DRYLAB KNOWLEDGE MANAGEMENT DOCUMENT

UPLC Mehod for Amlodipine: Aquity BEH C18

by R. Kormány, I. Molnár, H.J. Rieger

Summary

Project Description

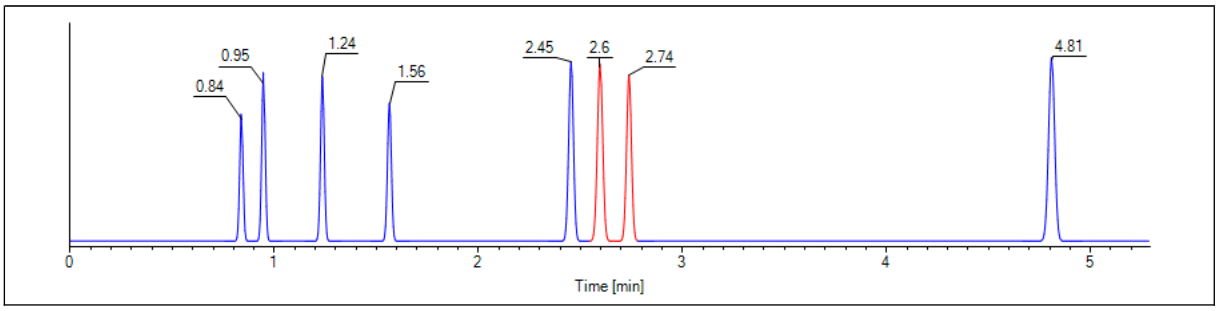
Exchange an old HPLC method against a better and faster method.

Analytical Target Profile (ATP)

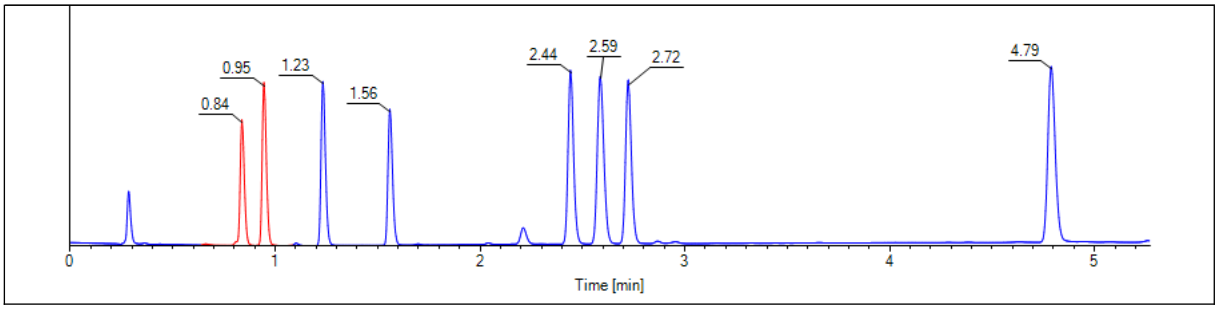
Separate all the drug substance from all the impurities with a minimum resolution of 1.5. The limit of the quantification must be less than 0.05% of the drug substance (reporting limit according ICH Q3A).

Confirmation Run vs Predicted Run

Predicted Run



Confirmation Run



Conclusions

In reference to the analytical target profile (ATP) the selected working point shows a separation off all impurities from each other and from the drug substance with more than resolution of 1.5 (2.6). The separation is good enough to guarantee the quantification limit 0.05%.
The new proposed method has a run time minor than 6 min and it is compared to the old method (60) 10 times faster.

Signature:
Name:

Table of Contents

DryLab Knowledge Management Document 2
Project Description 2
Analytical Target Profile (ATP) 2
Risk Management 2
Documentation of the initial experiments 3
3D Experimental Space 11
Selected Working Point (Set Point) 12
Method Robustness Calculation 13
Confirmation Run vs Predicted Run 14
Conclusions 15
References and Comments 15
Annex 16

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Project Description

Exchange an old HPLC method against a better and faster method.

Analytical Target Profile (ATP)

Separate all the drug substance from all the impurities with a minimum resolution of 1.5. The limit of the quantification must be less than 0.05% of the drug substance (reporting limit according ICH Q3A).

Risk Management

The Critical Quality Attribute (CQA) is: sufficient separation of all the impurities according to the ATP.
Based on prior knowledge and experience with similar projects, the parameters: gradient time tG (3-9 [min]), pH (2 - 2.5 - 3), temperature T (15-45 [°C]) and ternary composition of the eluent B are the most probable potentially critical separation parameters.

Initial Conditions

The three parameters gradient time tG (3-9 [min]), temperature T(15-45 [ºC]) and pH (2-2.5-3) were optimized. The stationary phase Acquity HSS C18 was selected based on a previous test plan [1].

min	ºC	
tG1: 3.00	T1: 15.00	pH1: 2.00
tG2: 9.00	T2: 45.00	pH2: 2.50
		pH3: 3.00

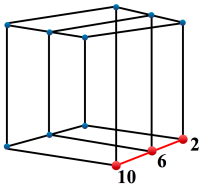
Column Data	
Name: Acquity BEH C18	
Diameter [mm]:	2.10
Length [mm]:	50.00
Particle size [µm]:	1.70
to [min]:	0.21
FlowRate [mL/min]:	0.50
Injection Volumen [µL]:	1.00

Instrument Data	
Name: N/A	
Dwell volume [mL]:	0.125
Extracol volume [mL]:	0.002
Time constant [s]:	0.10
Wavelength [nm]:	230

Eluent Data
Eluent A :
1: 100 % Water (H2O)
Eluent B :
1: 100 % Acetonitrile (ACN)

Documentation of the initial experiments

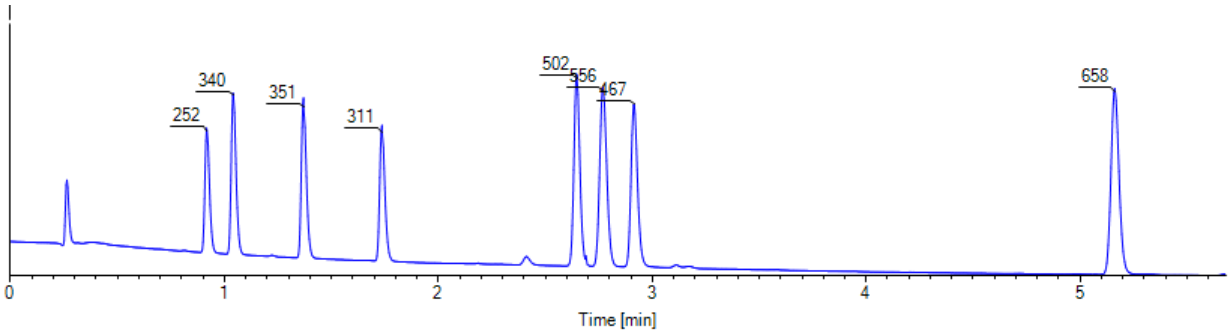
Reference runs: pH (2.00, 2.50, 3.00)



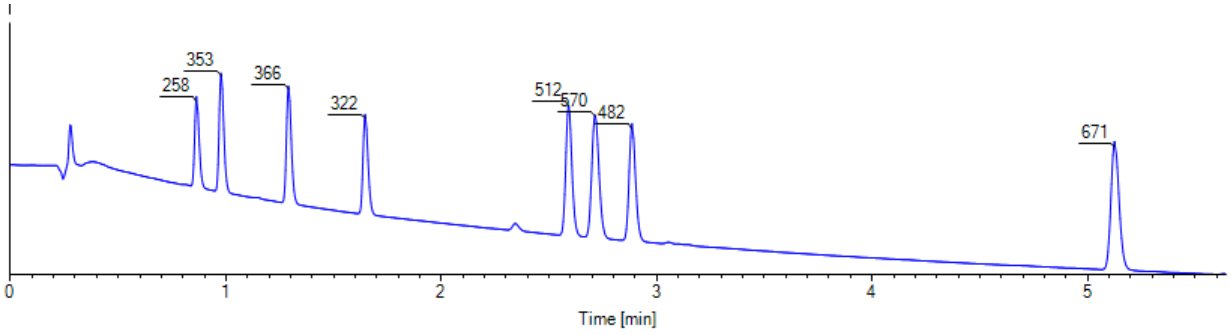
Run	tG[min]	T[°C]	pH
2	9.00	15.00	2.00
6	9.00	15.00	2.50
10	9.00	15.00	3.00

Chromatograms of the Reference Runs

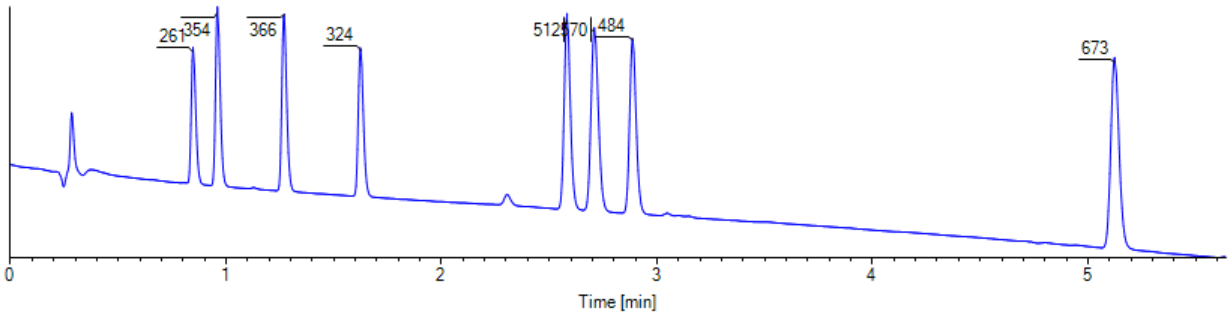
Run 10



Run 6



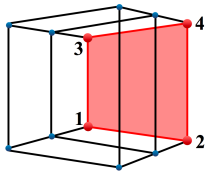
Run 2



Peak Tracking of the References Runs

			Run 2		Run 6		Run 10	
#	Name	SDev	tR [min]	Area	tR [min]	Area	tR [min]	Area
1	ImpD	1.36	0.85	261	0.86	258	0.92	252
2	ImpF	1.73	0.96	354	0.98	353	1.04	340
3	Amlodipine	2.00	1.27	366	1.29	366	1.37	351
4	ImpE	1.83	1.62	324	1.65	322	1.74	311
5	ImpB	0.94	2.58	512	2.59	512	2.65	502
6	ImpG	1.16	2.71	570	2.71	570	2.77	556
7	ImpH	1.55	2.89	484	2.89	482	2.92	467
8	ImpA	0.99	5.13	673	5.13	671	5.16	658
	Σ Areas	1.36	18	3543	18	3534	19	3437

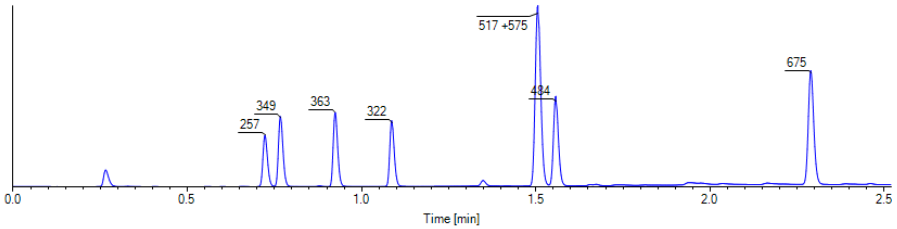
tG-T-Plane 1 (pH1 2.00)



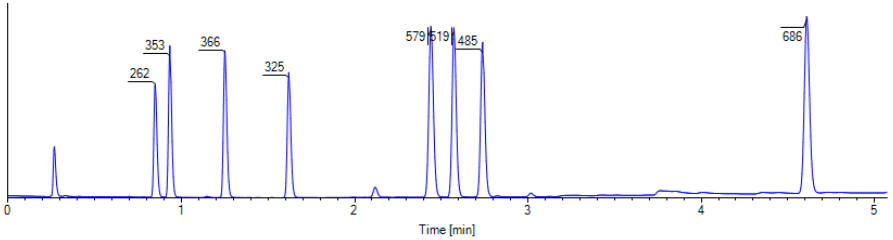
Run	tG[min]	T[°C]	pH
1	3.00	15.00	2.00
2	9.00	15.00	2.00
3	3.00	45.00	2.00
4	9.00	45.00	2.00

Chromatograms of tG-T-Plane 1

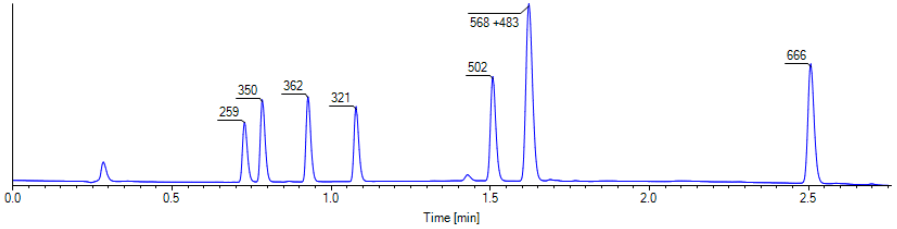
Run 3



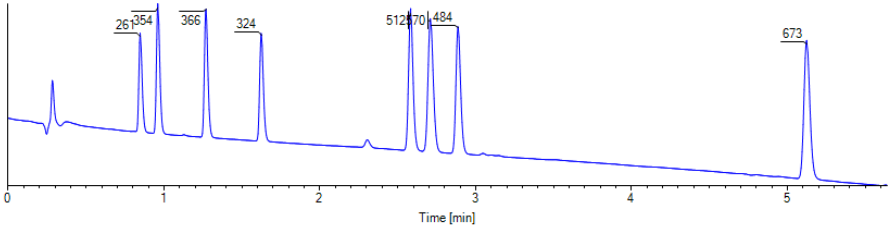
Run 4



Run 1



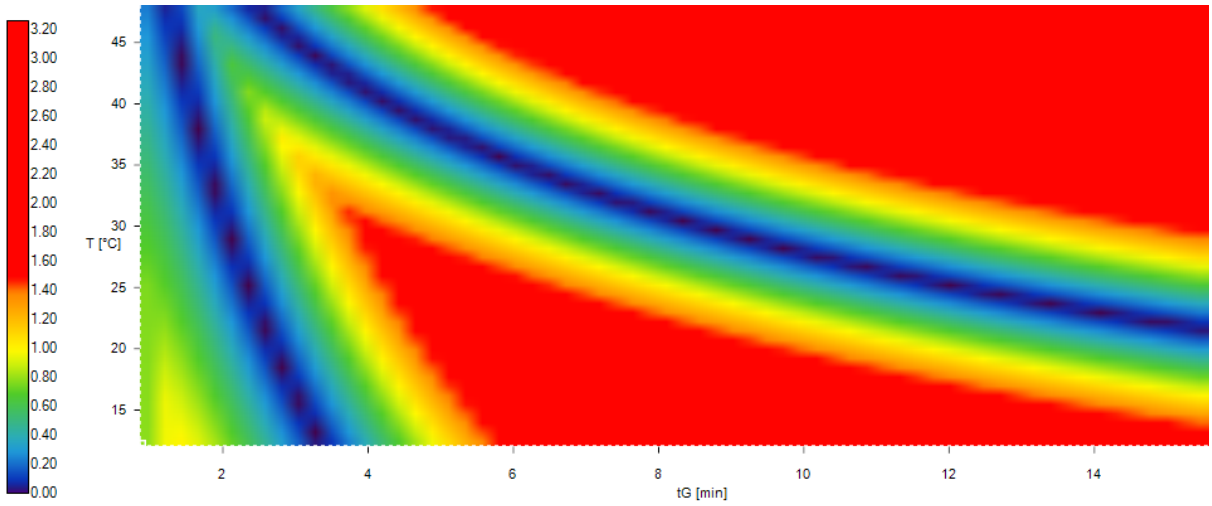
Run 2



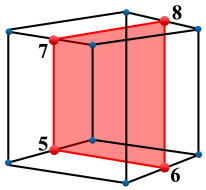
Peak Tracking of the tG-T-Plane 1

			Run 1		Run 2		Run 3		Run 4	
#	Name	SDev	tR [min]	Area	tR [min]	Area	tR [min]	Area	tR [min]	Area
1	ImpD	0.61	0.73	259	0.85	261	0.72	257	0.85	262
2	ImpF	0.57	0.78	350	0.96	354	0.77	349	0.93	353
3	Amlodipine	0.54	0.93	362	1.27	366	0.92	363	1.25	366
4	ImpE	0.46	1.08	321	1.62	324	1.09	322	1.62	325
5	ImpB	1.29	1.51	502	2.58	512	1.51	517	2.57	519
6	ImpG	0.75	1.62	568	2.71	570	1.51	575	2.44	579
7	ImpH	0.19	1.62	483	2.89	484	1.56	484	2.74	485
8	ImpA	1.08	2.51	666	5.13	673	2.29	675	4.61	686
	Σ Areas	0.64	11	3511	18	3543	10	3542	17	3575

Original Resolution Map Plane 1



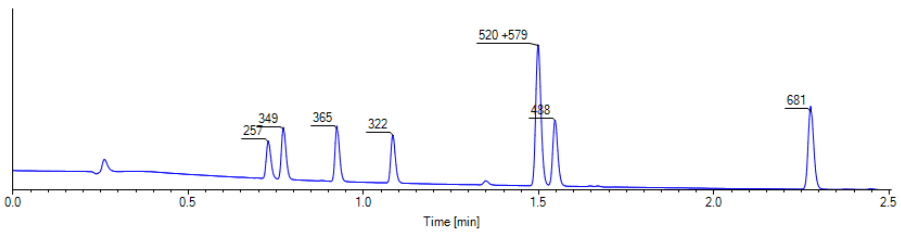
tG-T-Plane 2 (pH2 2.50)



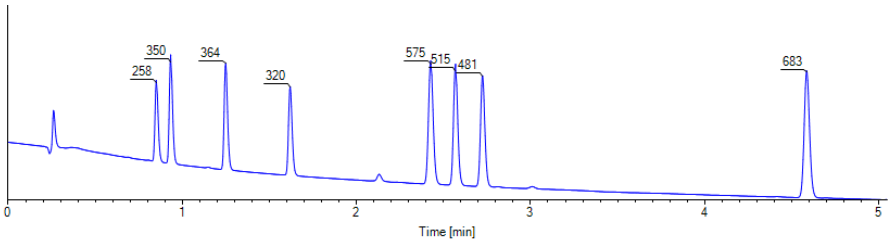
Run	tG[min]	T[°C]	pH
5	3.00	15.00	2.50
6	9.00	15.00	2.50
7	3.00	45.00	2.50
8	9.00	45.00	2.50

Chromatograms of tG-T-Plane 2

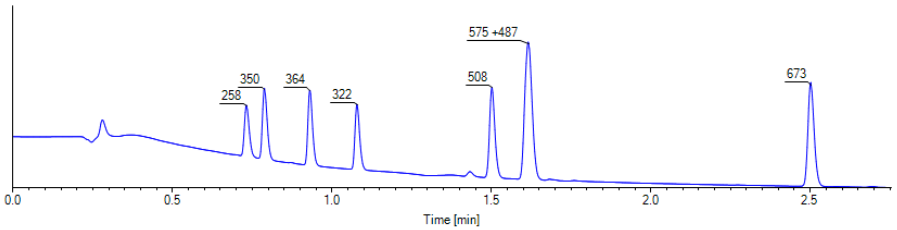
Run 7



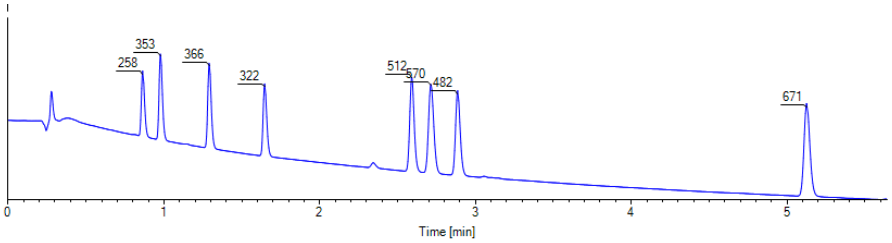
Run 8



Run 5



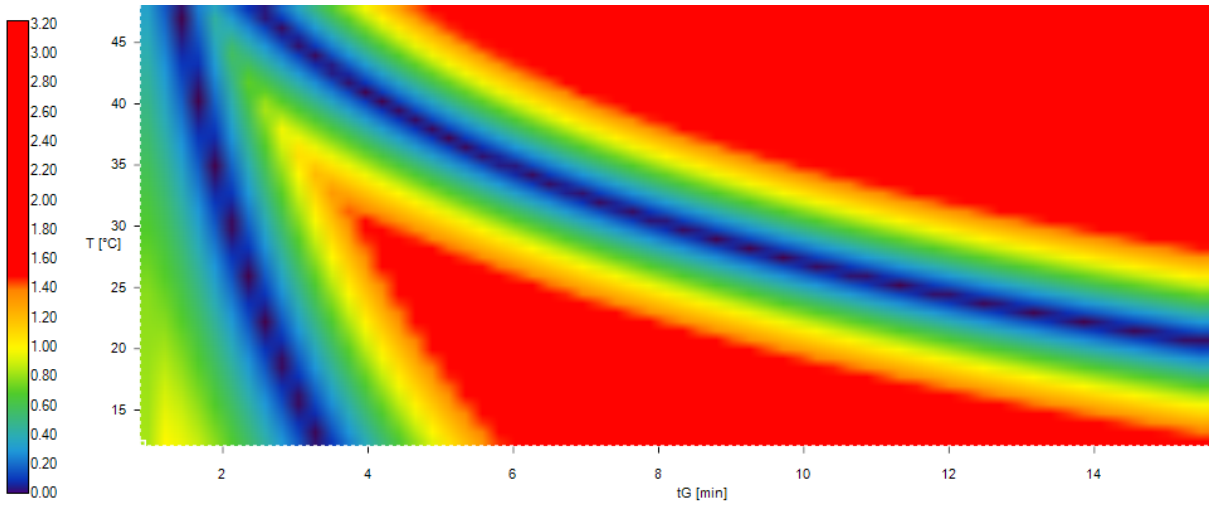
Run 6



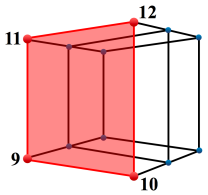
Peak Tracking of the tG-T-Plane 2

			Run 5		Run 6		Run 7		Run 8	
#	Name	SDev	tR [min]	Area	tR [min]	Area	tR [min]	Area	tR [min]	Area
1	ImpD	0.28	0.73	258	0.86	258	0.73	257	0.85	258
2	ImpF	0.35	0.79	350	0.98	353	0.77	349	0.93	350
3	Amlodipine	0.16	0.93	364	1.29	366	0.92	365	1.25	364
4	ImpE	0.29	1.08	322	1.65	322	1.08	322	1.62	320
5	ImpB	0.87	1.50	508	2.59	512	1.50	520	2.57	515
6	ImpG	0.59	1.62	575	2.71	570	1.50	579	2.43	575
7	ImpH	0.60	1.62	487	2.89	482	1.55	488	2.72	481
8	ImpA	0.72	2.50	673	5.13	671	2.28	681	4.59	683
	Σ Areas	0.29	11	3539	18	3534	10	3561	17	3547

Original Resolution Map Plane 2



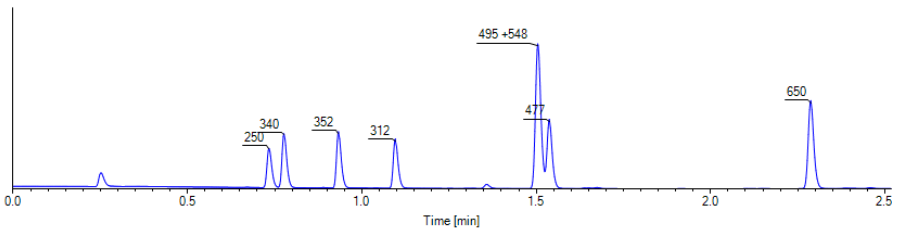
tG-T-Plane 3 (pH3 3.00)



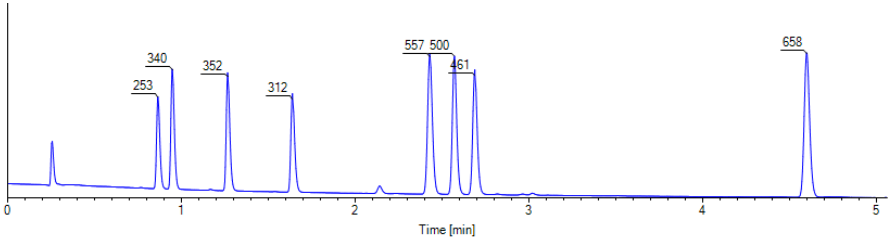
Run	tG[min]	T[ºC]	pH
9	3.00	15.00	3.00
10	9.00	15.00	3.00
11	3.00	45.00	3.00
12	9.00	45.00	3.00

Chromatograms of tG-T-Plane 3

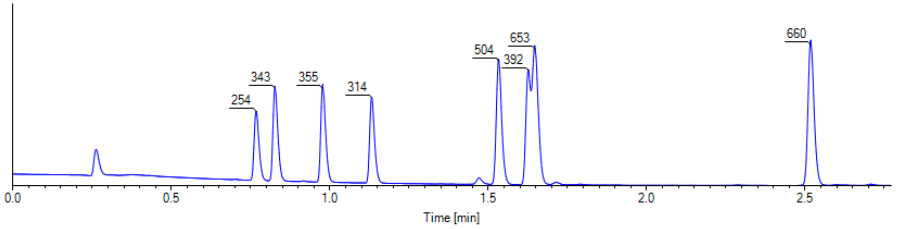
Run 11



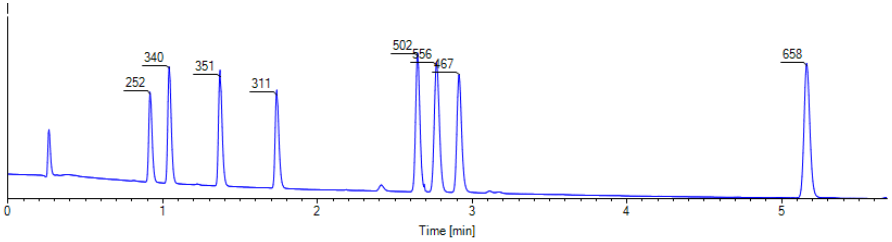
Run 12



Run 9



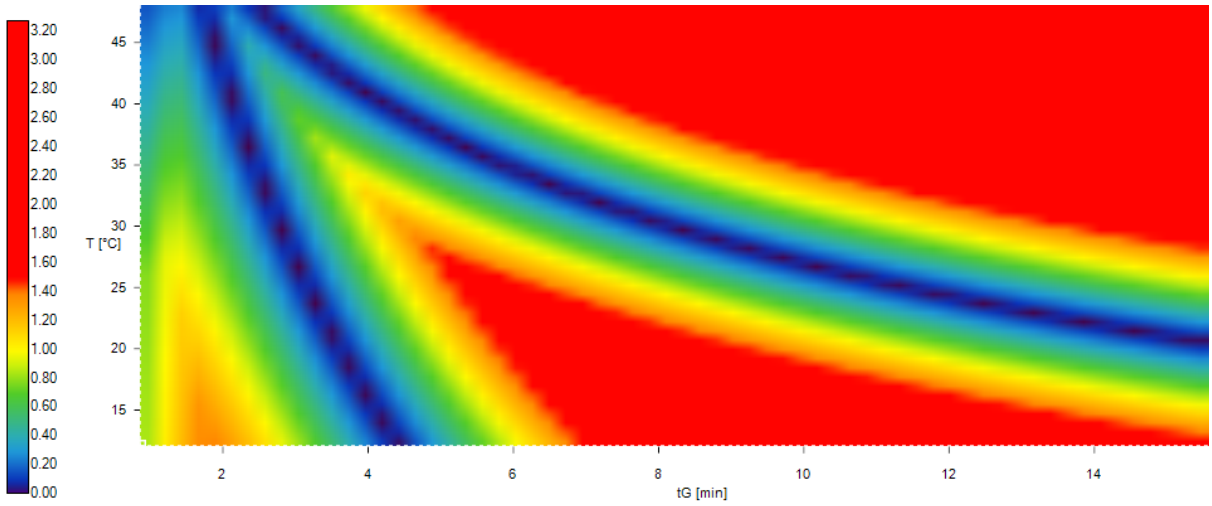
Run 10



Peak Tracking of the tG-T-Plane 3

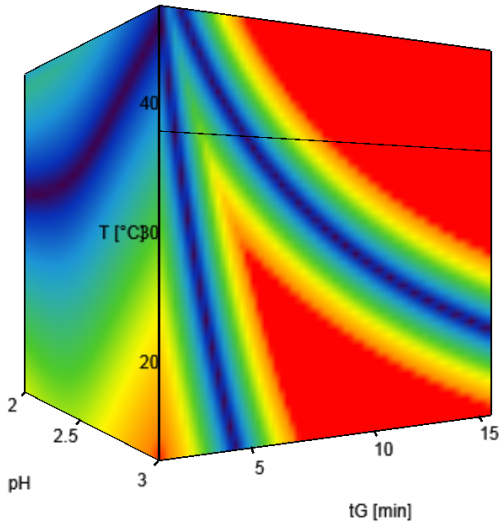
			Run 9		Run 10		Run 11		Run 12	
#	Name	SDev	tR [min]	Area	tR [min]	Area	tR [min]	Area	tR [min]	Area
1	ImpD	0.59	0.77	254	0.92	252	0.73	250	0.86	253
2	ImpF	0.41	0.83	343	1.04	340	0.78	340	0.94	340
3	Amlodipine	0.40	0.98	355	1.37	351	0.93	352	1.26	352
4	ImpE	0.39	1.13	314	1.74	311	1.10	312	1.64	312
5	ImpB	0.67	1.53	504	2.65	502	1.51	495	2.57	500
6	ImpG	7.47	1.65	653	2.77	556	1.51	548	2.43	557
7	ImpH	7.51	1.63	392	2.92	467	1.54	477	2.69	461
8	ImpA	0.58	2.52	660	5.16	658	2.29	650	4.60	658
	Σ Areas	0.56	11	3474	19	3437	10	3423	17	3432

Original Resolution Map Plane 3



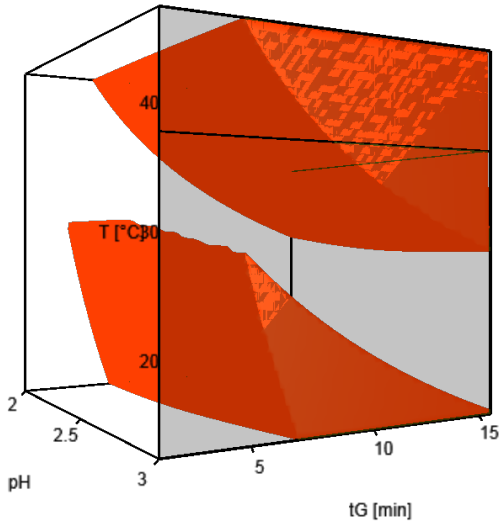
3D Experimental Space

Max Crit. Resolution: 3.35



Method Operable Design Region (MODR)

Critical Resolution Level: 1.5



Selected Working Point (Set Point)

The working point from the previously constructed Design Space was selected because it had the highest critical resolution (Rs, crit).

Final Conditions

Column Data	
Name: Acquity BEH C18	
Diameter [mm]:	2.10
Length [mm]:	50.00
Particle size [µm]:	1.70
to [min]:	213.00
FlowRate [mL/min]:	0.50
Injection Volumen [µL]:	1.00

Instrument Data	
Name: N/A	
Dwell volume [mL]:	0.125
Extracol volume [mL]:	0.002
Time constant [s]:	0.10
Wavelength [nm]:	230

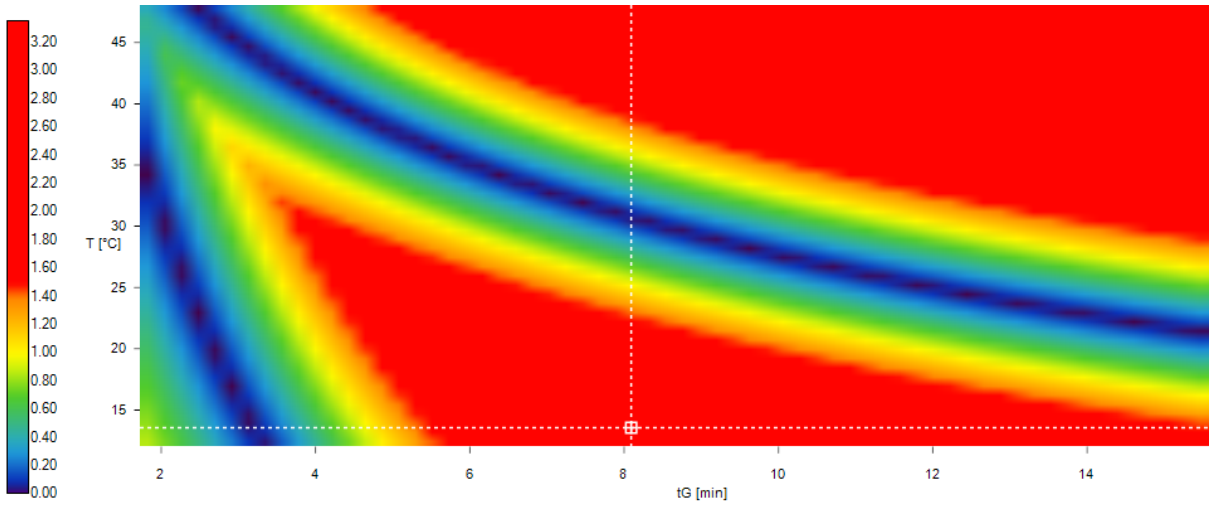
Additional Column Data	
Observed t0 [min]:	213.000
at a Flow Rate of [mL/min]:	0.50
Pore Diameter [nm]:	10.00
A-value:	0.80
Plate Number:	0.00

Status	
8.10	tG [min]
13.50	T [°C]
2.10	pH

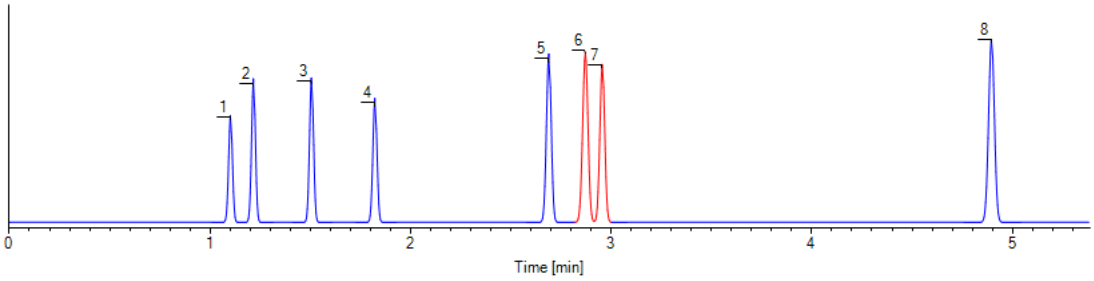
Gradient Table		
Time [min]	[%B]	Rate [%B/min]
0.00	30.00	
8.10	90.00	7.41

Pressure [psi]:	6207
Plate Number:	10606 (calculated)
Rs,crit:	2.96
Crit. Peak Pair:	6, 7
Run Time [min]:	6.00
Eluent Used [mL]:	3.00

Resolution Map for the Selected Working point



Chromatogram Selected Working point



Results Table

#	Name	tR [min]	Area	Avg. k	Width	Resolution
1	ImpD	1.10	259.96	3.22	0.04	3.08
2	ImpF	1.22	352.93	3.31	0.04	7.65
3	Amlodipine	1.51	365.74	3.64	0.04	8.12
4	ImpE	1.82	323.13	3.95	0.04	20.33
5	ImpB	2.69	509.08	5.63	0.05	3.76
6	ImpG	2.87	568.10	6.91	0.05	1.74
7	ImpH	2.96	485.59	5.83	0.05	38.00
8	ImpA	4.90	670.50	8.04	0.06	0.00

Method Robustness Calculation

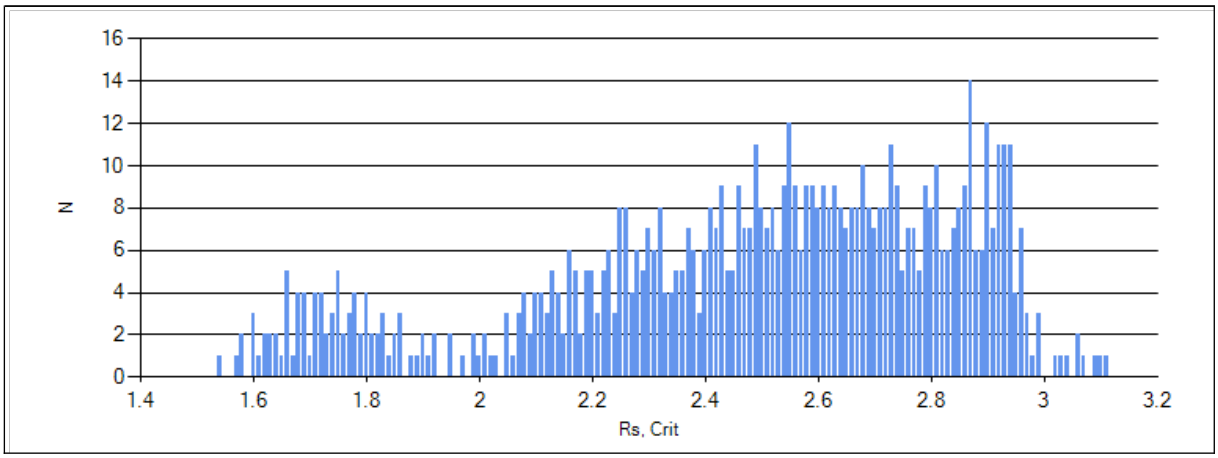
1.-Frequency distribution:
Out of the 729 model simulation no result was less than 1.52. This is good to guarantee an adequate robustness.
2.-Regression Coefficients:
From the selected set of potentially critical separation parameters as selected from the quality risk management evaluation, only the parameter tG and Flow have a significant and positive influence on critical resolution The influence of T, Start %B, End %B or pH is small and probably not significant.
As a consequence the parameters tG and Start %B are confirmed to be critical separation parameters.

T	13.5 ± 1	[°C]
pH	2.1 ± 0.1	
Flow Rate	0.5 ± 0.1	[mL/min]

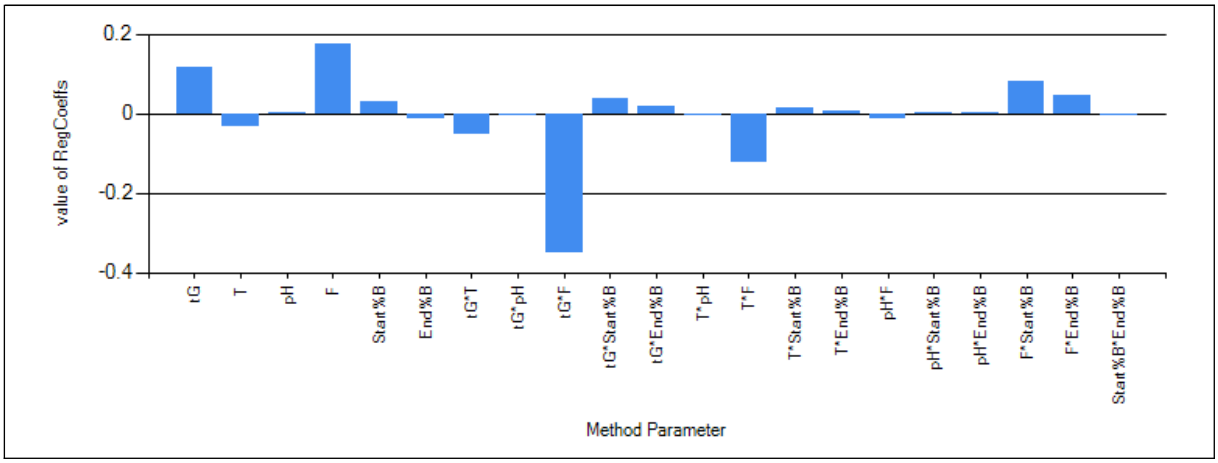
Start-%B	30 ± 1	%B
tG	8.1 ± 1	[min]
End-%B	90 ± 1	%B
Dwell Volume	0.12 ± 0	[mL]

Required Resolution	1.5
Successful Experiments	729
Success Rate	100 %
No of Factors	6
No of Levels	3
No of Experiments	729

Frequency Distribution

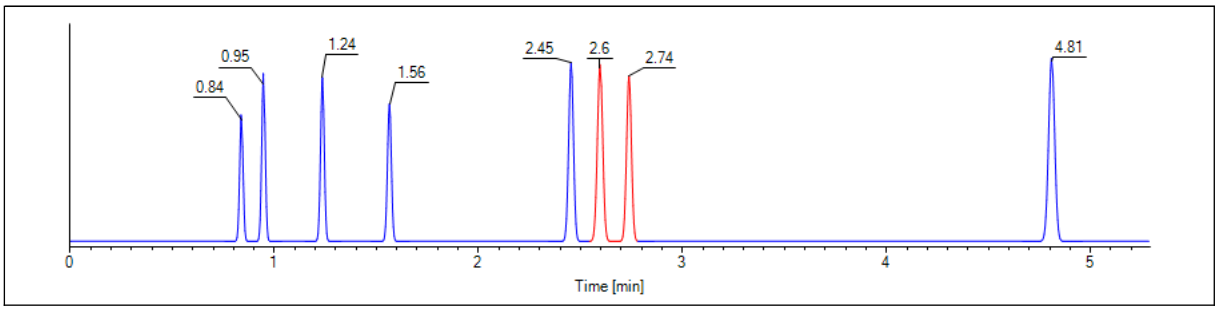


Regression Coefficients



Confirmation Run vs Predicted Run

Predicted Run



Confirmation Run

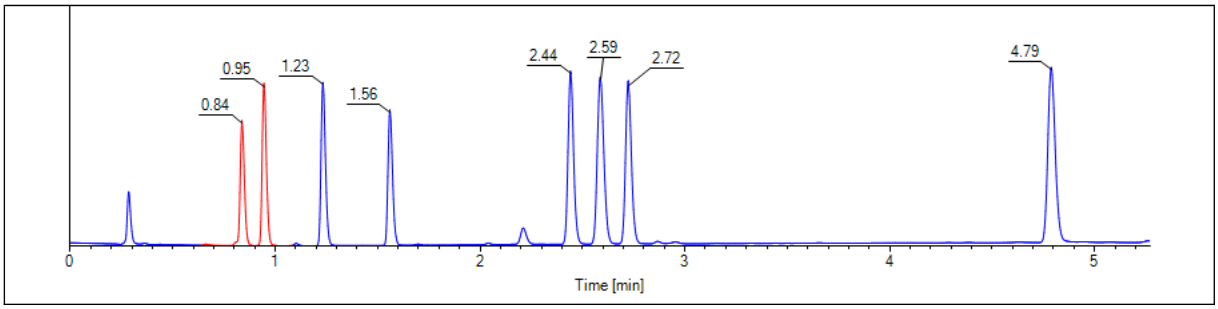
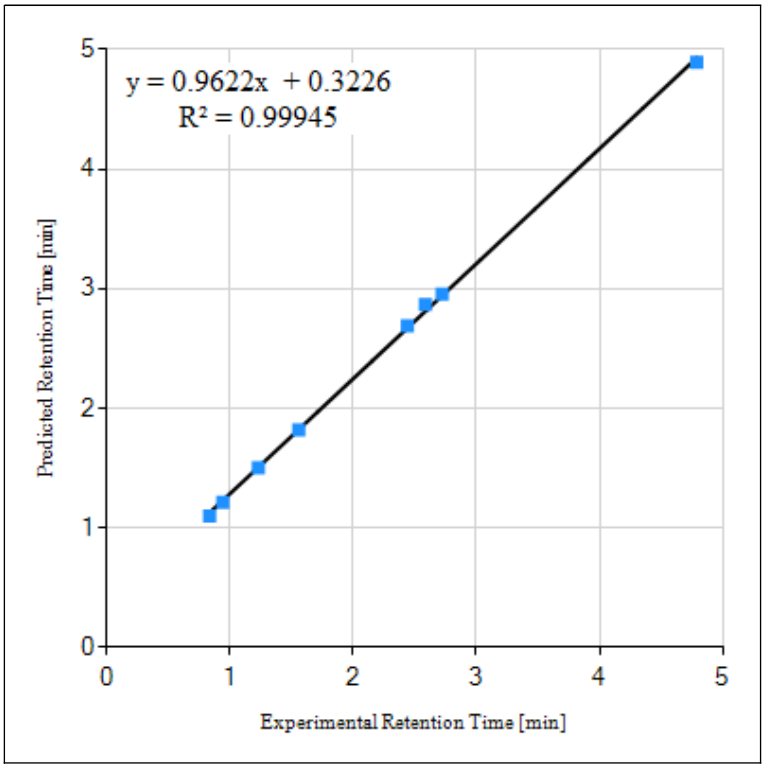


Table Confirmation vs Prediction

#	Name	Experimental tR [min]	Predicted tR [min]	Difference [min]	Error [%]
1	ImpD	0.84	1.10	-0.26	-23.95
2	ImpF	0.95	1.22	-0.27	-22.28
3	Amlodipine	1.23	1.51	-0.27	-18.09
4	ImpE	1.56	1.82	-0.26	-14.34
5	ImpB	2.44	2.69	-0.25	-9.15
6	ImpG	2.59	2.87	-0.28	-9.85
7	ImpH	2.72	2.96	-0.23	-7.82
8	ImpA	4.79	4.90	-0.11	-2.15

Chart Confirmation vs Prediction



Conclusions

In reference to the analytical target profile (ATP) the selected working point shows a separation off all impurities from each other and from the drug substance with more than resolution of 1.5 (2.6). The separation is good enough to guarantee the quantification limit 0.05%.
The new proposed method has a run time minor than 6 min and it is compared to the old method (60) 10 times faster.

References and Comments

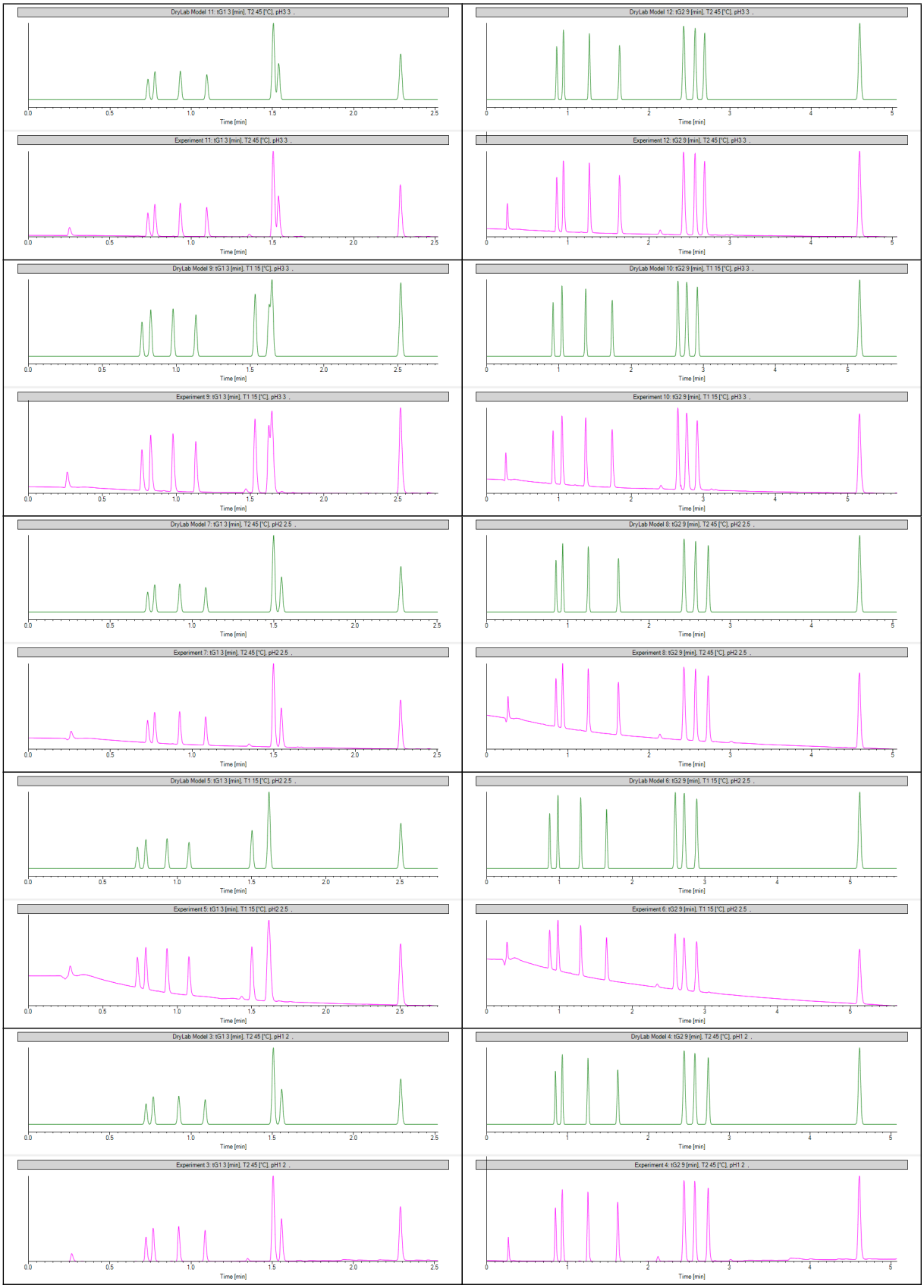
[1] Table 1 in Reference 1.

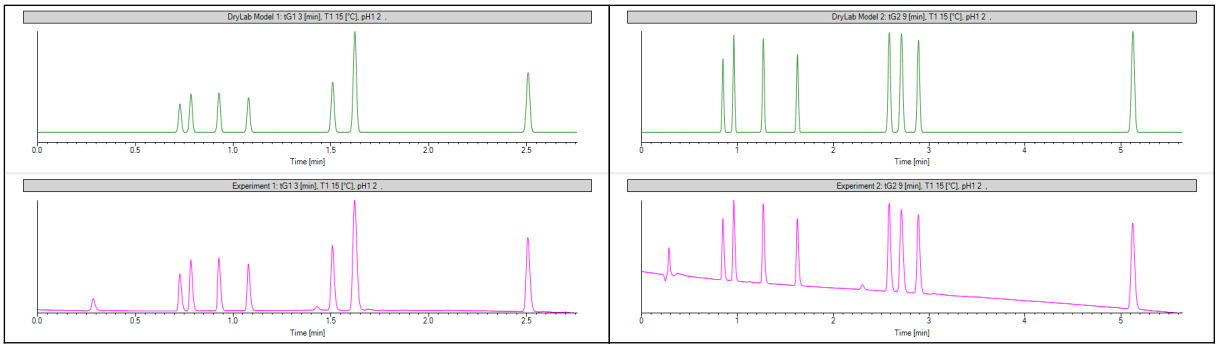
References:
1.- Exploring better column selectivity choices in ultra-high performance liquid chromatography using Quality by Design principles,R. Kormány, I. Molnár, H. J. Rieger /Journal of Pharmaceutical and Biomedical Analysis 80 (2013) 79-88

Model and document created with DryLab® version 4.3.1

Annex

Comparison




DryLab Model 1: tG1 3 [min], T1 15 [°C], pH1 2 .
DryLab Model 2: tG2 9 [min], T1 15 [°C], pH1 2 .
Experiment 1: tG1 3 [min], T1 15 [°C], pH1 2 .
Experiment 2: tG2 9 [min], T1 15 [°C], pH1 2 .
Time [min]