What makes YMC-Triart Phenyl advantageous?

Alkyl ligand phases such as C18 or C8 are by far the most popular columns in reversed phase chromatography and give good results for many of separations. But sometimes there are those challenging analyses where there it is not possible to improve the resolution further by method optimisation. This is the time to consider a change in column selectivity, especially when working with aromatic compounds.

Phenyl stationary phases show a different selectivity to aliphatic phases. Typically, aromatic compounds and conjugated systems are analysed on phenyl phases which show a changed elution profile due to their additional interactions present. Hydrophobic and π-π interactions contribute to different retention times. Figure 2 illustrates the differences in selectivity for several aromatic compounds such as steroids or tricyclic antidepressants: A peak pattern different to that from C18 and C8 columns is obtained.

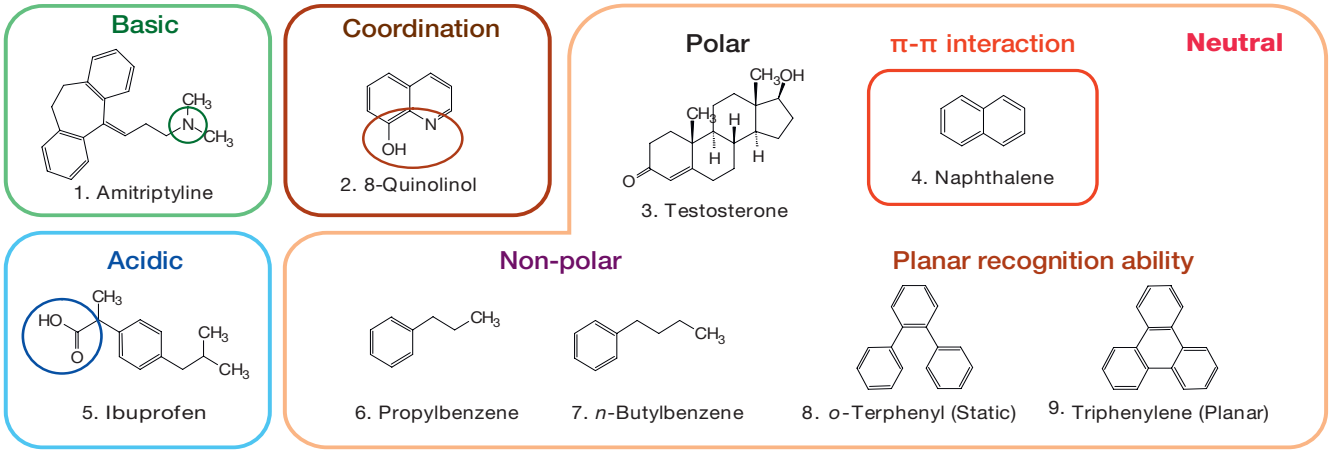
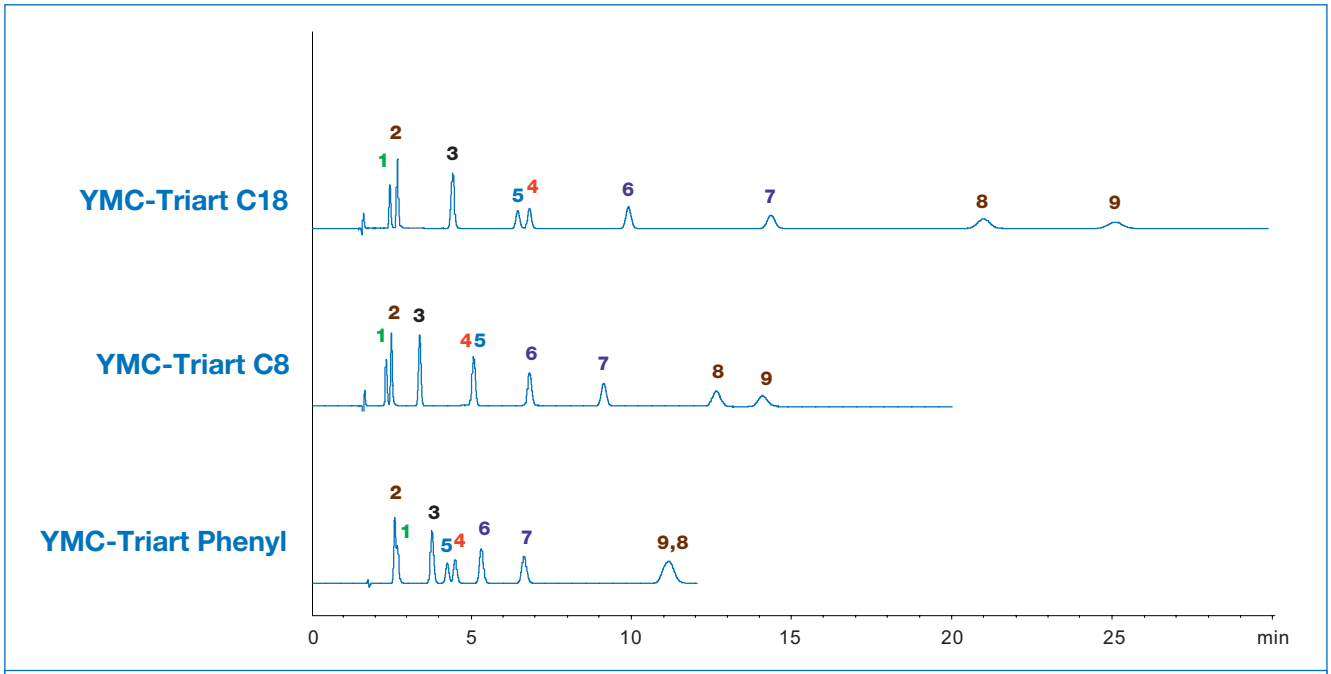


Figure 1: The compounds with different properties separated in Figure 2 and 3.



Columns:	YMC-Triart C18 (5 µm, 12 nm) 150 x 3 mm ID YMC-Triart C8 (5 µm, 12 nm) 150 x 3 mm ID YMC-Triart Phenyl (5 µm, 12 nm) 150 x 3 mm ID	Eluent:	20 mM H_3PO_4-KH_2PO_4 (pH 3.1)/methanol (25/75)
Part Nos.:	TA12S05-1503PTH TO12S05-1503PTH TPH12S05-1503PTH	Flow rate: Temperature: Detection: Injection:	0.425 mL/min 40°C UV at 265 nm 4 µL

Figure 2: Separation of compounds with different properties using YMC-Triart C18, C8 and Phenyl columns.

Wide Range of Phenyl Column Types

There are many different phenyl stationary phases available on the market. But why are there differences between the columns from different manufacturers? And how critical are the analytes' properties in their analysis?
The chemistry of phenyl modified stationary phases can be quite different. The aromatic group itself can be a mono or biphenyl group, the linkage to the silica surface can vary in its carbon chain length, the silica can be endcapped or not and different side chains can be attached to the ligands to name only some of the differences. In this study, stationary phases from different manufacturers varying in base particle chemistry (hybrid silica vs. silica vs. silica with a charges surface) and length of spacer (no linker vs. butyl vs. hexyl) are compared analysing compounds with different properties, such as acidic, coordinating and basic compounds.

Table 1: YMC columns evaluated.

	YMC-Triart C18	YMC-Triart C8	YMC-Triart Phenyl	YMC-Pack Ph
Base material	hybrid silica	hybrid silica	**hybrid silica**	silica
Modification	C18	C8	**phenyl-butyl**	phenyl
Particle size [µm]	5*	5*	**5***	5*
Pore size [nm]	12	12	**12**	12
Specific surface area [m^2/g]	360	360	**360**	330
Carbon content [%]	20	17	**17**	9
Bonding	trifunctional	trifunctional	**trifunctional**	monofunctional
Endcapping	yes	yes	**yes**	yes
Column dimension	150 x 3 mm ID	150 x 3 mm ID	**150 x 3 mm ID**	150 x 3 mm ID
Part number	TA12S05-1503PTH	TO12S05-1503PTH	**TPH12S05-1503PTH**	PH12S05-1503WT

**further particle sizes available (YMC-Triart: 1.9 and 3 µm; YMC-Pack Ph: 3 µm)*

Table 2: Competitor columns evaluated.

	XBridge BEH Phenyl	XSelect CSH Phenyl-Hexyl	ZORBAX Eclipse Plus Phenyl-Hexyl	Ascentis Phenyl
Manufacturer	Waters	Waters	Agilent	Supelco
Base material	hybrid silica	hybrid silica	silica	silica
Modification	phenyl-hexyl	phenyl-hexyl	phenyl-hexyl	phenyl-butyl
Particle size [µm]	5*	5*	5*	5
Pore size [nm]	13	13.5	9.5	10
Specific surface area [m^2/g]	185	185	160	450
Carbon content [%]	15	14	9	19
Bonding	trifunctional	trifunctional	N/A	N/A
Endcapping	yes	yes	yes	yes
Column dimension	150 x 4.6 mm ID	150 x 4.6 mm ID	150 x 4.6 mm ID	150 x 4.6 mm ID

**further particle sizes available (XBridge BEH Phenyl and XSelect CSH Phenyl-Hexyl: 2.5 and 3.5 µm; ZORBAX Eclipse Plus Phenyl-Hexyl: 1.8 and 3.5 µm)*

Selectivities of the Evaluated Columns

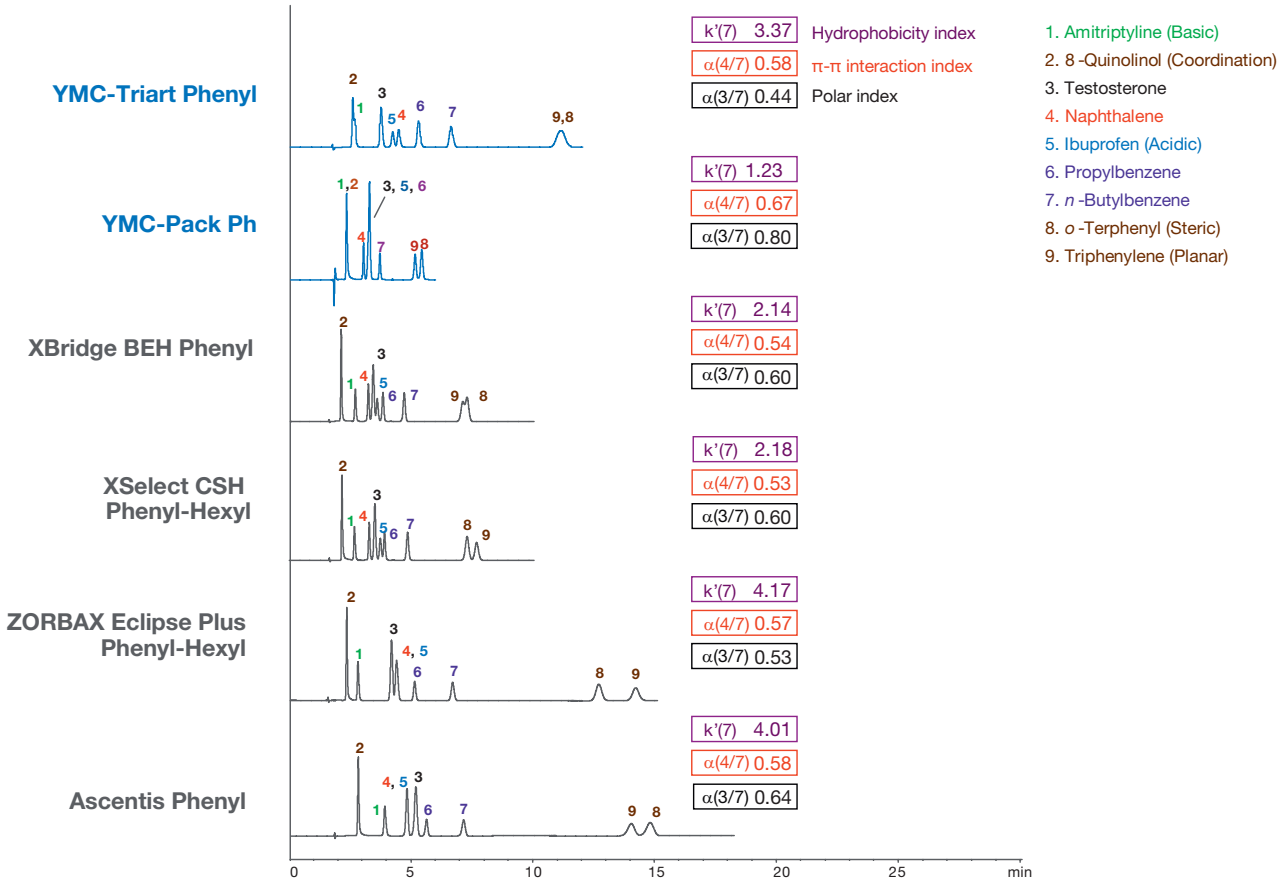
In order to obtain an overall impression about the columns' selectivities a mixture containing acidic, basic, coordinating, aromatic, non-polar and sterically different compounds was analysed on all the columns. Methanol was used as organic solvent which is often preferred over acetonitrile when using phenyl columns since the π-electrons from the acetonitrile compete with the analytes for the π-π interactions with the stationary phase reducing the interactions between the analyte and the stationary phase.

The capacity factor k' and the separation factor α are used to describe the chromatographic behaviour. k' gives a value for the intensity of the interactions between an analyte and the stationary phase, whereas α describes the ability to distinguish between two analytes. If α is below 0 the separation factor improves the smaller the value is, whereas if it is above 0 it gets better the greater the value is. Both k' and α were calculated for all columns. YMC-Pack Ph showed the least hydrophobic interaction based on its hydrophobicity index k'(7) as it has no spacer between the phenyl group and the stationary phase that could interact with the analyte. For the other phases which either have a butyl or hexyl spacer the hydrophobicity index is generally higher but there is no direct correlation with the alkyl chain length.

The π-π interaction indices turned out to be comparable for the phases containing alkyl spacers and slightly better compared to YMC Pack Ph with no spacer. This emphasises that the aromatic selectivity is not affected by the alkyl chain length of four or six carbon atoms but is affected to a small extent if there is no spacer.

As for the polar selectivity YMC-Triart Phenyl showed the best results. Whilst the phase without spacer provided the lowest polar selectivity, no clear trend could be observed for the other spacer lengths indicating that other phase characteristics play an additional role.

For further investigations YMC-Pack Ph was no longer considered in order to compare only the most similar stationary phases.



Eluent: 20 mM H_3PO_4-KH_2PO_4 (pH 3.1)/methanol (25/75)
Flow rate: 0.425 mL/min for 3 mm ID
1.0 mL/min for 4.6 mm ID

Temperature: 40° C
Detection: UV at 265 nm
Injection: 4 µL for 3 mm ID
9 µL for 4.6 mm ID

Figure 3: Separation of compounds with different properties using several YMC and competitor columns.

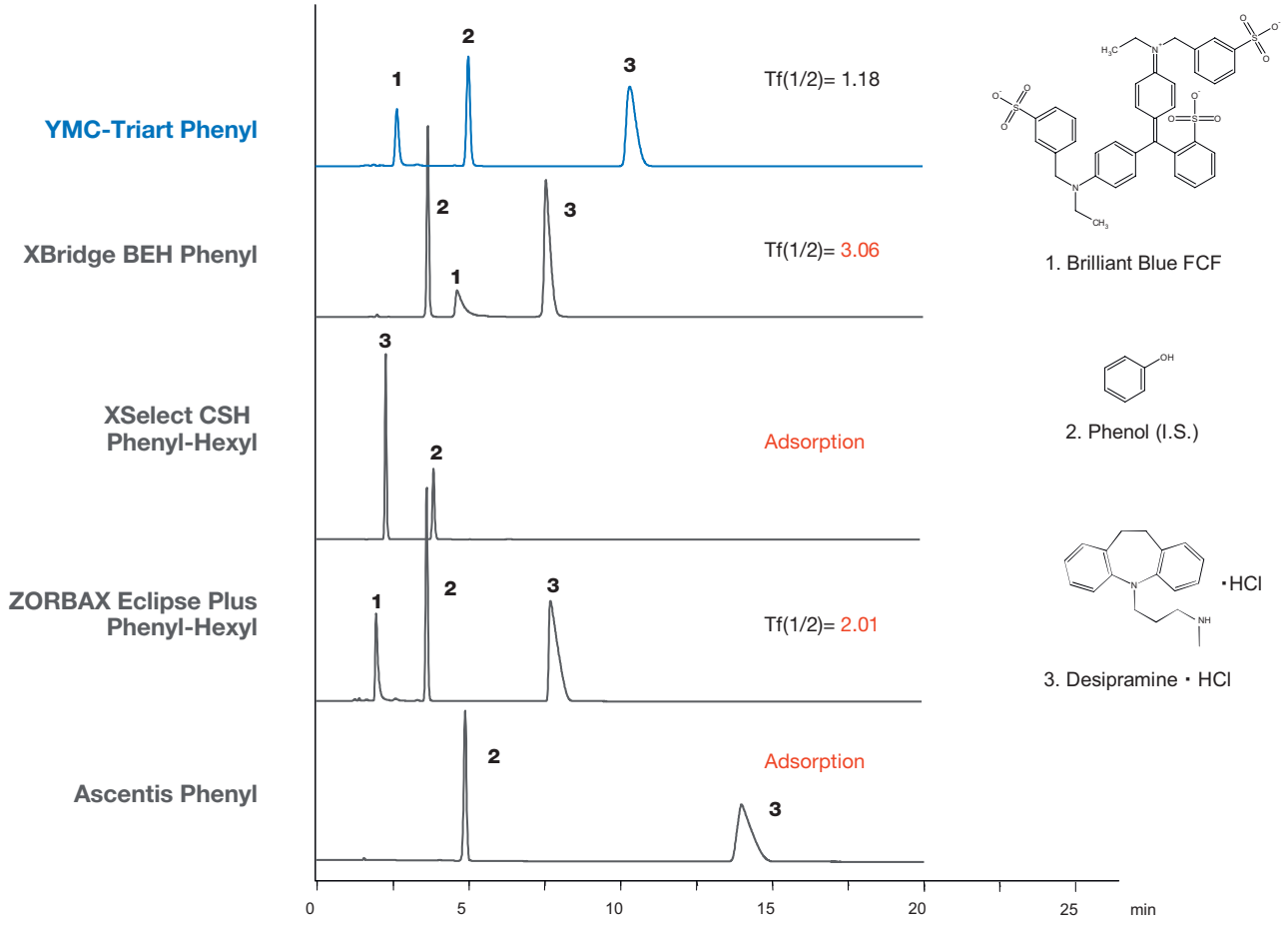
High Recovery of Strongly Acidic Compound Using YMC-Triart Phenyl

Brilliant Blue FCF was analysed together with desipramine HCl to evaluate the phase properties using acidic eluent conditions of 0.1% H_3PO_4. Brilliant Blue FCF is a strongly acidic compound due to its sulphonic acid functional groups and remains unprotonated under these conditions. Brilliant Blue FCF can easily adsorb onto the silica surface if the material is slightly basic resulting in poor peak shape. High tailing factors could be observed for the two competitor columns XBridge BEH Phenyl and ZORBAX Eclipse Plus Phenyl-Hexyl.

As for other columns the results were even worse as the Brilliant Blue FCF was not eluted at all. Due to the positively charged surface of XSelect CSH Phenyl-Hexyl the electrostatic interaction between the analyte and silica surface is too strong to allow elution of the compound.

For the basic compound desipramine which was added to the sample mixture the results do not look any better. Due to the surface charge the cationic desipramine is repelled and shows low retention times. For ZORBAX Eclipse Plus Phenyl-Hexyl and Ascentis Phenyl the peaks also show a significant tailing behaviour indicating the presence of secondary interactions between the analyte and the stationary phase.

Using YMC-Triart Phenyl peak shape is excellent for both the acidic and basic compound. Due to the extended endcapping process and the highly inert particles of YMC-Triart no secondary interactions occur providing great results for the analysis of both Brilliant Blue FCF and desipramine using acidic conditions.



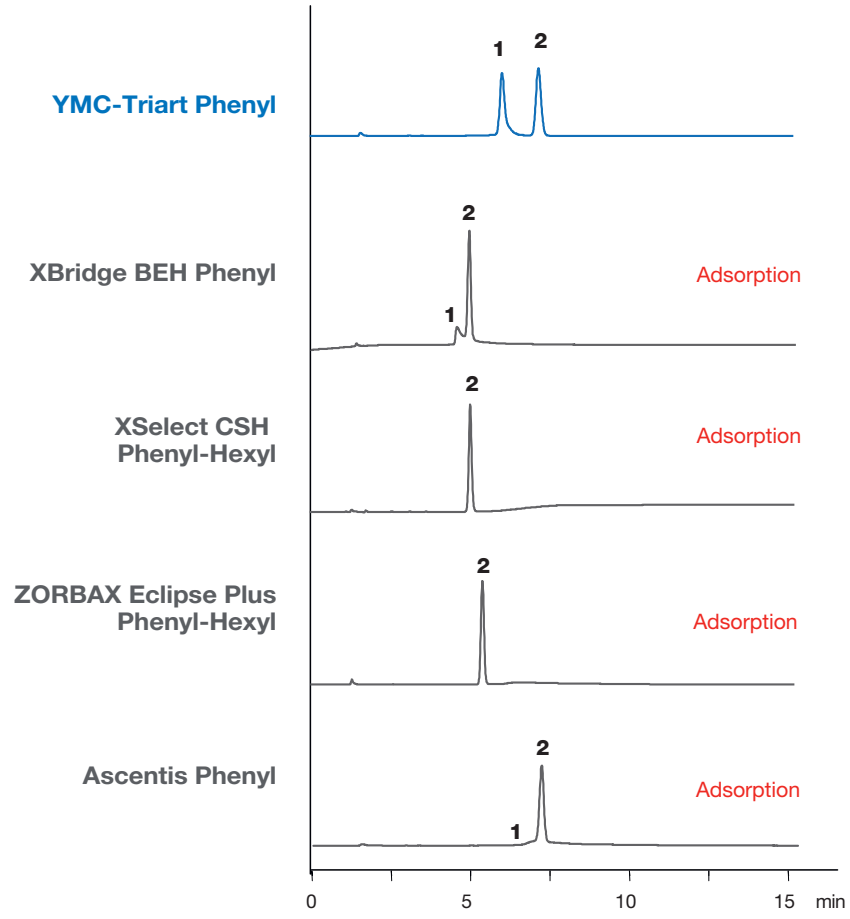
Eluent: acetonitrile/0.1% H_3PO_4 (30/70)
Flow rate: 0.425 mL/min for 3 mm ID
1.0 mL/min for 4.6 mm ID
Temperature: 40°C
Detection: UV at 254 nm
Injection: 2 µL for 3 mm ID
5 µL for 4.6 mm ID

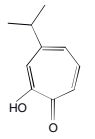
Figure 4: Separation of strongly acidic and basic compound using YMC-Triart Phenyl and competitor columns.

No Adsorption of Coordinating Compounds Using YMC-Triart Phenyl

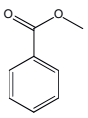
Hinokitiol which is used in oral and skin care products is known to be a coordinating compound making its analysis quite challenging. If the stationary phase has a high amount of metal impurities complexes can be formed resulting in no elution. Using the competitor's stationary phases this indeed was the case.

For XSelect CSH Phenyl-Hexyl and ZORBAX Eclipse Plus Phenyl-Hexyl there was no elution possible even after the third run. Using YMC-Triart Phenyl no coordination could be observed due to its extremely low level of metal impurities ensuring excellent peak shapes for coordinating compounds.





1. Hinokitiol



2. Methyl benzoate (I.S.)

Eluent:	acetonitrile/0.1% H_3PO_4 (40/60)
Flow rate:	0.425 mL/min for 3 mm ID 1.0 mL/min for 4.6 mm ID
Temperature:	40°C
Detection:	UV at 254 nm
Injection:	4 µL for 3 mm ID 9 µL for 4.6 mm ID

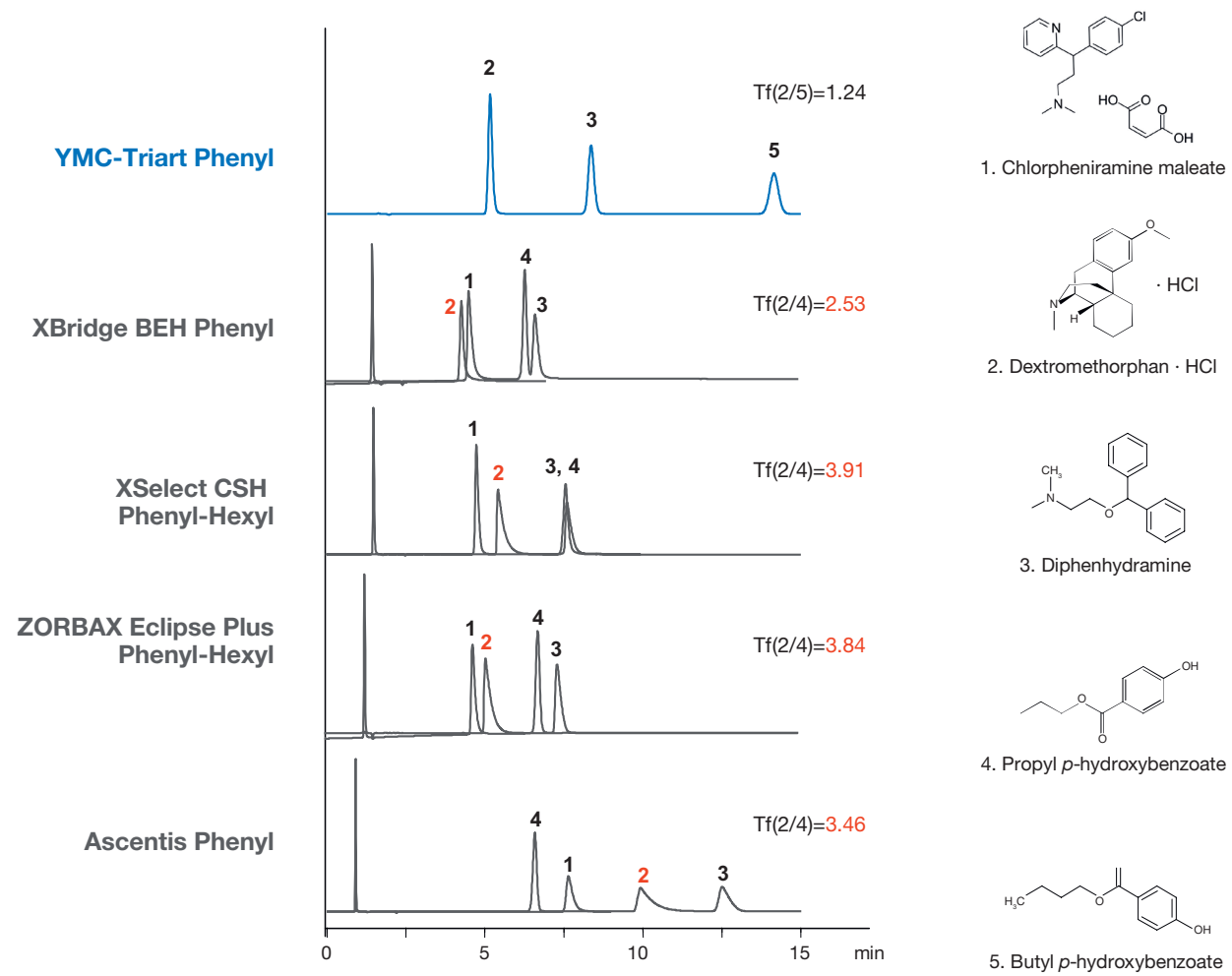
Figure 5: Separation of coordinating compound and internal standard using YMC-Triart Phenyl and competitor columns.

Excellent Peak Shape of Strongly Basic Compounds Using YMC-Triart Phenyl

In addition to acidic and coordinating compounds, the chromatographic behaviour of strongly basic compounds typically used in cold medications was investigated. A mixture of dextromethorphan, diphenhydramine and butyl p-hydroxybenzoate was separated using YMC-Triart Phenyl, whereas a mixture of chlorpheniramine maleate, dextromethorphan, diphenhydramine and propyl p-hydroxybenzoate was analysed using the competitor columns.

In order to evaluate the endcapping quality, neutral mobile phase conditions were chosen where the analytes are positively charged. The magnitude of the interaction between the charged analytes and residual silanols is described by the tailing factor.

Due to the multi-stage endcapping of YMC-Triart, excellent peak shapes and high resolution were obtained using YMC-Triart Phenyl for the analysis of strongly basic compounds. This was different for the other phases evaluated as significant tailing was observed. The tailing factors were at least double compared to YMC-Triart Phenyl, and even more than triple for XSelect CSH Phenyl-Hexyl and ZORBAX Eclipse Plus Phenyl-Hexyl.



Eluent:	20 mM KH_2PO_4-K_2HPO_4 (pH 6.9)/acetonitrile (65/35)
Flow rate:	0.425 mL/min for 3 mm ID 1.0 mL/min for 4.6 mm ID
Temperature:	40 °C
Detection:	UV at 235 nm
Injection:	2 µL for 3 mm ID 5 µL for 4.6 mm ID

Figure 6: Separation of basic compounds using YMC-Triart Phenyl and competitor columns.

5. Conclusions

The chromatographic behaviour of acidic, coordinating and basic compounds was evaluated using several YMC and competitor phenyl-type columns. It was shown that YMC-Triart Phenyl has significant advantages over the other stationary phases for all types of compounds evaluated. In addition to high recovery, excellent peak shapes could be obtained with high resolution.

	Advantages of YMC-Triart Phenyl
Acidic compounds	High recovery Sharp and symmetrical peaks
Coordinating compounds	No adsorption
Basic compounds	Excellent peak shapes High resolution