Decitabine in mobile phase	
Pressure Drop:	30 Bar (435 psi)	



Chromatographic Data

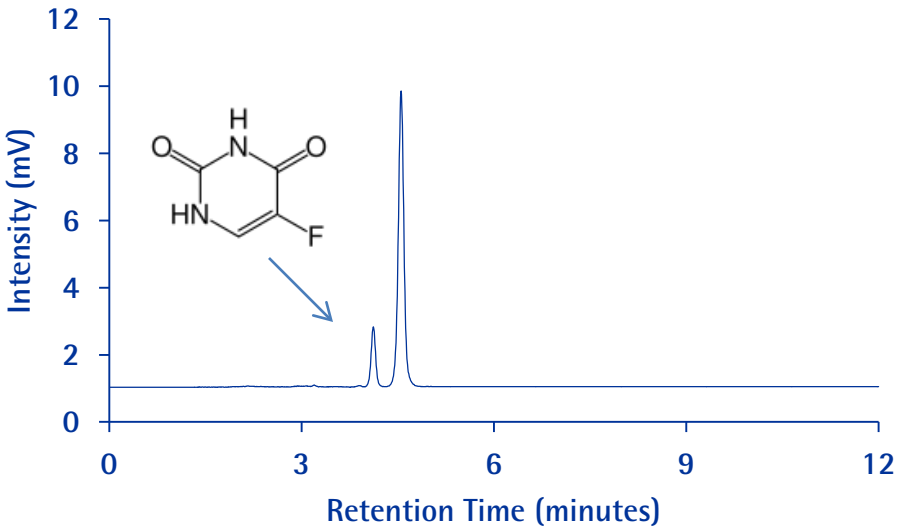
No.	Compound	Time	Tailing Factor	Retention Factor (k')	Resolution
1	α- Anomer	4.7	1.1	1.4	-
	Decitabine	5.2	1.1	1.6	2.2

5-Fluorouracil and 5-Fluorouracil Dimer

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 150 x 4.6 mm	1.50455.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence, UV@254 nm	
Cell:	10 µl	
Flow Rate:	0.5 mL/min	
Mobile Phase (v/v):	Buffer: Dissolve 1.54 g of ammonium acetate in 1L water (20 mM). Mix acetonitrile and buffer by volume 80:20.	
Temperature:	25°C	
Diluent	Mobile phase	
Sample:	Weigh 50 mg of each substance in 100 ml volumetric flask. Dilute upto mark with mobile phase. Pipette out 10 ml of the above solution & dilute to 100 ml with mobile phase.	
Pressure Drop:	29 Bar (418 psi)	



Chromatographic Data

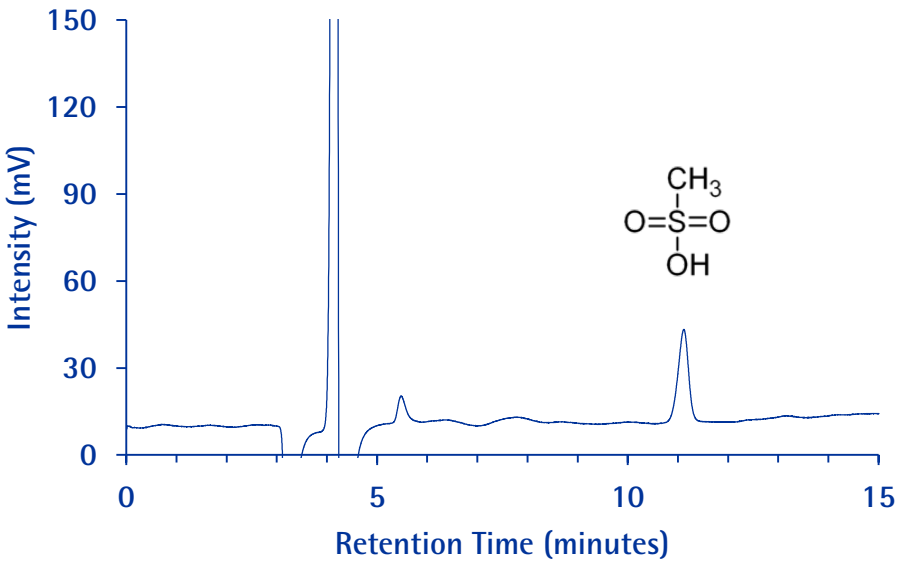
No.	Compound	Time	Tailing Factor	Resolution
1	5-Fluorouracil	4.1	1.1	
2	5-Fluorouracil Dimer	4.6	1.1	2.6

Methanesulfonic Acid in Busulfan Injection

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (3.5 µm, 200 Å) PEEK 150 × 4.6 mm	1.51444.0001
Injection:	20 µL	
Detection:	Shimadzu Prominence, RI (cell temperature 40 °C, Range 1.0)	
Cell:	10 µl	
Flow Rate:	0.5 mL/min	
Mobile Phase:	Buffer: Dissolve 7.7 g of ammonium acetate in 1000 ml water (100 mM). Adjust pH to 4.5 with glacial acetic acid. Mix Acetonitrile and Buffer 80:20 (v/v)	
Temperature:	40 °C	
Diluent	Mobile phase	
Sample:	Weigh 20 mg of sample in 100 ml volumetric flask. Dilute up to the mark with mobile phase.	
Pressure Drop:	57 bar (826 psi)	



Chromatographic Data

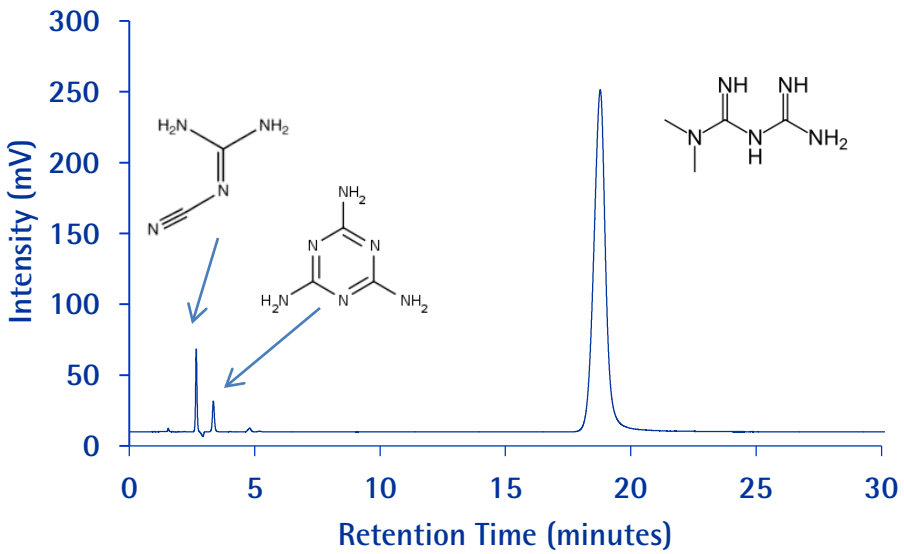
No.	Compound	Time	Theoretical Plates	Tailing Factor
1	Methanesulfonic Acid	11.1	12917	1.0

Metformin and Related Impurities

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 250 × 4.6 mm	1.51458.0001
Injection:	10 µL	
Detection:	Shimadzu LC-10, UV 218 nm	
Cell:	10 µl	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Buffer: Dissolve 0.77 g of ammonium acetate in 1000 ml water (10 mM). Mix Acetonitrile and Buffer 85:15 (v/v)	
Temperature:	30 °C	
Diluent	Mobile phase	
Sample:	500 ppm metformin and 4 ppm of each impurity in mobile phase	
Pressure Drop:	63 bar (913 psi)	



Chromatographic Data

No.	Compound	Time	Theoretical Plates	Tailing Factor
1	Cyanoguanidine	2.7	11209	1.1
2	Melamine	3.3	8937	1.1
3	Metformin	18.8	8042	1.0

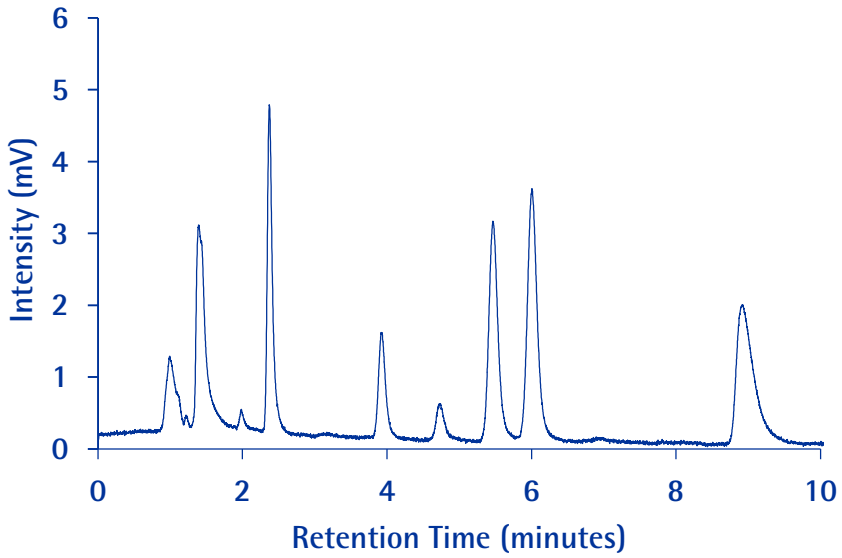
Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
http://www.merckmillipore.com/chromatography
chromatography@merckgroup.com

Organic Acids

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC (3 µm, 100 Å) PEEK 150 x 2.1 mm	1.50658.0001
Injection:	5 µL	
Detection:	UV at 200 nm. Shimadzu LC-10Vp equipped with 2.5µL semi-micro flow-cell	
Flow Rate:	0.3 mL/min.	
Mobile Phase:	Acetonitrile and 25mM Potassium Phosphate buffer pH 6.0 (75:25 v/v)	
Temperature:	30 °C	
Diluent	Mobile phase	
Sample:	10ppm mix of each analyte diluted in mobile phase	



Chromatographic Data

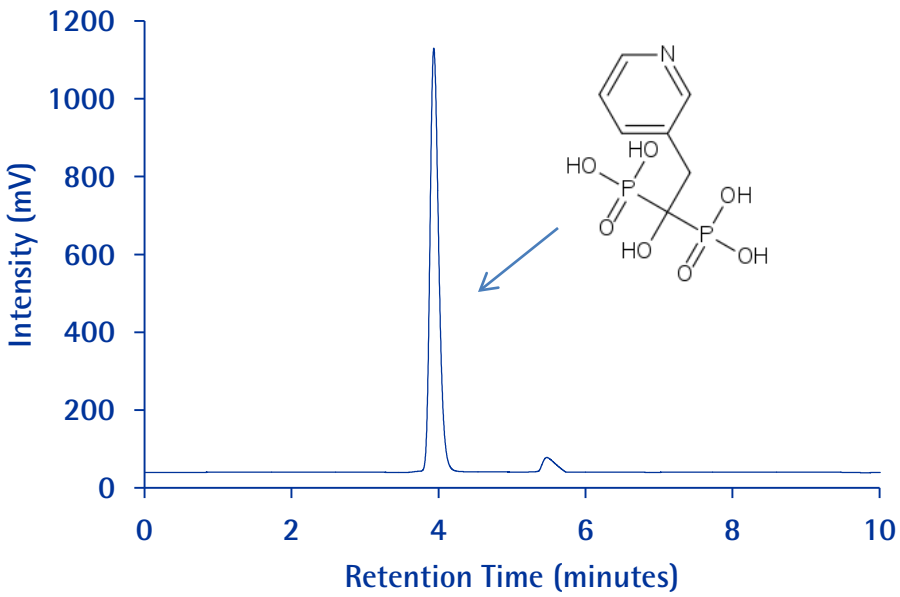
No.	Compound	Time (min)	Retention Factor
	Void volume (t0)	1	-
1	Acetic acid	2.4	1.4
2	Succinic acid	3.9	2.9
3	Malic acid	5.5	4.5
4	Tartaric acid	6.0	5.0
5	Citric acid	8.9	7.9

Risedronate sodium and Related impurities

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 100 × 4.6 mm	1.51453.0001
Injection:	5 µL	
Detection:	Shimadzu LC 2010, UV 226 nm	
Cell:	10 µl	
Flow Rate:	0.5 mL/min	
Mobile Phase:	Dissolve 1.54 g of ammonium acetate in 1L water (20 mM). Mix Acetonitrile and Buffer 80:20 (v/v)	
Temperature:	25 °C	
Diluent	Mobile phase	
Sample:	Weigh 100 mg of sample in 100 ml volumetric flask. Dilute upto the mark with mobile phase. Pipette out 10 ml of the above solution & dilute to 100 ml with mobile phase.	
Pressure Drop:	46 bar (667 psi)	



Chromatographic Data

No.	Compound	Time	Tailing Factor	Theoretical Plate
1	Risedronate Na	3.9	1.4	5212

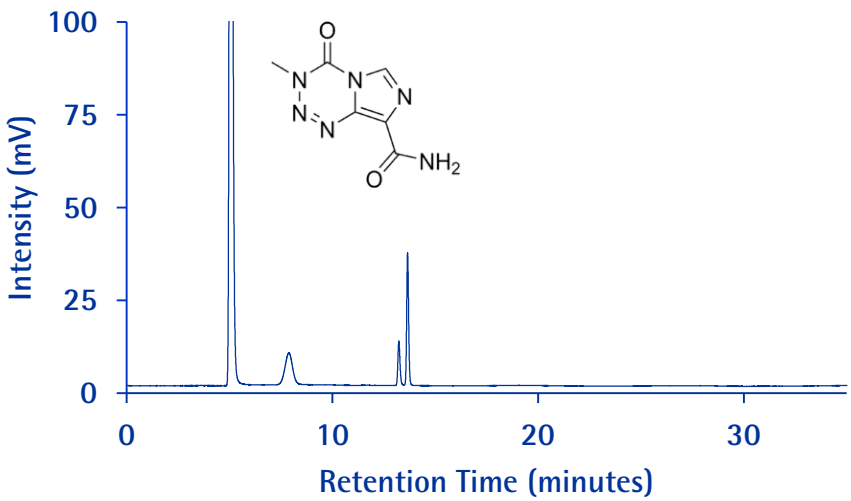
Temozolomide and Related Impurities

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 250 × 4.6 mm	1.51458.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence, UV 254 nm	
Cell:	10 µl	
Flow Rate:	0.8 mL/min	
Mobile Phase (v/v):	A: Dissolve 3.08 g of ammonium acetate in 1000 ml water (40 mM). B: 100% Acetonitrile	
Gradient:	See Table:	
Temperature:	25 °C over column and autosampler cooler set at 15 °C	
Diluent	Mobile phase	
Sample:	400 ppm of Temozolomide and 1 ppm of each impurity A, B and C in acetonitrile. Keep the solution for 4 hrs in amber glassware before analysis for stabilization. Use amber coloured vial for analysis.	

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	Tailing Factor	Resolution*
1	Temozolomide	5.1	1.3	0.0
2	Impurity C	7.9	1.0	6.3
3	Impurity B	13.2	1.1	12.5
4	Impurity A	13.7	1.1	2.4

Recommended Reagents and Chemicals

Name of the chemical	Ordering information
Ammonium acetate LiChropur®	61855405001730 *
Formic acid 100 % GR	10026405001730 *
Acetonitrile Gradient Grade for Chromatography LiChrosolv®	1.00030.4000 **
Acetonitrile for Chromatography LiChrosolv®	60003025001730 *
Acetic acid for Chromatography LiChrosolv®	61866510001730 *

* Chemical manufactured in India
** Chemical manufactured in Darmstadt, Germany