DRYLAB KNOWLEDGE MANAGEMENT DOCUMENT

UPLC Method for Ebastine

by Alexander H. Schmidt, Imre Molnár

SUMMARY

Project Description

Exchange an old EP HPLC method of 160 min analysis time against a better and faster method.

Analytical Target Profile (ATP)

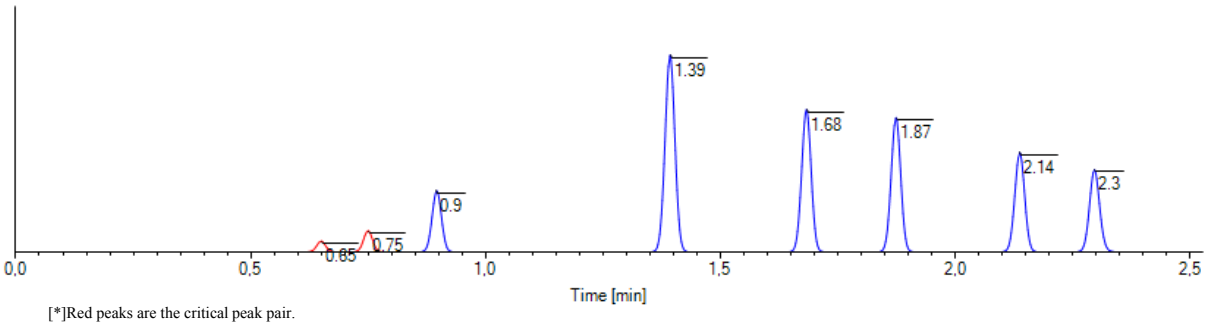
Separate the drug substance from all the impurities with a minimum resolution of 2.0. The limit of the quantification must be less than 0.05% of the drug substance (reporting limit according ICH Q3A).

Proposed Working Point

This working point was found at tG = 3 [min], T = 60 [°C] and tC = 50 [% B2 in B1].

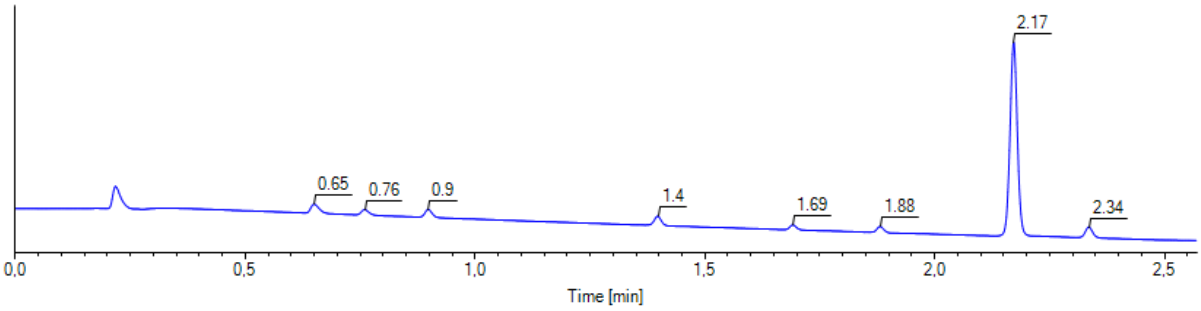
Comparison: Confirmation Run vs Predicted Run

Predicted Run



[*]Red peaks are the critical peak pair.

Confirmation Run



Conclusion

In reference to the analytical target profile (ATP) the selected working point shows a separation of all the impurities from each other and from the drug substance with more than resolution of 2.0 (2.18-3.18). This allows for some aging of the separation column. The separation is good enough to guarantee the quantification limit 0.05%. The new proposed method has a run time of 2.5 min and it is compared to the original EP method (150 min) 60 times faster.

Project Manager: _____
Date: Signature

Approval: _____
Date: Signature

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PROJECT DESCRIPTION

Exchange an old EP HPLC method of 160 min analysis time against a better and faster method.

ANALYTICAL TARGET PROFILE (ATP)

Separate the drug substance from all the impurities with a minimum resolution of 2.0. The limit of the quantification must be less than 0.05% of the drug substance (reporting limit according ICH Q3A).

QUALITY RISK MANAGEMENT (QRM)

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 phase the parameters gradient time tG (2-6 [min]), pH, temperature T (35-70 [°C]) and ternary composition of the eluent B are the most probable potentially critical separation parameters.

The pH 6.2 (10mM acetate buffer) and the separation column were selected based on preliminary experiments.

INITIAL CONDITIONS

Based on preliminary results and quality risk assessment, the stationary phase Acquity UPLC® BEH C18 and

ACN/isopropanol as mobile phase B were selected. The three parameters gradient time tG (2-6 [min]), temperature T (35-70 [°C]) and ternary composition of the eluent B (100% ACN, resp. 70% ACN - 30% isopropanol, resp. 40% ACN - 60% isopropanol) were optimized. The pH 6.2 (10mM acetate buffer) was selected based on previous experiences[1].

Design: LC - RP Gradient/Temperature/Ternary (12 runs)

Project File: Z:\Kunden\Steiner\Ebastin\Ebastin 3D-1.dlproj

min	ºC	%B2 in B1
tG1: 2,00	T1: 35,00	tC1: 0,00
tG2: 6,00	T2: 70,00	tC2: 30,00
		tC3: 60,00

Gradient Range 30 to 90 [%B]

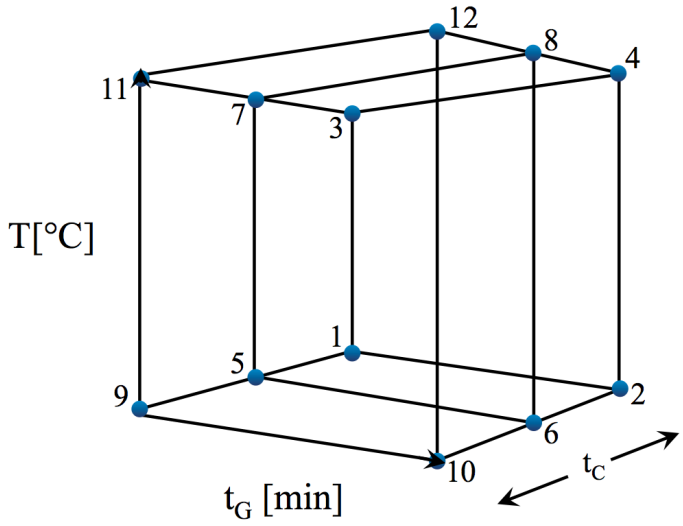
%B = 100% - %A

Column Data	
Name: Acquity UPLC BEH C18	
Particle size [µm]:	1,70
Diameter [cm]:	0,21
Length [cm]:	5,00
to [min]:	0,21
FlowRate [mL/min]:	0,50
Injection Volumen [µL]:	5,00

Instrument Data	
Name: Acquity UPLC H-Class	
Dwell volume [mL]:	0,120
Extracol volume [mL]:	0,004
Time constant [s]:	0,10
Wavelength [nm]:	210

Eluent Data
Eluent A :
1: 100 % Water (H2O)
1: 10 mM acetate
pH : 6,2
Eluent B1 :
1: 100 % Acetonitrile (ACN)
Eluent B2 :
1: 100 % Methanol (MeOH)

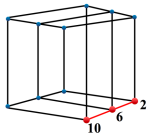
Design of Experiments



AIA-Chromatograms	
Run	File Name
1	1 Acn 2min 35C
2	2 Acn 6min 35C
3	3 Acn 2min 70C
4	4 Acn 6min 70C
5	5 Acn-Iso 7-3 2min 35C
6	Acn-Iso 7-3 6min 35C_cdf(AD1-Ch1)
7	7 Acn-Iso 7-3 2min 70C
8	8 Acn-Iso 7-3 6min 70C
9	9 Acn-Iso 4-6 2min 35C
10	10 Acn-Iso 4-6 6min 35C
11	11 Acn-Iso 4-6 2min 70C
12	12 Acn-Iso 4-6 6min 70C

DOCUMENTATION OF THE INITIAL EXPERIMENTS

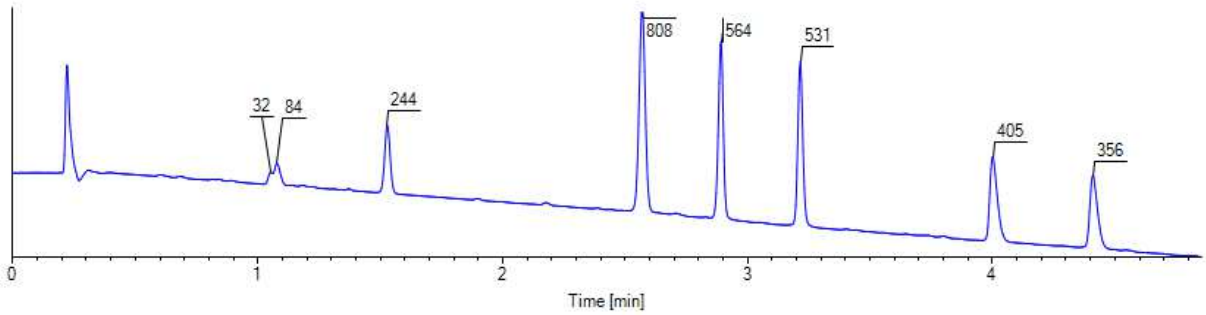
Reference runs: tC (0,00, 30,00, 60,00[%B2 in B1])



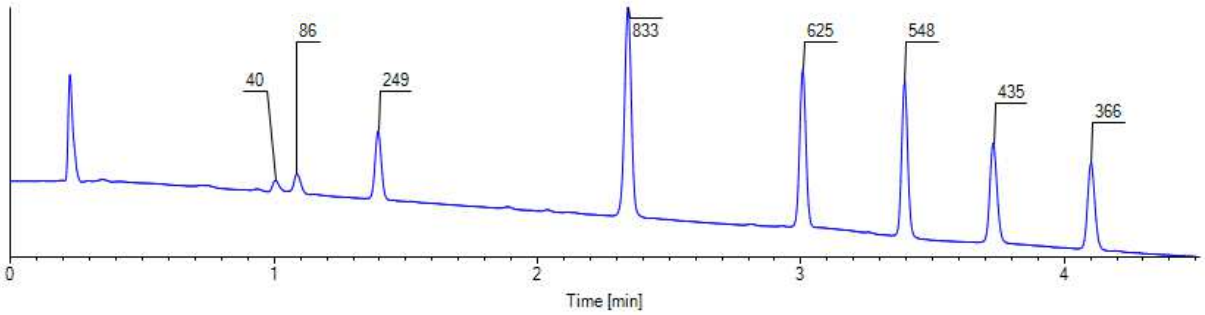
Run	tG[min]	T[ºC]	tC[%B2 in B1]
2	6,00	35,00	0,00
6	6,00	35,00	30,00
10	6,00	35,00	60,00

Chromatograms of the Reference Runs

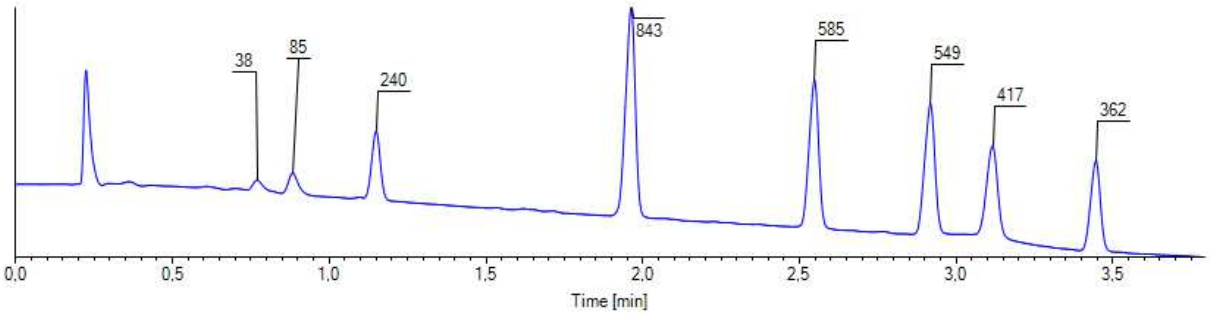
Run 2



Run 6



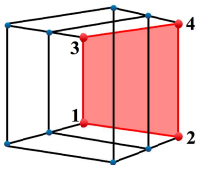
Run 10



Peak Tracking of the Reference Runs

			Run 2		Run 6		Run 10	
#	Name	SDev	tR [min]	Area	tR [min]	Area	tR [min]	Area
1	Imp. C	9,28	1,05	32	1,01	40	0,77	38
2	Imp.D	0,94	1,08	84	1,09	86	0,88	85
3	Imp.A	1,58	1,53	244	1,39	249	1,15	240
4	Imp.F	1,77	2,57	808	2,34	833	1,96	843
5	Imp.B	4,26	2,89	564	3,01	625	2,55	585
6	Imp.G	1,56	3,22	531	3,39	548	2,92	549
7	Ebastin	2,88	4,00	405	3,73	435	3,12	417
8	Imp.E	1,14	4,41	356	4,10	366	3,45	362
	Σ Areas	2,08		3025		3183		3119

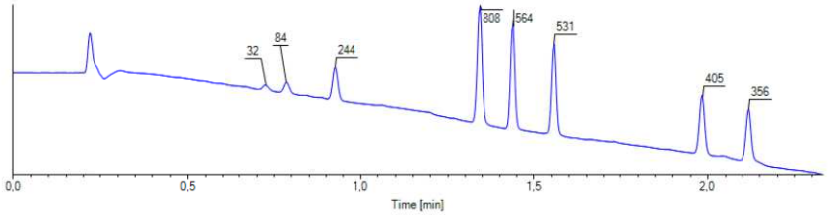
tG-T-Plane 1 (tC 1 0,00[%B2 in B1])



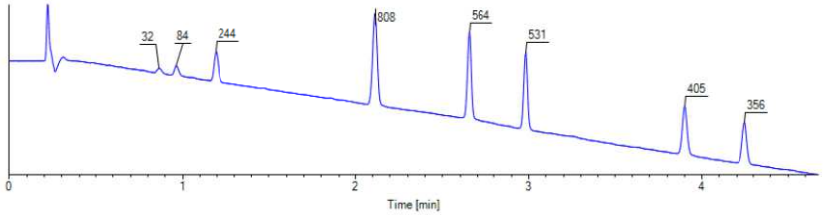
Run	tG[min]	T[ºC]	tC[%B2 in B1]
1	2,00	35,00	0,00
2	6,00	35,00	0,00
3	2,00	70,00	0,00
4	6,00	70,00	0,00

Chromatograms of tG-T-Plane 1

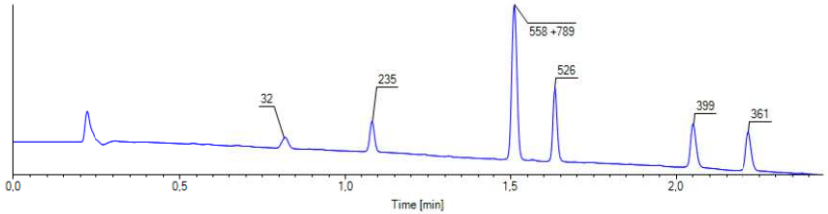
Run 3



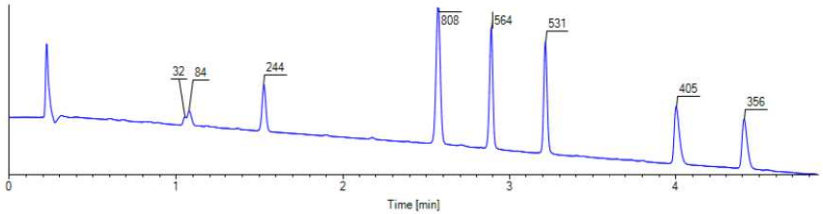
Run 4



Run 1



Run 2



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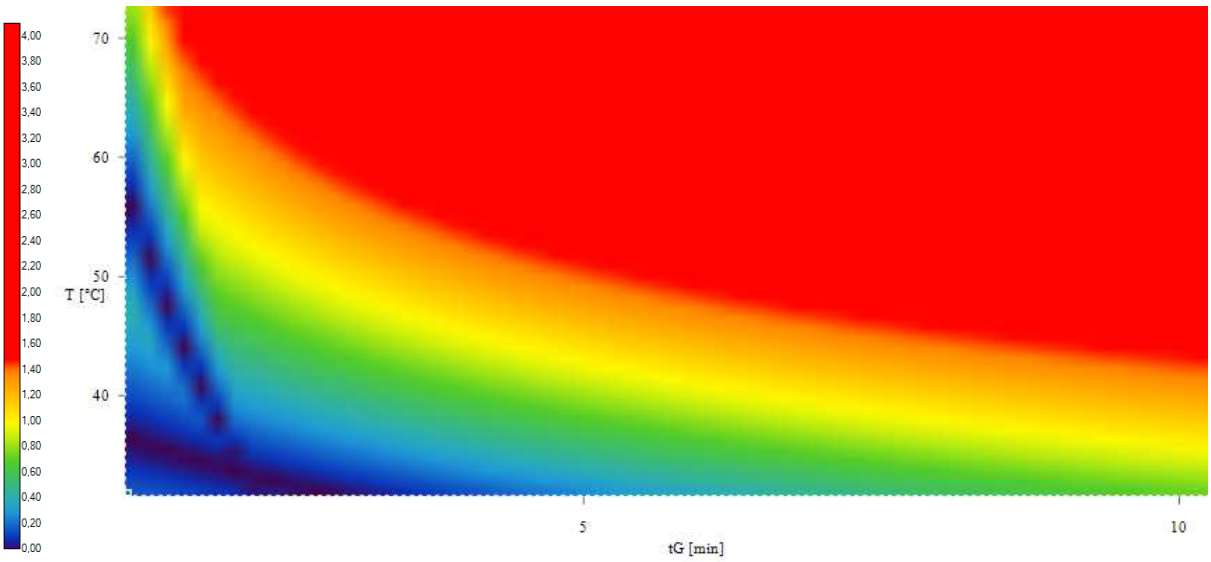
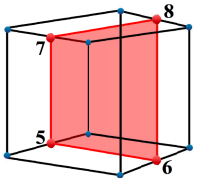
Peak Tracking of the tG-T-Plane 1

			Run 1		Run 2		Run 3		Run 4	
#	Name	SDev	tR [min]	Area	tR [min]	Area	tR [min]	Area	tR [min]	Area
1	Imp. C	0,02	0,82	32	1,05	32	0,73	32	0,86	32
2	Imp.D	6,35	0,82	72	1,08	84	0,79	84	0,96	84
3	Imp.A	1,55	1,08	235	1,53	244	0,93	244	1,19	244
4	Imp.F	1,03	1,51	789	2,57	808	1,34	808	2,11	808
5	Imp.B	0,47	1,51	558	2,89	564	1,44	564	2,65	564
6	Imp.G	0,44	1,63	526	3,22	531	1,56	531	2,98	531
7	Ebastin	0,63	2,05	399	4,00	405	1,98	405	3,90	405
8	Imp.E	0,67	2,22	361	4,41	356	2,12	356	4,25	356
	Σ Areas	0,74		2973		3025		3024		3024

Resolution Map for tG-T-Plane 1 (tC 0,00[%B2 in B1])



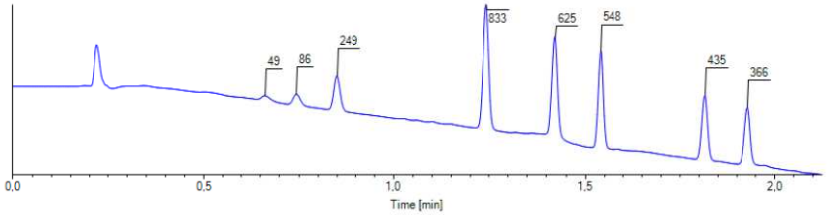
tG-T-Plane 2 (tC 2 30,00[%B2 in B1])



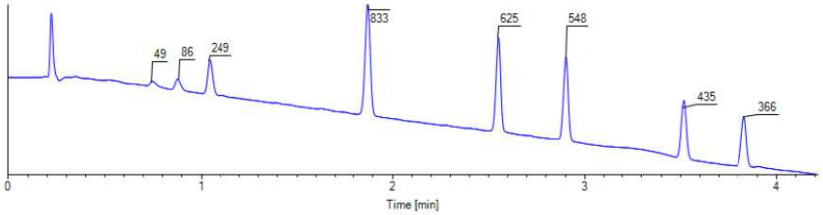
Run	tG[min]	T[ºC]	tC[%B2 in B1]
5	2,00	35,00	30,00
6	6,00	35,00	30,00
7	2,00	70,00	30,00
8	6,00	70,00	30,00

Chromatograms of the tG-T-Plane 2

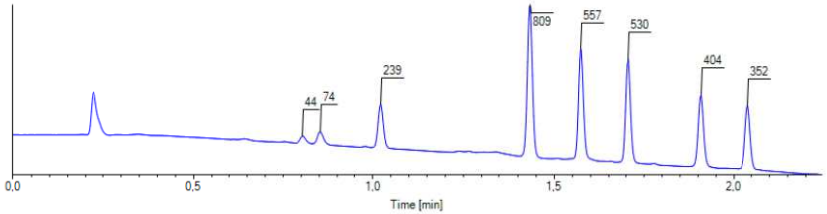
Run 7



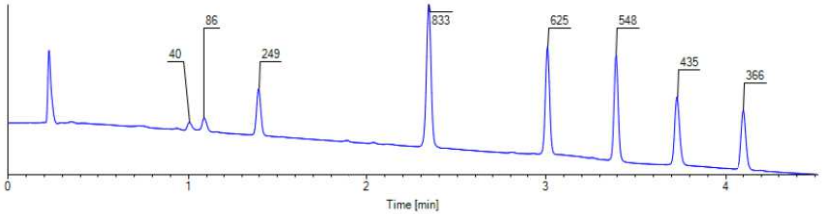
Run 8



Run 5



Run 6



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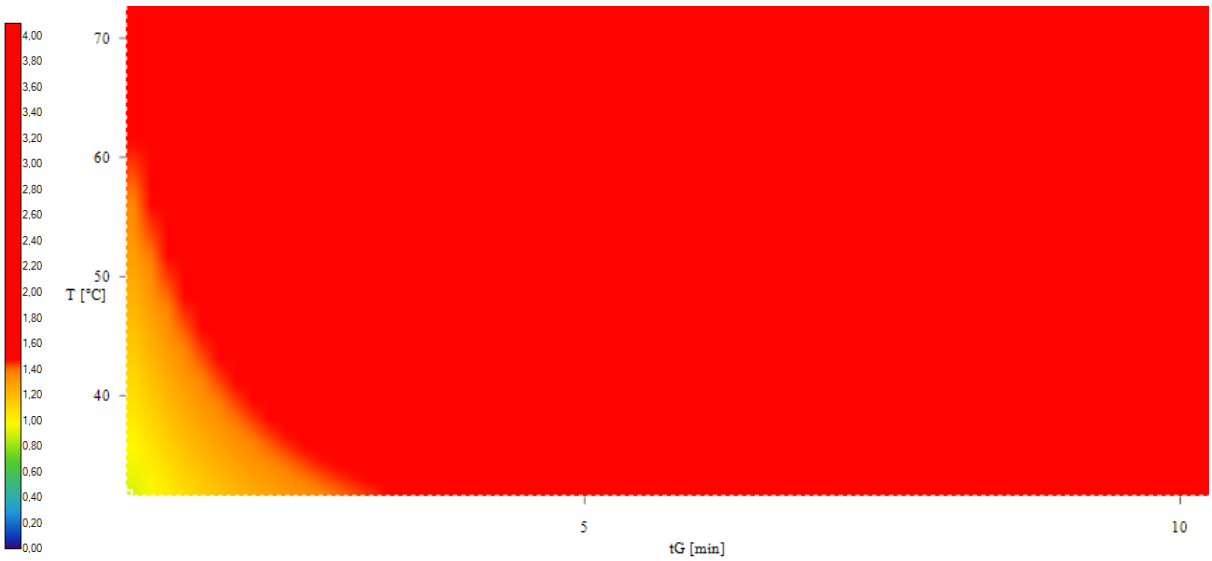
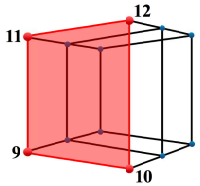
Peak Tracking of the tG-T-Plane 2

			Run 5		Run 6		Run 7		Run 8	
#	Name	SDev	tR [min]	Area	tR [min]	Area	tR [min]	Area	tR [min]	Area
1	Imp. C	8,30	0,80	44	1,01	40	0,66	49	0,74	49
2	Imp.D	6,31	0,85	74	1,09	86	0,74	86	0,88	86
3	Imp.A	1,69	1,02	239	1,39	249	0,85	249	1,04	249
4	Imp.F	1,28	1,43	809	2,34	833	1,24	833	1,87	833
5	Imp.B	4,85	1,57	557	3,01	625	1,42	625	2,55	625
6	Imp.G	1,47	1,71	530	3,39	548	1,54	548	2,90	548
7	Ebastin	3,11	1,91	404	3,73	435	1,82	435	3,51	435
8	Imp.E	1,67	2,04	352	4,10	366	1,93	366	3,83	366
	Σ Areas	2,47		3009		3183		3191		3191

Resolution Map for tG-T-Plane 2 (tC 30,00[%B2 in B1])



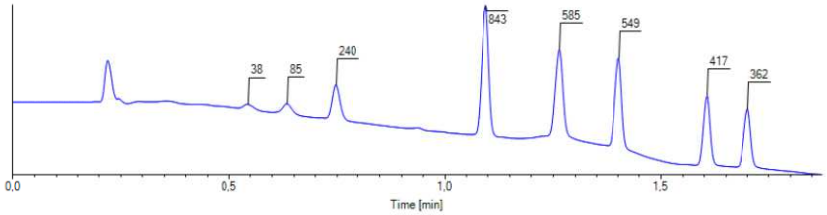
tG-T-Plane 3 (tC 3 60,00[%B2 in B1])



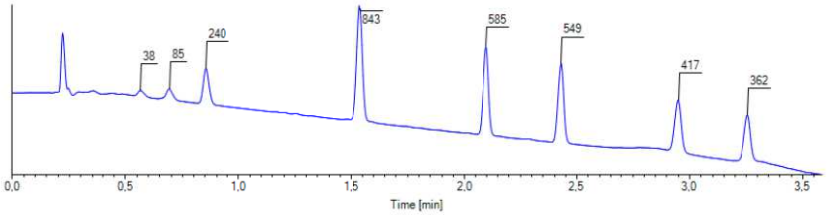
Run	tG[min]	T[ºC]	tC[%B2 in B1]
9	2,00	35,00	60,00
10	6,00	35,00	60,00
11	2,00	70,00	60,00
12	6,00	70,00	60,00

Chromatograms of the tG-T-Plane 3

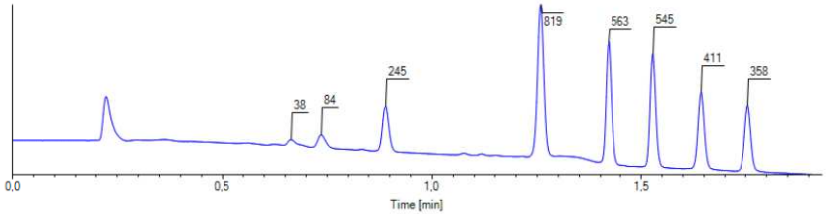
Run 11



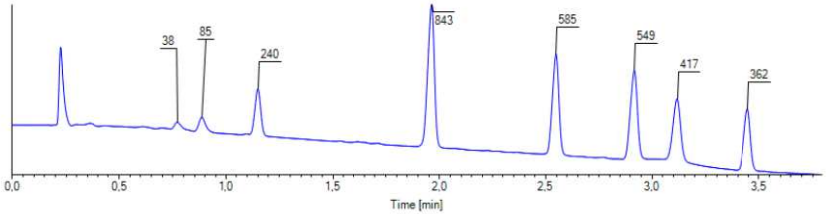
Run 12



Run 9



Run 10



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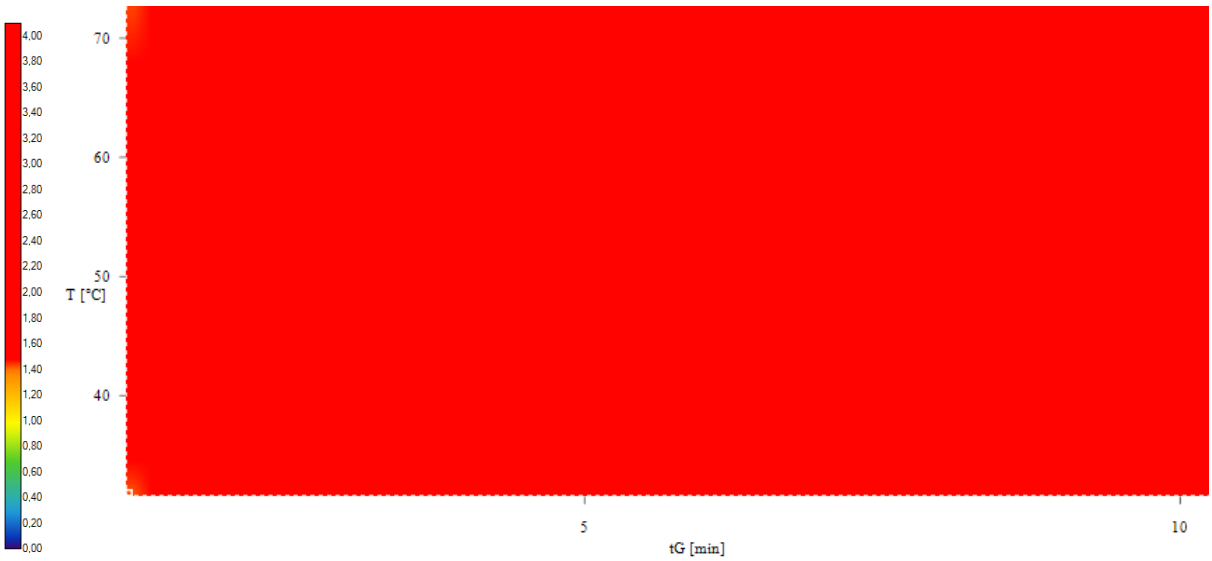
Peak Tracking of the tG-T-Plane 3

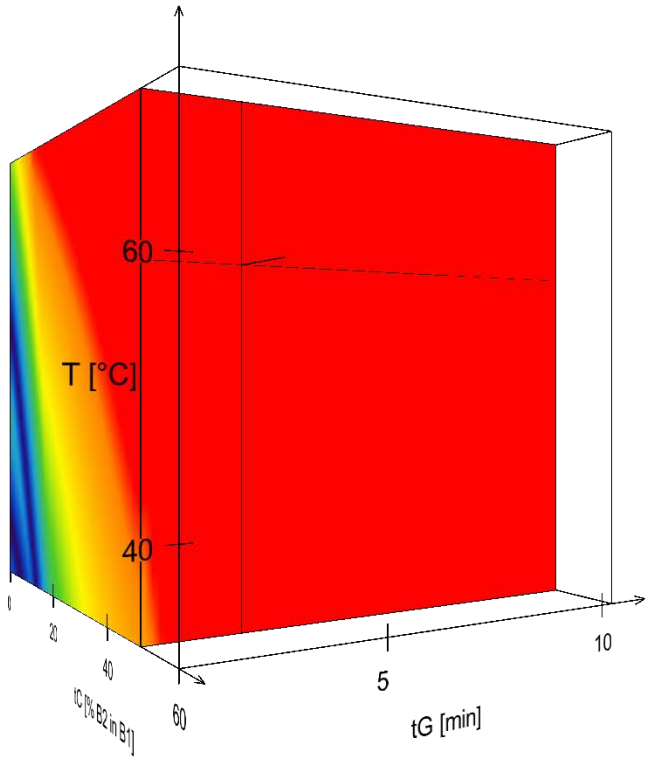
			Run 9		Run 10		Run 11		Run 12	
#	Name	SDev	tR [min]	Area	tR [min]	Area	tR [min]	Area	tR [min]	Area
1	Imp. C	0,50	0,66	38	0,77	38	0,54	38	0,57	38
2	Imp.D	0,70	0,74	84	0,88	85	0,63	85	0,69	85
3	Imp.A	0,96	0,89	245	1,15	240	0,75	240	0,85	240
4	Imp.F	1,22	1,26	819	1,96	843	1,09	843	1,54	843
5	Imp.B	1,63	1,42	563	2,55	585	1,26	585	2,10	585
6	Imp.G	0,35	1,53	545	2,92	549	1,40	549	2,43	549
7	Ebastin	0,63	1,64	411	3,12	417	1,61	417	2,95	417
8	Imp.E	0,52	1,75	358	3,45	362	1,70	362	3,26	362
	Σ Areas	0,79		3062		3119		3119		3119

Resolution Map for tG-T-Plane 3 (tC 60,00[%B2 in B1])



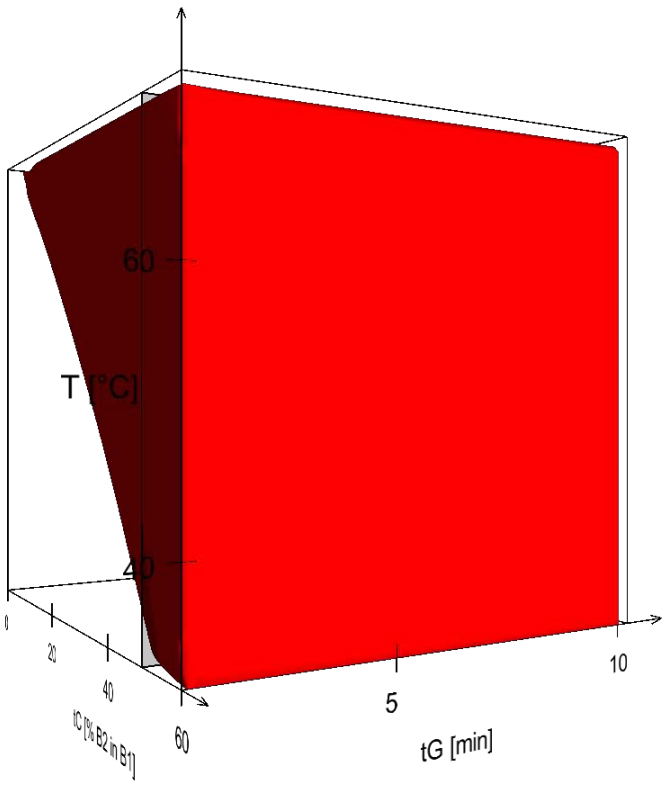
3D EXPERIMENTAL SPACE

Max Crit. Resolution: 4,1



Method Operation Design Region (MODR)

Critical Resolution Level: 1,5



SELECTED WORKING POINT (SET POINT)

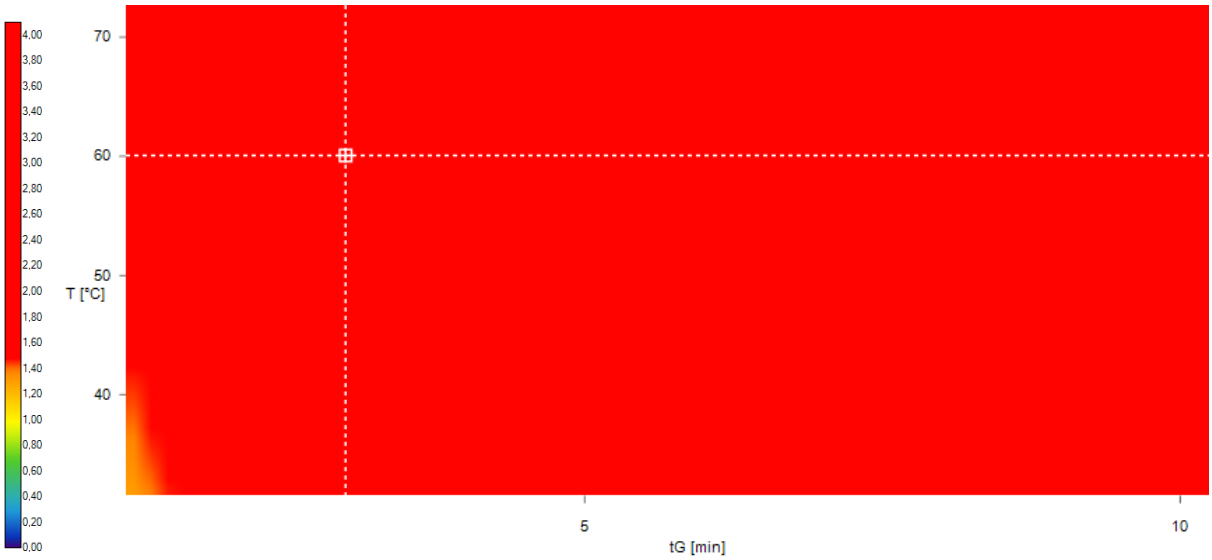
From the previously constructed Resolution maps, the working point was selected by visual examination looking for a high critical resolution (Rs,crit), good robustness and fast separation of the method. At this point small changes of the critical parameters gradient time,temperature and ternary composition of the eluent B as well as flow rate, gradient slope and shape, column dimensions and dwell volume have no negative influence on the separation of all peaks.This working point was found in the cube at tG = 3.0 min, T = 60 °C and tC = acetonitrile/2-propanol (50:50, v/v) .

Status	
3,00	tG [min]
60,00	T [°C]
50,00	tC [% B2 in B1]

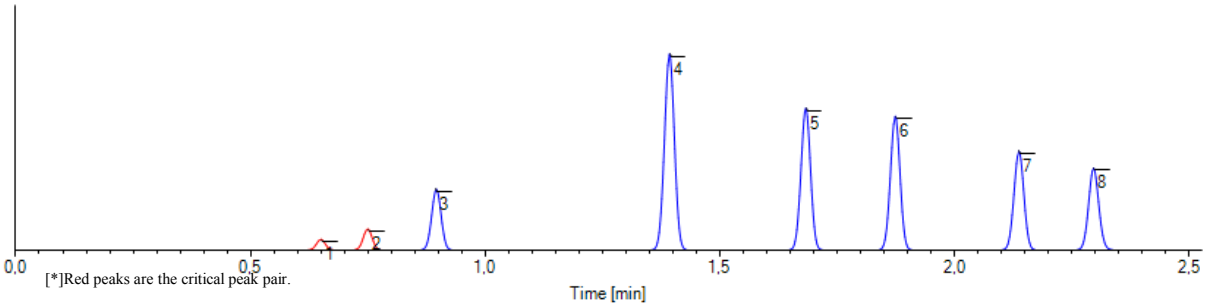
Gradient Table		
Time [min]	%B	Rate [%B/min]
0,00	30,00	
3,00	90,00	20,00

Pressure [psi]:	3303
Plate Number:	5158 (calculated)
Rs,crit:	2,73
Crit. Peak Pair:	1, 2
Run Time [min]:	3,00
Eluent Used [mL]:	1,50

Resolution Map for the Selected Working Point



Chromatogram at the Selected Working Point



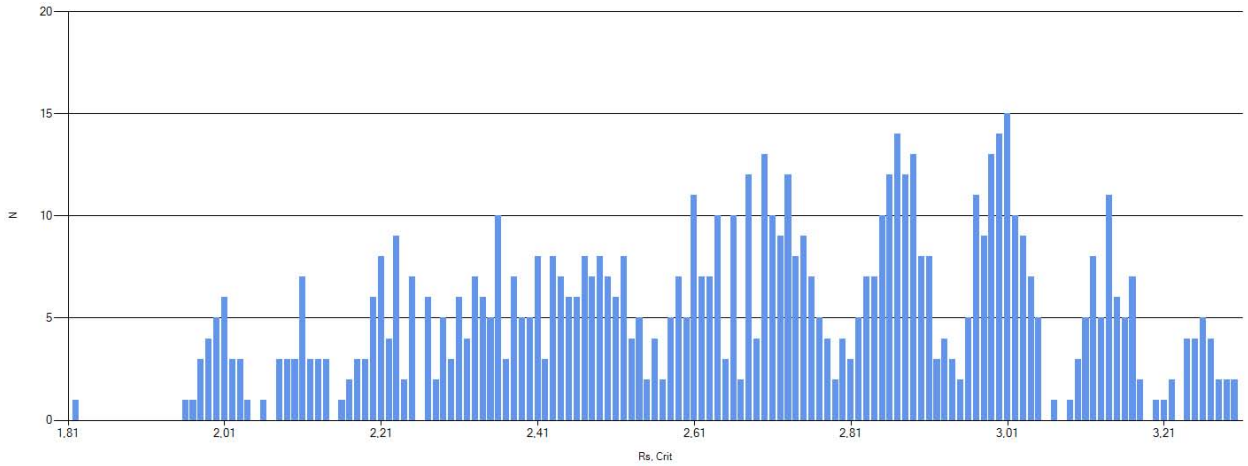
[*]Red peaks are the critical peak pair.

METHOD ROBUSTNESS CALCULATION

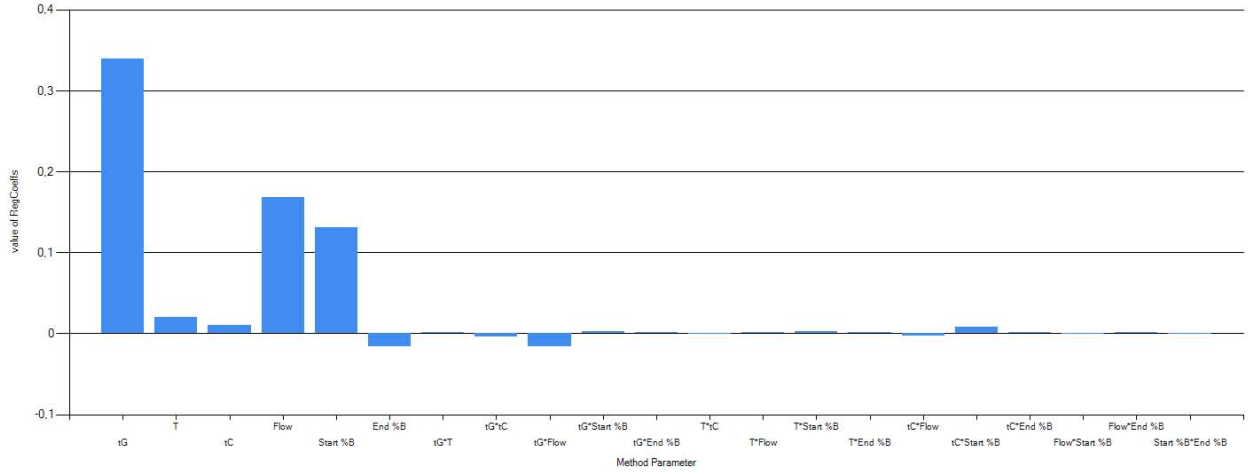
tG	3	+/-	1	[min]	Flow Rate	0.5	+/-	0.1	[mL/min]
T	60	+/-	1	[°C]	Start %B	30	+/-	1	[%B]
tC	50	+/-	1	[% B2 in B1]	End %B	90	+/-	1	[%B]

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



1.-Frequency distribution:

Out of the 729 models no result was having less than Rs,crit of 2.18. This is good value to guarantee an adequate robustness.

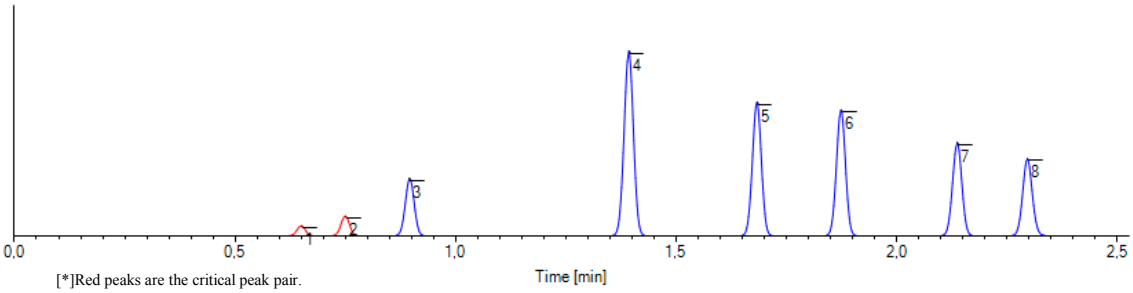
2.-Regression Coefficients:

From the selected set of potentially critical separation parameters as selected from the quality risk management evaluation, the parameters tG, Flowrate and Start-%B have a significant and positive influence on the critical resolution. The influence of End-%B is small and slightly negative, but it is probably not a significant factor.

As a consequence, the parameters tG, Flow and Start %B are confirmed to be critical separation parameters.

The flowrate tolerance of 0.1 mL/min is very genereous, as the manufacturers garantee at least 0.02 mL/min accuracy with a 0.5 mL/min flowrate. The seals of the pumps should be replaced every 6-12 months.

PREDICTED CHROMATOGRAM

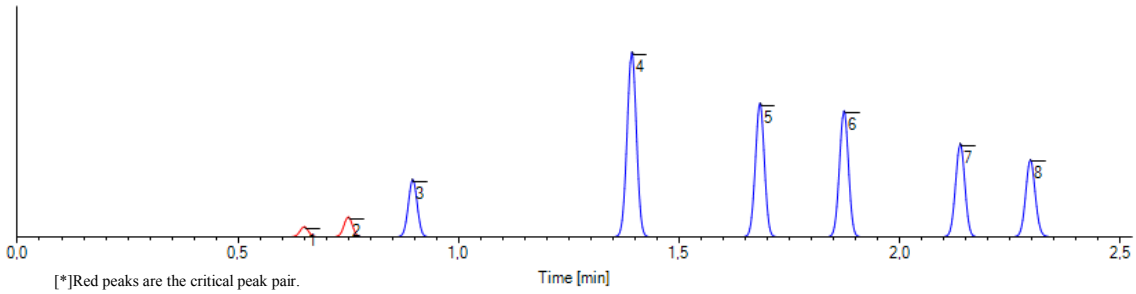


[*]Red peaks are the critical peak pair.

Result Table						
#	Name	tR [min]	Area	Avg. k	Width	Resolution
1	Imp. C	0,65	40,65	2,29	0,04	2,73
2	Imp.D	0,75	81,19	2,69	0,04	3,90
3	Imp.A	0,90	243,40	3,43	0,04	12,71
4	Imp.B	1,39	822,63	4,34	0,04	7,41
5	Imp.F	1,68	571,94	3,57	0,04	4,95
6	Imp.G	1,87	541,72	3,64	0,04	6,72
7	Ebastin	2,14	412,90	4,26	0,04	4,00
8	Imp.E	2,30	357,14	4,21	0,04	

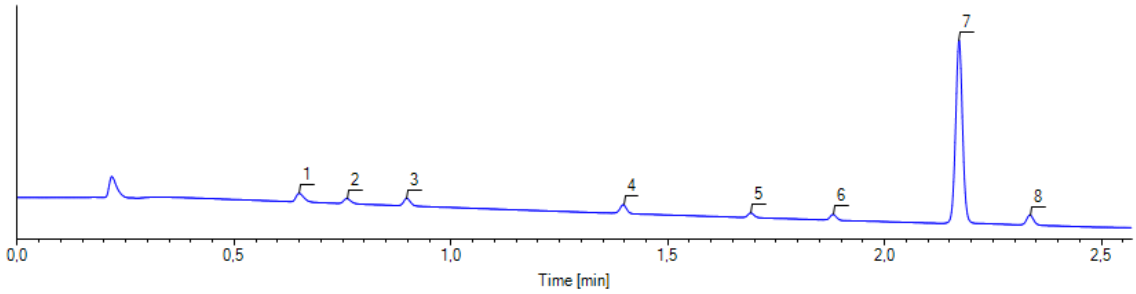
COMPARISON: CONFIRMATION RUN VS PREDICTED RUN

Predicted Run



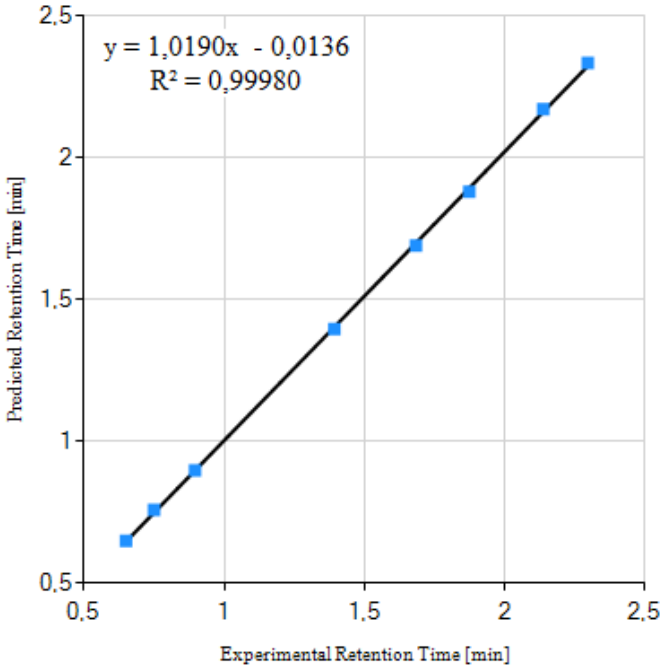
[*]Red peaks are the critical peak pair.

Confirmation Run

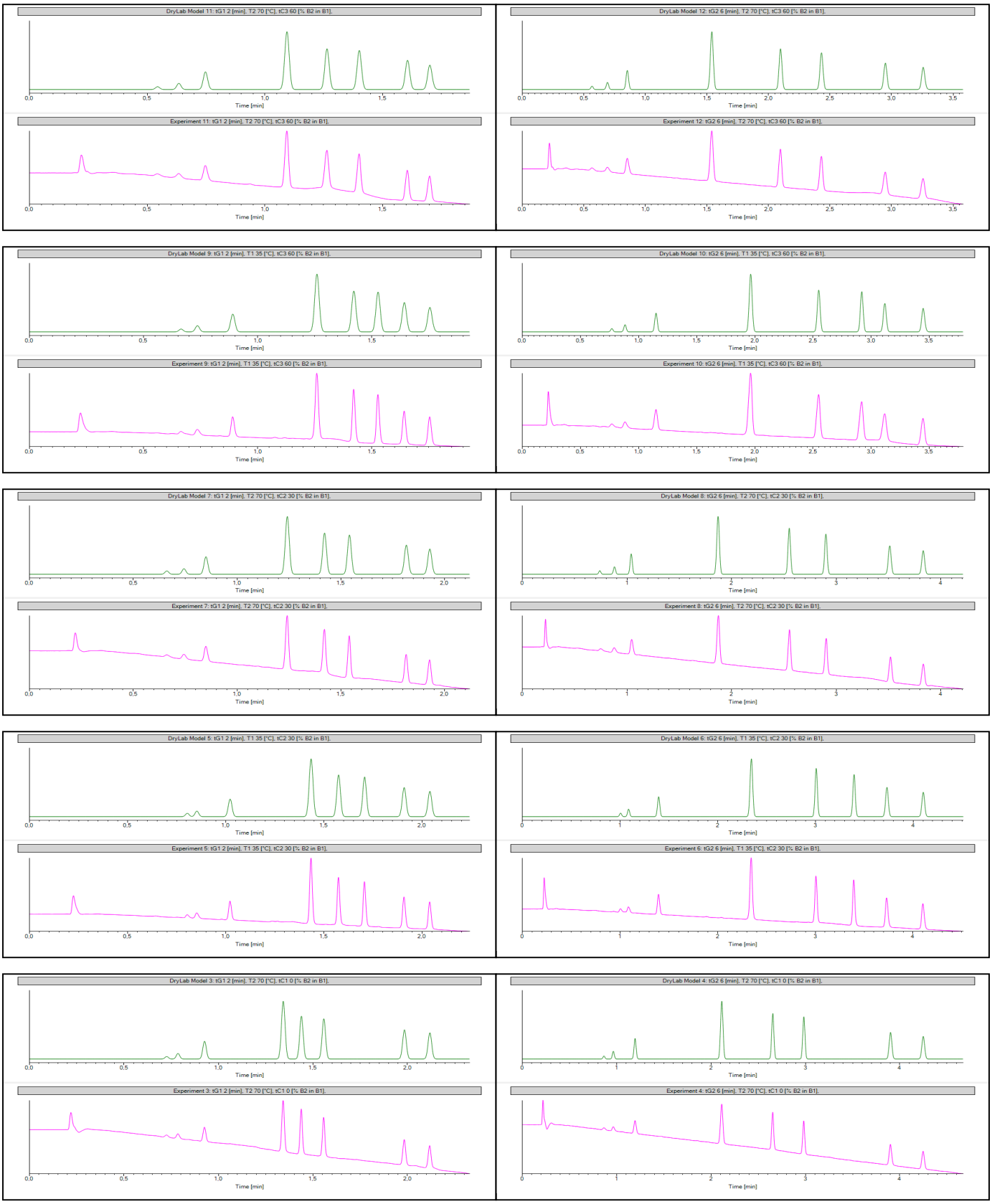


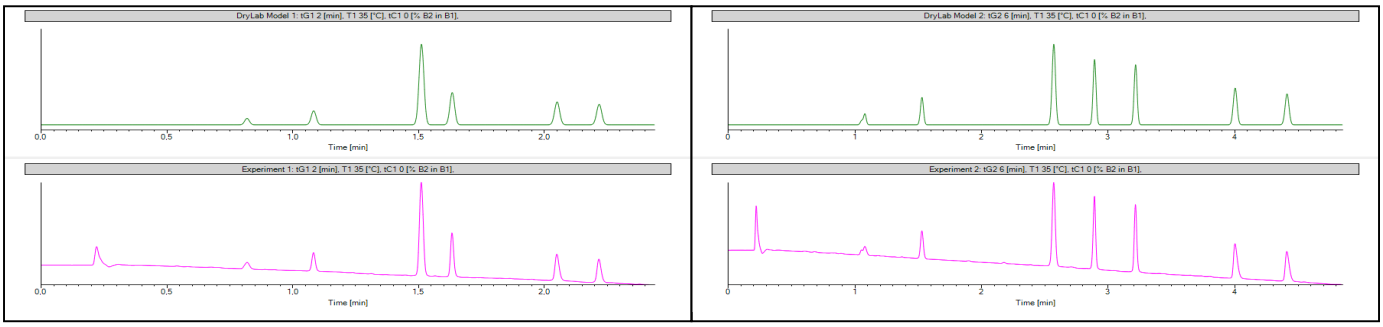
Confirmation Run filename: working point.cdf

#	Name	Experimental tR [min]	Predicted tR [min]	Difference [min]	Error [%]
1	Imp. C	0,65	0,65	0,00	0,04
2	Imp.D	0,76	0,75	0,01	1,39
3	Imp.A	0,90	0,90	0,00	0,28
4	Imp.B	1,40	1,39	0,01	0,38
5	Imp.F	1,69	1,68	0,01	0,55
6	Imp.G	1,88	1,87	0,01	0,47
7	Ebastin	2,17	2,14	0,04	1,68
8	Imp.E	2,34	2,30	0,04	1,76



COMPARISON


DryLab Model 11: tG1 2 [min], T2 70 [°C], tC3 60 [% B2 in B1],
Experiment 11: tG1 2 [min], T2 70 [°C], tC3 60 [% B2 in B1],
DryLab Model 12: tG2 6 [min], T2 70 [°C], tC3 60 [% B2 in B1],
Experiment 12: tG2 6 [min], T2 70 [°C], tC3 60 [% B2 in B1],
DryLab Model 9: tG1 2 [min], T1 35 [°C], tC3 60 [% B2 in B1],
Experiment 9: tG1 2 [min], T1 35 [°C], tC3 60 [% B2 in B1],
DryLab Model 10: tG2 6 [min], T1 35 [°C], tC3 60 [% B2 in B1],
Experiment 10: tG2 6 [min], T1 35 [°C], tC3 60 [% B2 in B1],
DryLab Model 7: tG1 2 [min], T2 70 [°C], tC2 30 [% B2 in B1],
Experiment 7: tG1 2 [min], T2 70 [°C], tC2 30 [% B2 in B1],
DryLab Model 8: tG2 6 [min], T2 70 [°C], tC2 30 [% B2 in B1],
Experiment 8: tG2 6 [min], T2 70 [°C], tC2 30 [% B2 in B1],
DryLab Model 5: tG1 2 [min], T1 35 [°C], tC2 30 [% B2 in B1],
Experiment 5: tG1 2 [min], T1 35 [°C], tC2 30 [% B2 in B1],
DryLab Model 6: tG2 6 [min], T1 35 [°C], tC2 30 [% B2 in B1],
Experiment 6: tG2 6 [min], T1 35 [°C], tC2 30 [% B2 in B1],
DryLab Model 3: tG1 2 [min], T2 70 [°C], tC1 0 [% B2 in B1],
Experiment 3: tG1 2 [min], T2 70 [°C], tC1 0 [% B2 in B1],
DryLab Model 4: tG2 6 [min], T2 70 [°C], tC1 0 [% B2 in B1],
Experiment 4: tG2 6 [min], T2 70 [°C], tC1 0 [% B2 in B1],
Time [min]


DryLab Model 1: tG1 2 [min], T1 35 [°C], tC1 0 [% B2 in B1],
0,0
0,5
1,0
1,5
2,0
Time [min]
DryLab Model 2: tG2 6 [min], T1 35 [°C], tC1 0 [% B2 in B1],
0
1
2
3
4
Time [min]
Experiment 1: tG1 2 [min], T1 35 [°C], tC1 0 [% B2 in B1],
0,0
0,5
1,0
1,5
2,0
Time [min]
Experiment 2: tG2 6 [min], T1 35 [°C], tC1 0 [% B2 in B1],
0
1
2
3
4
Time [min]

ANALYTICAL METHOD CONTROL STRATEGY

Based on the knowledge and understanding of the separation gained during DoE experiments and QRM a simple control strategy based on a System Suitability Test (SST) is proposed. The SST should test for the critical separation of impurities 1 and 2 with the acceptance criteria of not less than 1.5. The SST should also test for the LOQ for the impurities with the acceptance criteria of the S/N of one of the critical impurities at the level of the reporting limit according ICH Q3A of not less than 10.

The critical separation parameters tG, T, tC, Flow and Start %B are all controlled by the instrument calibration program and their influence to separation is indirectly tested with the SST.

CONCLUSION

In reference to the analytical target profile (ATP) the selected working point shows a separation of all the impurities from each other and from the drug substance with more than resolution of 2.0 (2.18-3.18). This allows for some aging of the separation column. The separation is good enough to guarantee the quantification limit 0.05%. The new proposed method has a run time of 2.5 min and it is compared to the original EP method (150 min) 60 times faster.

REFERENCES

[1] Figure 7 in Reference 1.

References:

1.- Using an innovative Quality-by-Design approach for development of a stability indicating UHPLC method for ebastine in the API and pharmaceutical formulations, A.H. Schmidt, I. Molnár / Journal of Pharmaceutical and Biomedical Analysis 78– 79 (2013) 65– 74

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