

# Aurorasil<sup>™</sup> MIXED-MODE CHROMATOGRAPHIC COLUMN

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## MIXED-MODE CHROMATOGRAPHY, MMC

With the advancement of chromatographic techniques, Mixed-Mode Chromatography (MMC) has emerged as a novel liquid chromatography approach that allows for various forms of interactions with solutes. MMC is a chromatographic method in which multiple forces or separation mechanisms are applied on a single column to retain and separate solutes from the stationary phase. Its functionalities often exhibit complementarity or synergy.



The primary advantage of Mixed-Mode Chromatography over conventional single-mode chromatography lies in its ability to achieve multiple types of separation on a single column. Due to the presence of multiple forces, MMC can significantly enhance separation selectivity and peak shape, achieving effects that traditional single-mode chromatography cannot attain. Another advantage of MMC is its flexibility in adjusting the retention and selectivity of analytes by altering the pH, salt concentration, or organic solvent concentration in the mobile phase, enabling faster and more efficient separations. Additionally, MMC typically does not require the use of ion-pairing reagents, making it highly compatible with the widely used Electrospray Ionization Mass Spectrometry (ESI-MS) technique.

Compared to traditional reverse-phase, hydrophilic interaction, and ion exchange chromatographic columns with single retention modes, Dikma Aurorasil<sup>™</sup> columns possess a unique surface chemical structure. This allows them to offer multiple retention mechanisms on a single bonded phase, thereby optimizing selectivity to the maximum extent. By adjusting the buffer salt concentration, pH, and organic phase ratio, Aurorasil<sup>™</sup> columns can simultaneously retain strongly polar, moderately polar, and weakly polar compounds. They can also separate polar, non-polar, acidic, basic, and neutral compounds.

### Features of Aurorasil<sup>™</sup> Columns

- Capable of retaining various types of samples on a single column.
- Multiple retention mechanisms greatly enhance separation selectivity and peak shape.
- Excellent performance in terms of selectivity, resolution, and retention.
- Enable rapid and highly efficient separation analysis.
- Outstanding batch-to-batch reproducibility and stability.
- Suitable for the separation of complex mixtures such as drug molecules, active pharmaceutical ingredients (APIs), positional and geometric isomers, metabolites, strongly hydrophilic and polar compounds, natural products, acidic and basic compounds, amino acids, peptides, proteins, proteomics, and metabolomics.



Aurorasil<sup>™</sup> columns are based on high-purity silica gel. They incorporate Dikma Technology's independently designed and developed multifunctional ligand silane, unique stationary phase crosslinking and multiple bonding techniques, as well as advanced end-capping technology. While maintaining the hydrophobicity of the stationary phase, the new type of multifunctional ligand silane provides various forms of interactions between the stationary phase and analytes. This imparts the series of chromatographic columns with multiple retention mechanisms, granting the stationary phase unique selectivity within an extremely wide elution range. This results in excellent separation of polar compounds, acidic and basic compounds, isomers, and various complex systems, simplifying method development.

Aurorasil<sup>™</sup> columns offer a variety of stationary phases such as MMP-G, MMP-H, MMP-L, MMP-M, MMP-Q, MMP-Y, and MMP-Z, meeting a wide range of separation requirements and standing as the best choice for method development and complex sample separation.

#### Aurorasil<sup>™</sup> Material Characteristics

Bonded phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m²/g)	Purity (%)	Phase Density (µmol/m²)	Carbon Loading (%)	pH Range	Endcapping
MMP-G	3,5	200	200	> 99.999	5.5	17	1.5 – 10.0	Yes
MMP-H	3,5	200	200	> 99.999	5.5	17	2.0 – 9.5	Yes
MMP-L	3,5	200	200	> 99.999	5.5	17	2.0 – 7.5	No
MMP-M	3,5	200	200	> 99.999	5.5	16	2.0 – 7.5	No
MMP-Q	3,5	200	200	> 99.999	5.5	17	2.0 – 7.5	No
MMP-Y	3,5	100	440	> 99.999	2.1	17	1.5 – 9.0	Yes
MMP-Z	3,5	100	440	> 99.999	2.6	20	1.5 – 9.5	Yes

#### Simultaneous Analysis of Different Compound Types Polar, Non-Polar, Acidic, Basic, and Neutral Compounds

When dealing with multi-component mixed samples, especially those containing complex components like acids, bases, polar, and non-polar substances, it often requires complex mobile phase conditions and longer analysis times to simultaneously analyze a variety of substances on a single column. Additionally, obtaining ideal chromatographic peak shapes and resolutions can be challenging. Aurorasil<sup>™</sup> columns possess a unique surface chemical structure, providing multiple retention mechanisms on a single stationary phase. This allows for the simultaneous analysis of various compound types using simple chromatographic conditions. As shown in the figure below, compared to columns from other brands, Aurorasil<sup>™</sup> columns exhibit superior selectivity and separation capabilities, along with excellent resolution, perfect peak shapes, and high detection efficiency.





#### Phenol and Phenolic Compounds

A group of phenol and phenolic compounds were selected as analytes. Due to the different positions of methyl and phenolic hydroxyl groups, multiple positional isomers were formed. To address the separation challenge of these compounds, complex additives are usually added to the mobile phase system, which increases the equilibration time of the chromatographic column and reduces experimental efficiency. Aurorasil<sup>™</sup> columns significantly enhance the bonding density and isomer resolution of the stationary phase through unique cross-linking bonding technology and capping process, thus achieving efficient separation of various phenolic isomers. More importantly, in many cases, conventional chromatographic columns simply cannot capture small changes in the position of these functional groups. However, Aurorasil<sup>™</sup> columns can distinguish small differences in the spatial position of functional groups or shifts in dipole moments in the analyte.



#### Phenol and Phenolic Compounds

Column:	Listed on chromatograms	Dikm
Dimension:	150 x 4.6 mm	
Mobile Phase:	A: 0.1% HCOOH in H <sub>2</sub> O	
	B: 0.1% HCOOH in MeCN	
socratic:	A:B = 68:32	
Flow rate:	1.0 mL/min	
Temperature:	30 °C	
Detection:	UV 280nm	Ū
Sample:	1. Hydroquinone	
	2. Resorcinol	Water
	3. 2-Methylresorcinol	
	4.2,5-Dimethylresorcinol	
	5.4-Methylcatechol	
	6. Phenol	
	7. <i>m</i> -Cresol	
	8. <i>o</i> -Cresol	0
	9. 3,4-Dimethylphenol	
	10. 2,6-Dimethylphenol	Sigma-
	11. 2,5-Dimethylphenol	
	12. 2,4-Dimethylphenol	



In this application, 15 phenolic compounds with different substituents were selected as analytes to investigate the selectivity and retention ability of Aurorasil<sup>™</sup> MMP-Z for phenolic compounds. As seen in the figure below, Aurorasil<sup>™</sup> MMP-Z exhibits better separation performance for phenolic compounds compared to the C18 chromatographic column.

Column:	Listed on chromatograms
Dimension:	150 x 4.6 mm
Mobile Phase:	A: 0.1% HCOOH in MeCN
	B: 0.1% HCOOH in H <sub>2</sub> O
	A:B = 40:60
Flow rate:	1.0 mL/min
Temperature:	Ambient
Detection:	UV 280 nm

#### Sample: 1. 3,4-Dimethoxyphenol 2. Phenol 3. 3,5-Dimethoxyphenol 4. 4-Nitrophenol 5. 2-Chlorophenol 6. 4-Chlorophenol 7. 3,5-Dimethylphenol 8. 2-Nitrophenol

- 9. 2,6-Dimethylphenol
- 10. 4-Chloro-3-methylphenol
- 11. 2,3-Dichlorophenol
- 12. 4-Chloro-2-methylphenol
- 13. 3,4-Dichlorophenol
- 14. 3,5-Dichlorophenol
- 15. 2,4,6-Trichlorophenol



Dikma Aurorasil<sup>™</sup> 5 µm MMP-F

Agilent Polaris 5 Amide-C18

Time (min)

Time (min)

10



#### Strongly Alkaline Compounds

Tricyclic and benzodiazepine antidepressants are both classified as strongly alkaline compounds. When using conventional chromatographic columns to analyze such compounds, under neutral conditions, the alkaline groups on the compound are prone to interact with the residual silicone hydroxyl groups on the surface of the silica gel, causing the chromatographic peak to tail off or the resolution to decrease. Aurorasil<sup>™</sup> columns use unique cross-linking bonding technology and capping process to minimize the interaction between residual silicon hydroxyl groups on the surface of silica gel and analytes, significantly improving the separation ability and chromatographic peak tailing issues for alkaline compounds, and achieving efficient separation of alkaline compounds.

Column:	Listed on chromatograms
Dimension:	150 x 4.6 mm
Mobile Phase:	A: 20 mM K <sub>2</sub> HPO <sub>4</sub> (pH 7.0)
	B: MeOH
Isocratic:	A:B = 20:80
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Detection:	UV 254 nm

#### Sample: 1. Alprazolam

- 2. Chlordiazepoxide
- 3. Desipramine hydrochloride
- 4. Nortriptyline hydrochloride
- 5. Imipramine hydrochloride
- 6. Amitriptyline hydrochloride
- 7. Trimipramine maleate



Column:	Listed on chromatograms
Dimension:	150 x 4.6 mm
Mobile Phase:	A: 20 mM Na <sub>2</sub> HPO <sub>4</sub> + NaH <sub>2</sub> PO <sub>4</sub> (pH 7.0)
	B: MeCN
	A:B = 37:63
Isocratic:	1.0 mL/min
Flow Rate:	30 °C
Temperature:	UV 254 nm
Detection:	1. Nortriptyline hydrochloride
Sample:	2. Estazolam
	3. Clonazepam
	4. Imipramine hydrochloride
	5. Diazepam
	6. Amitriptyline hydrochloride

- 7. Clomipramine hydrochloride



#### Strongly Alkaline Compounds

Isocratic:

Flow Rate:

Temperature: 30 °C

A:B = 35:65

1.0 mL/min

Column:	Listed on chromatograms	Dikma Aurorasil <sup>™</sup> 5µm MMP-M	Thermo Acclaim <sup>™</sup> Polar Advantage C16 5 μm
Dimension:	150 x 4.6 mm	4 5	2+3
Mobile Phase:	A: 20 mM K₂HPO₄ (pH 7.0) B: MeOH		4 1
Isocratic:	A:B = 38:62		i I A A
Flow Rate:	1.0 mL/min		
Temperature:	30 °C		
Detection:	UV 220 nm	Time (min)	Time (min)
Sample:	1. Landiolol hydrochloride		
	2. Alprazolam		
	3. Oxazepam		
	4. Chlordiazepoxide		
	5. Diazepam		
Column:	Listed on chromatograms	Sample: 1. Nadolol	
Dimension:	150 x 4.6 mm	2. Landiolol hydroch	loride
Mobile Phase:	A: 20 mM K <sub>2</sub> HPO <sub>4</sub> (pH 7.0)	3. Clonazepam	
	B: MeOH	4. Triazolam	

Detection: UV 220 nm		
Dikma Aurorasil <sup>™</sup> 5 µm MMP-Q	Agilent Polaris 5 Amide-C18	Sigma-Aldrich Discovery® RP Amide C16 5µm
Time (min)	Time (min)	Time (min)

5. Oxazepam

7. Diazepam

6. Chlordiazepoxide

Traditional C18 columns cannot achieve effective separation of compounds 2 and 3, while phenyl columns cannot achieve effective separation of compounds 3 and 4. Due to the ability of Aurorasil<sup>™</sup> MMP-Z chromatography column to interact with analytes in various forms, it can significantly improve the separation of compounds 2, 3, and 3, 4, thereby achieving perfect separation of these compounds.

2. Benzylamine

3. Procainmide 4. Salbutamol

5. Phenol







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#### Analysis of Nucleoside Compounds

Nucleoside compounds are a group of highly polar compounds that typically require analysis under high water flow conditions. Aurorasil<sup>™</sup> columns use unique polarity modification technology to enable the column to operate in 100% aqueous phase, with good retention of nucleoside compounds, excellent selectivity, and resolution.





Column:	Listed on chromatograms
Dimension:	150 x 4.6 mm
Mobile Phase:	10 mM HCOONH <sub>4</sub> , pH 3.6
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Detection:	UV 254 nm
Sample:	1. Cytosine
	2. Uracil
	3. Guanine
	4. Uridine
	5. Thymine



#### Separation of Isomeric Halogenated Phenols

Halogenated phenolic compounds are important chemical intermediates but also toxic and hard-to-degrade organic pollutants. The addition of halogen atoms in the molecule can enhance the polarity of the compound, which in turn increases the difficulty of separating these compounds. In this experiment, a group of halogenated phenolic compounds were selected as analytes. Due to the different positions of halogen and hydroxyl groups in the benzene ring, multiple positional isomers were formed. Since the structures of these isomers are very similar, complex mobile phase systems are often used to improve separation selectivity. In this experiment, Aurorasil<sup>™</sup> columns were used, and with a simple mobile phase system, complete separation of various positional isomers of halogenated phenols was achieved. Compared to other chromatographic columns, Aurorasil<sup>™</sup> columns offer faster analysis and higher separation efficiency.





Column:	Listed on chromatograms
Dimension:	150 x 4.6 mm
Mobile Phase:	A: 0.1% HCOOH in $H_2O$
	B: 0.1% HCOOH in MeCN
Isocratic:	A:B = 62:38
Flow rate:	1.0 mL/min
Temperature:	30 °C
Detection:	UV 275 nm
Sample:	1. 2-Fluorophenol
	2. 3-Fluorophenol
	3. 2-Chlorophenol
	4. 4-Chlorophenol
	5. 3-Chlorophenol
	6. 4-Chloro-2-Fluorophenol
	7. 4-Chloro-3-Fluorophenol
	8. 2,3-Dichlorophenol
	9. 2,4-Dichlorophenol











#### Additives

Except for caffeine, all compounds in this group are polar compounds containing hydroxyl groups, each with different levels of polarity. The aim of this experiment was to investigate the polarity of the stationary phase and its separation ability for polar compounds. By adjusting the structural design of the Aurorasil<sup>™</sup> stationary phase and controlling the types and quantities of introduced functional groups, the stationary phase was endowed with characteristics of fast analysis and strong separation ability.



#### Antibiotics

Sulfonamide drugs are broad-spectrum antibiotics mainly used in clinical practice for the prevention and treatment of infectious diseases. The active moiety in sulfonamide antibiotics is the sulfonamido group, and there are also a certain number of basic groups. The differences in the number and types of basic groups lead to variations in the polarity and hydrophilicity of each compound. The polar groups in the Aurorasil<sup>™</sup> stationary phase enhance the polar interactions between sulfonamide compounds and the stationary phase. Additionally, the hydrophobic interactions, dipole-dipole interactions, and hydrogen bonding interactions of functional groups in the stationary phase further enhance the separation ability of the chromatographic column.

Column:	Listed on chromatograms	Dikma Aurorasil <sup>™</sup> 5µm MMP-H
Dimension:	150 x 4.6 mm	2
Mobile Phase:	A: 0.1% HCOOH in H <sub>2</sub> O	1 3 67
	B: 0.1% HCOOH in MeCN-MeOH	
	(6/4, v/v)	
Isocratic:	A:B = 81:19	
Flow Rate:	1.0 mL/min	
Temperature:	30 °C	Time (min)
Detection:	UV 254 nm	
Sample:	1. Sulfanilamide	Thermo Acclaim <sup>™</sup> Polar Advantage C16 5µm
	2. Sulfadiazine	5+6
	3. Sulfapyridine	1 2
	4. Sulfamerazine	
	5. Sulfamethazine	
	6. Thiamphenicol	
	7. Sulfamethoxypyridazine	0 2 4 6 8
		Time (min)





Column:	Listed on chromatograms
Dimension:	150 x 4.6 mm
Mobile Phase:	A: 0.1% HCOOH in H <sub>2</sub> O
	B: 0.1% HCOOH in MeCN-MeO
	(6/4, v/v)
Isocratic:	A:B = 84:16
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Detection:	UV 254 nm
Sample:	1. Sulfanilamide
	2. Sulfadiazine
	3. Sulfapyridine
	4. Sulfamerazine
	5. Sulfamethazine
	6. Thiamphenicol
	7. Sulfamethoxypyridazine



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#### Water-Soluble Vitamins

Water-soluble vitamins are a class of highly polar compounds among vitamins. These compounds are poorly retained on conventional chromatographic columns, and often require the addition of additives such as ion-pairing reagents to the mobile phase. This increases the complexity of the experiment and causes irreversible damage to the chromatographic column, greatly reducing its lifespan. Aurorasil<sup>™</sup> MMP-H chromatographic column can interact with water-soluble vitamins in various ways, enhancing the retention of hydrophilic and polar compounds. This allows for effective separation of water-soluble vitamin compounds under simple experimental conditions.



In this application, seven water-soluble vitamins were selected as analytes to investigate the retention ability of Aurorasil<sup>™</sup> MMP-Z for strongly polar compounds. Due to the high polarity of these compounds, conventional C18 columns exhibit weak retention, and baseline separation of some components is difficult to achieve. Aurorasil<sup>™</sup> MMP-Z can interact with water-soluble vitamins in various ways, enhancing the retention of hydrophilic and polar compounds, and providing unique selectivity.



#### Separation of Methoxybenzene Isomers

This application selected methoxybenzene isomers with different substituents as analytes, including methoxybenzene, dimethoxybenzene, and trimethoxybenzene isomers, with toluene as a neutral reference compound. The aim was to investigate the separation ability of Aurorasil<sup>TM</sup> MMP-Y for isomers. Compared to the Sunniest 5  $\mu$ m PFP&C18 column, Aurorasil<sup>TM</sup> MMP-Y, due to its multiple retention mechanisms, enhances the selectivity for positional isomers, enabling easy separation of methoxybenzene and its various substituted isomers.



#### Separation of Dinitrobenzene Isomers

This application selected dinitrobenzene isomers with different substituents as analytes, with toluene as a neutral reference compound. Compared to traditional C18 columns, Aurorasil<sup>™</sup> MMP-Y exhibits stronger separation ability for isomers, while also showing different selectivity.

Column:	Listed on chromatograms
Dimension:	150 x 4.6 mm
Mobile Phase:	$MeOH:H_2O = 40:60$
Flow Rate:	1.0 mL/min
Temperature:	40 °C
Detection:	UV 254 nm
Sample:	1. 1,4-Dinitrobenzene
	2. 1,3-Dinitrobenzene
	3. 1,2-Dinitrobenzene
	4. Toluene







#### Catecholamines

Catecholamines are a class of sympathomimetic amine drugs with high polarity. Reverse-phase chromatography typically requires operation in 100% aqueous phase to enhance their retention, which poses a significant challenge to the water resistance of traditional C18 and PFP columns. Due to its unique bonded phase cross-linking, multiple bonding techniques, and advanced capping technology, Aurorasil<sup>™</sup> MMP-Y can introduce a variety of different functional groups on the silica surface. This allows it to handle high water flow conditions and enhance the retention of strongly polar compounds. In this comparative experiment, the separation performance of Aurorasil<sup>™</sup> MMP-Y column was significantly superior to other brand columns.



Sample: 1. Norepinephrine 2. Levodopa 3. Epinephrine 4. *L*-Tyrosine 5. Dopamine



#### Fluorobenzene

Aurorasil<sup>™</sup> MMP-Y has a unique surface chemical structure, providing multiple retention mechanisms on a single stationary phase. This enhances its ability to recognize halogenated aromatic compounds, improving selectivity and resolution for both halogenated and non-halogenated aromatic compounds. Experimental results demonstrate the unparalleled advantages of Aurorasil<sup>™</sup> MMP-Y in this application.

Column:	Listed on chromatograms	Dikma Aurorasil <sup>™</sup> 5µm MMP-Y	Dikma Inspire® 5 µm C18
Dimension:	150 x 4.6 mm		
Mobile Phase:	$MeOH:H_2O = 60:40$	2	1+2
Flow Rate:	1.0 mL/min	3 4	
Temperature:	40 °C		4
Detection:	UV 250 nm		3
Sample:	1. Benzene		
	2. Fluorobenzene		
	3. Toluene	0 2 4 6 8 10 12 14 16	0 2 4 6 8 10 12 14 16
	4. <i>α,α,α</i> -Trifluorotoluene	Time (min)	Time (min)

#### Aromatic Nitrobenzenes

This application selected nitrobenzenes with varying numbers of nitro substituents as analytes, with toluene as a neutral reference compound. The aim was to investigate the selectivity, retention ability, and hydrophobicity of Aurorasil<sup>™</sup> MMP-Z for aromatic compounds. Aurorasil<sup>™</sup> MMP-Z exhibits selectivity distinct from C18 and phenyl columns.



#### Analgesics

Aurorasil<sup>™</sup> MMP-Z has a unique surface chemical structure, providing multiple retention mechanisms on a single stationary phase. Compared to traditional C18 columns, it demonstrates excellent selectivity and separation efficiency.



 $\mathcal{D}\mathcal{H}(\mathbf{A})$ 

#### Sulfur-Containing Compounds

Traditional C18 columns face challenges in effectively separating structurally similar compounds due to their single hydrophobic retention mechanism. Aurorasil<sup>™</sup> MMP-Z can offer multiple retention mechanisms, optimizing selectivity to the fullest extent. This allows for perfect separation of this class of compounds, effectively addressing the separation issues that C18 columns struggle with.



#### Aurorasil<sup>™</sup> Ordering Information

#### $5 \mu m$ Analytical Columns

Phases	150 x 4.6 mm	250 x 4.6 mm
MMP-G	89001	89002
MMP-H	89003	89004
MMP-L	89005	89006
MMP-M	89007	89008
MMP-Q	89009	89010
MMP-Y	89011	89012
MMP-Z	89013	89014



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