Supporting Information

Replacing DMF in solid-phase peptide synthesis: Varying the composition of green binary solvent mixtures as a tool to mitigate common side-reactions

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1. General information

All reagents and solvents were obtained from standard suppliers of raw materials for peptide synthesis and were used as received. All reactions were carried out under ambient temperature.

1.1 HPLC, UPLC, and LC-MS instruments and methods

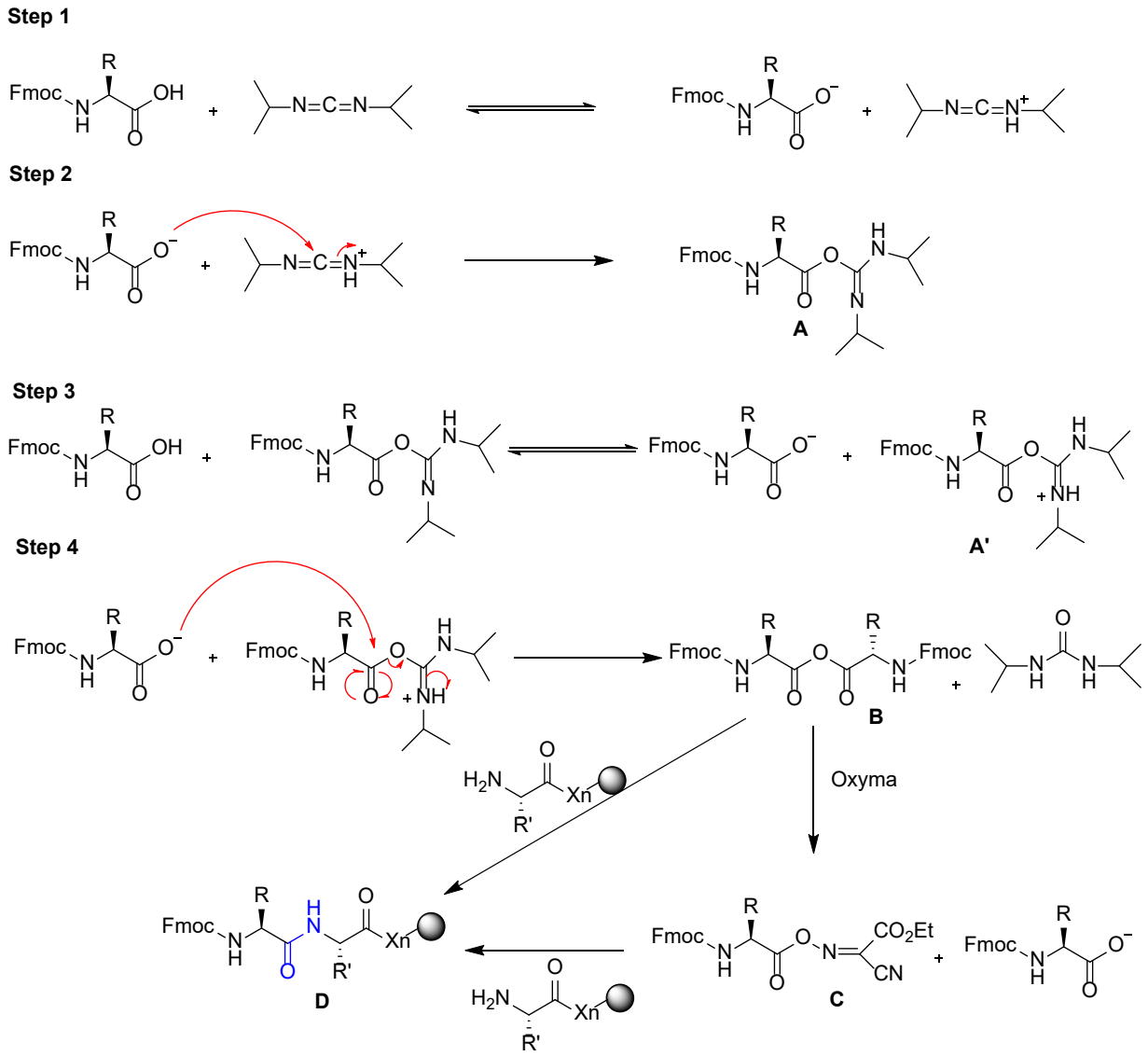
Analytical HPLC was performed on Thermo Scientific™ Vanquish™ UHPLC system using AQUITY UPLC BEH 130 C18 (1.7 µm, 2.1 × 150 mm) column, with a flow rate of 0.4 mL/min and UV detection at 220 nm. The MS analysis was performed on UHPLC coupled with Bruker maXis II™ spectrometer with ultra-high resolution QTOF technology equipped with electron-transfer dissociation (ETD) capabilities. Buffer A: 0.05% TFA in MeCN/H_2O (1:99), v/v/v and buffer B: 0.05% TFA in MeCN, v/v. **Method A**: 0%-100% B in 30 min. **Method B**: 30%-100% B in 20 min. **Method C**: 5%-30% B in 12 min.

Analytical HPLC was performed on Waters Acquity UPLC H-Class using Waters Column Acquity BEH UPLC C18 1.7 µm, 2.1 × 50 mm with a flowrate of 0.5 mL/min and UV detection at 214 nm and 280 nm. Buffer A: 0.1% TFA in MeCN/H_2O (10:90), v/v/v Buffer B: 0.1% TFA in MeCN/H_2O (90:10), v/v/v. **Method D**: 0-100% B in 6 min. **Method E**: 5-100% B in 7.5 min. **Method F**: 0-85% B in 7.5 min.

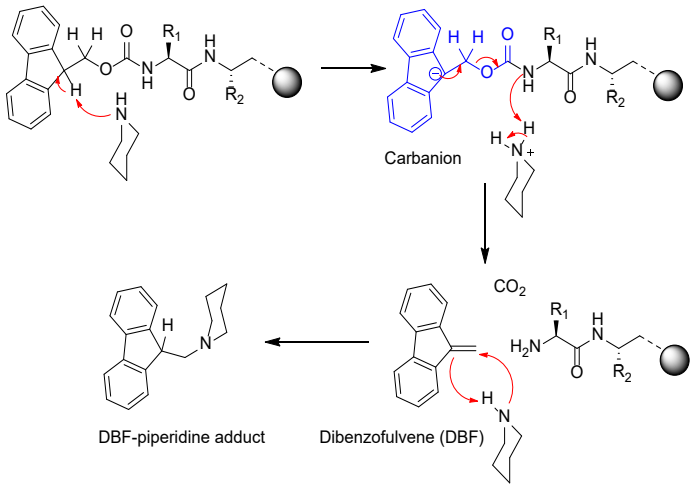
Analytical HPLC was performed on Waters Acquity UPLC H-Class using Waters Column Acquity BEH UPLC C18 1.7 µm, 2.1 × 50 mm with a flowrate of 0.5 mL/min and UV detection at 214 nm. Buffer A: 0.1% TFA in H_2O, v/v/v. Buffer B: 0.1% TFA in MeCN/H_2O (90:10), v/v/v. **Method G**: 0-12.5% in 4 min and then isocratic for 15 min.

Analytical HPLC was performed on Thermo Scientific™ Scientific Ultimate UHPLC system using Kinetex UPLC column C18 100Å 1.7 µm, 150 × 2.1 mm, with a flow rate of 0.5 mL/min and UV detection at 210 nm. The MS analysis was performed on MSQ Plus Mass spectrometer, operating in positive mode ESI. Buffer A: 0.1% HCOOH in MeCN/H_2O (10:90), v/v/v. Buffer B: 0.1% HCOOH in MeCN/H_2O (90:10), v/v/v. **Method H**: 0-60% B in 12 min followed by 60-100% B in 2 min.

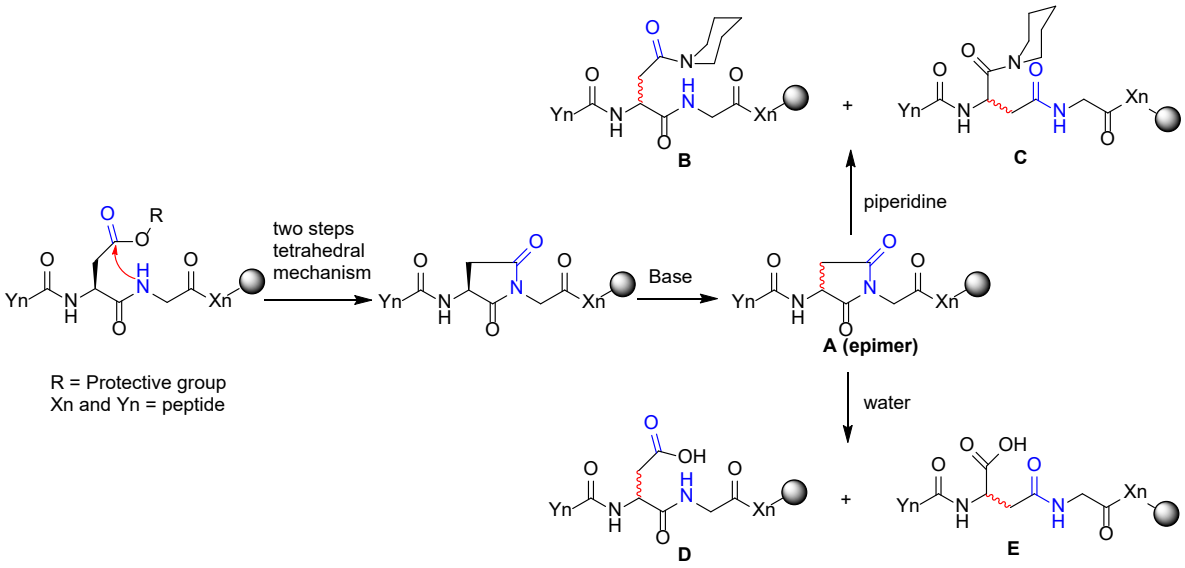
2. Supplementary Figures – Reaction Mechanisms



Supplementary Figure S1. Plausible reaction mechanism of DIC/Oxyma-mediated amide bond formation in SPPS



Supplementary Figure S2. Plausible mechanism (E1cB) of piperidine mediated Fmoc-removal



Supplementary Figure S3. Aspartimide formation and the subsequent epimerisation, *α*-*β*-peptide isomerisation, and *α*-*β*-piperidide formation during SPPS

3. Experimental methods and results

3.1 Amino acid stability

A solution of Fmoc-amino acids containing Oxyma Pure (0.3 M each) was kept sealed for 14 days, after which 10 µL was extracted, diluted with 1 mL MeCN/H_2O/TFA (50/50/0.1, v/v/v) and analysed by HPLC (Method D) at 214 nm. The results were divided into three categories, no apparent change (>99% remaining Fmoc-amino acid), minor degradation (95-99%) and significant degradation (<95%) (Supplementary Table S1). The most common degradation products were loss of side chain protecting groups or oxidation.

Supplementary Table S1. Remaining (%) Fmoc-amino acids after 14 days in the designated solvent containing OxymaPure (0.3 M). Initial Fmoc-amino acid concentration 0.3 M[a]

Fmoc-AA-OH	DMF	DMSO/2-Me-THF (3:7)	DMSO/EtOAc (4:6)	DMSO/DOL (3:7)	NBP/DOL (4:6)	NFM/DOL (2:8)
Fmoc-Ala-OH	>99%	>99%	>99%	>99%	>99%	>99%
Fmoc-Cys(Trt)-OH	99%	>99%	97%	>99%	>99%	98%
Fmoc-Asp(OtBu)-OH	>99%	>99%	>99%	>99%	>99%	>99%
Fmoc-Glu(OtBu)-OH	>99%	>99%	>99%	>99%	>99%	>99%
Fmoc-Phe-OH	>99%	99%	>99%	97%	>99%	>99%
Fmoc-His(Trt)-OH	88%	96%	95%	Insoluble	Insoluble	Insoluble
Fmoc-His(Boc)-OH	73%	80%	77%	78%	76%	74%
Fmoc-Ile-OH	>99%	>99%	>99%	>99%	>99%	>99%
Fmoc-Lys(Boc)-OH	>99%	>99%	97%	99%	>99%	>99%
Fmoc-Met-OH	>99%	99%	99%	94%	95%	99%
Fmoc-Asn(Trt)-OH	>99%	>99%	>99%	>99%	>99%	>99%
Fmoc-Pro-OH	>99%	>99%	97%	>99%	>99%	>99%
Fmoc-Gln(Trt)-OH	>99%	>99%	98%	>99%	>99%	>99%
Fmoc-Arg(Pbf)-OH	>99%	>99%	>99%	>99%	>99%	>99%
Fmoc-Ser(tBu)-OH	99%	>99%	98%	>99%	>99%	>99%
Fmoc-Thr(tBu)-OH	>99%	>99%	95%	>99%	>99%	>99%
Fmoc-Trp(Boc)-OH	99%	99%	99%	99%	95%	96%
Fmoc-Tyr(tBu)-OH	>99%	>99%	>99%	99%	98%	99%

[a] Green: >99% remaining Fmoc-amino acid. Yellow: 99-95% remaining Fmoc-amino acid. Red: <95% remaining Fmoc-amino acid. Grey: The Fmoc-amino acid was not fully soluble at 0.3 M in this solvent mixture.

3.2 Automated SPPS of Bivalirudin on 7.5 mmol scale

- Bivalirudin (H-fPRPGGGGNGDFEEIPEEYL-OH)

The synthesis of Bivalirudin was carried out on 7.5 mmol scale using an automated synthesizer (Sonata, Gyros Protein Technologies). H-Leu-2-CTR resin (9.26 g, loading 0.81 mmol/g) was pre-swelled in the indicated binary solvent mixture. Fmoc-protected amino acid derivatives (2 equiv.) were pre-activated for 5 min using DIC/Oxyma (2 equiv. each) and the resin was treated with the reagent mixture for 90 min, except for tyrosine which was coupled using a TBTU/DIPEA (2 and 4 equiv. respectively) activation protocol. At the end of each coupling reaction, the resin was drained and capped using a solution (150 mL) of Ac_2O and collidine in the candidate solvent. The resin was then filtered and washed with the indicated solvent. Fmoc-removal was carried out using a solution of 20% (v/v) piperidine in the corresponding solvent (10 + 5 min). After the final cycle, the resin was washed with the indicated solvent (150 mL) and IPA (150 mL) and dried under vacuum before the resin weight gain was calculated. The dry resin (100 mg) was treated with TFA/H_2O (95:5, v/v; 1 mL) for 2 h at ambient temperature. The cleavage solution was filtered, and the crude peptide was precipitated as a white to off-white powder using cold DIPE. The obtained crude peptide was subjected to HPLC and LC-MS analysis (Method A).

3.3 Arg-lactamisation

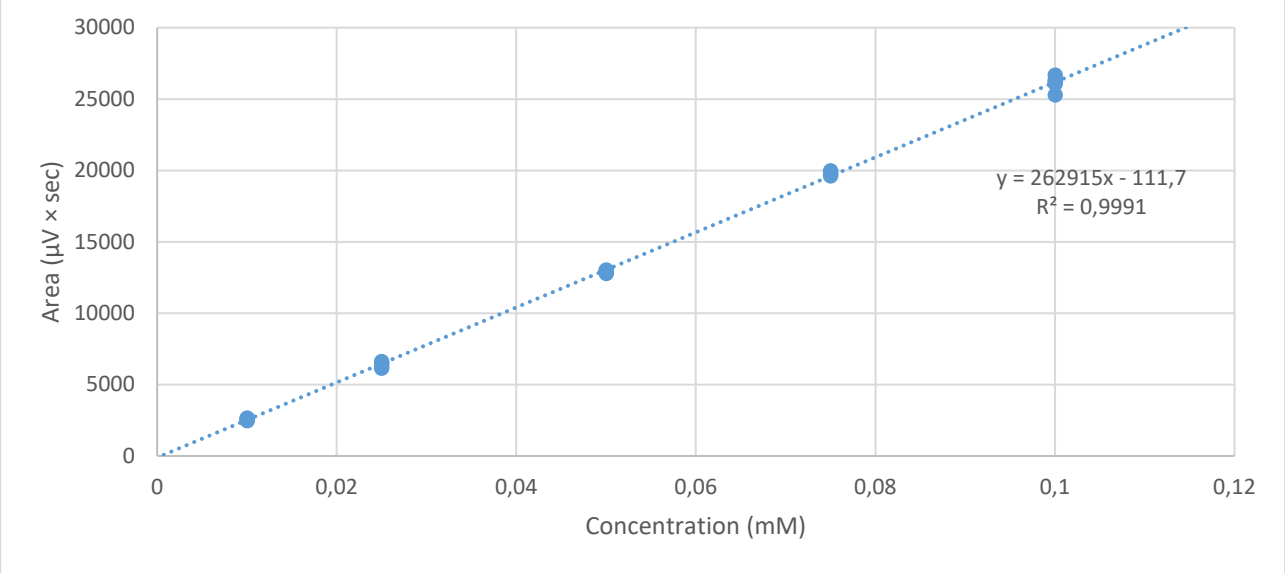
DIC (157 µL, 1 mmol) was added to a solution of Fmoc-Arg(Pbf)-OH (649 mg, 1 mmol) and Oxyma Pure (142 mg, 1 mmol) in the candidate solvent (10 mL). The reaction was allowed to stir for 90 min at ambient temperature. After 90 min, the reaction mixture (3 µL) was diluted to 1 mL with MeOH. The solution in MeOH was vortexed for 30 seconds and directly subjected to HPLC analysis (Method B).

3.4 Diketopiperazine (DKP) formation

H-Arg(Pbf)-OH was dissolved in MeCN/H_2O/TFA (50/50/0.1, v/v/v) at five different concentrations with five samples at each concentration HPLC analysis (Method E) at 280 nm was used to determine the area under the curve (Supplementary Table S2), and the data was plotted to generate a standard curve for the absorption of H-Arg(Pbf)-OH (Supplementary Figure S4).. The standard curve was then used to determine the concentration of DKP formed from Fmoc-Arg(Pbf)-Gly-Wang-resin, assuming similar absorption of H-Arg(Pbf)-OH and the Arg(Pbf)-Gly DKP.

Supplementary Table S2. Measured AUC at 280 nm for various concentrations of H-Arg(Pbf)-OH

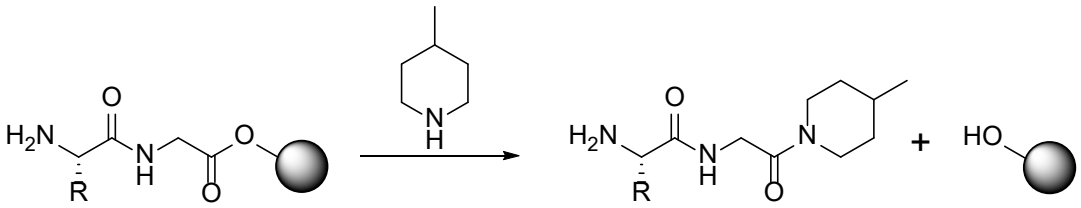
Concentration (mM)	Area 1 (μV × sec)	Area 2 (μV × sec)	Area 3 (μV × sec)	Area 4 (μV × sec)	Area 5 (μV × sec)	Area Avg. (μV × sec)
0.01	2607	2648	2570	2457	2621	2581
0.025	6475	6126	6548	6608	6275	6406
0.05	12963	12866	13009	12767	12962	12913
0.075	19979	19701	19768	19875	19603	19785
0.1	26673	26057	26232	26320	25287	26114



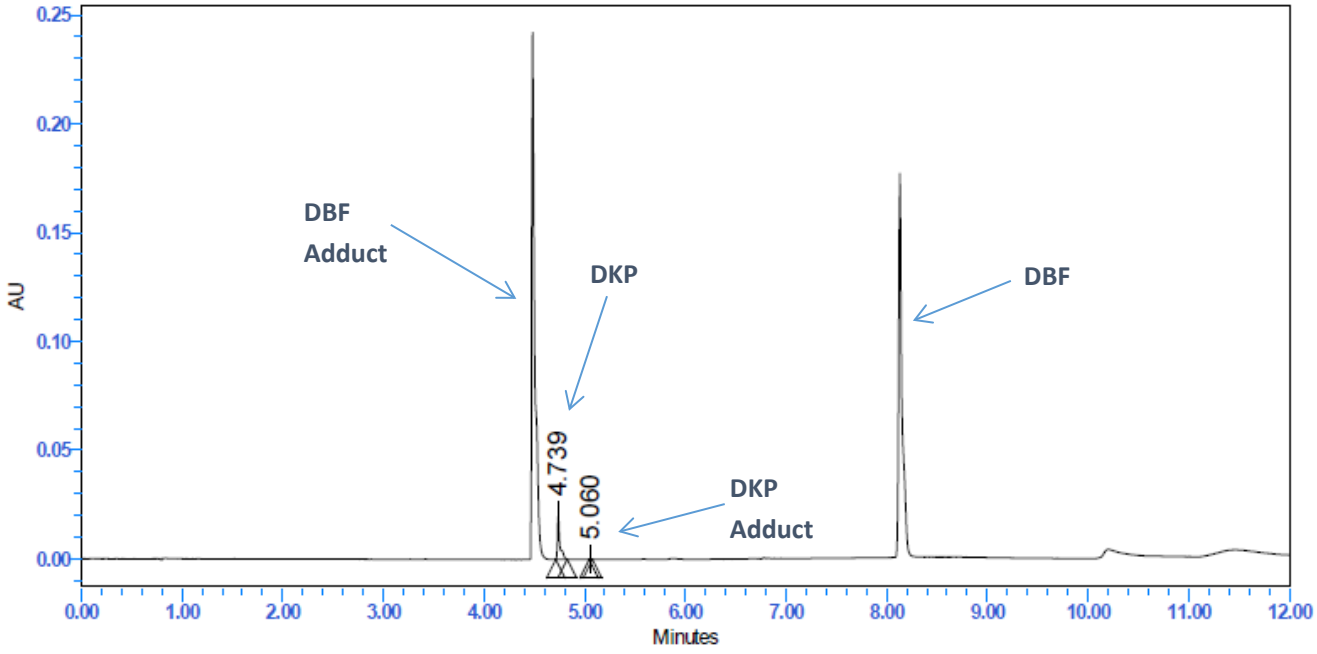
Supplementary Figure S4. Standard curve for the measured AUC at 280 nm as a function of the concentration of H-Arg(Pbf)-OH

The candidate solvent (8 mL/g resin) was added to Fmoc-Arg(Pbf)-Gly-Wang-resin (~30 mg, loading 0.47 mmol/g) which was swelled for 60 min at ambient temperature. Base (2 mL/g resin) was added the mixture left shaking for another 10 or 30 min (Supplementary Table S3). Reaction mixture (10 μL) was extracted and diluted with MeCN/H_2O/TFA (75/25/0.1, v/v/v; 490 μL) and analysed by HPLC (Method E) at 280 nm. All experiments were carried out in duplicate or more (Supplementary Table S4). When used as base, 4-Me-piperidine was in some solvents able to react with the ester linkage and form an adduct (Supplementary

Figure S5). This was only observed in a few cases, and consisted of less than 1% by-product compared to DKP.



Supplementary Figure S5. Reaction between H-Arg(Pbf)-Gly-O-Wang resin and 4-Me-piperidine



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		4.739	33034	20508		98.91
2		5.060	364	385		1.09

Supplementary Figure S6. DKP-formation. HPLC analysis (λ = 280 nm) after 30 min deprotection with piperidine in DMF.

Supplementary Table S3. Conditions for DKP formation on Fmoc-Arg(Pbf)-Gly-Wang-PS resin

	Method A	Method B	Method C

Time (min)	30	30	10
Base	Piperidine	4-Me-Piperidine	Piperidine

Supplementary Table S4. DKP formation for Fmoc-Arg(Pbf)-Gly-Wang-PS resin in binary solvent mixtures and DMF

	Method A		Method B		Method C	
Solvent	Area (μV × sec) [a]	%DKP	Area (μV × sec) [a]	%DKP	Area (μV × sec) [a]	%DKP
DMF	30514	16.12	27422	14.49	11027	5.82
	33398	17.64	31145	16.45	12919	6.82
DMSO/2-Me-THF (3:7)	42443	22.42	40037	21.15	15203	8.03
	46604	24.62	39077	20.64	15821	8.36
DMSO/2-Me-THF (4:6)	41142	21.73	46355	24.29	16624	8.78
	41499	21.92	36167	19.11	16507	8.72
DMSO/EtOAc (4:6)	52574	27.77	50791	26.83	13880	7.33
	35638	18.83	54010	28.53	14811	7.82
	43637	23.05				
DMSO/EtOAc (6:4)	19437	10.27	29037	15.34	12617	6.67
	28984	15.31	33330	17.61	11999	6.34
DMSO/DOL (3:7)	33544	17.72	37517	19.82	13231	6.99
	28936	15.29	31979	16.89	11733	6.20
DMSO/DOL (4:6)	34709	18.34	31845	16.82	11716	6.19
	34549	18.25	32095	16.95	12580	6.65
NBP/DOL (4:6)	38306	20.24	32787	17.32	12673	6.69
	38969	20.59	30431	16.08	9805	5.18
NFM/DOL (2:8)	38139	20.15	37266	19.69	12741	6.73
	34339	18.14	35977	19.00	10330	5.46

[a] In cases where the base reacts with the linker and forms an adduct, the area is the sum of the two compounds.

3.5 Aspartimide formation (with OMpe aspartyl protection)

The model Scorpion Toxin II peptidyl resin, H-Val-Lys(Boc)-Asp(OMpe)-Gly-Tyr(*t*Bu)-Ile-Wang resin, was synthesised in DMF using standard Fmoc-SPPS chemistry at 4 mmol scale. The dry peptidyl resin (100 mg) was transferred to a syringe (2 mL) fitted with porous frit. The peptidyl resin was swelled (2 × 10 min) with a candidate solvent. The 1 mL of 20% piperidine (v/v) prepared in the corresponding candidate solvent was added to this pre-swelled peptidyl resin and the reaction was allowed to proceed by agitating the syringe

at ambient temperature. Parallel experiments were performed to simulate the base treatment for different periods (45, 225, and 405 min). At each time point, the piperidine solution was filtered off from the resin and the resin bed was washed (3 × 1 mL) alternating with DMF and isopropyl alcohol (IPA). Finally, the peptidyl resin was washed again with IPA (3 × 1 mL). The resin was dried under vacuum overnight and the peptide was cleaved from the resin using a TFA/H_2O (95:5, v/v; 1 mL) solution for 1.5 h. The crude peptide was precipitated using DIPE (10 mL) and dried under vacuum. The crude peptide (1 mg) was dissolved in 5% AcOH solution and analysed via HPLC (Method C). The untreated peptidyl resin (without piperidine treatment) was used as a reference peptide in this study.

3.6 Aspartimide formation (with O*t*Bu aspartyl protection)

Fmoc-Val-Lys(Boc)-Asp(O*t*Bu)-Gly-Tyr(tBu)-Ile-Wang resin (100 mg, loading 0.60 mmol/g) was swelled in the indicated solvent (1 mL) for 1 h in a 5 mL fritted syringe. A solution of 20% (v/v) piperidine in the indicated solvent (1 mL) was added to the resin, which was agitated for 4 hrs. The solvent was drained, and the resin was washed with DMF (4 × 3 mL). The peptide was capped with a solution of DMF/Ac_2O/DIPEA (120:4.73:10.45, v/v/v; 5 mL) for 2 × 5 min. Experiments without Oxyma Pure were performed in triplicates, while the experiments with Oxyma Pure were only performed once. Approximately 10 mg peptide resin was cleaved with TFA/TIPS/H_2O (95/2.5/2.5, v/v/v; 400 µL) for 1 h. Afterwards MeCN (1.2 mL) and 7.0 M NH_4OAc (0.68 mL) were added, and the sample was filtered and analysed by LC-MS (Method H) at 210 nm.

To quantify the amount of *α*- and *β*-isomers the cleaved peptide samples were analysed by HPLC (Method G) at 214 nm. In order to ensure that no co-elution between the *α*- and *β*-isomer was present, each sample was subsequently co-injected with a reference sample of the *β*-isomer.

Supplementary Table S5. Aspartimide formation without Oxyma Pure

Solvent	Target peptide	Aspartimides	Piperidides
	87%	6%	7%
DMF	85%	6%	9%
	87%	6%	7%
	88%	4%	8%
DMSO/2-Me-THF (3:7)	88%	5%	7%
	88%	6%	6%
	88%	6%	6%
DMSO/2-Me-THF (4:6)	88%	6%	6%
	89%	6%	5%
	88%	5%	7%
DMSO/EtOAc (4:6)	89%	5%	6%
	89%	4%	7%
	87%	6%	7%
DMSO/EtOAc (6:4)	88%	7%	5%
	87%	6%	7%
	88%	5%	7%
DMSO/DOL (3:7)	86%	4%	10%
	90%	4%	6%
	81%	10%	9%
DMSO/DOL (4:6)	86%	6%	8%
	88%	5%	7%
	90%	3%	7%
NBP/DOL (4:6)	90%	4%	6%
	91%	3%	6%
	90%	3%	7%
NFM/DOL (2:8)	89%	3%	8%
	89%	5%	6%

Supplementary Table S6. Aspartimide formation with Oxyma Pure

Solvent	Target peptide	Aspartimides	Piperidides
DMF	86%	9%	5%
DMSO/2-Me-THF (3:7)	88%	8%	4%
DMSO/2-Me-THF (4:6)	89%	9%	2%
DMSO/EtOAc (4:6)	90%	7%	3%
DMSO/EtOAc (6:4)	89%	7%	4%
DMSO/DOL (3:7)	93%	5%	2%
DMSO/DOL (4:6)	89%	8%	3%
NBP/DOL (4:6)	92%	4%	4%
NFM/DOL (2:8)	92%	4%	4%

Supplementary Table S7. Formation of the *β*-isomer

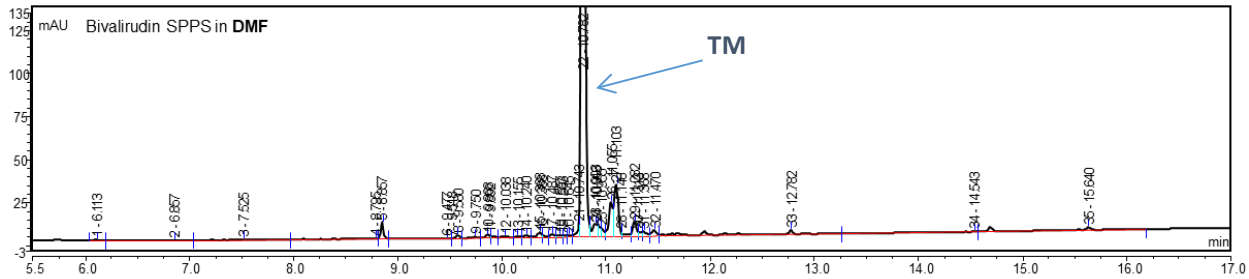
Solvent	%β-isomer without Oxyma Pure	%β-isomer with 0.1M Oxyma Pure
DMF	0.33%	<0.01%
DMSO/2-Me-THF (3:7)	0.35%	<0.01%
DMSO/2-Me-THF (4:6)	0.01%	<0.01%
DMSO/EtOAc (4:6)	<0.01%	<0.01%
DMSO/EtOAc (6:4)	<0.01%	<0.01%
DMSO/DOL (3:7)	<0.01%	<0.01%
DMSO/DOL (4:6)	<0.01%	<0.01%
NBP/DOL (4:6)	<0.01%	<0.01%
NFM/DOL (2:8)	<0.01%	<0.01%

3.7 Amino acid racemisation

The model peptide, H-Val-Gly-Rink Amide AMPS resin, was synthesised in DMF using standard Fmoc-SPPS chemistry. Coupling of the amino acid, Fmoc-Cys(Trt)-OH, Fmoc-His(Boc)-OH or Fmoc-His(Trt), was carried out manually with 500 mg resin, using a 5 mL fritted syringe at room temperature. The synthesis was undertaken using 3 equiv. of amino acid, DIC and Oxyma Pure dissolved in the investigated solvent (concentration 0.25 M) with 1 min pre-activation. The reaction was shaken for 80 min, with a second addition of 1 equiv. of DIC after 20 min. Fmoc-removal was performed using 20% piperidine in DMF (v/v) solution (2 × 15 min). The peptide was then capped on solid support using a solution of DMF/Ac_2O/DIPEA (120:4.73:10.45, v/v/v; 5 mL) for 2 × 10 min. Approximately 10 mg peptide resin was cleaved with 400 µL TFA/TIPS/H_2O (95/2.5/2.5, v/v/v) for 1 h. Afterwards MeCN (1.2 mL) and 7.0 M NH_4OAc (0.68 mL) were added and the sample was filtered and analysed by HPLC (Method F) at 214 nm. In order to ensure that there was no co-elution of the D- and L-isomers, each sample was subsequently co-injected with a reference sample of the D-isomer.

4. Chromatograms

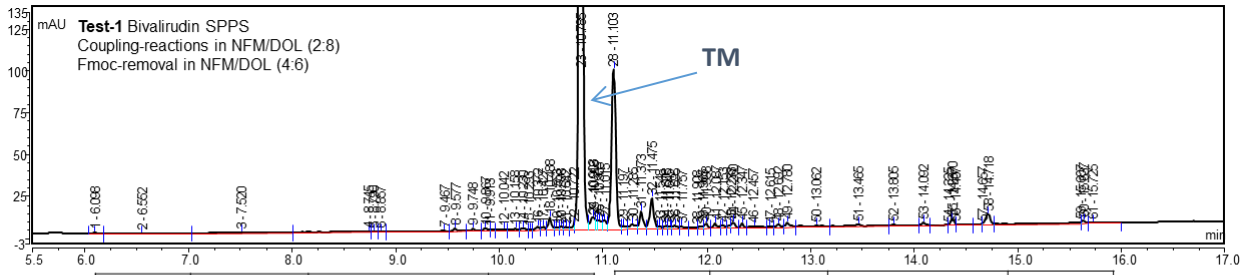
4.1 Automated SPPS of Bivalirudin (H-fPRPGGGGNGDFEEIPEEYL-OH) on 7.5 mmol scale



Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
5	8.857	Ac-[Glu^{17}-Leu^{20}]-OH	1.20
22	10.782	Bivalirudin	73.82
23	10.903	des[Gly-Gly]	1.54
25	10.955	des[Gly]	0.62
26	11.055	Ac-[Gly^{7}-Leu^{20}]-OH	3.25

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
27	11.103	Ac-[Pro^{4}-Leu^{20}]-OH	5.64
29	11.282	Ac-[Asn^{9}-Leu^{20}]-OH	1.40
31	11.368	des[Arg-Pro]	0.53
32	11.470	des[Arg^{3}]	0.70
other peaks			11.3

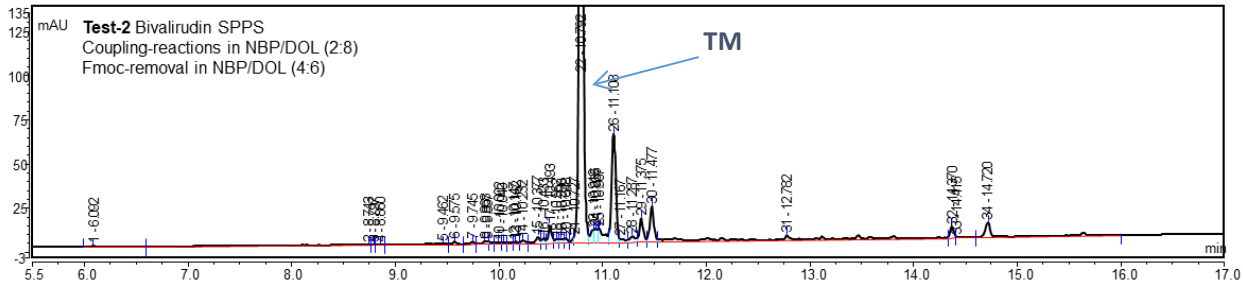
Supplementary Figure S7. Chromatogram depicting the impurity profile of Bivalirudin SPPS in DMF. The relative percentage of selected peaks are tabulated.



Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
18	10.488	des[Pro]	1.03
23	10.785	Bivalirudin	60.18
24	10.903	des[Gly-Gly]	1.21
28	11.103	Ac-[Pro^4-Leu^{20}]-OH	16.58
31	11.373	des[Arg-Pro]	1.63

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
32	11.475	des[Arg^3]	3.10
55	14.370	Fmoc-[Gly^5-Leu^{20}]-OH	0.50
58	14.718	Fmoc-[Pro^4-Leu^{20}]-OH	1.25
other peaks			14.52

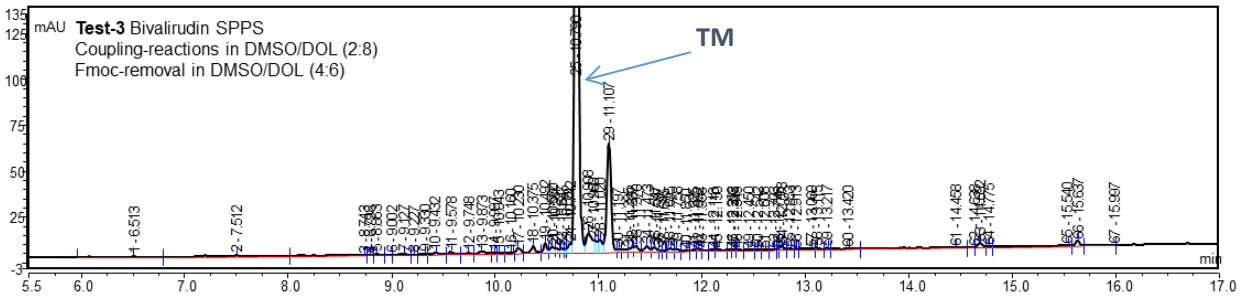
Supplementary Figure S8. Chromatogram depicting the impurity profile of Bivalirudin SPPS (entry 1) with coupling reactions in NFM/DOL (2:8) and Fmoc-removal in NFM/DOL (4:6). The relative percentage of selected peaks are tabulated.



Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
17	10.493	des[Pro]	1.45
22	10.792	Bivalirudin	63.17
23	10.912	des[Gly-Gly]	1.08
26	11.108	Ac-[Pro^4-Leu^{20}]-OH	10.74
29	11.375	des[Arg-Pro]	2.18

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
30	11.477	des[Arg^3]	3.46
32	14.370	Fmoc-[Gly^5-Leu^{20}]-OH	0.92
34	14.720	Fmoc-[Pro^4-Leu^{20}]-OH	2.54
other peaks			14.46

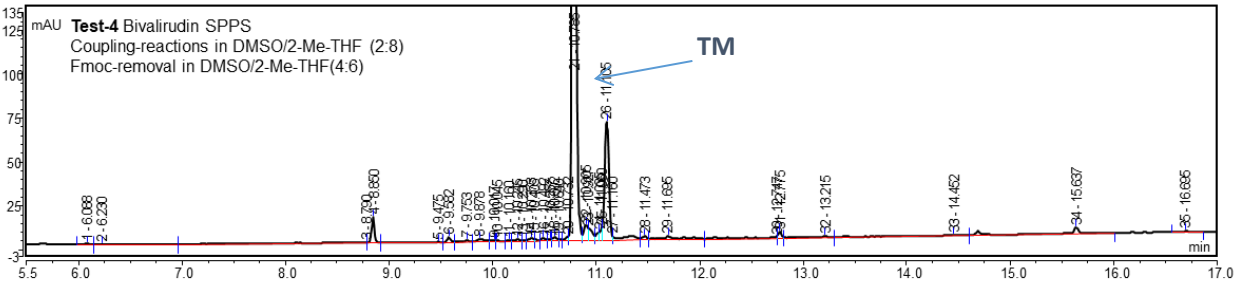
Supplementary Figure S9. Chromatogram depicting the impurity profile of Bivalirudin SPPS (entry 2) with coupling reactions in NBP/DOL (2:8) and Fmoc-removal in NBP/DOL (4:6). The relative percentage of selected peaks are tabulated.



Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
25	10.790	Bivalirudin	64.95
26	10.908	des[Gly-Gly]	3.01
28	11.020	des[Gly]	1.40

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
29	11.107	Ac-[Pro^4-Leu^{20}]-OH	11.99
other peaks			18.65

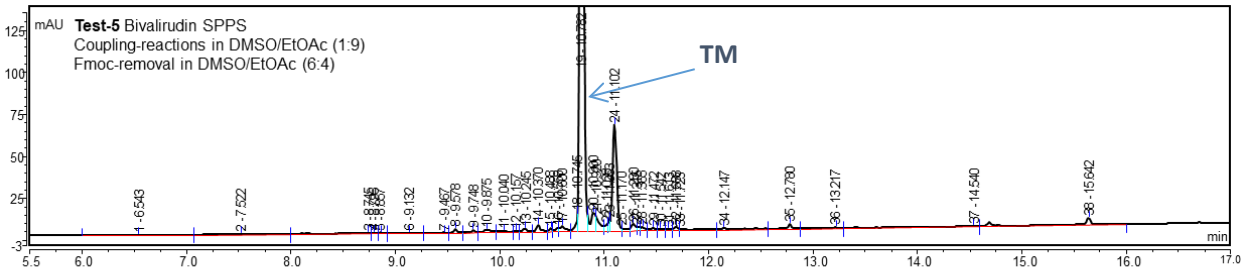
Supplementary Figure S10. Chromatogram depicting the impurity profile of Bivalirudin SPPS (entry 3) with coupling reactions in DMSO/DOL (2:8) and Fmoc-removal in DMSO/DOL (4:6). The relative percentage of selected peaks are tabulated.



Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
4	8.850	Ac-[Glu^{17}-Leu^{20}]-OH	1.74
21	10.785	Bivalirudin	70.49
22	10.905	des[Gly-Gly]	1.50

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
24	11.035	des[Gly]	0.60
26	11.105	Ac-[Pro^4-Leu^{20}]-OH	12.37
other peaks			13.3

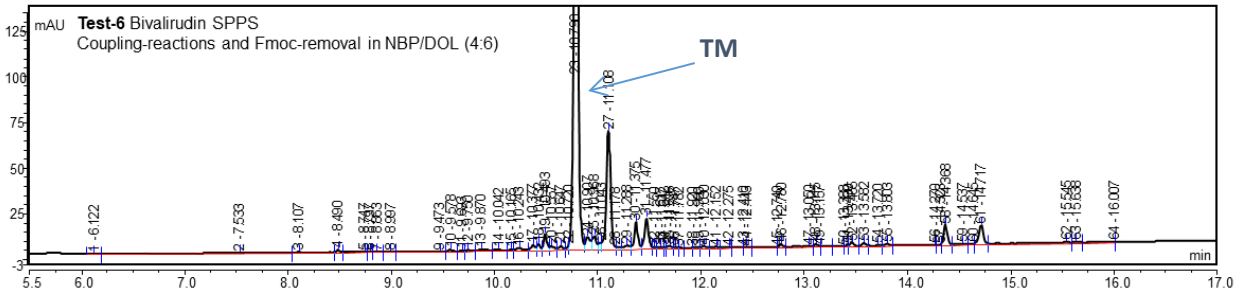
Supplementary Figure S11. Chromatogram depicting the impurity profile of Bivalirudin SPPS (entry 4) with coupling reactions in DMSO/2-Me-THF (2:8) and Fmoc-removal in DMSO/2-Me-THF (4:6). The relative percentage of selected peaks are tabulated.



Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
19	10.782	Bivalirudin	70.53
20	10.900	des[Gly-Gly]	2.31
21	10.920	des[Gly]	1.50
24	11.102	Ac-[Pro^4-Leu^{20}]-OH	11.94

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
26	11.280	Ac-[Asn^9-Leu^{20}]-OH	0.78
28	11.365	des[Arg-Pro]	0.42
29	11.472	des[Arg^3]	0.35
other peaks			12.17

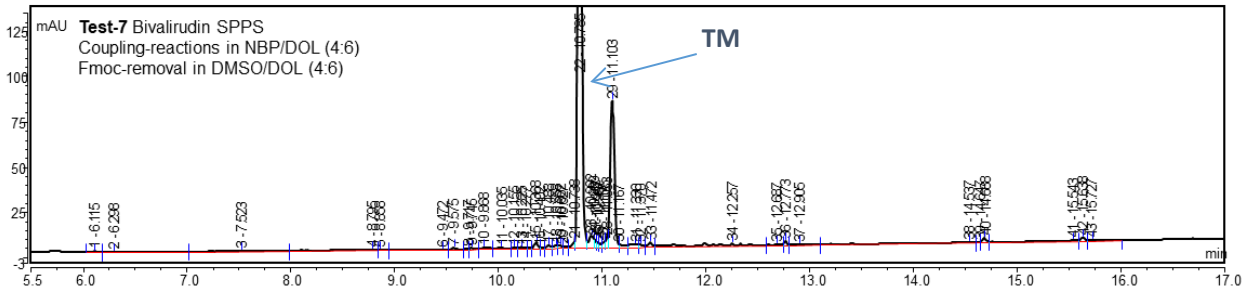
Supplementary Figure S12. Chromatogram depicting the impurity profile of Bivalirudin SPPS (entry 5) with coupling reactions in DMSO/EtOAc (1:9) and Fmoc-removal in DMSO/DOL (6:4). The relative percentage of selected peaks are tabulated.



Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
19	10.493	des[Pro]	1.34
23	10.790	Bivalirudin	62.53
24	10.907	des[Gly-Gly]	1.50
26	11.043	des[Gly]	0.52
27	11.108	Ac-[Pro^4-Leu^{20}]-OH	12.85
30	11.375	des[Arg-Pro]	2.55

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
31	11.477	des[Arg^3]	3.21
58	14.368	Fmoc-[Gly^5-Leu^{20}]-OH	1.81
61	14.717	Fmoc-[Pro^4-Leu^{20}]-OH	2.12
other peaks			11.57

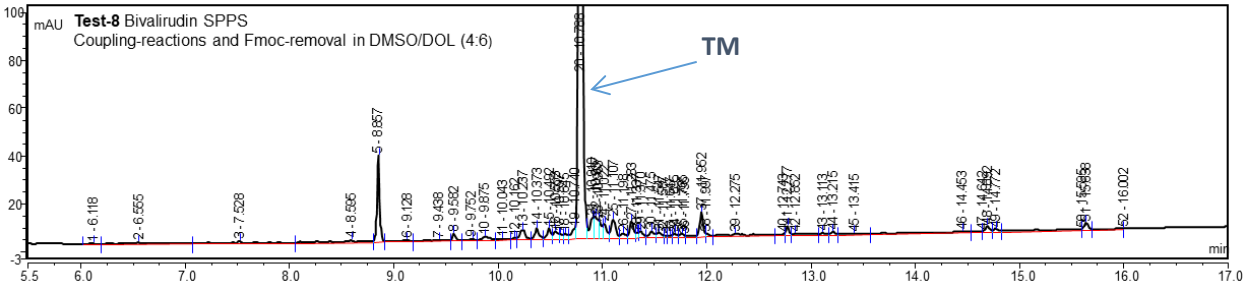
Supplementary Figure S13. Chromatogram depicting the impurity profile of Bivalirudin SPPS (entry 6) with coupling reactions and Fmoc-removal in NBP/DOL (4:6). The relative percentage of selected peaks are tabulated.



Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
22	10.785	Bivalirudin	67.68
29	11.103	Ac-[Pro^{4}-Leu^{20}]-OH	17.8
32	11.370	des[Arg-Pro]	0.35

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
33	11.472	des[Arg^{3}]	0.57
40	14.688	Fmoc-[Pro^{4}-Leu^{20}]-OH	0.46
other peaks			13.14

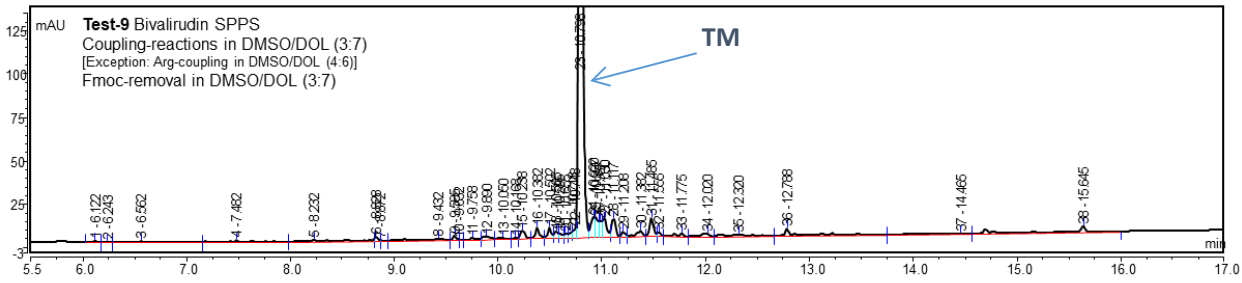
Supplementary Figure S14. Chromatogram depicting the impurity profile of Bivalirudin SPPS (entry 7) with coupling reactions in NBP/DOL (4:6) and Fmoc-removal in DMSO/DOL (4:6). The relative percentage of selected peaks are tabulated.



Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
5	8.857	Ac-[Glu^{17}-Leu^{20}]-OH	5.01
20	10.788	Bivalirudin	67.72
21	10.910	des[Gly-Gly]	1.67
24	11.022	des[Gly]	1.26

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
25	11.107	Ac-[Pro^{4}-Leu^{20}]-OH	1.86
27	11.283	Ac-[Asn^{9}-Leu^{20}]-OH	1.24
37	11.952	Ac-[Asp^{11}-Leu^{20}]-OH	1.78
other peaks			19.46

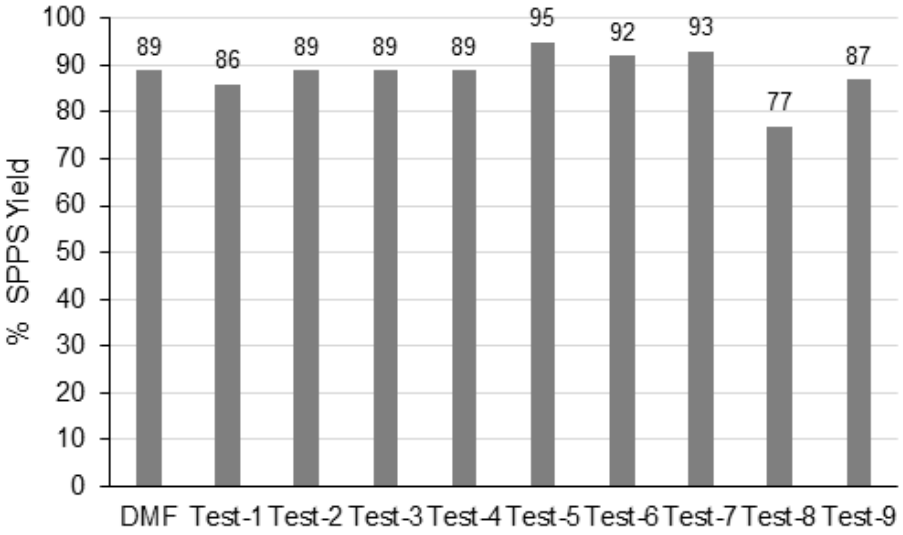
Supplementary Figure S15. Chromatogram depicting the impurity profile of Bivalirudin SPPS (entry 8) with coupling reactions and Fmoc-removal in DMSO/DOL (4:6). The relative percentage of selected peaks are tabulated.



Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
6	8.828	Ac-[Glu^{17}-Leu^{20}]-OH	0.29
23	10.798	Bivalirudin	72.49
24	10.920	des[Gly-Gly]	1.88
27	11.030	des[Gly]	2.12

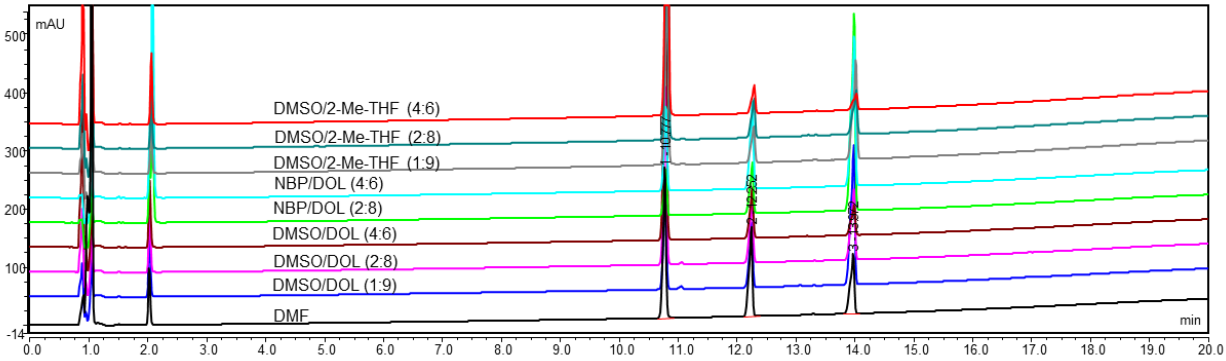
Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
28	11.117	Ac-[Pro^{4}-Leu^{20}]-OH	1.93
31	11.485	des[Arg^{3}]	1.90
other peaks			19.36

Supplementary Figure S16. Chromatogram depicting the impurity profile of Bivalirudin SPPS (Figure 6) with coupling reactions and Fmoc-removal in DMSO/DOL (3:7), except for the coupling of Fmoc-Arg(Pbf)-OH that was performed in DMSO/DOL (4:6). The relative percentage of selected peaks are tabulated.



Supplementary Figure S17. The crude yield (%) of Bivalirudin SPPS based on corresponding resin weight gain (%) for synthesis entries 1-8 (Test-1-8), and the synthesis in DMSO/DOL (3:7) with the coupling of Fmoc-Arg(Pbf)-OH in DMSO/DOL (4:6) (Test-9).

4.2 Arg-lactamisation

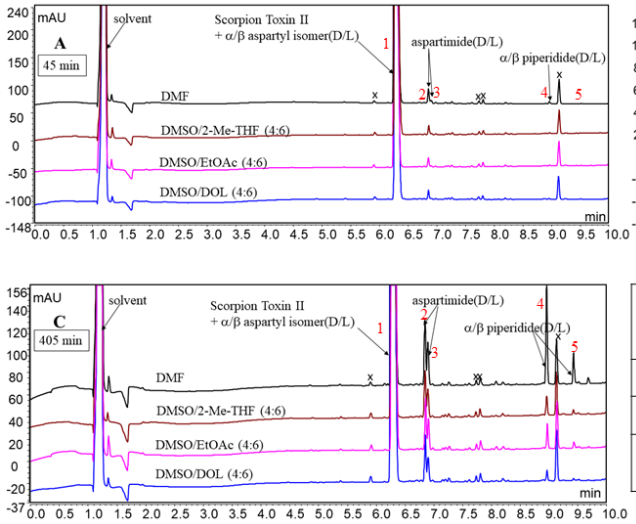


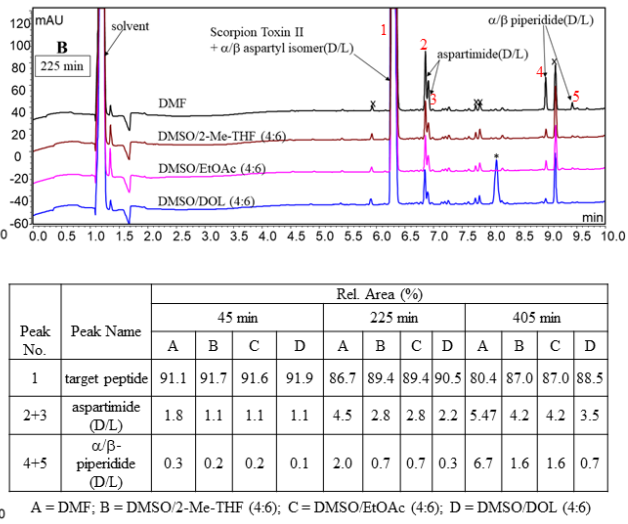
Solvent	Rel. Area %		
	Peak No.1 Ret.Time = 10.7 min Fmoc-Arg(Pbf)-OH	Peak No. 2 Ret.Time = 12.2 min Fmoc-Arg(Pbf)-OMe	Peak No. 3 Ret.Time = 13.9 min Fmoc-Arg(Pbf)-δ-lactam
DMF	45.24	30.86	23.89
DMSO/DOL (1:9)	28.01	20.49.	51.50
DMSO/DOL (2:8)	50.78	22.18	27.04
DMSO/DOL (4:6)	75.10	14.74	10.16
NBP/DOL (2:8)	12.34	15.62	72.04

Solvent	Rel. Area %		
	Peak No.1 Ret.Time = 10.7 min Fmoc-Arg(Pbf)-OH	Peak No. 2 Ret.Time = 12.2 min Fmoc-Arg(Pbf)-OMe	Peak No. 3 Ret.Time = 13.9 min Fmoc-Arg(Pbf)-δ-lactam
NBP/DOL (4:6)	21.85	26.18	51.98
DMSO/2-Me-THF (1:9)	52.43	11.44	36.13
DMSO/2-Me-THF (2:8)	73.06	11.69	15.25
DMSO/2-Me-THF (4:6)	86.73	7.85	5.42

Supplementary Figure S18. Chromatograms depicting the Arg-lactamisation in various binary solvent mixtures and DMF. The relative percentage of unreacted Fmoc-Arg(Pbf)-OH, methyl ester (Fmoc-Arg(Pbf)-OMe) and δ-lactam is tabulated.

4.3 Aspartimide formation (with OMpe aspartyl protection)

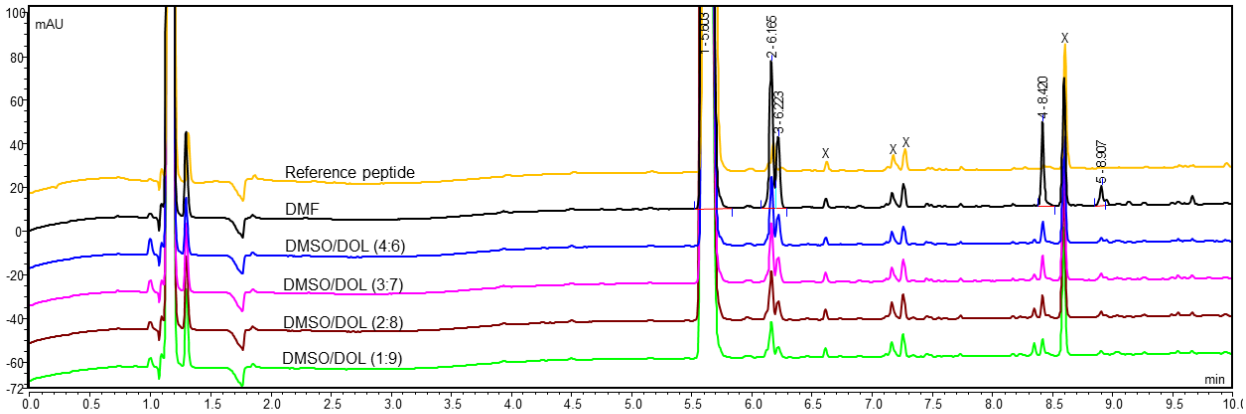




Peak No.	Peak Name	Rel. Area (%)											
		45 min				225 min				405 min			
		A	B	C	D	A	B	C	D	A	B	C	D
1	target peptide	91.1	91.7	91.6	91.9	86.7	89.4	89.4	90.5	80.4	87.0	87.0	88.5
2+3	aspartimide (D/L)	1.8	1.1	1.1	1.1	4.5	2.8	2.8	2.2	5.47	4.2	4.2	3.5
4+5	α/β-piperidide (D/L)	0.3	0.2	0.2	0.1	2.0	0.7	0.7	0.3	6.7	1.6	1.6	0.7

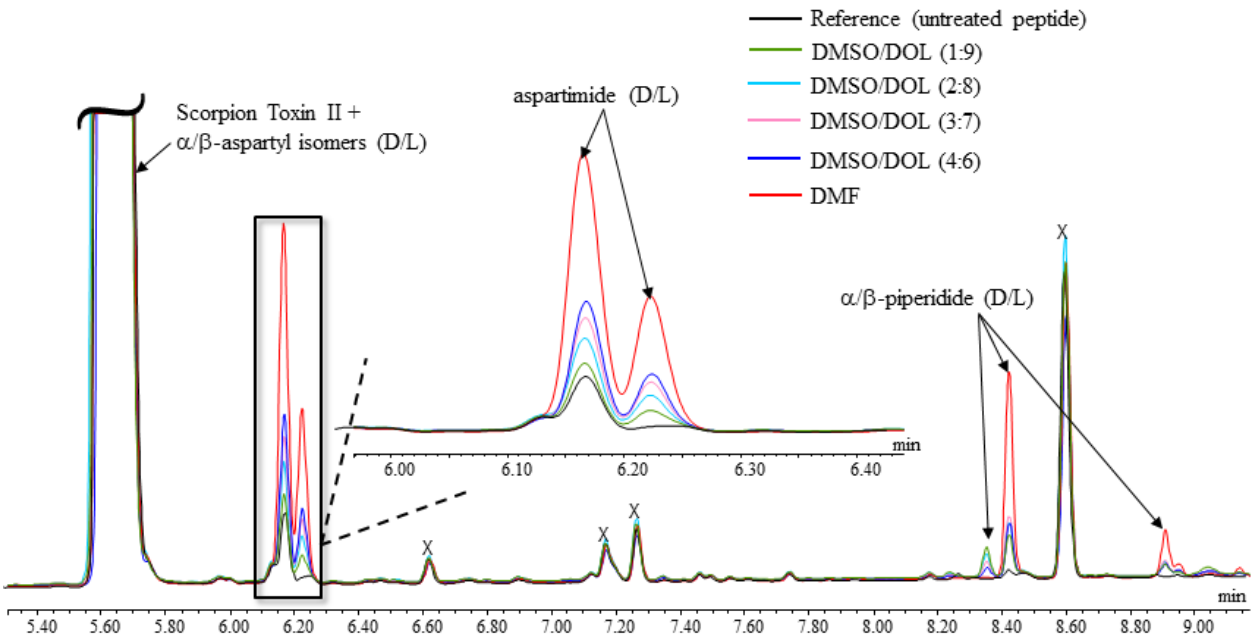
A = DMF; B = DMSO/2-Me-THF (4:6); C = DMSO/EtOAc (4:6); D = DMSO/DOL (4:6)

Supplementary Figure S19. Chromatograms depicting the aspartimide formation after A) 45 min, B) 225 min and C) 405 min of piperidine treatment (20%, v/v) in Scorpion Toxin II (with OMpe aspartyl protection) in binary solvent mixtures with similar polarity to DMF. The relative percentage of target peptide and aspartimide/piperidide is tabulated. The peaks marked with "X" are irrelevant to aspartimide/piperidide formation and originate from the reference peptide (peptide not treated with piperidine). The peak marked with "*" in DMSO/DOL (4:6) in B is induced during sample preparation for HPLC analysis and is irrelevant to aspartimide/piperidide formation.



			Rel. Area (%)					
Peak No.	Ret. Time (min)	Peak Name	Scorpion Toxin II - prior to base treatment (Reference)	DMF	DMSO/DOL (4:6)	DMSO/DOL (3:7)	DMSO/DOL (2:8)	DMSO/DOL (1:9)
1	5.6	Target peptide + α/β-aspartyl isomer (D/L), if any	99.08	93.14	96.53	97.21	97.90	98.27
2	6.1	aspartimide (D/L)	0.72	3.30	1.94	1.48	1.12	0.95
3	6.2		0.10	1.56	0.82	0.60	0.42	0.28
4	8.4	α/β-piperidide (D/L)	0.07	1.61	0.57	0.56	0.46	0.40
5	8.9		0.02	0.39	0.13	0.14	0.11	0.11

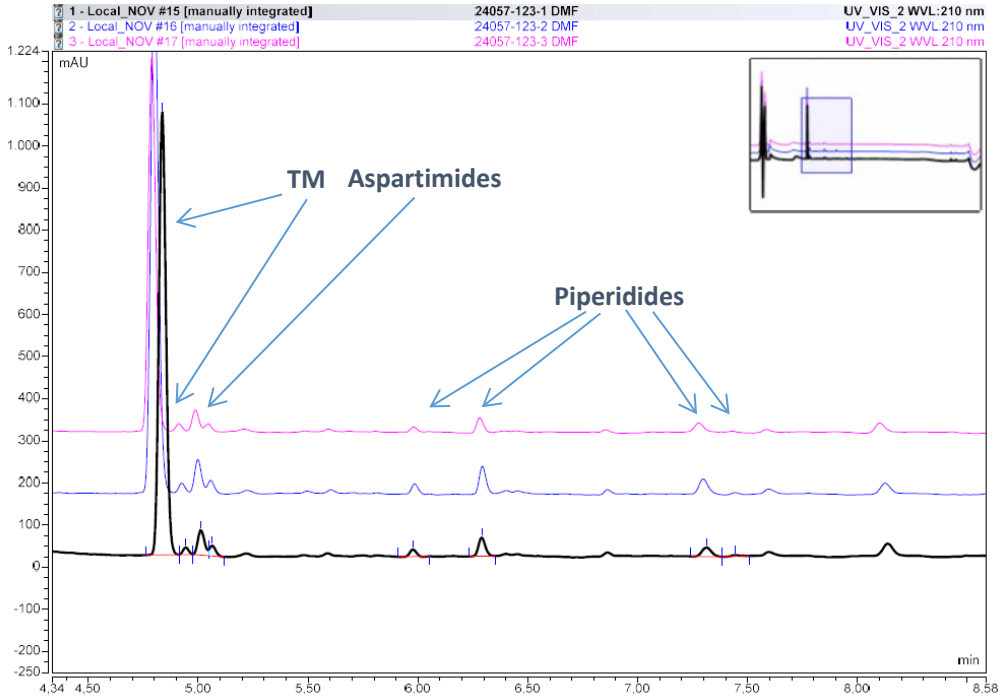
Supplementary Figure S20. Polarity scan depicting increasing aspartimide formation with increasing polarity of DMSO/DOL after 225 min of piperidine treatment (20%, v/v). DMF shows higher aspartimide/piperidide formation than all tested combinations of DMSO/DOL. The relative percentage of target peptide and aspartimide/piperidide is tabulated. The peaks marked with "X" are irrelevant to aspartimide/piperidide formation and are originated from reference peptide (peptide not treated with piperidine).



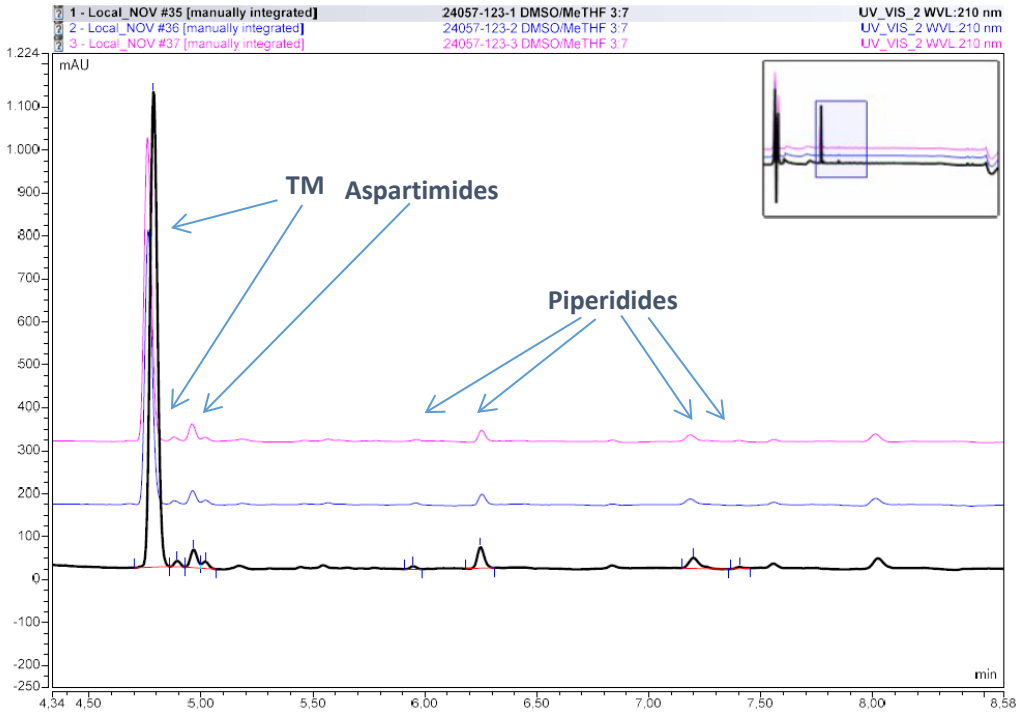
Supplementary Figure S21. Polarity scan depicting increasing aspartimide formation with increasing polarity of DMSO/DOL (polarity order 1:9 < 2:8 < 3:7 < 4:6) after 225 min of piperidine treatment (20%, v/v). In comparison, DMF shows higher aspartimide/piperidide formation than all other tested combinations of DMSO/DOL. The peaks marked with "X" are irrelevant to aspartimide/piperidide formation and originate from the reference peptide (peptide not treated with piperidine).

4.4 Aspartimide formation without Oxyma Pure (with O*t*Bu aspartyl protection)

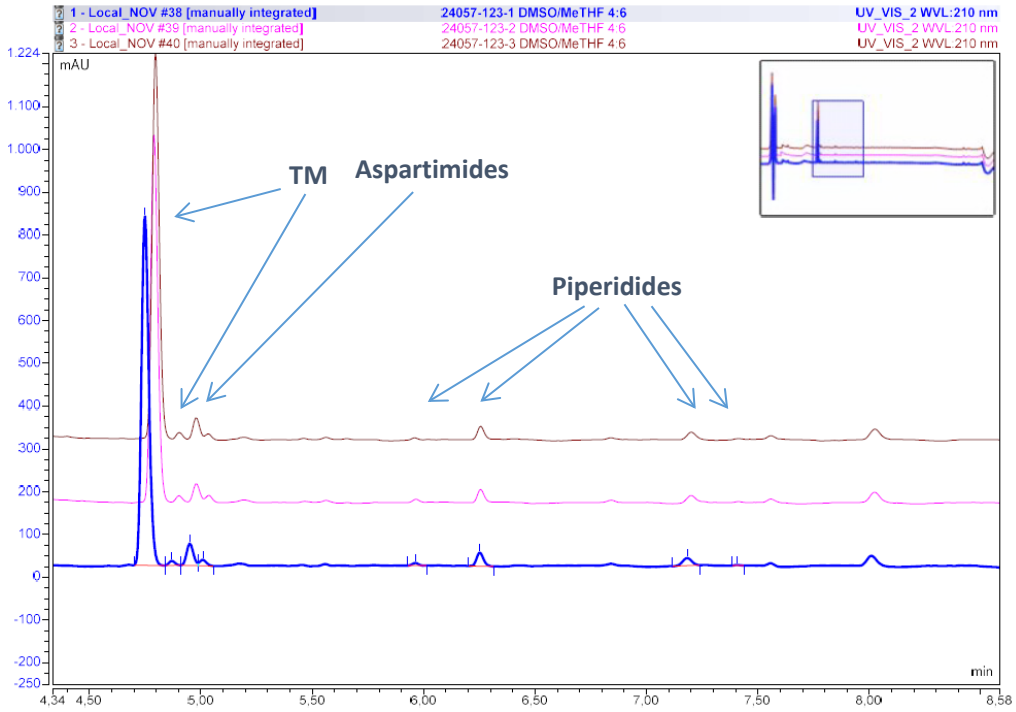
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMF, repeated three times, method H



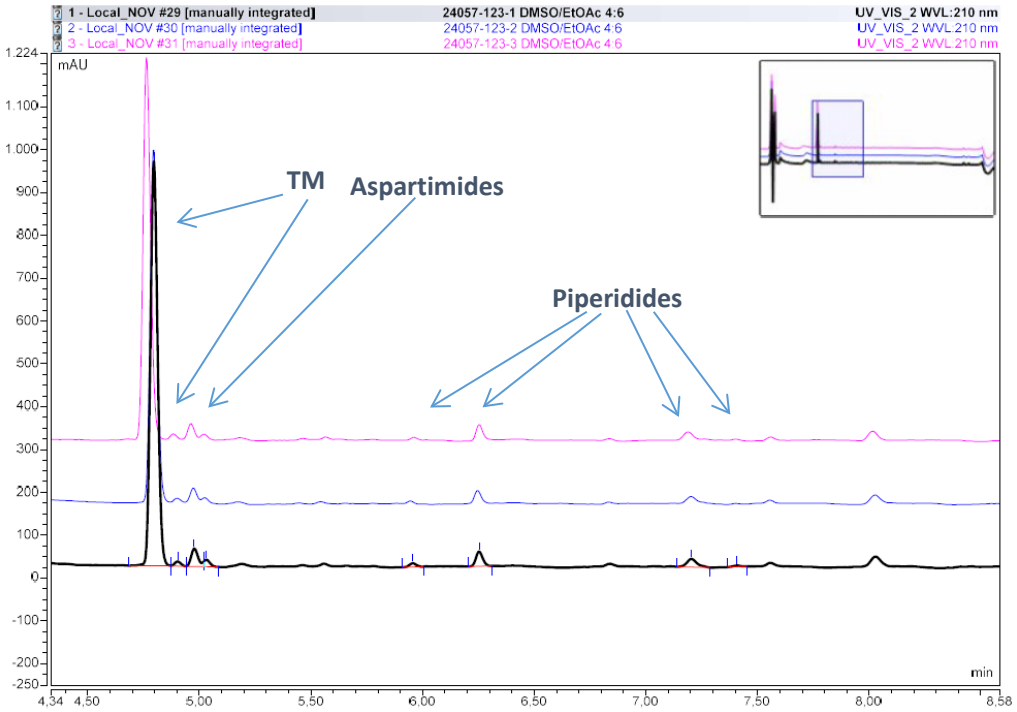
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMSO/2-Me-THF (3:7), repeated three times, method H



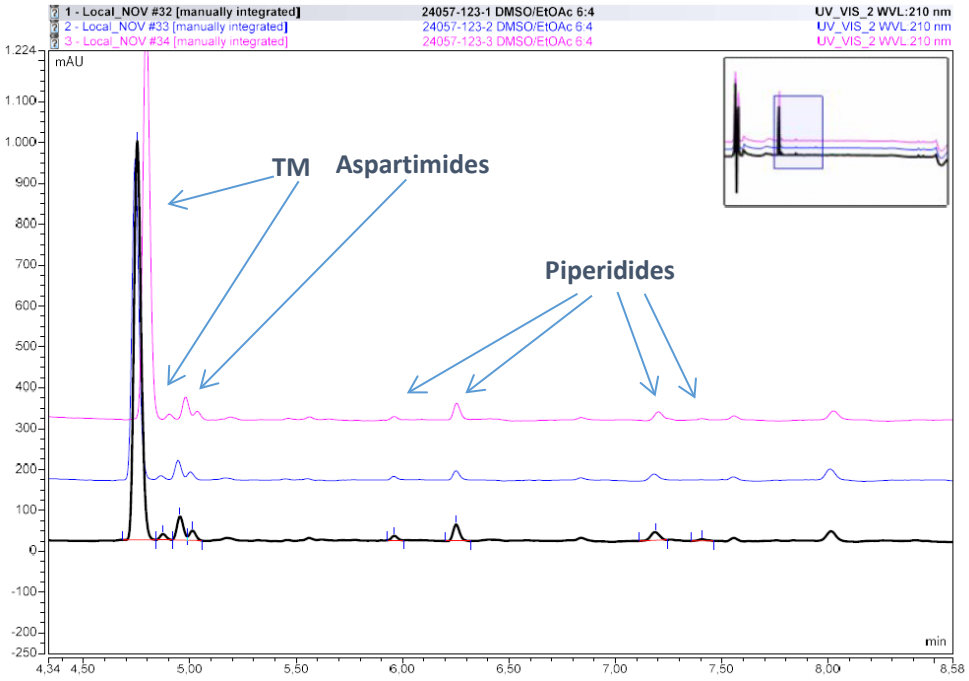
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMSO/2-Me-THF (4:6), repeated three times, method H



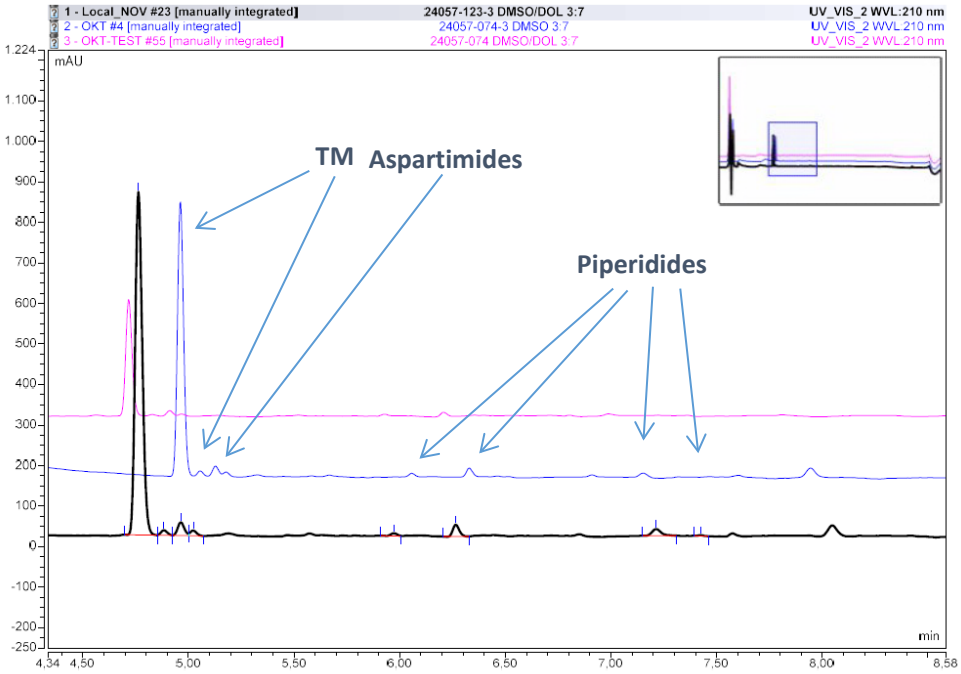
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMSO/EtOAc (4:6), repeated three times, method H



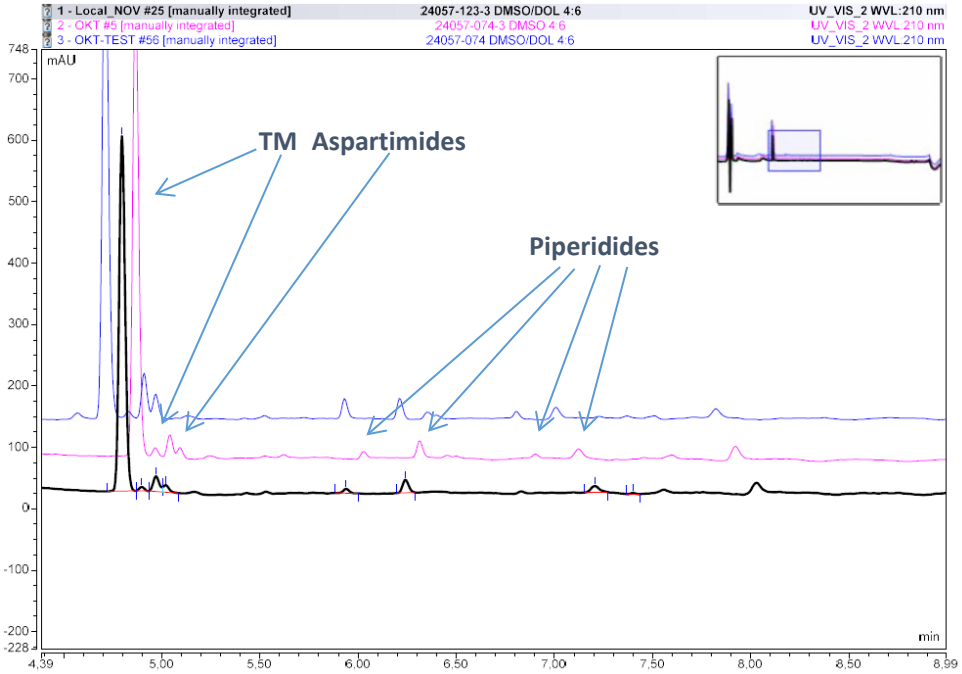
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMSO/EtOAc (6:4), repeated three times, method H



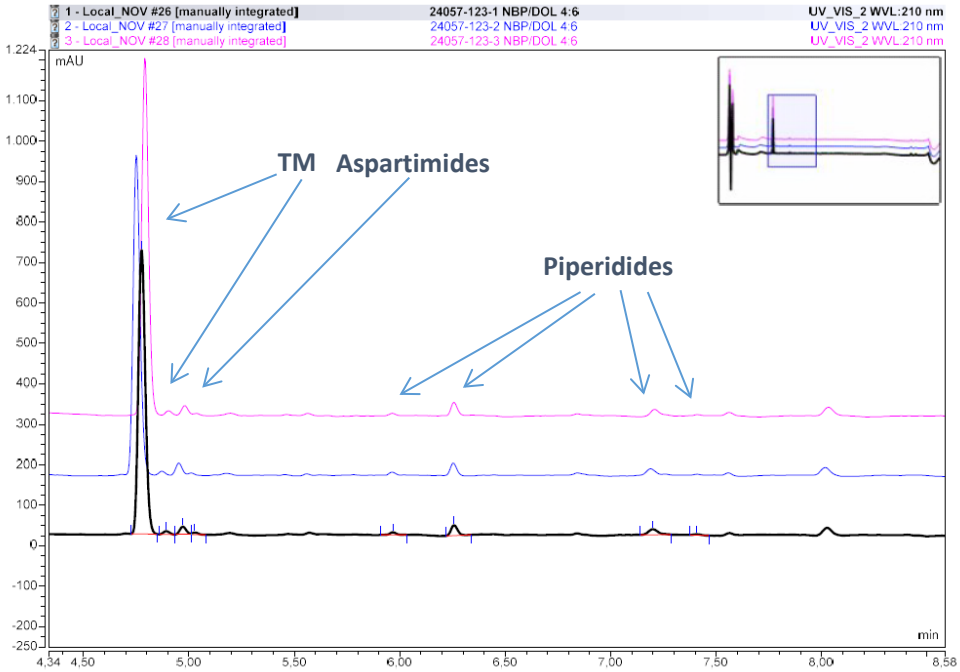
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMSO/DOL (3:7), repeated three times, method H



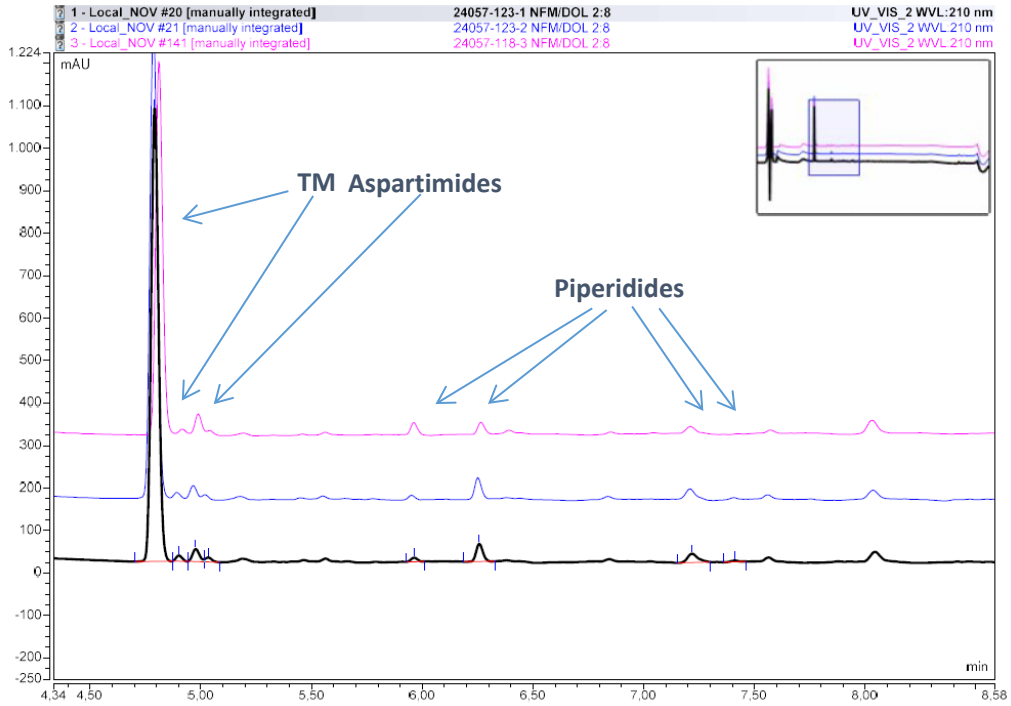
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMSO/DOL (4:6), repeated three times, method H



Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in NBP/DOL (4:6), repeated three times, method H

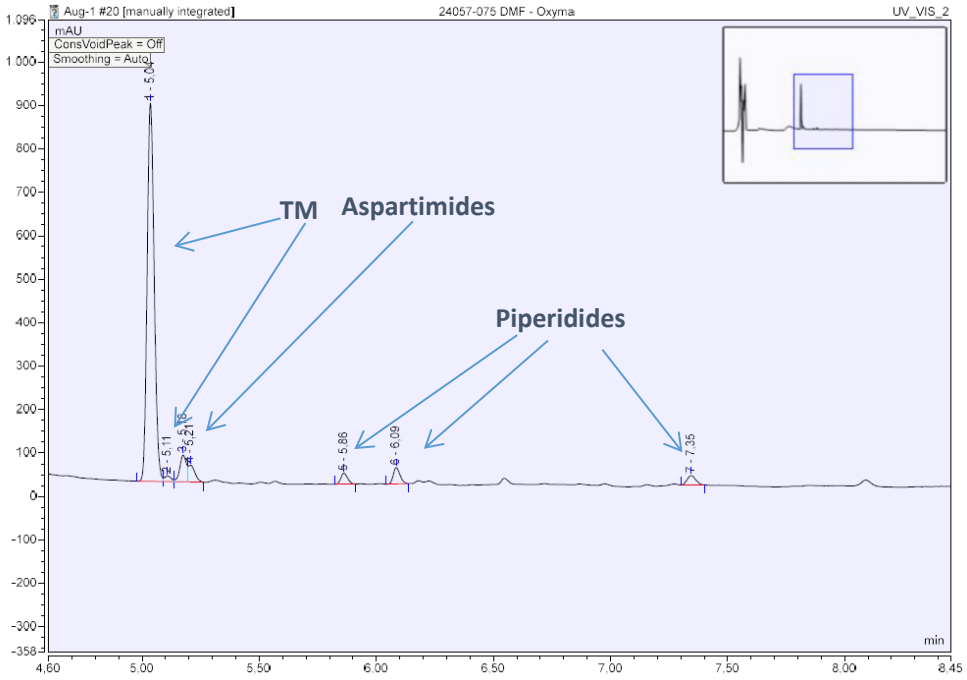


Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in NFM/DOL (2:8), repeated three times, method H

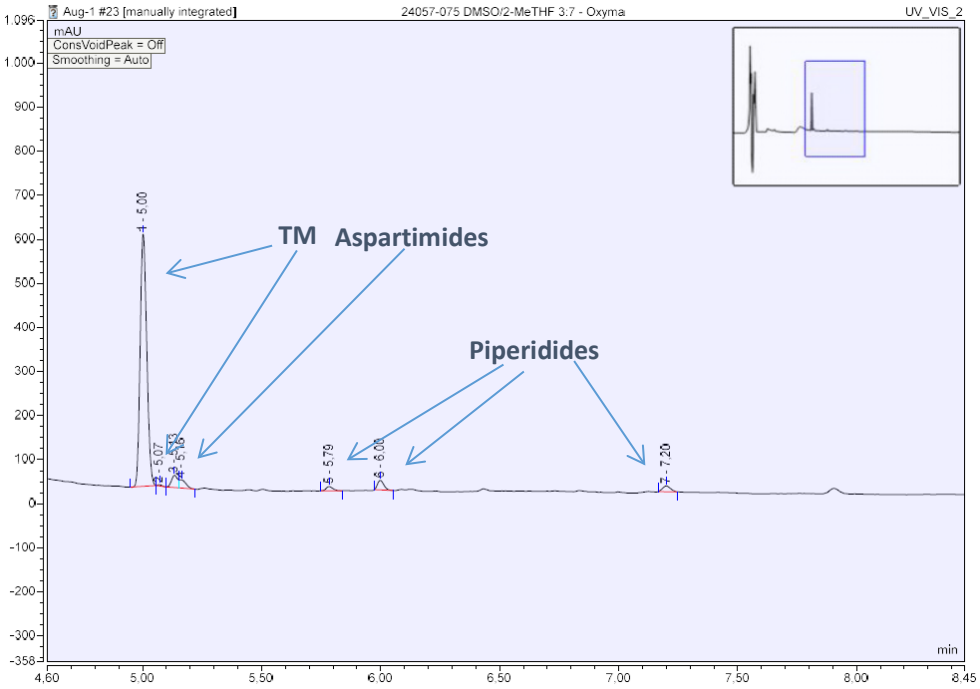


4.5 Aspartimide formation with Oxyma Pure (with O*t*Bu aspartyl protection)

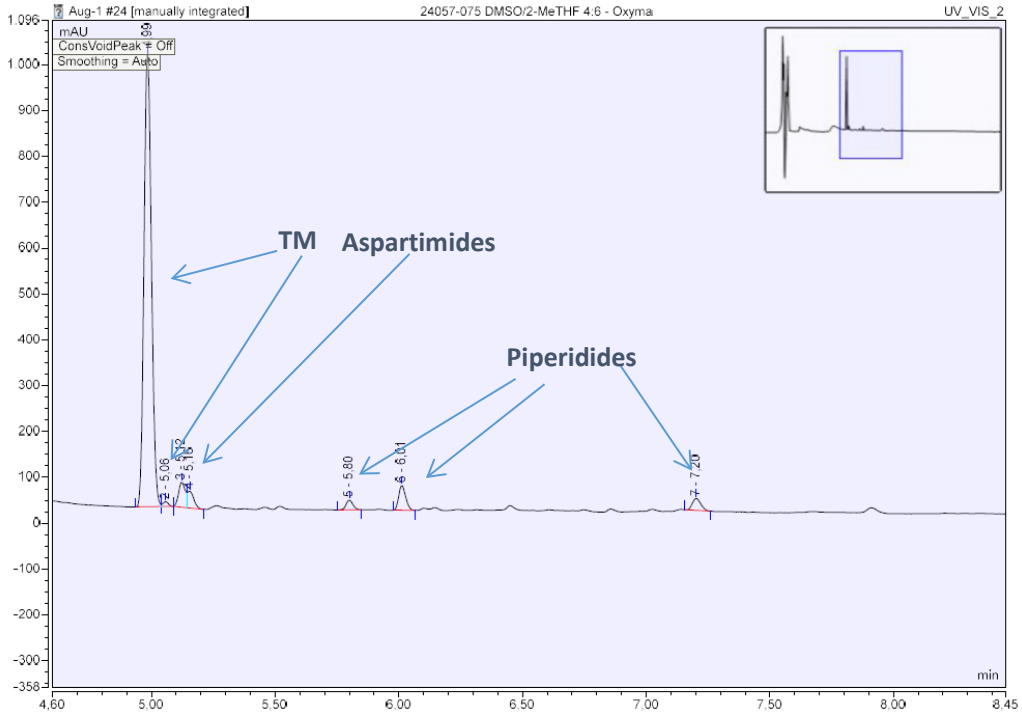
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMF, method H



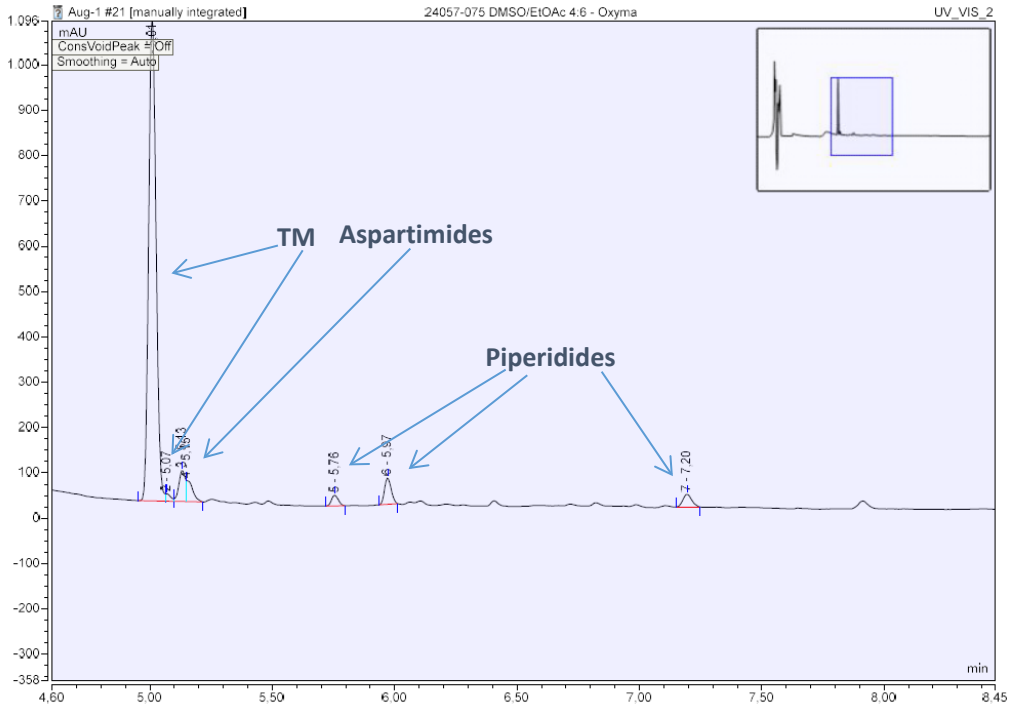
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMSO/2-Me-THF (3:7), method H



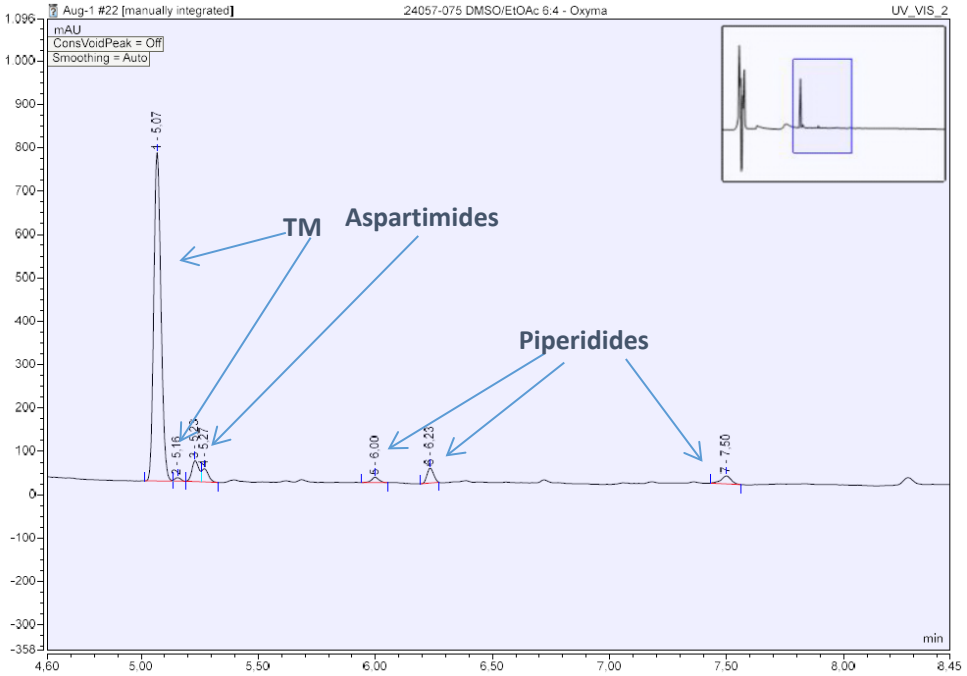
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMSO/2-Me-THF (4:6), method H



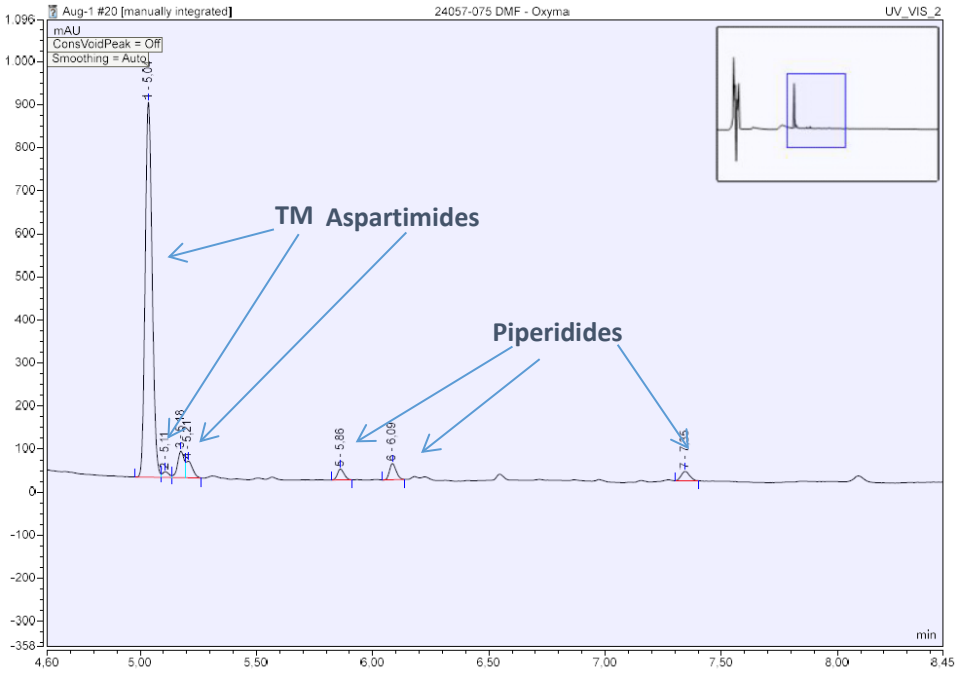
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMSO/EtOAc (4:6), method H



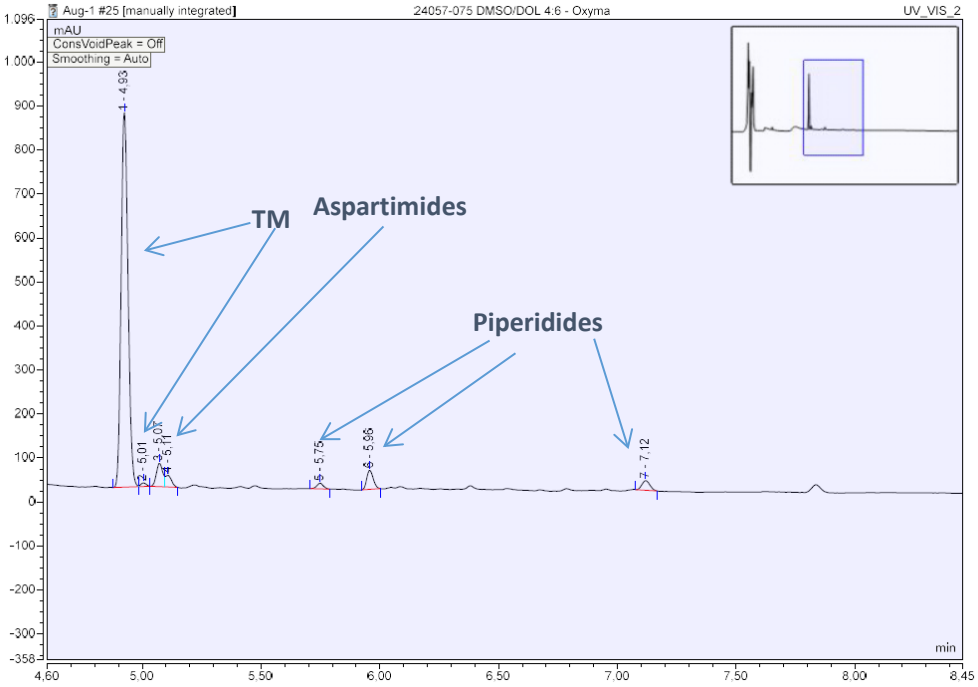
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMSO/EtOAc (6:4), method H



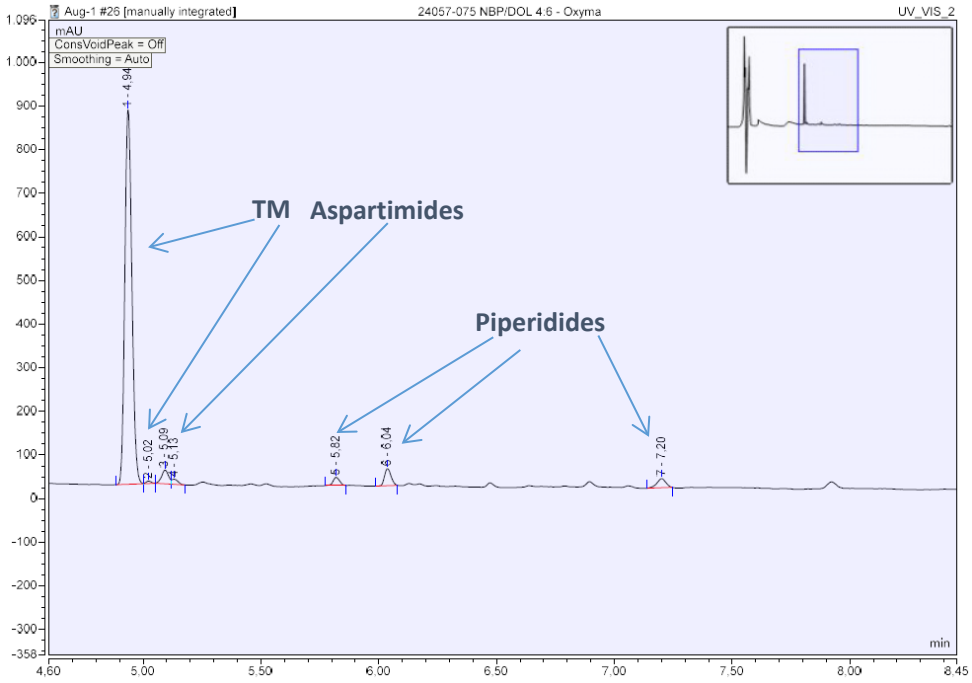
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMSO/DOL (3:7), method H



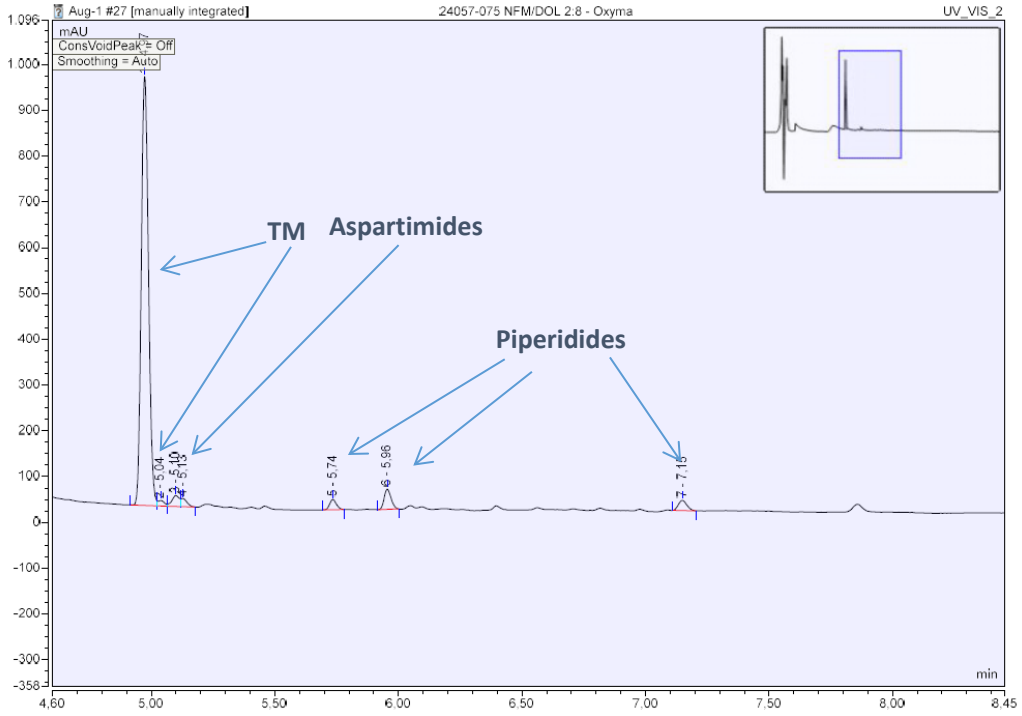
Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in DMSO/DOL (4:6), method H



Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in NBP/DOL (4:6), method H

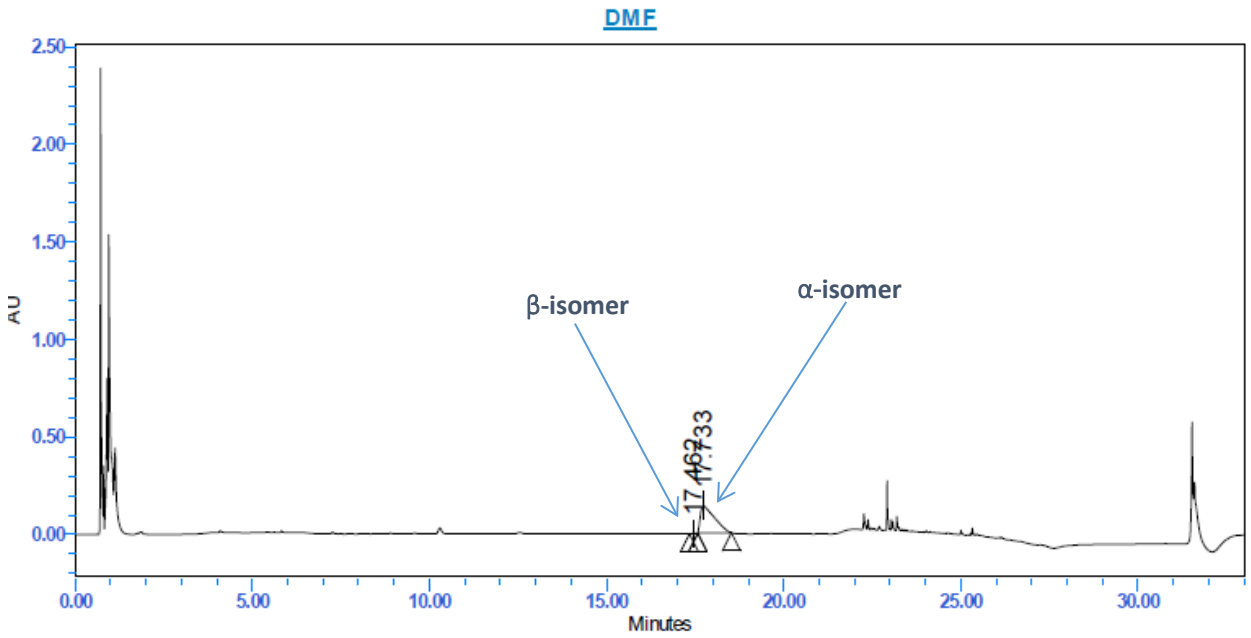


Aspartimide formation without Oxyma Pure (with OtBu aspartyl protection) in NFM/DOL (2:8), method H



4.6 Aspartimide formation with Oxyma Pure (with O*t*Bu aspartyl protection) (*α*-*β*-isomerisation)

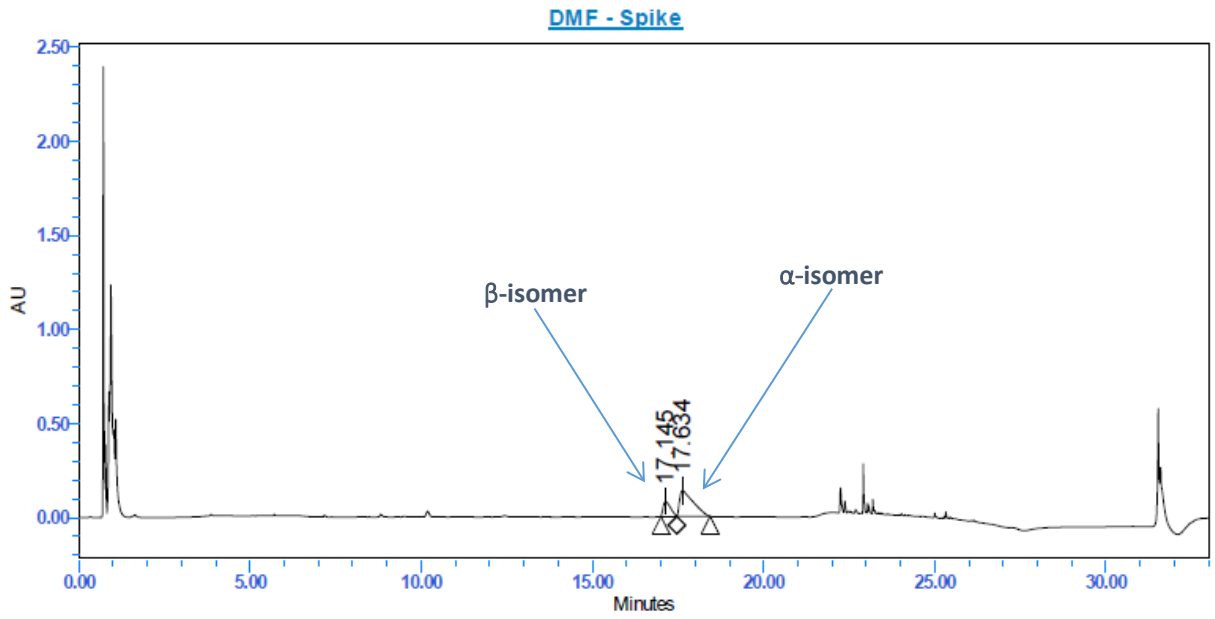
Aspartimide formation without Oxyma Pure (*α*-*β*-isomerisation) in DMF, without Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.462	12681	1915		0.33
2		17.733	3860004	140419		99.67

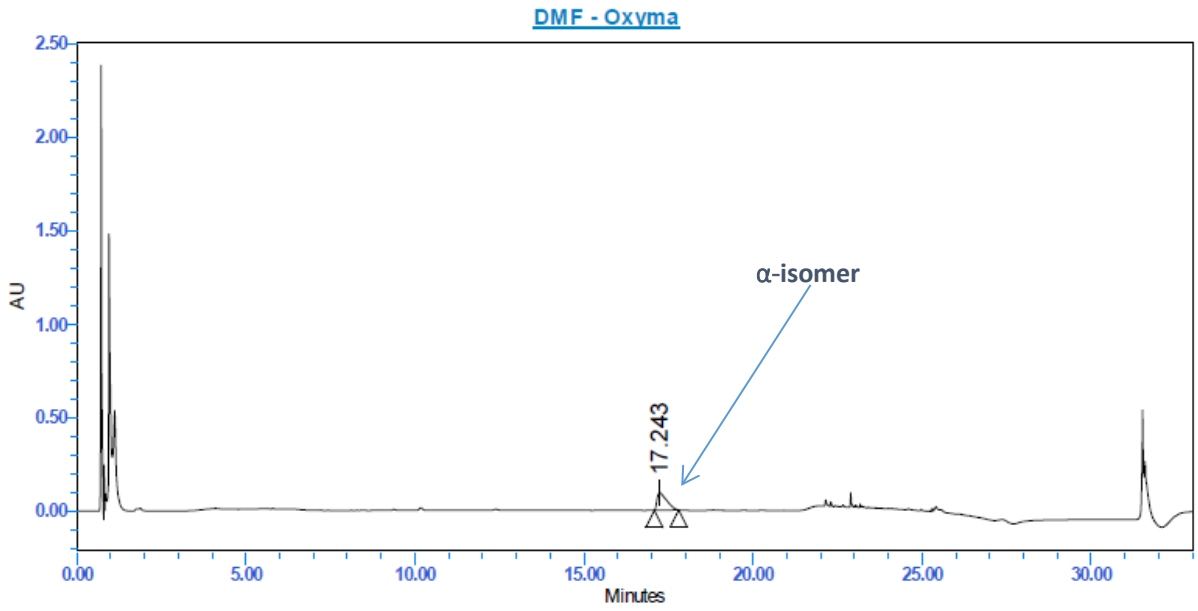
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMF, without Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.145	1171612	79577		23.68
2		17.634	3776602	137295		76.32

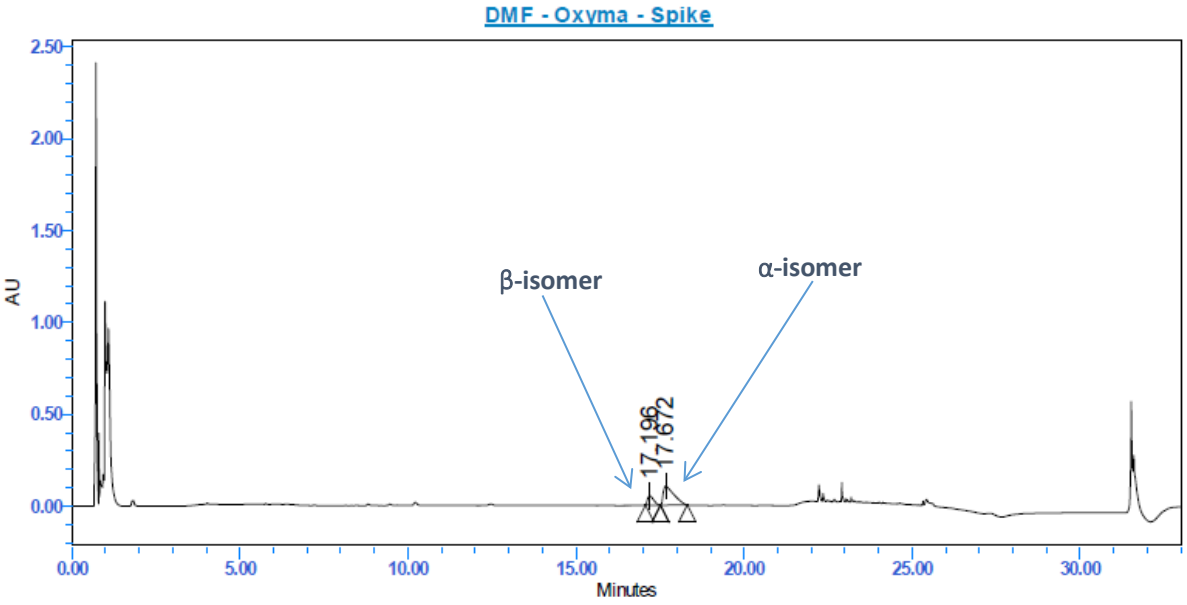
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMF, with Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.243	1849837	93727		100.00

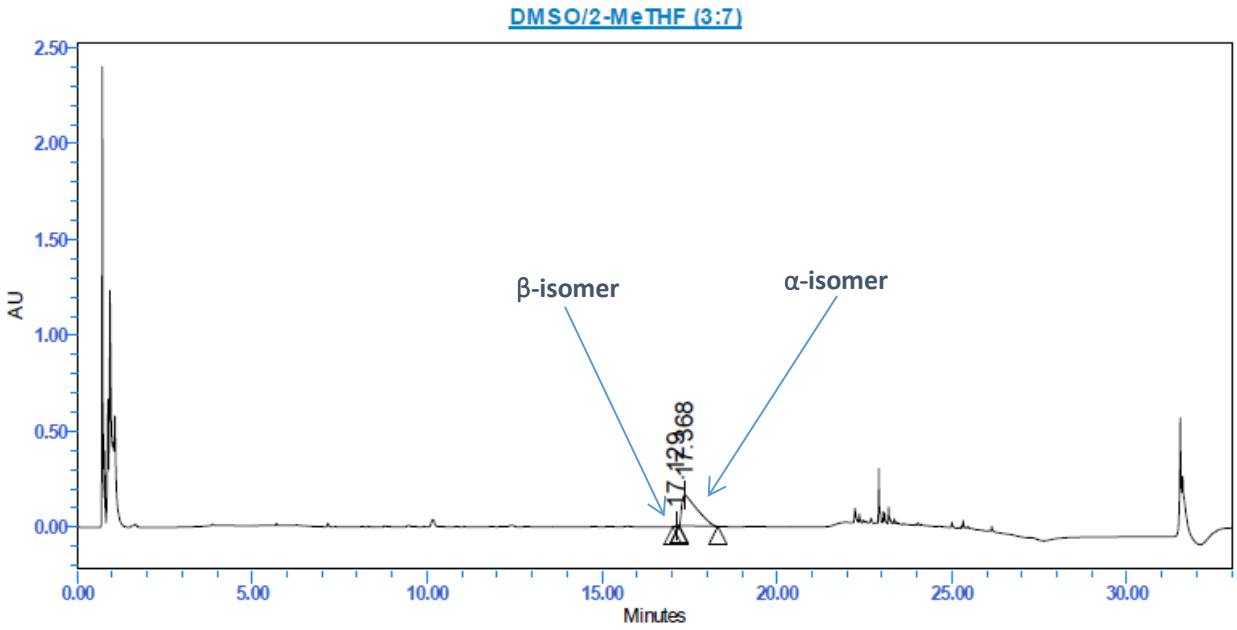
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMF, with Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.196	638883	51100		21.82
2		17.672	2288924	104336		78.18

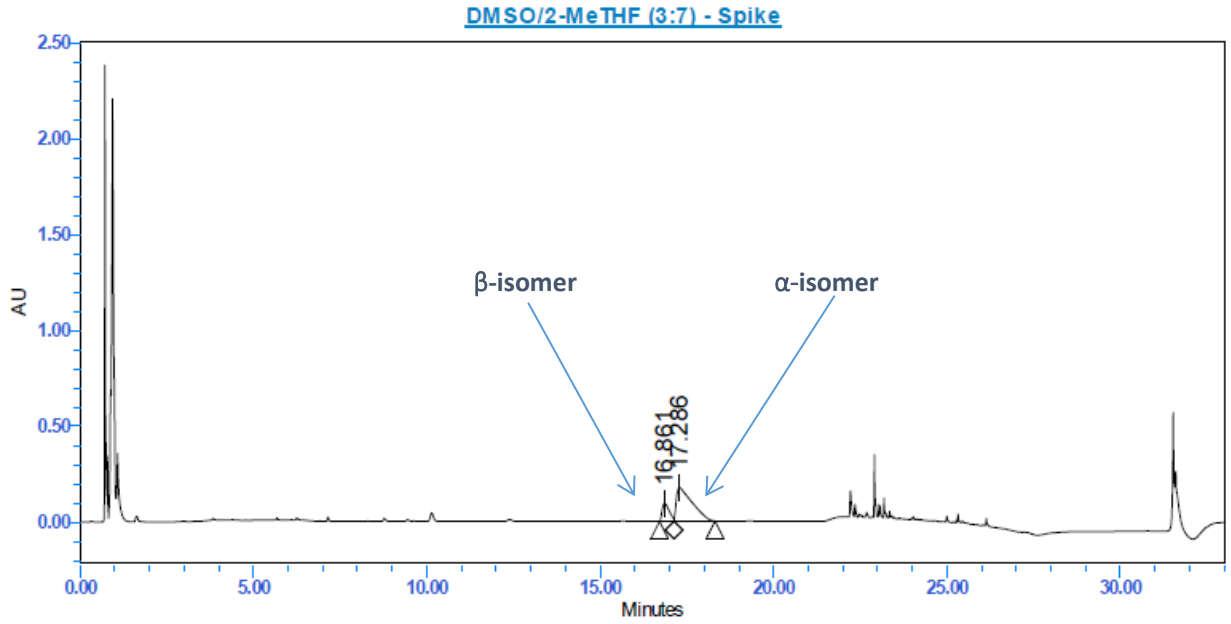
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/2-Me-THF (3:7), without Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.129	16766	3091		0.35
2		17.368	4797040	161187		99.65

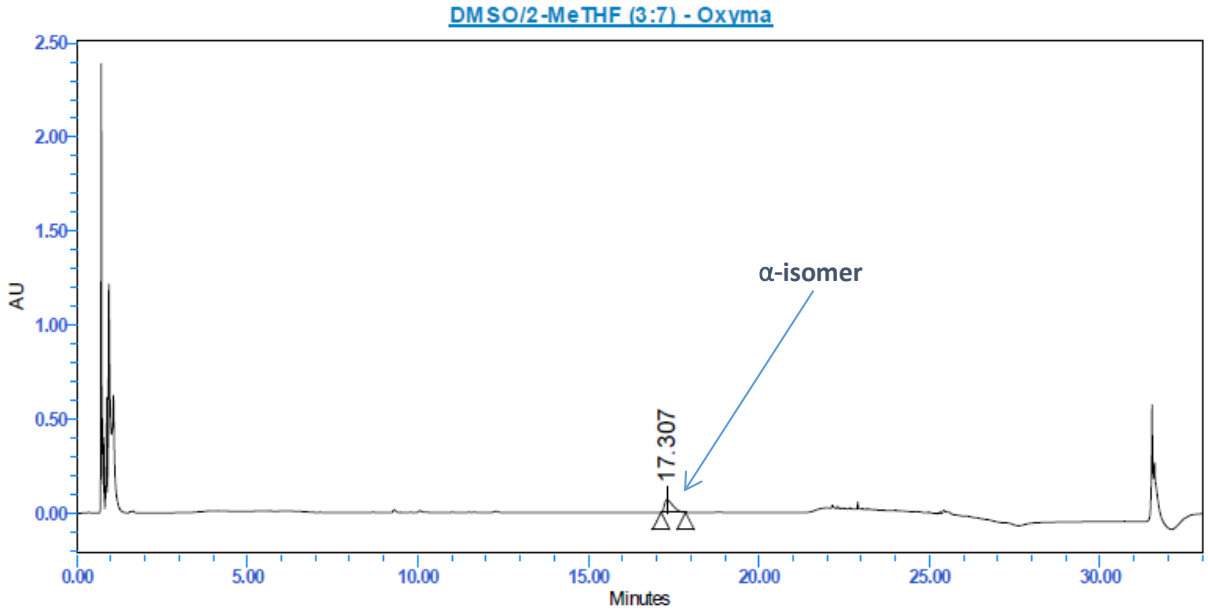
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/2-Me-THF (3:7), without Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		16.861	1324238	92797		19.18
2		17.286	5580584	175853		80.82

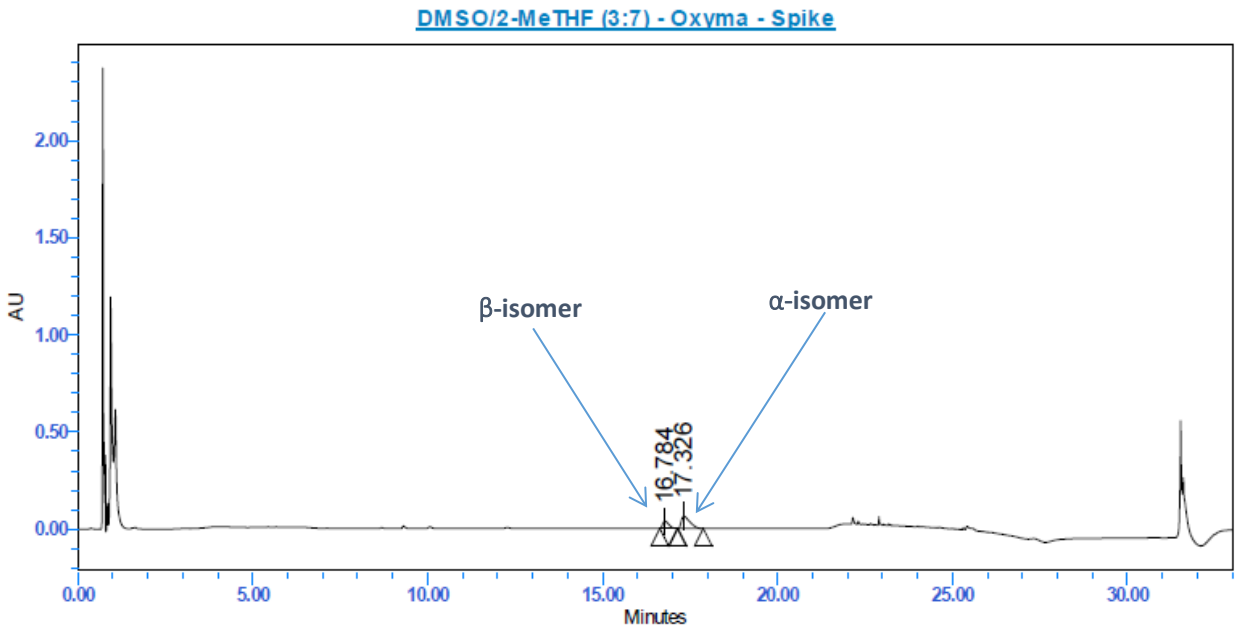
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/2-MeTHF (3:7), with Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.307	1156568	67824		100.00

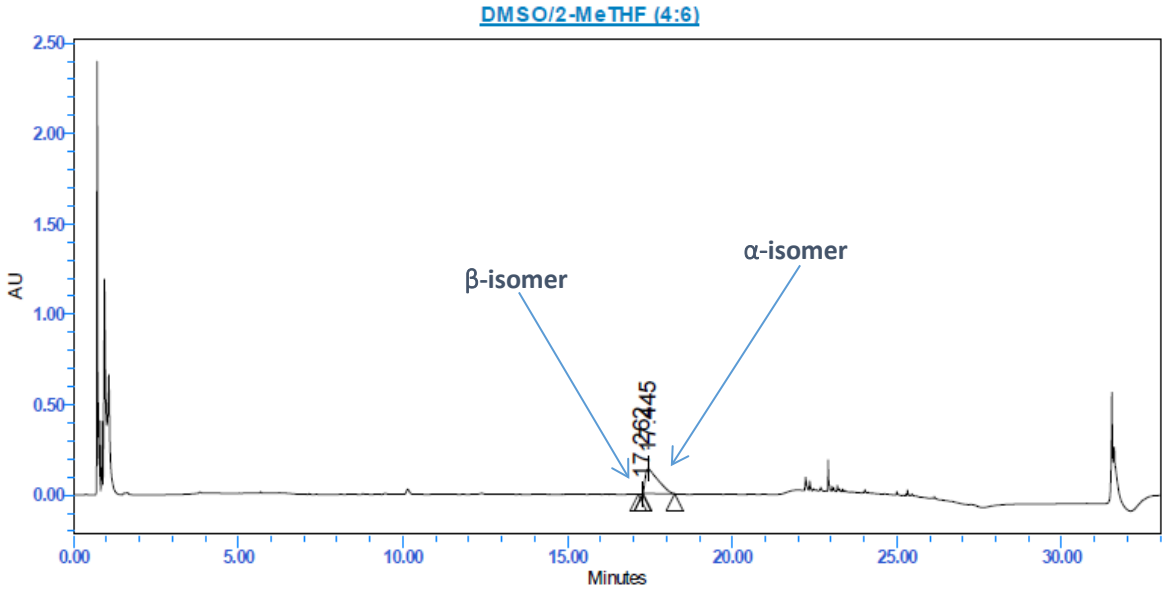
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/2-MeTHF (3:7), with Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		16.784	449504	36881		30.04
2		17.326	1046856	62340		69.96

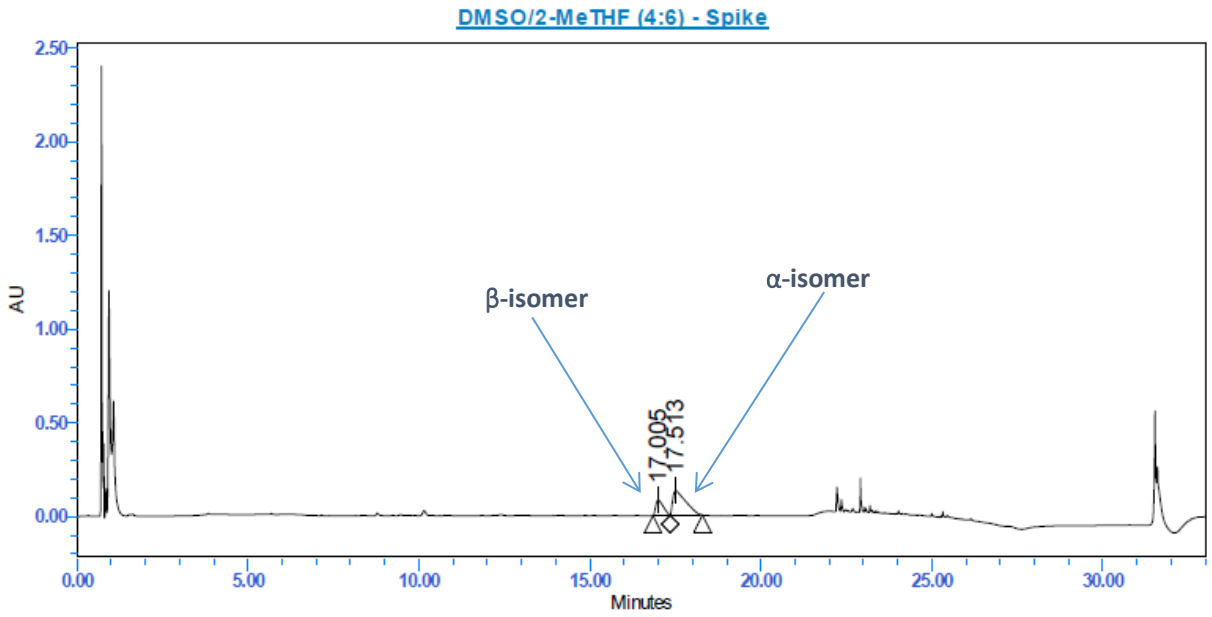
Aspartimide formation without Oxyma Pure (*α*-*β*-isomerisation) in DMSO/2-MeTHF (4:6), without Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.262	512	-192		0.01
2		17.445	3656734	137434		99.99

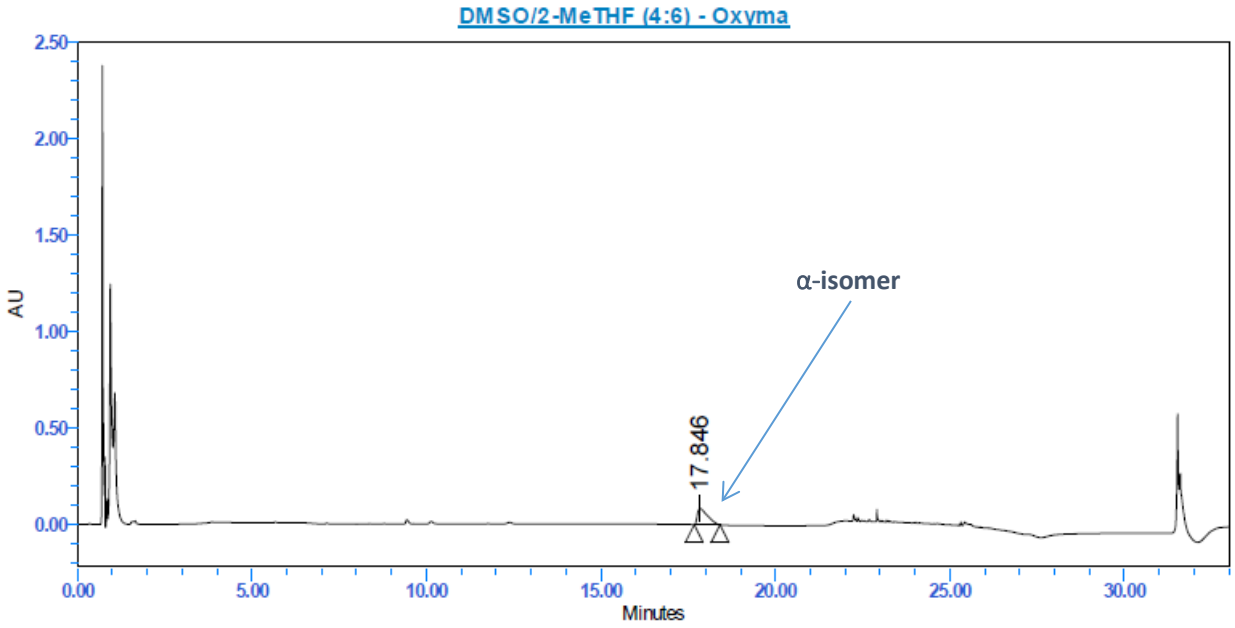
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/2-MeTHF (4:6), without Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.005	1281707	83072		26.72
2		17.513	3515228	133117		73.28

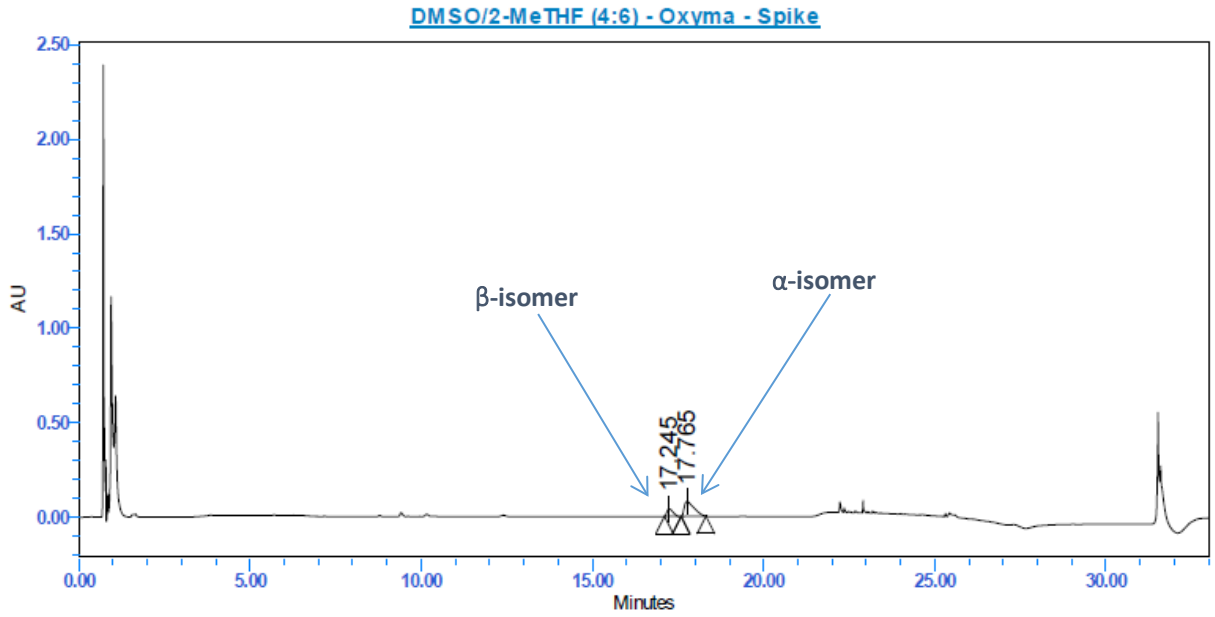
Aspartimide formation without Oxyma Pure (*α*-*β*-isomerisation) in DMSO/2-MeTHF (4:6), with Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.846	1691766	83928		100.00

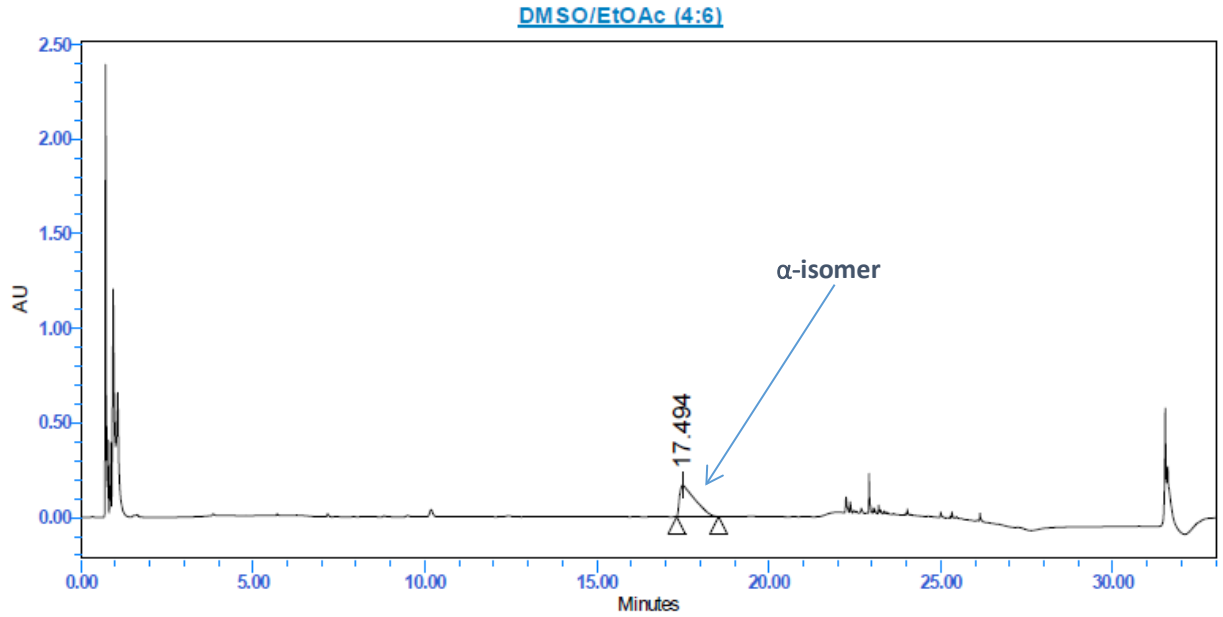
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/2-MeTHF (4:6), with Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.245	487634	39365		23.79
2		17.765	1562346	79204		76.21

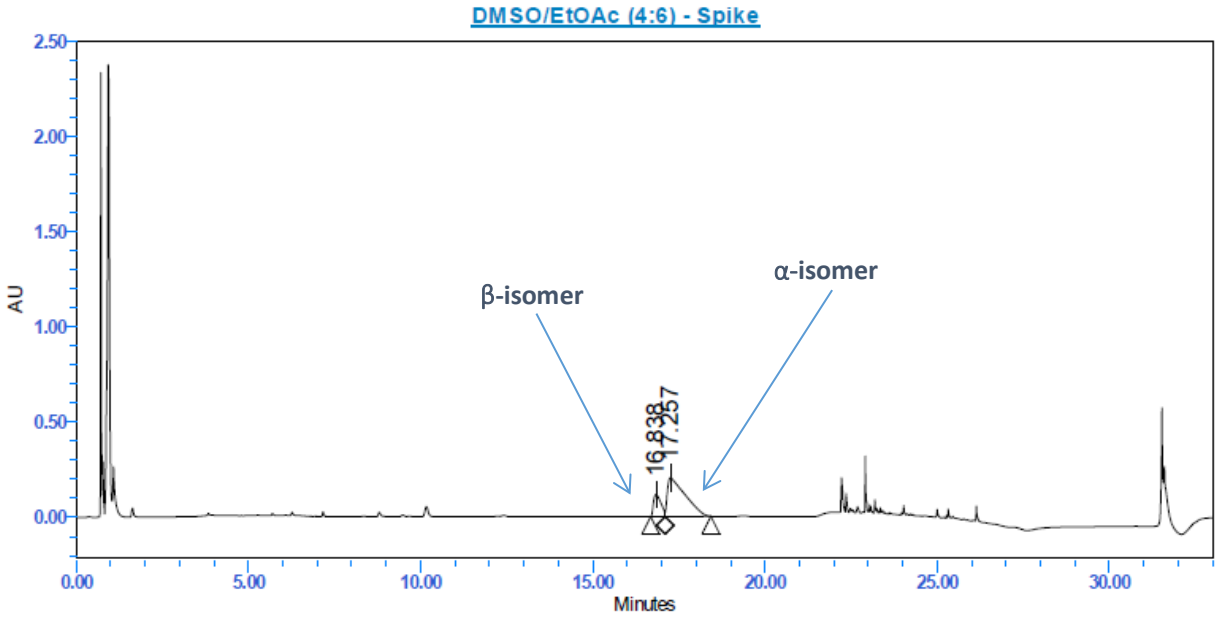
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/EtOAc (4:6), without Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.494	5159581	166304		100.00

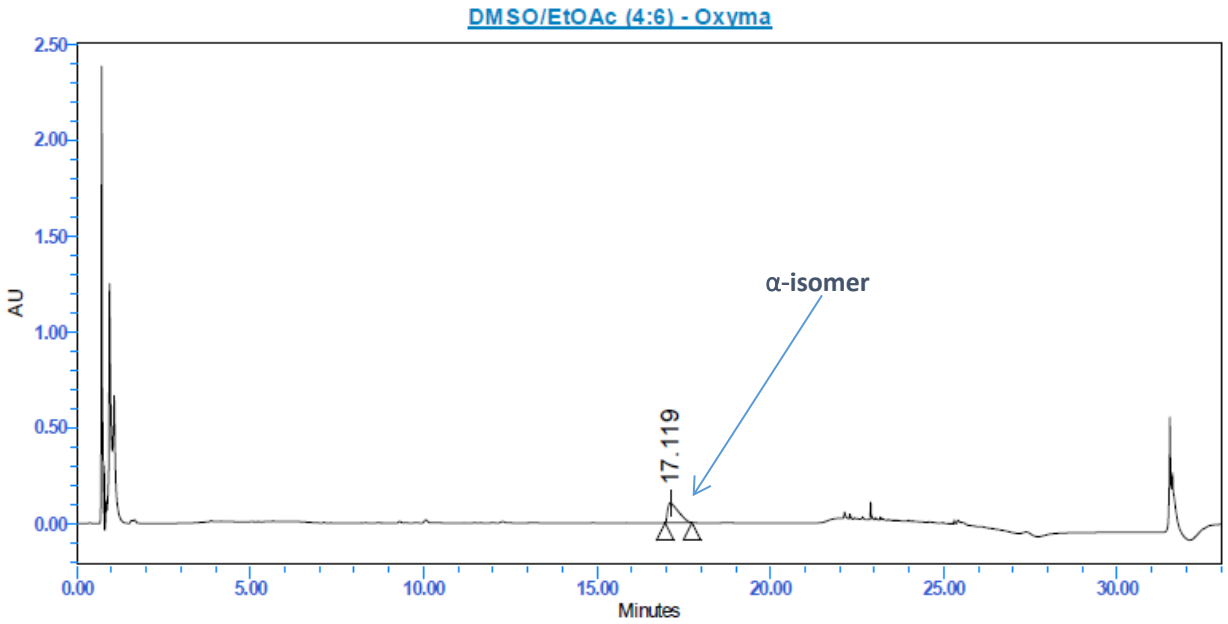
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/EtOAc (4:6), without Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		16.838	1757530	116762		19.37
2		17.257	7316642	204229		80.63

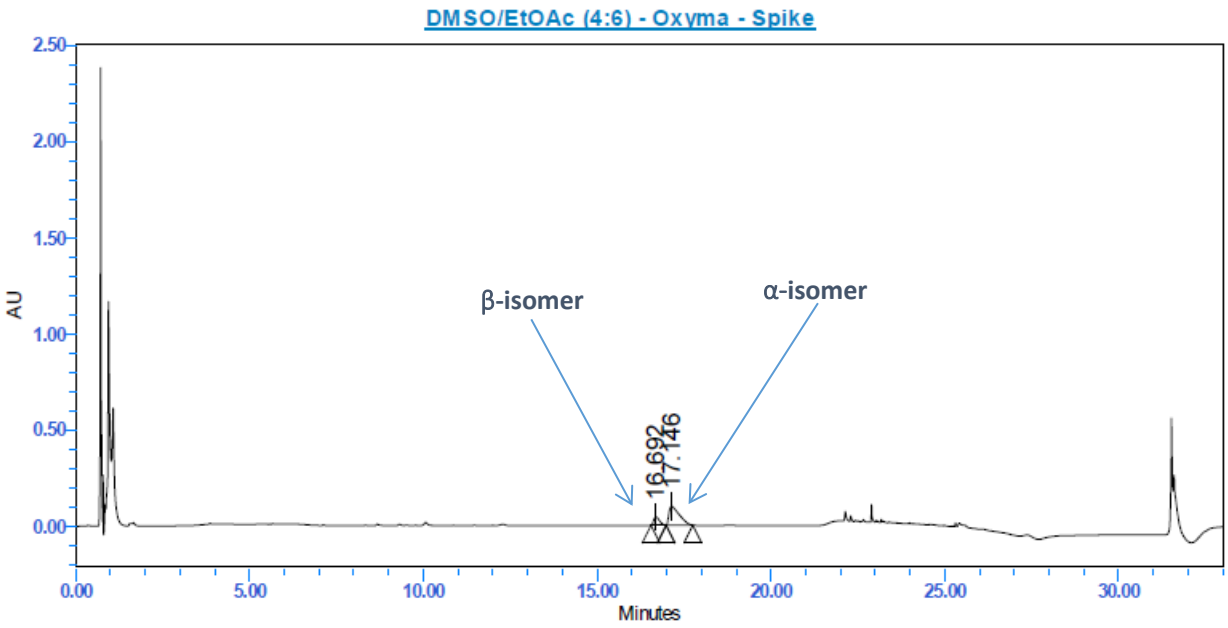
Aspartimide formation without Oxyma Pure (*α*-*β*-isomerisation) in DMSO/EtOAc (4:6), with Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.119	2226938	104851		100.00

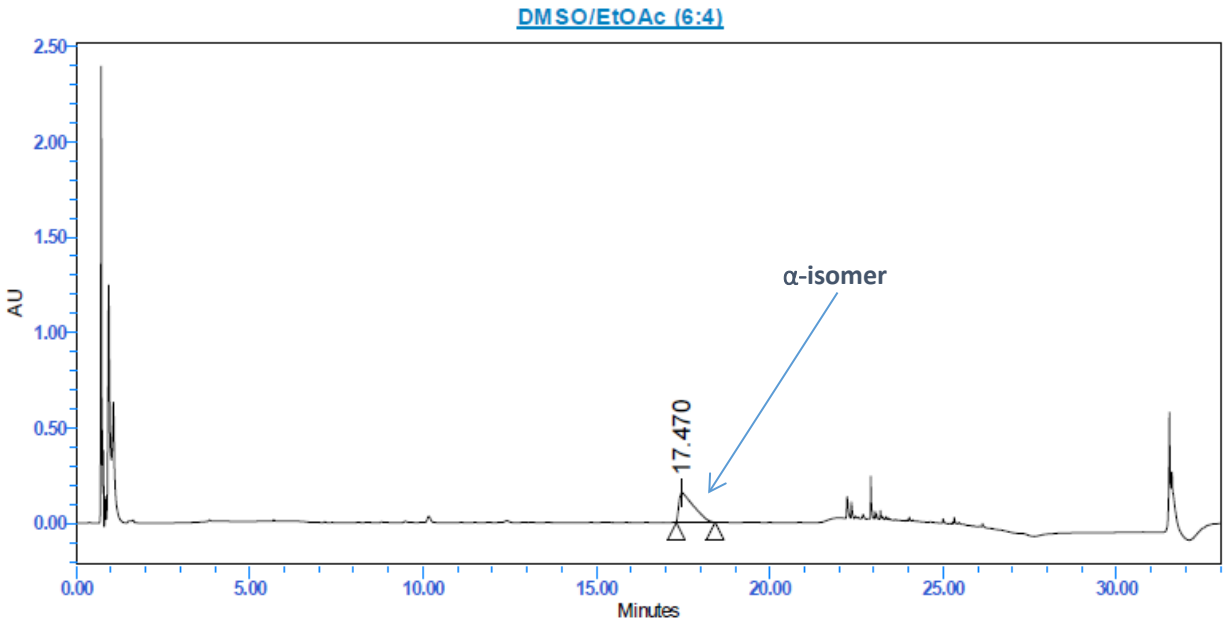
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/EtOAc (4:6), with Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		16.692	506388	43417		19.93
2		17.146	2034735	98954		80.07

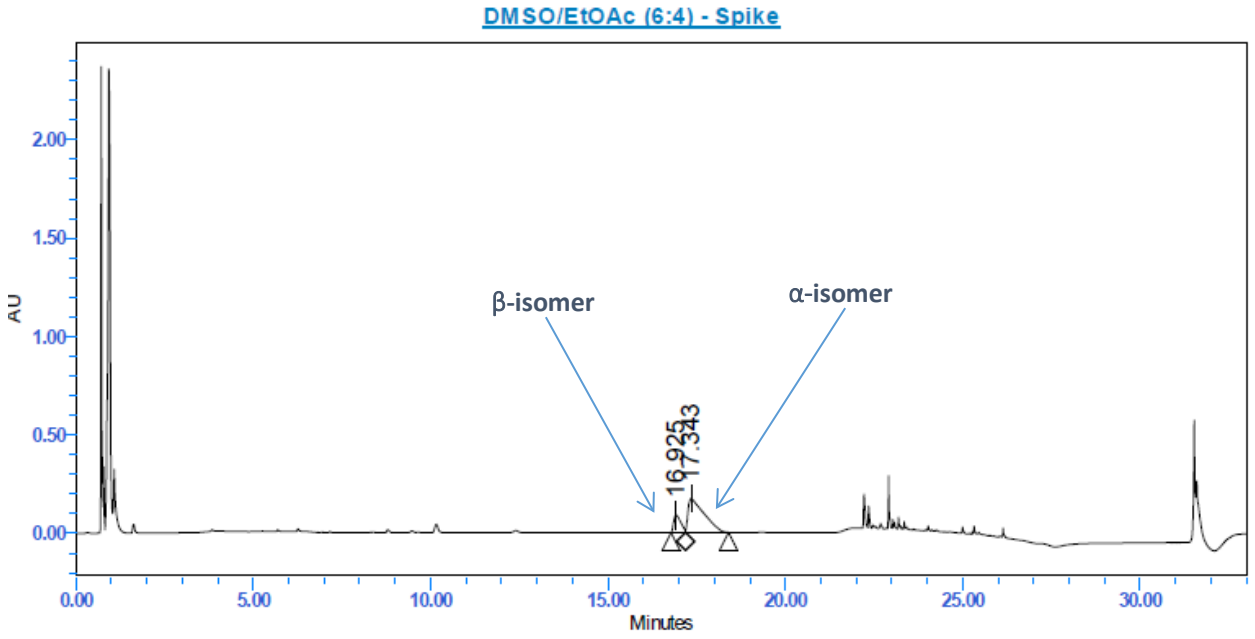
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/EtOAc (6:4), without Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.470	4563623	155171		100.00

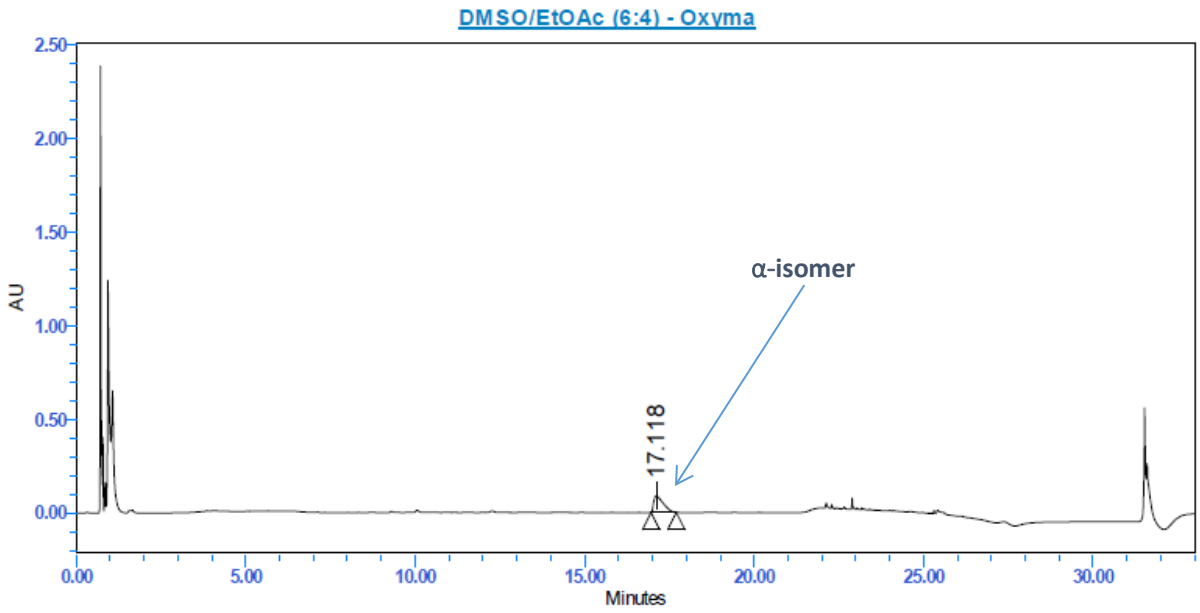
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/EtOAc (6:4), without Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		16.925	1229292	88057		18.48
2		17.343	5422386	172361		81.52

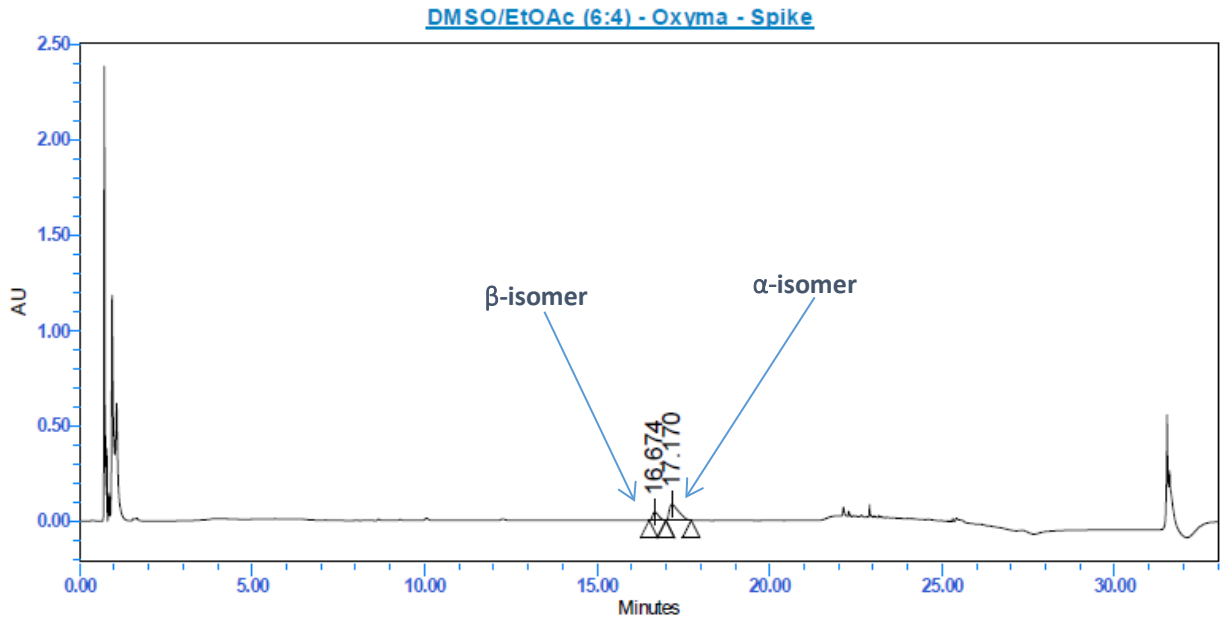
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/EtOAc (6:4), with Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.118	1717037	88794		100.00

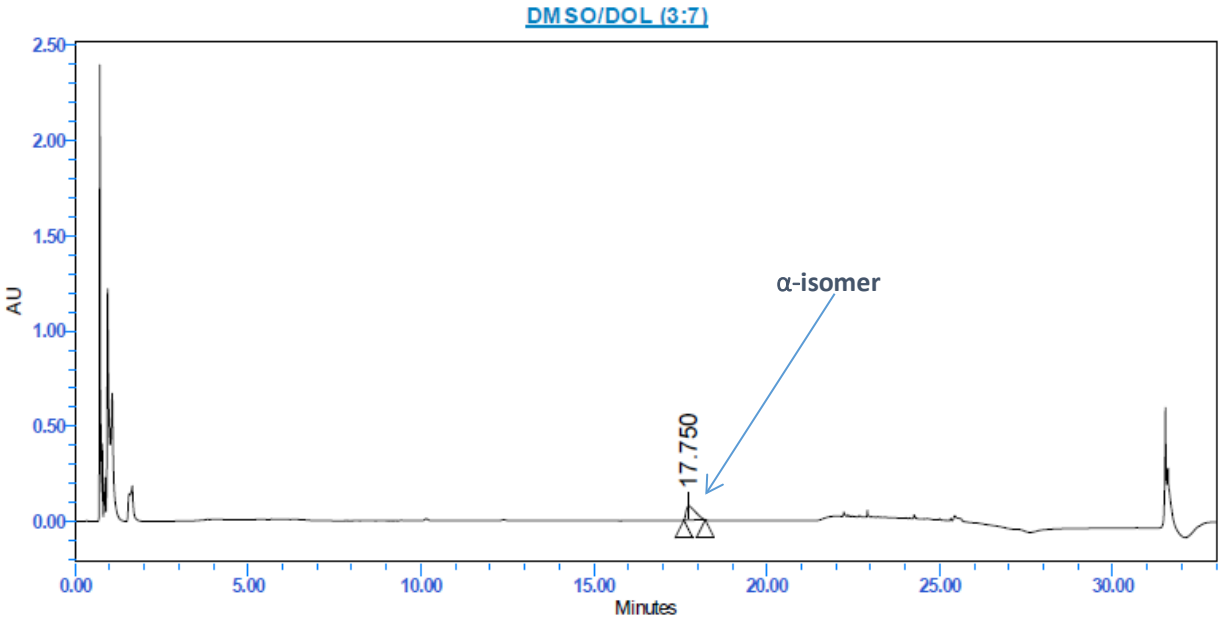
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/EtOAc (6:4), with Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		16.674	525050	43293		24.96
2		17.170	1578201	83723		75.04

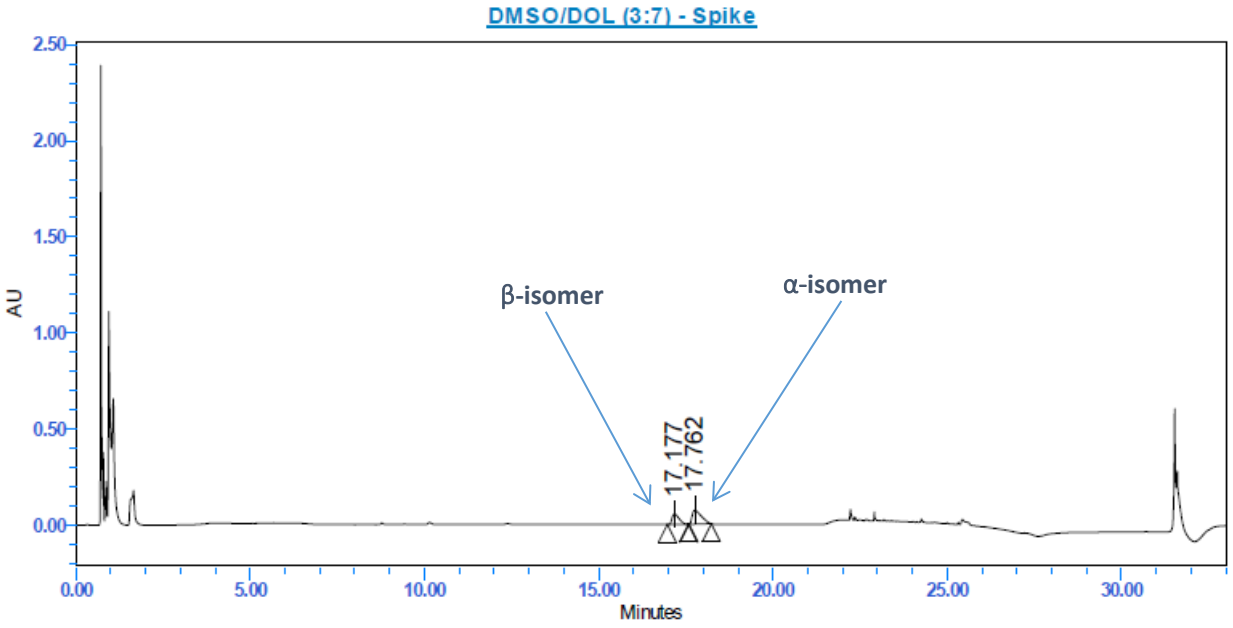
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/DOL (3:7), without Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.750	1386092	75223		100.00

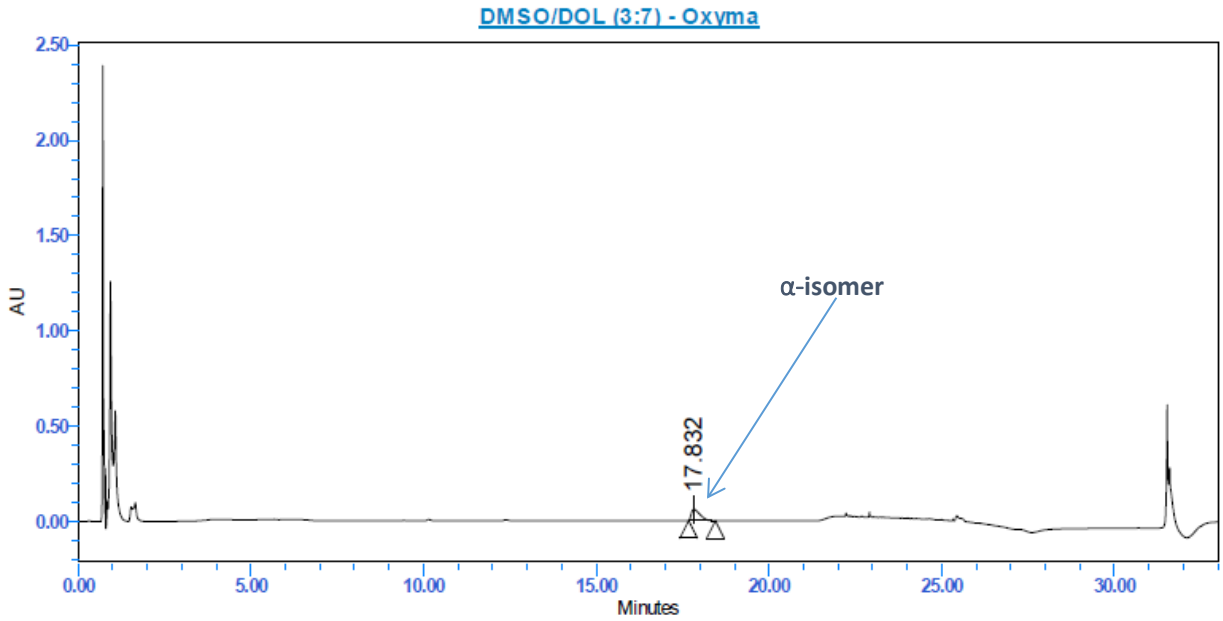
Aspartimide formation without Oxyma Pure (*α*-*β*-isomerisation) in DMSO/DOL (3:7), without Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.177	755524	54245		36.35
2		17.762	1322673	72867		63.65

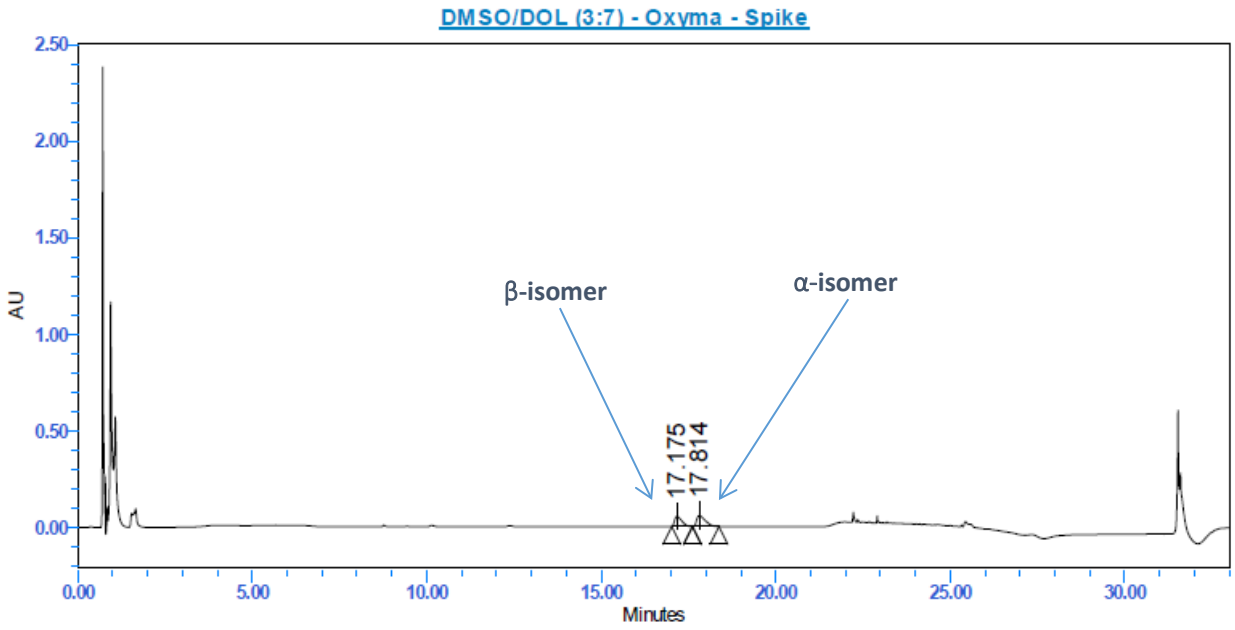
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/DOL (3:7), with Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.832	1033543	59806		100.00

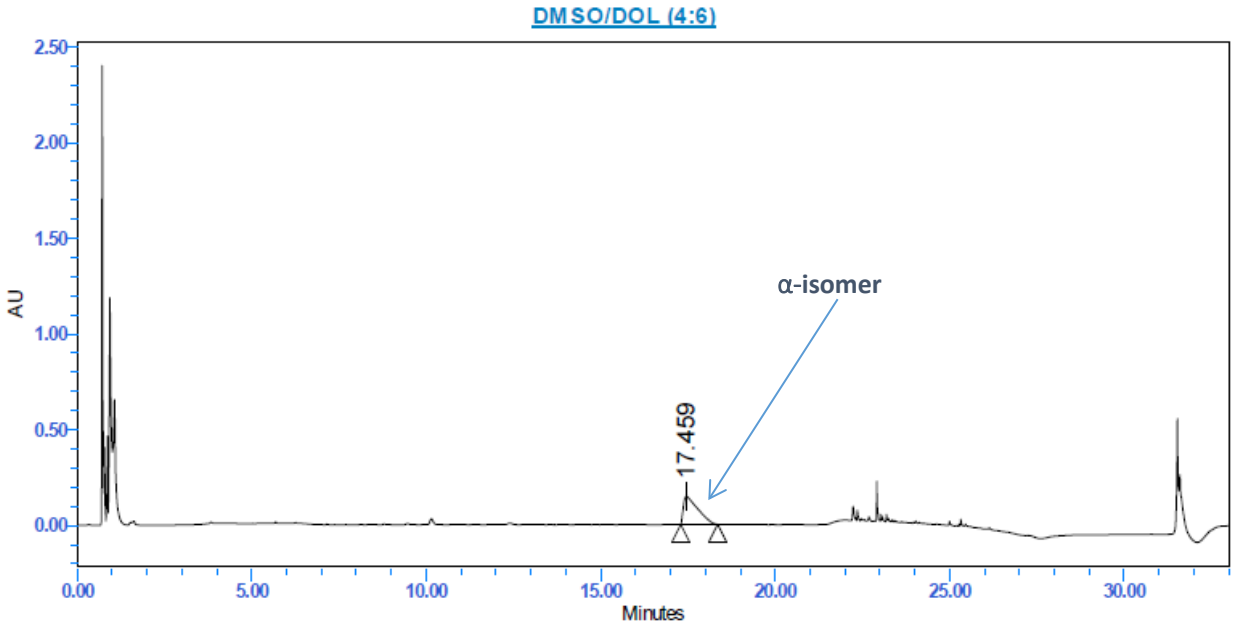
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/DOL (3:7), with Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.175	747863	52203		43.41
2		17.814	974979	56834		56.59

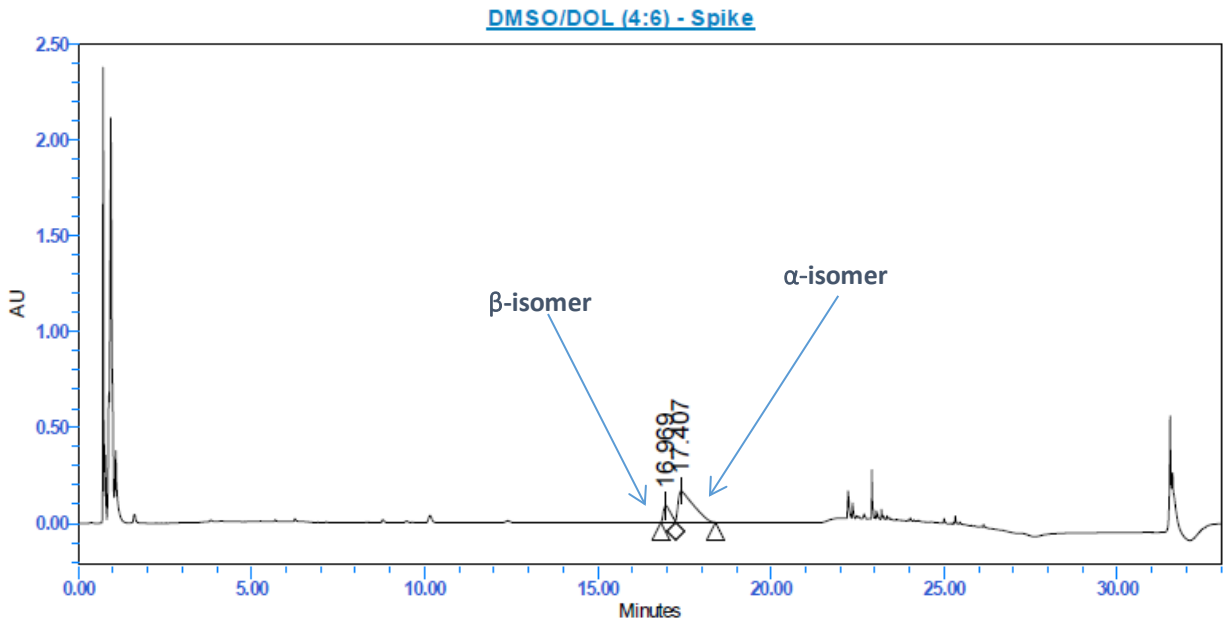
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/DOL (4:6), without Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.459	4201415	147630		100.00

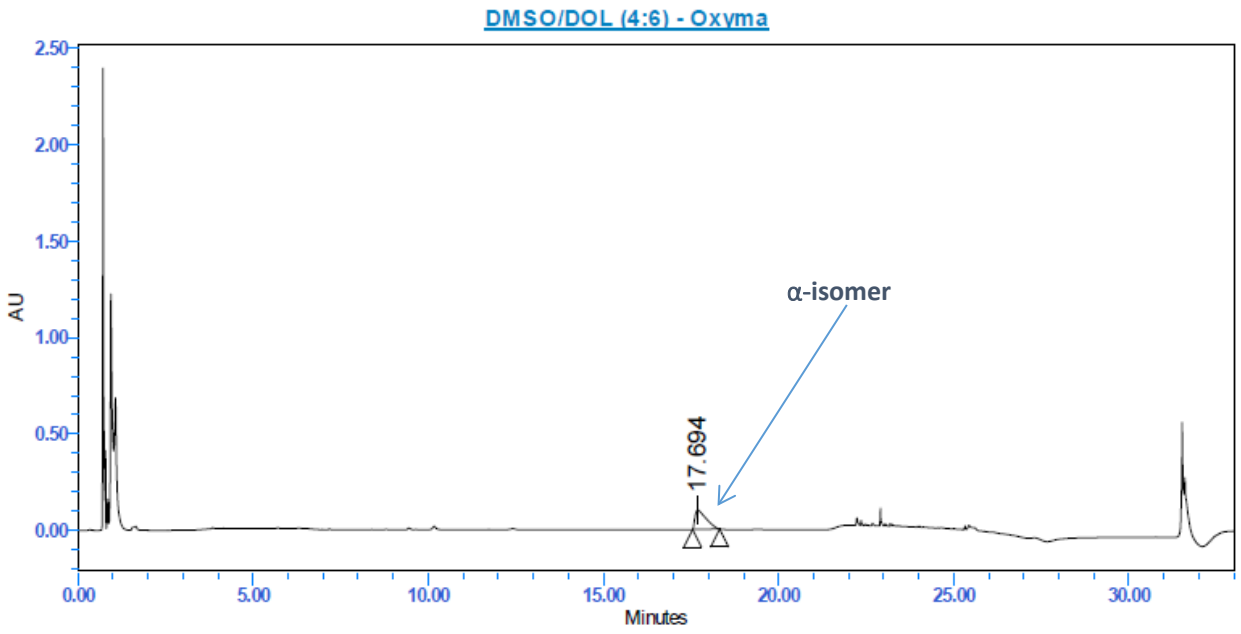
Aspartimide formation without Oxyma Pure (*α*-*β*-isomerisation) in DMSO/DOL (4:6), without Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		16.969	1247628	87235		19.86
2		17.407	5033716	164248		80.14

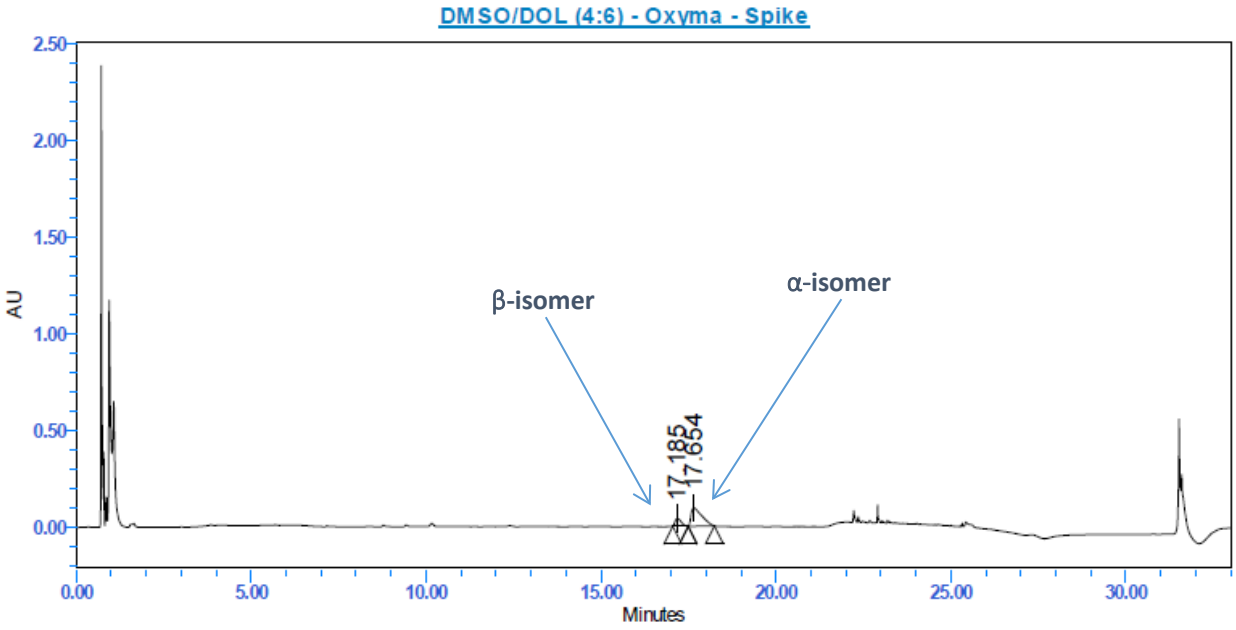
Aspartimide formation without Oxyma Pure (*α*-*β*-isomerisation) in DMSO/DOL (4:6), with Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.694	2193602	99834		100.00

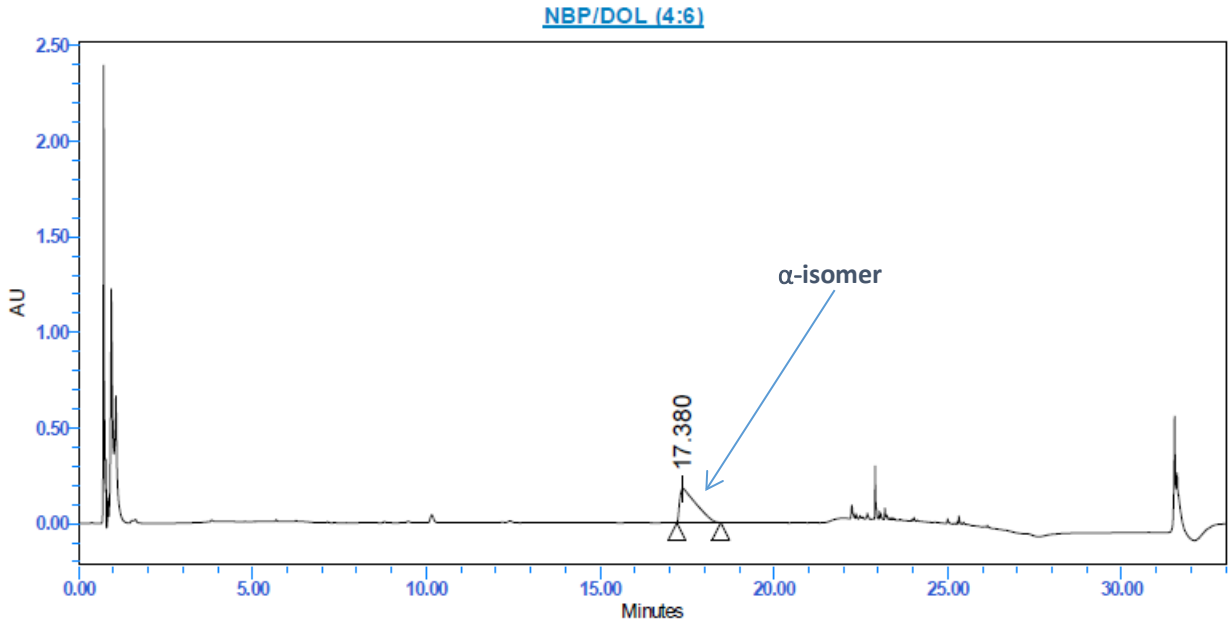
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in DMSO/DOL (4:6), with Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.185	491979	40905		19.40
2		17.654	2044173	95756		80.60

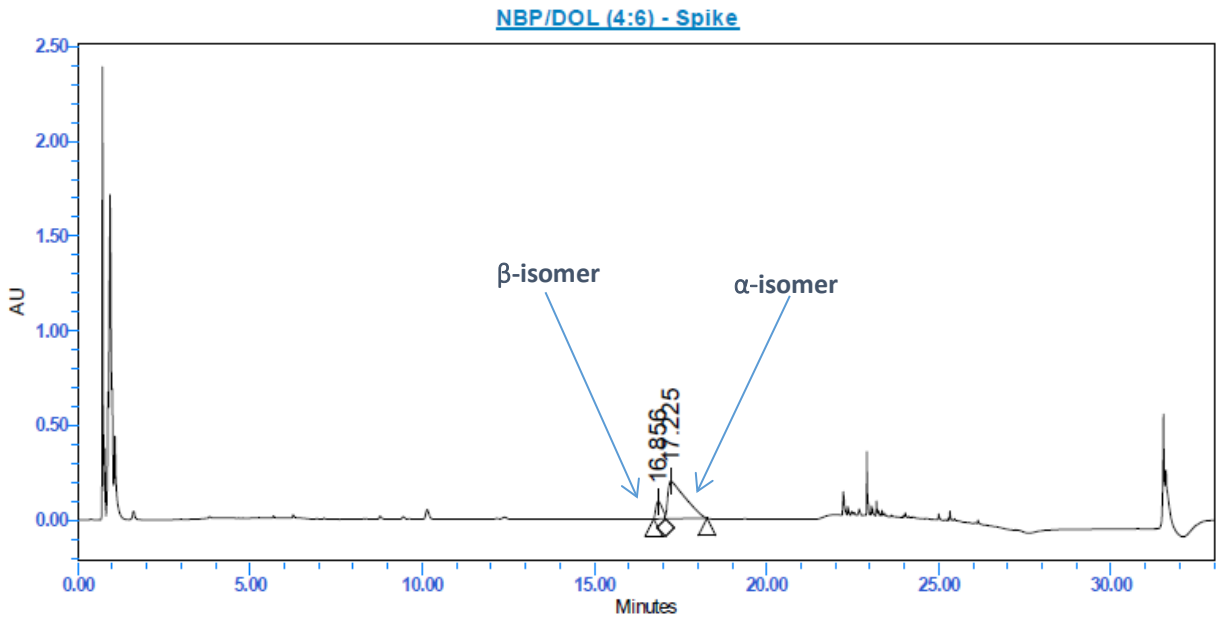
Aspartimide formation without Oxyma Pure (*α*-*β*-isomerisation) in NBP/DOL (4:6), without Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.380	5823822	178695		100.00

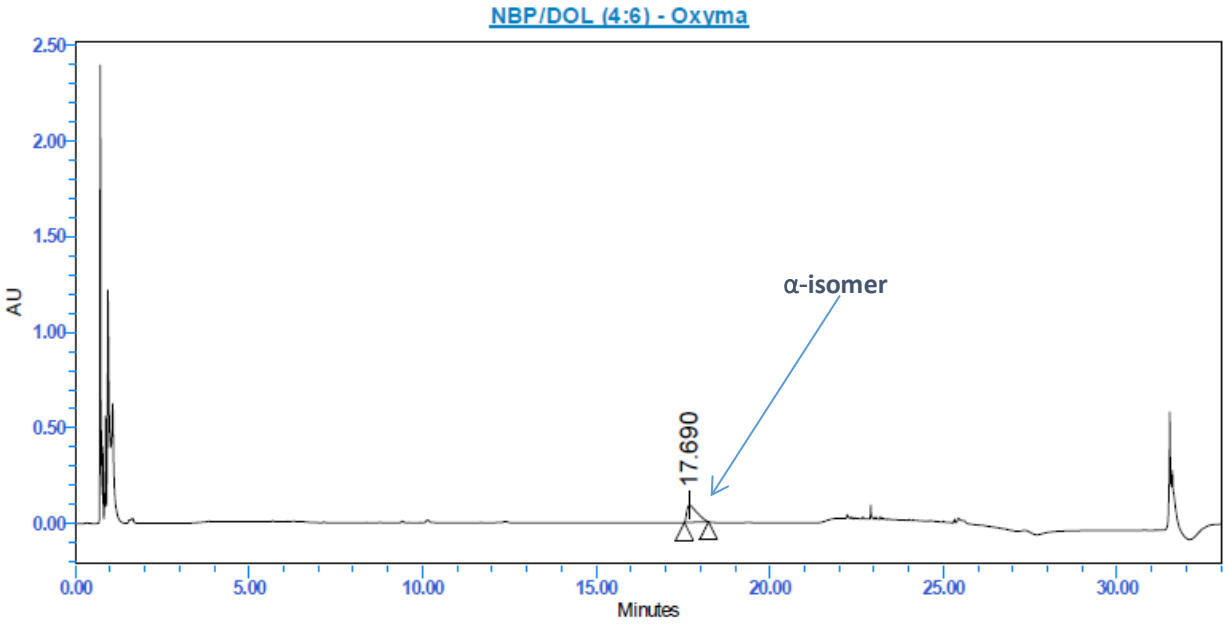
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in NBP/DOL (4:6), without Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		16.856	1182887	91919		14.67
2		17.225	6881198	199176		85.33

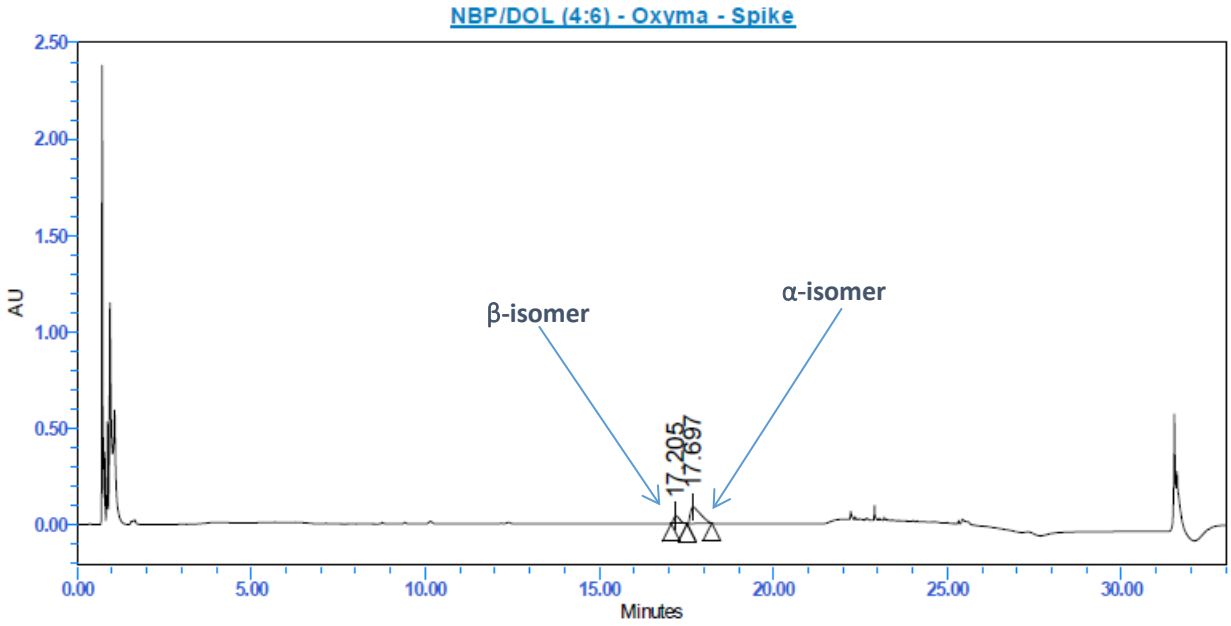
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in NBP/DOL (4:6), with Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.690	1829996	89985		100.00

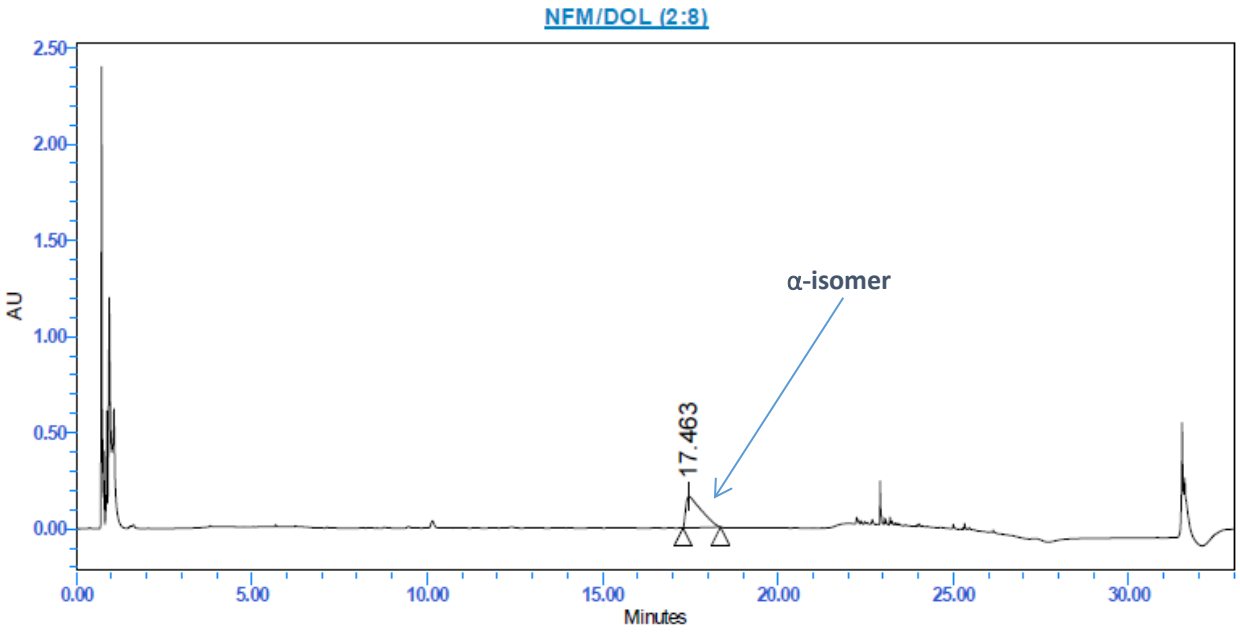
Aspartimide formation without Oxyma Pure (*α*-*β*-isomerisation) in NBP/DOL (4:6), with Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.205	487826	40254		22.00
2		17.697	1729336	86222		78.00

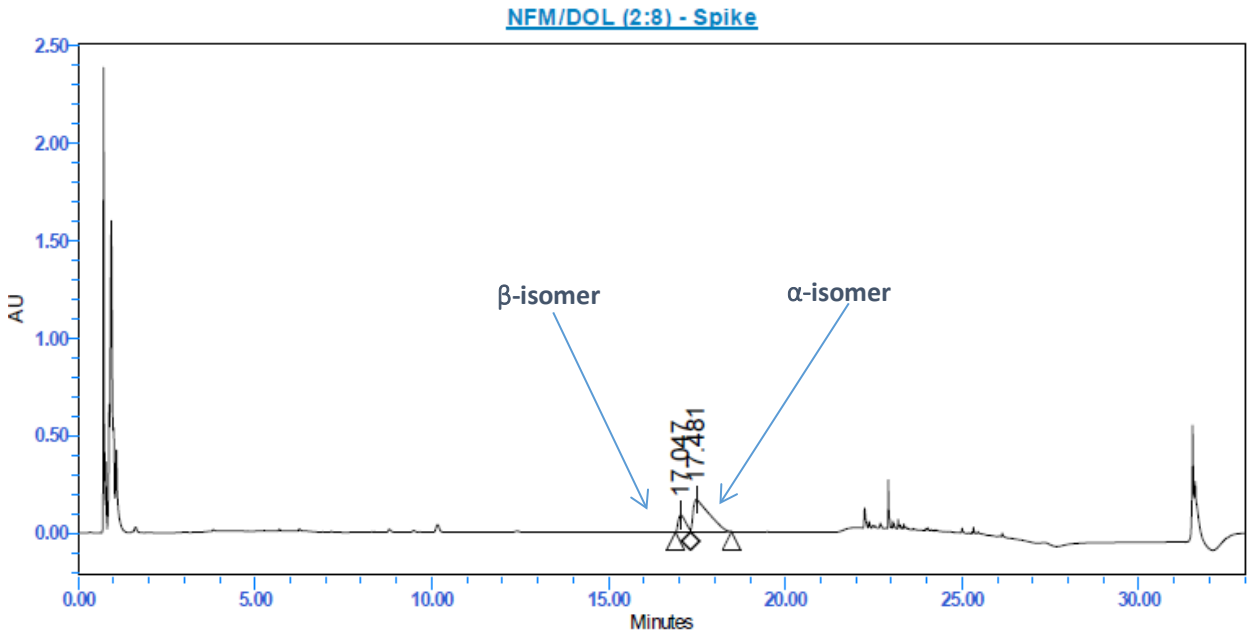
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in NFM/DOL (2:8), without Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.463	4989417	164683		100.00

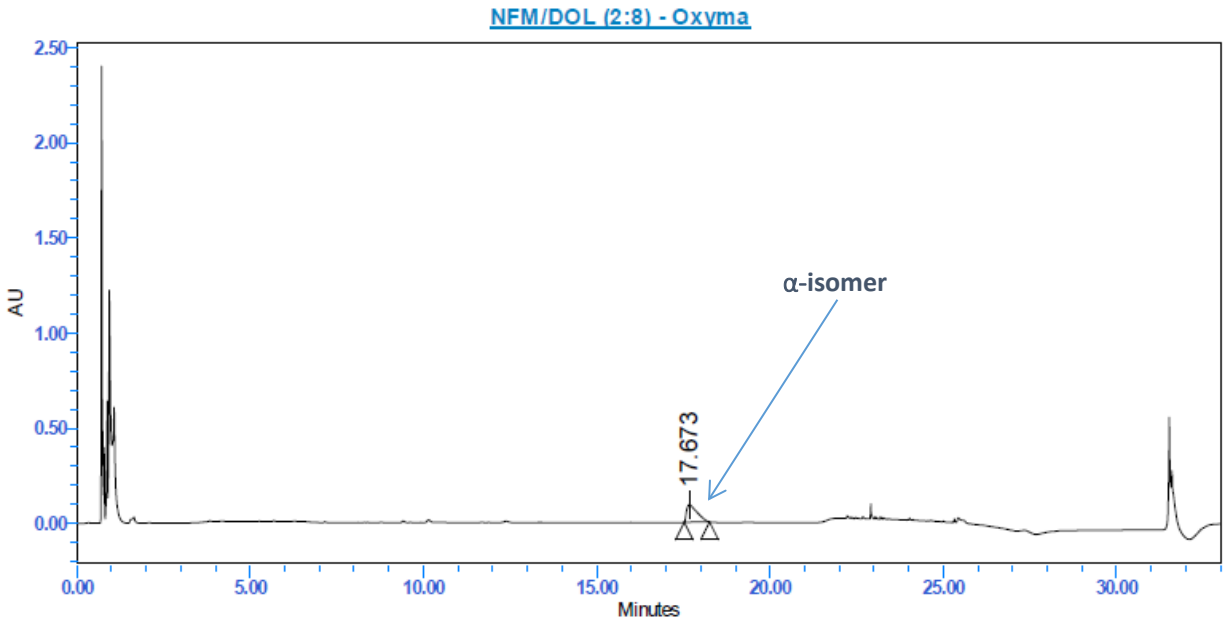
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in NFM/DOL (2:8), without Oxyma, with spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.047	1270863	87961		19.44
2		17.481	5265811	167063		80.56

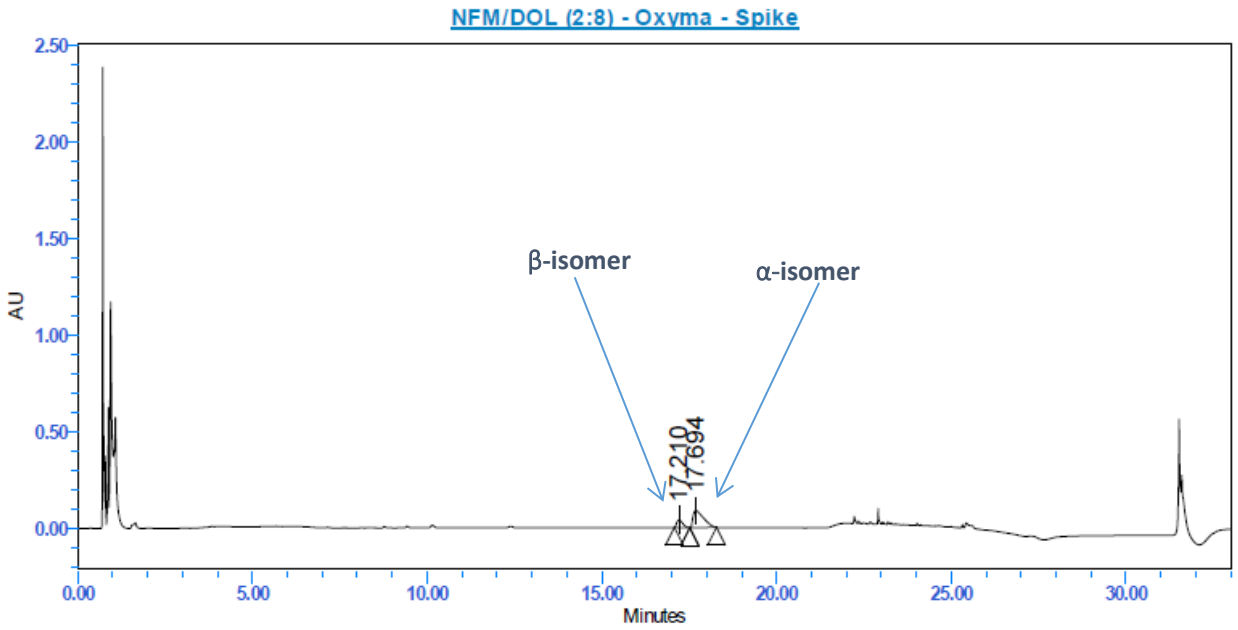
Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in NFM/DOL (2:8), with Oxyma, no spike, method H



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.673	1928392	92132		100.00

Aspartimide formation without Oxyma Pure (*α-β*-isomerisation) in NFM/DOL (2:8), with Oxyma, with spike, method H

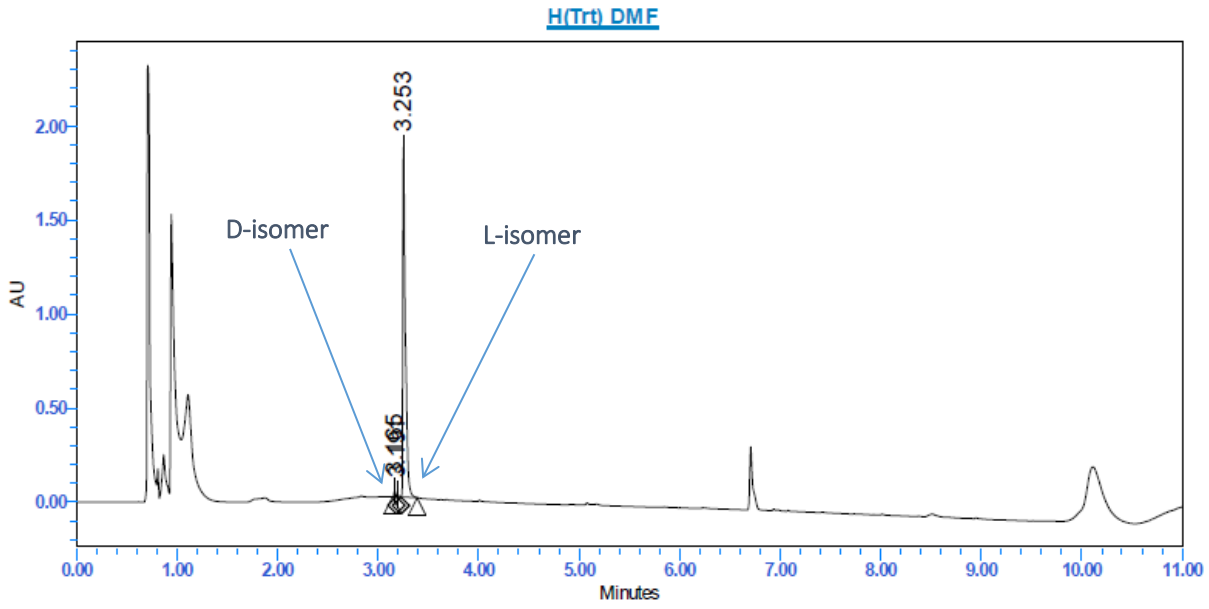


Peak Results

	Name	RT	Area	Height	Amount	% Area
1		17.210	463772	38938		20.04
2		17.694	1850247	89412		79.96

4.7 Amino acid racemisation

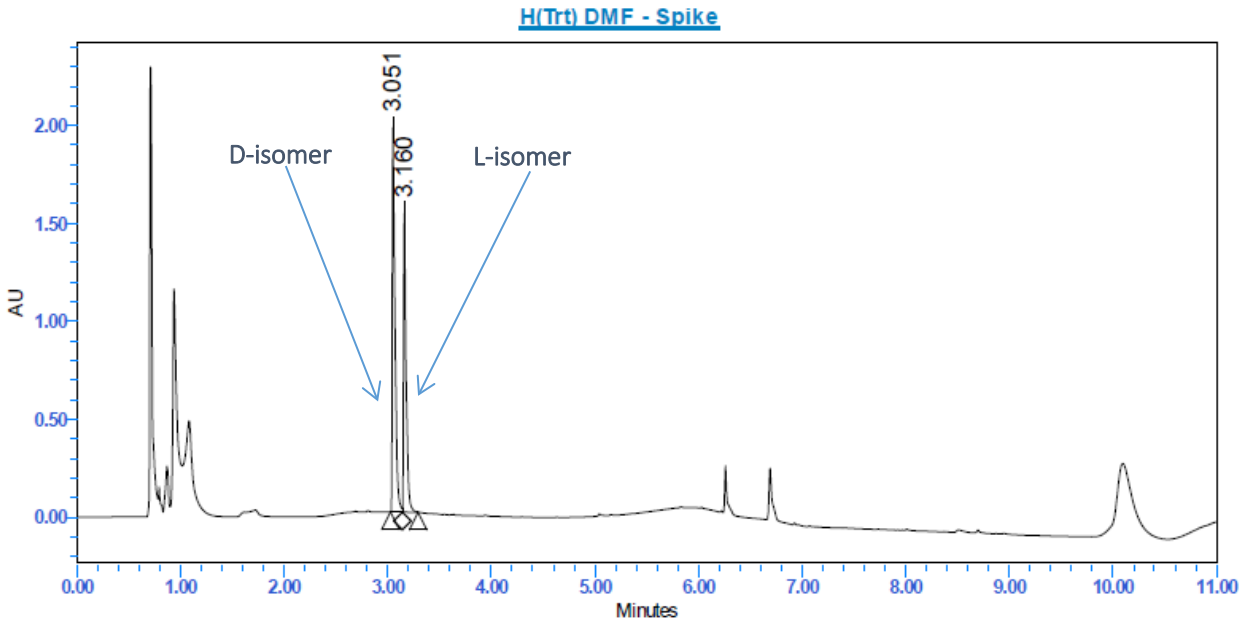
Amino acid racemisation in DMF, His(Trt), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.165	32578	30848		0.95
2		3.191	16559	11402		0.48
3		3.253	3370864	1861010		98.56

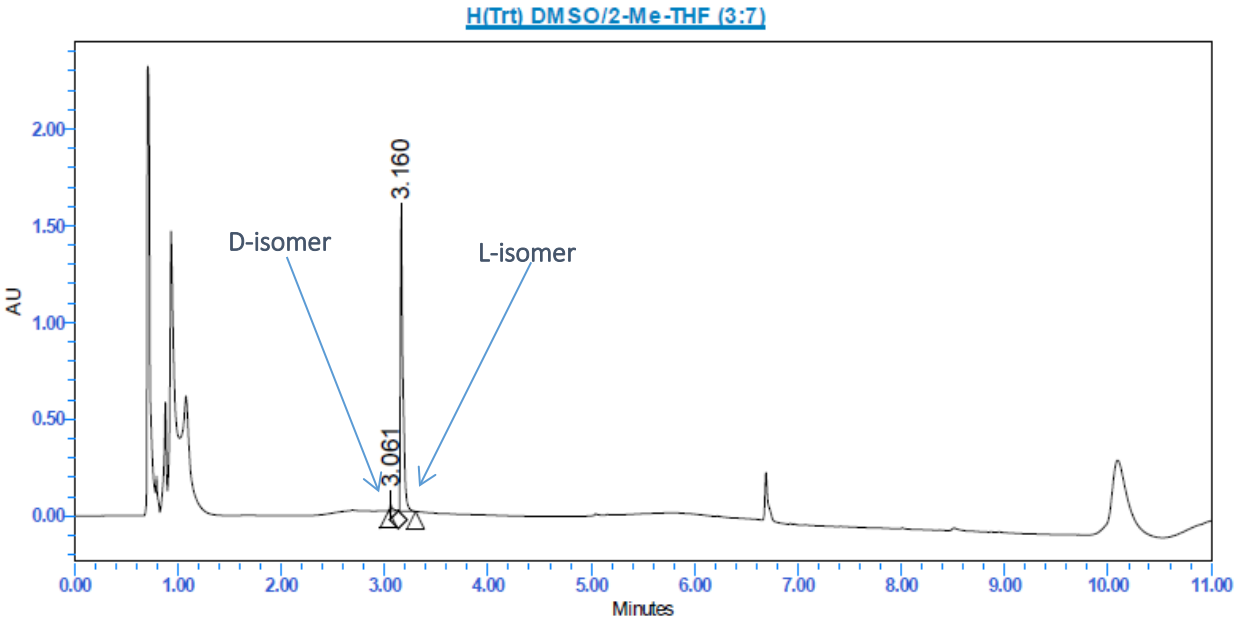
Amino acid racemisation in DMF, His(Trt), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.051	3647745	1962283		58.44
2		3.160	2593590	1525797		41.56

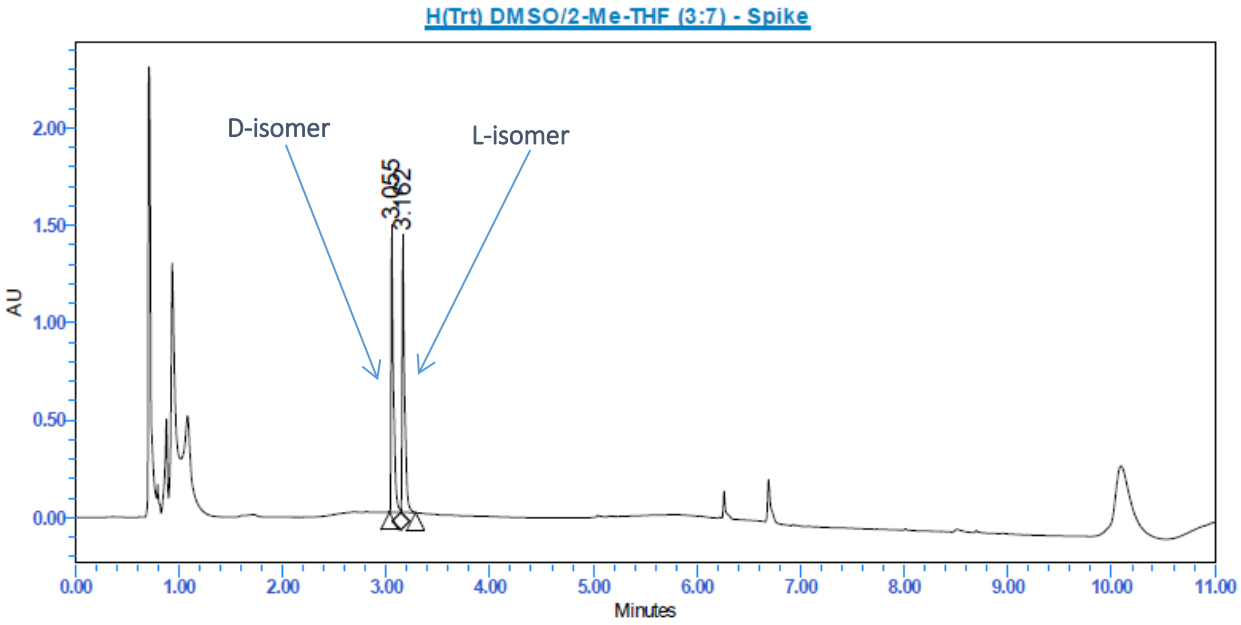
Amino acid racemisation in DMSO/2-Me-THF (3:7), His(Trt), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.061	53683	32378		2.03
2		3.160	2586951	1520379		97.97

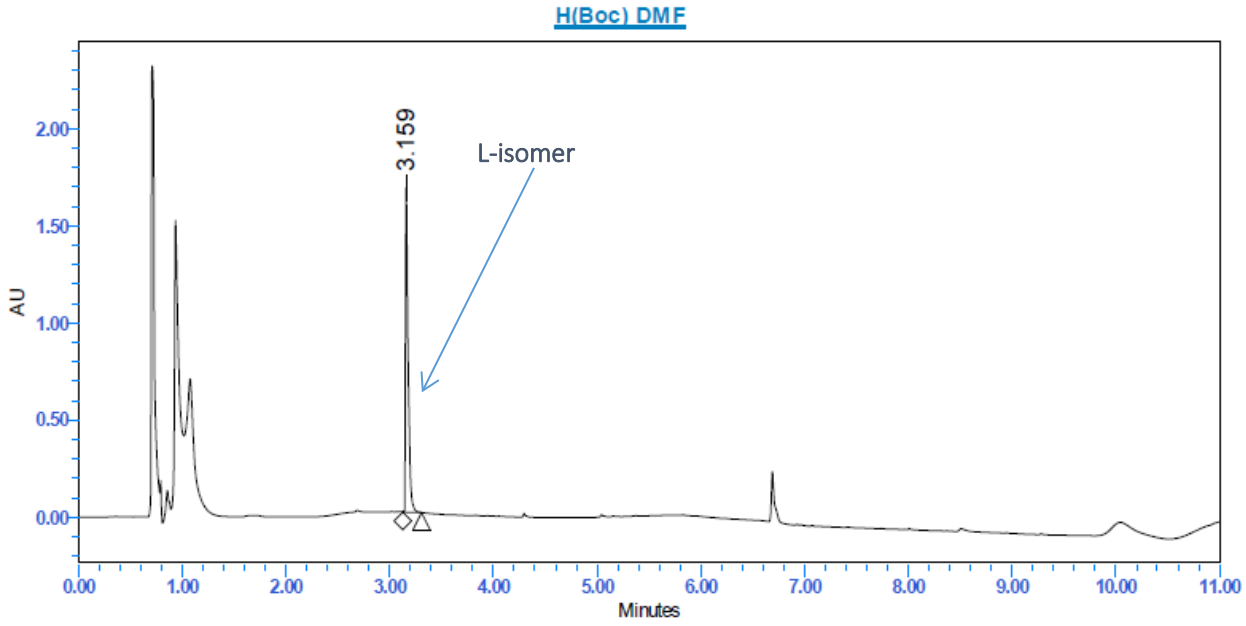
Amino acid racemisation in DMSO/2-Me-THF (3:7), His(Trt), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.055	2248592	1411238		50.30
2		3.162	2221502	1359087		49.70

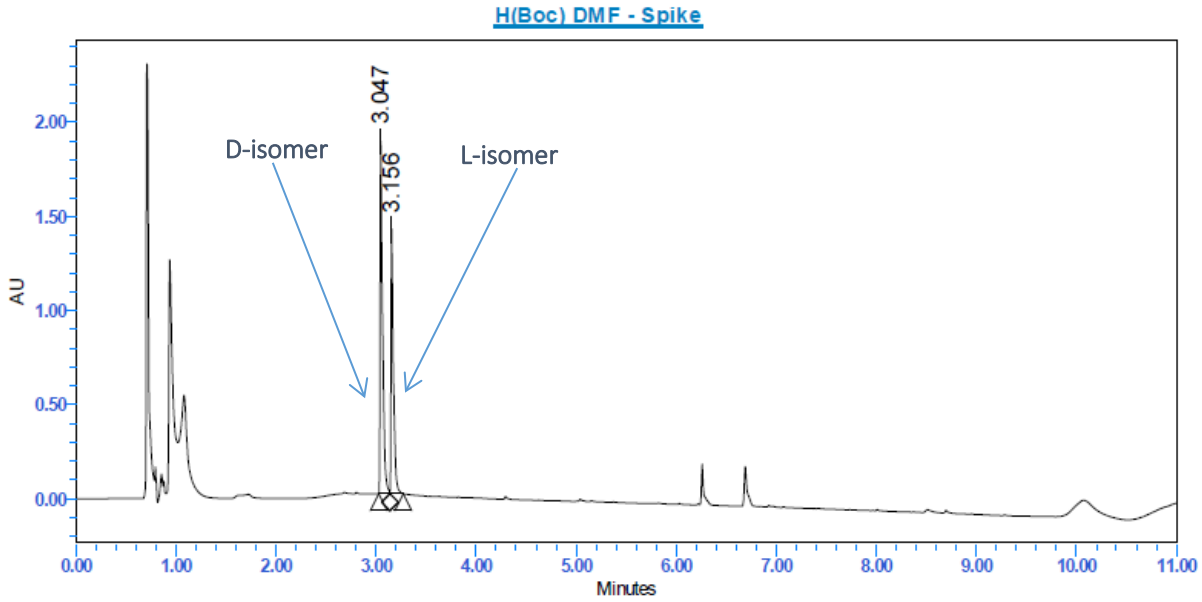
Amino acid racemisation in DMF, His(Boc), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.159	2970644	1671142		100.00

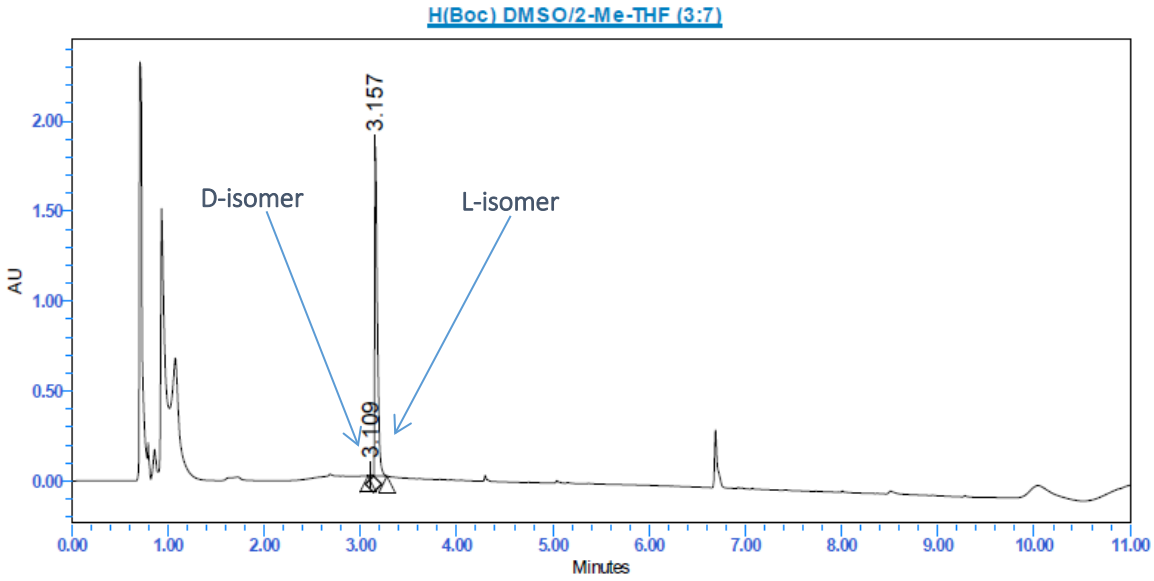
Amino acid racemisation in DMF, His(Boc), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.047	3316061	1872784		58.89
2		3.156	2314720	1408534		41.11

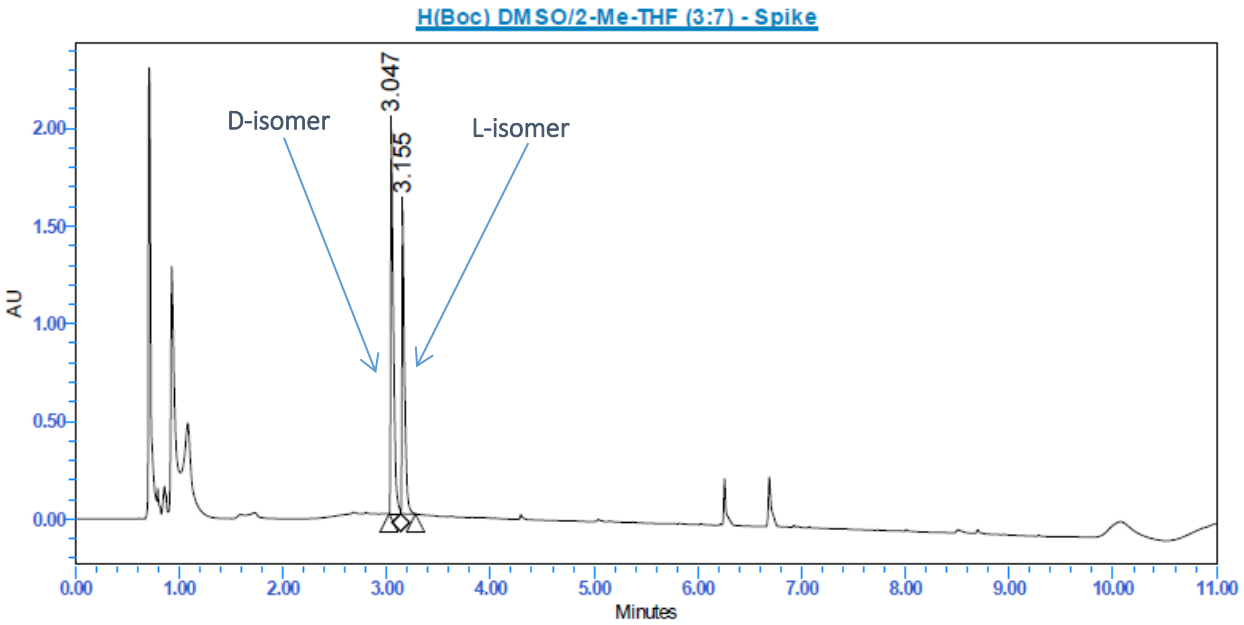
Amino acid racemisation in DMSO/2-Me-THF (3:7), His(Boc), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.109	13451	8634		0.39
2		3.157	3434680	1829909		99.61

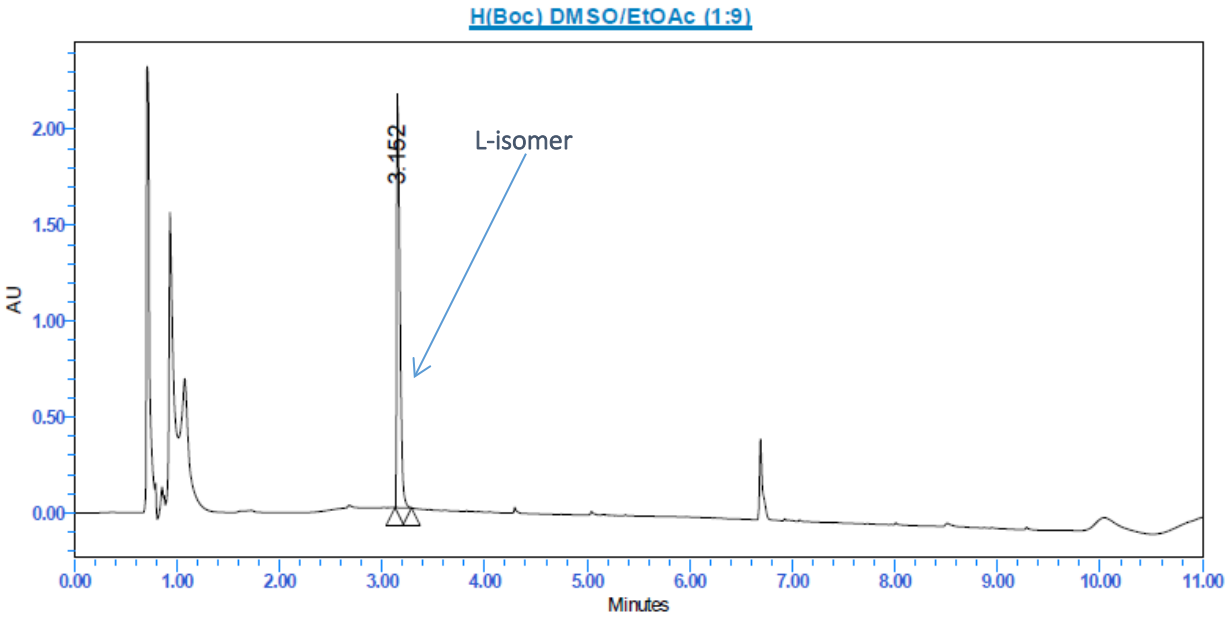
Amino acid racemisation in DMSO/2-Me-THF (3:7), His(Boc), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.047	3644308	1967155		57.98
2		3.155	2641564	1551705		42.02

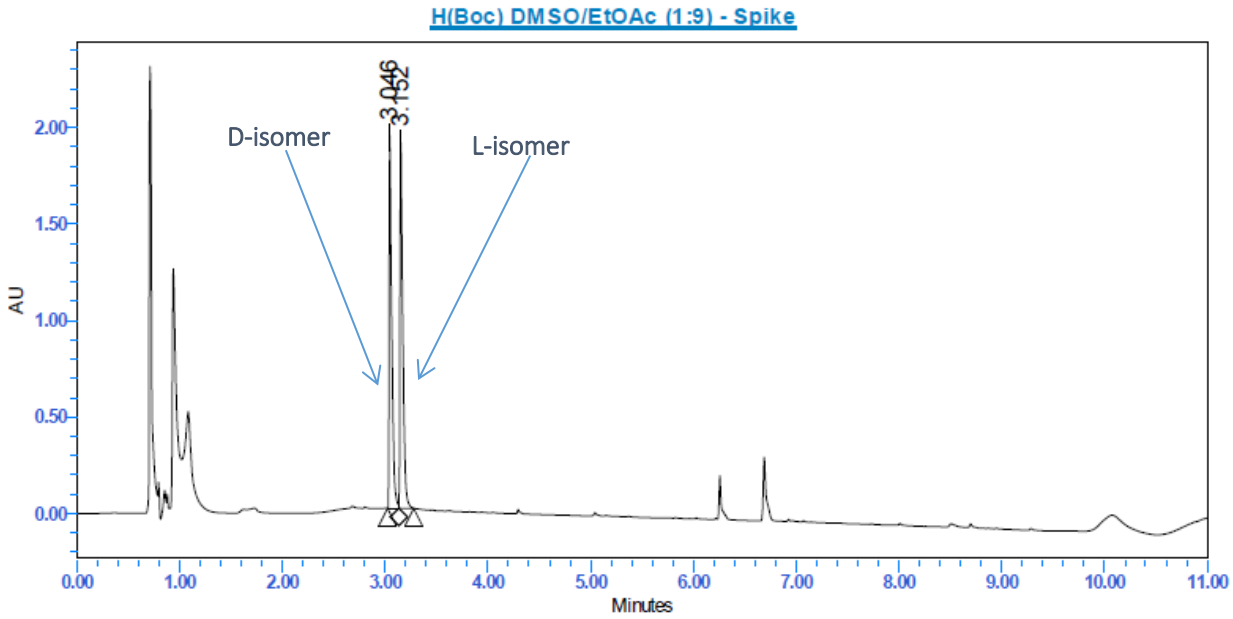
Amino acid racemisation in DMSO/EtOAc (1:9), His(Boc), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.152	4618065	2092638		100.00

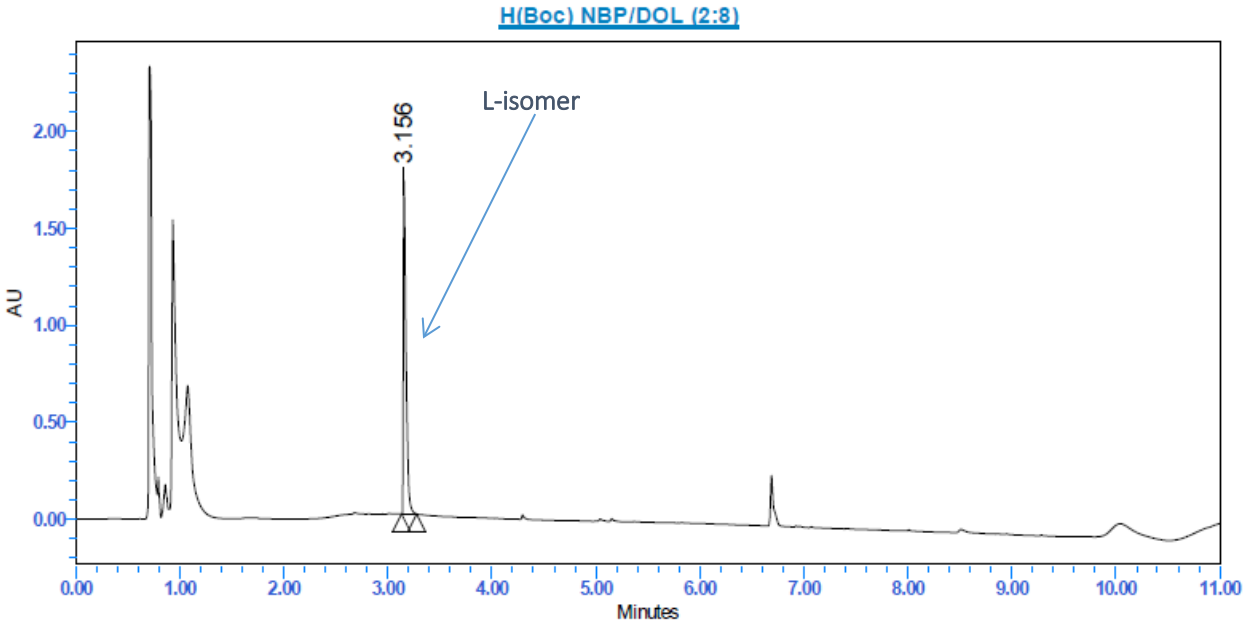
Amino acid racemisation in DMSO/EtOAc (1:9), His(Boc), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.046	3484506	1931301		48.93
2		3.152	3637489	1896250		51.07

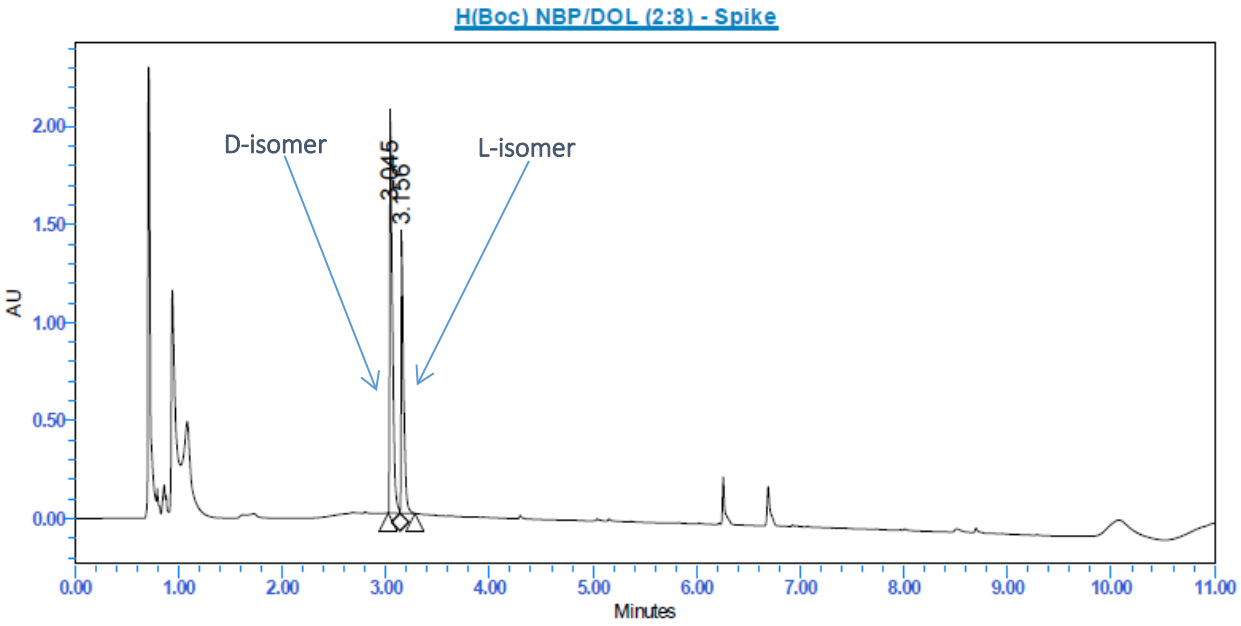
Amino acid racemisation in NBP/DOL (2:8), His(Boc), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.156	3089406	1718603		100.00

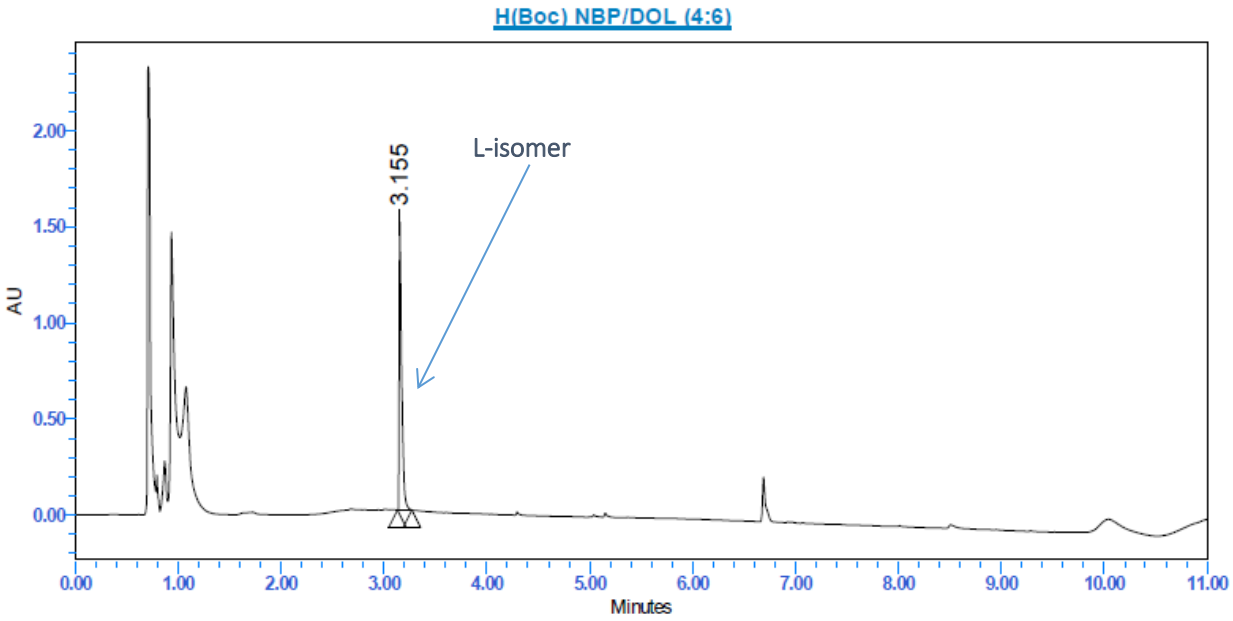
Amino acid racemisation in NBP/DOL (2:8), His(Boc), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.045	3765653	1998069		62.41
2		3.156	2268549	1388977		37.59

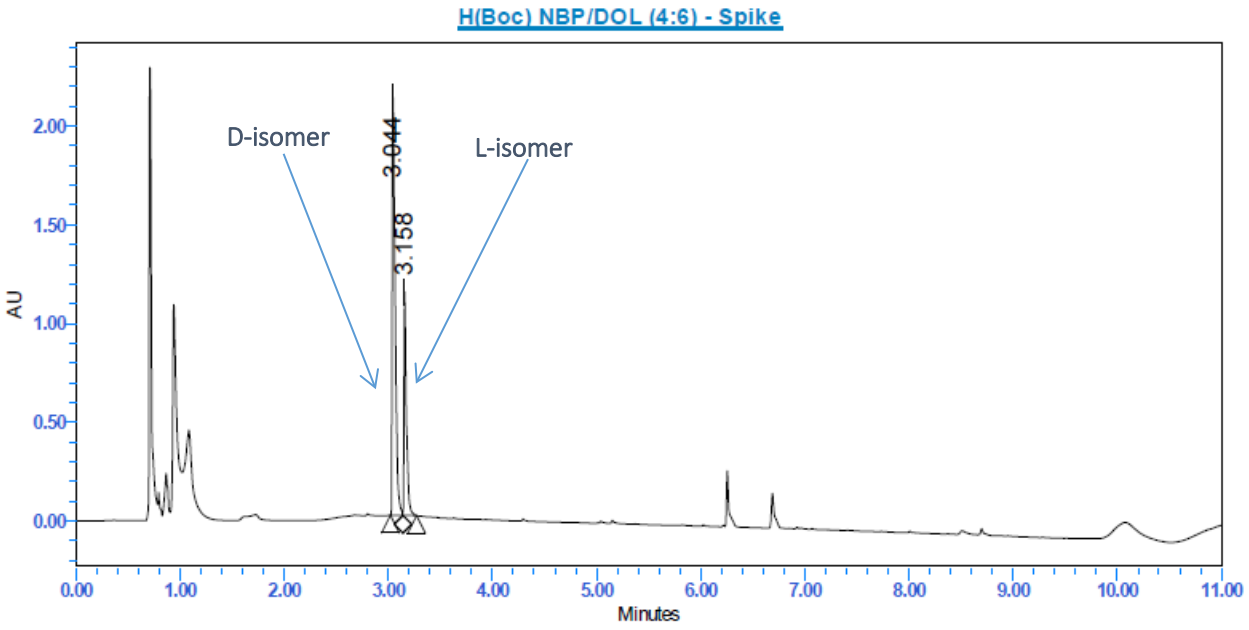
Amino acid racemisation in NBP/DOL (4:6), His(Boc), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.155	2518952	1497744		100.00

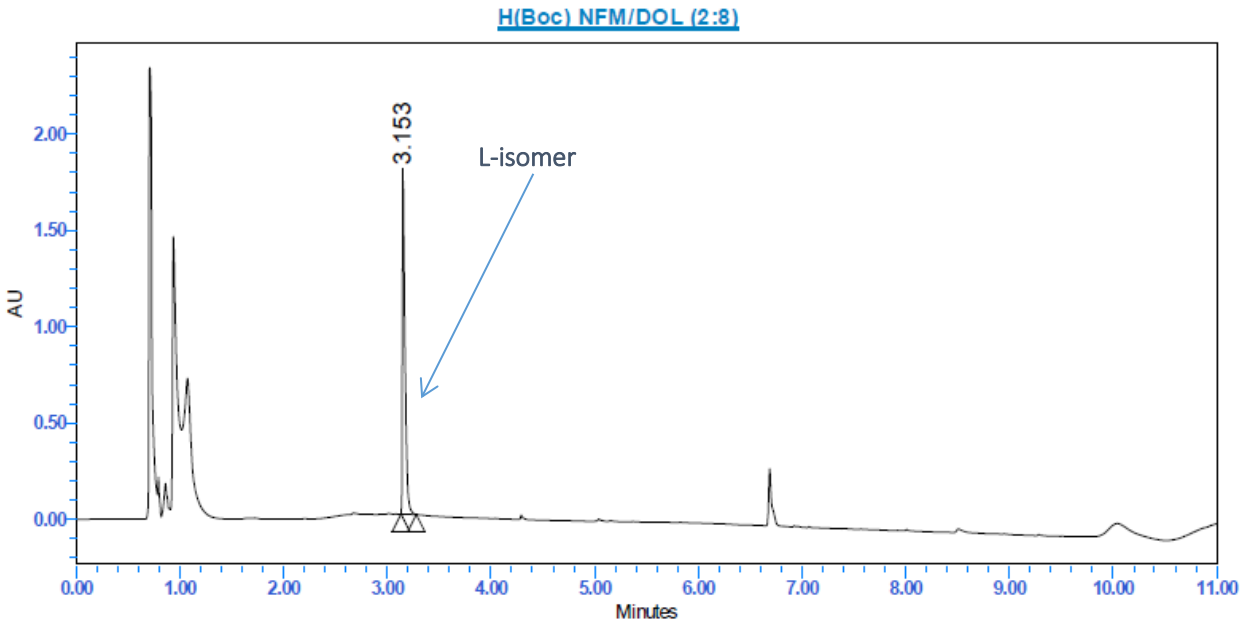
Amino acid racemisation in NBP/DOL (4:6), His(Boc), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.044	4360385	2118029		71.29
2		3.158	1756344	1132456		28.71

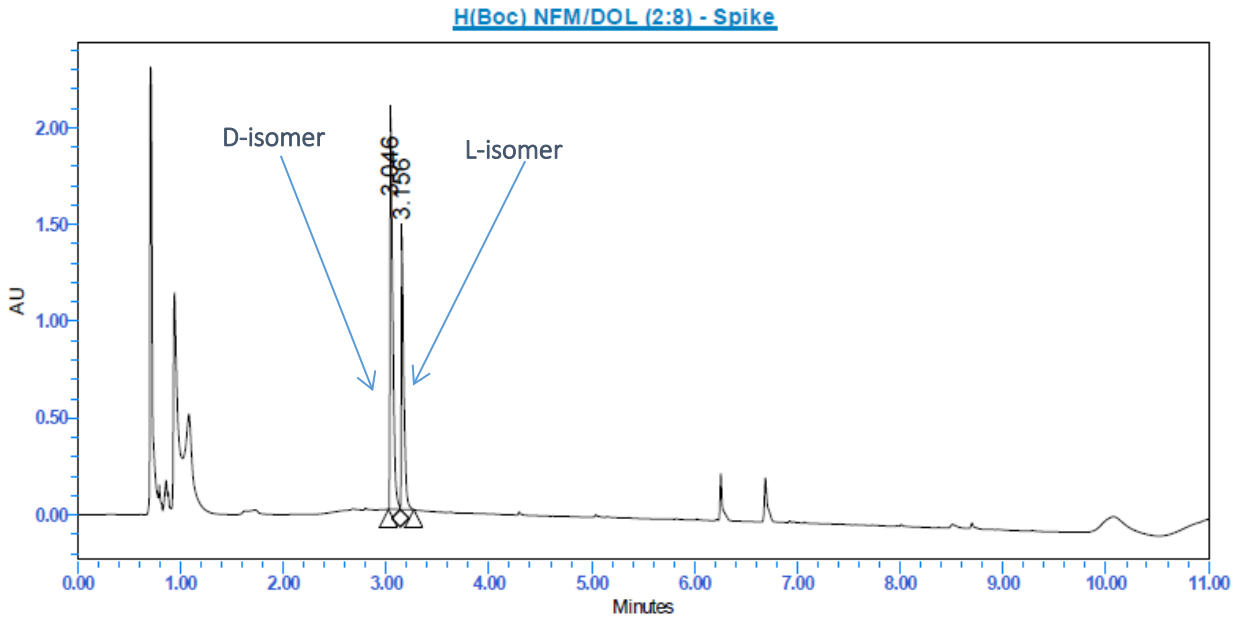
Amino acid racemisation in NFM/DOL (2:8), His(Boc), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.153	3115763	1730496		100.00

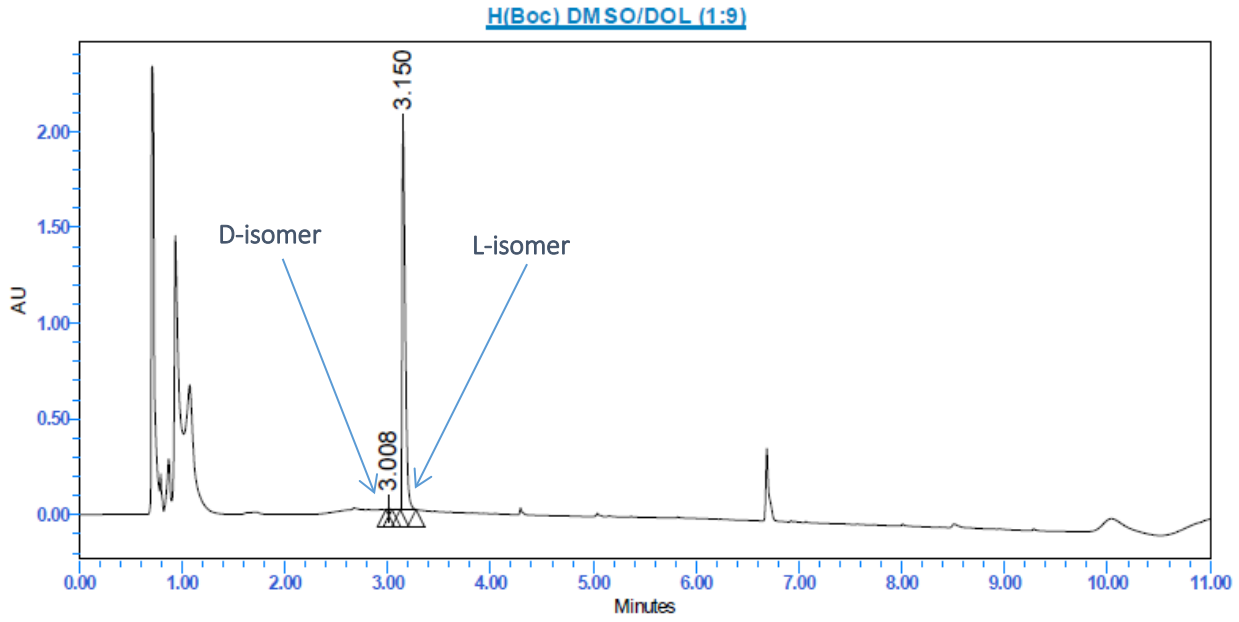
Amino acid racemisation in NFM/DOL (2:8), His(Boc), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.046	3839862	2019362		62.64
2		3.156	2290555	1407067		37.36

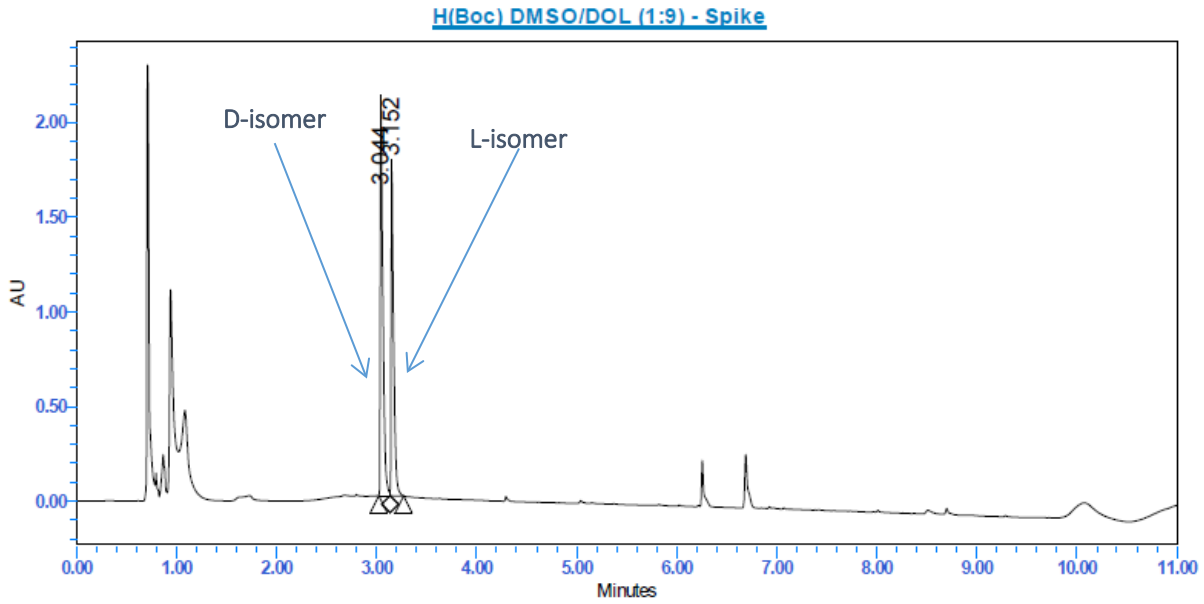
Amino acid racemisation in DMSO/DOL (1:9), His(Boc), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.008	6318	3487		0.15
2		3.150	4101470	2005526		99.85

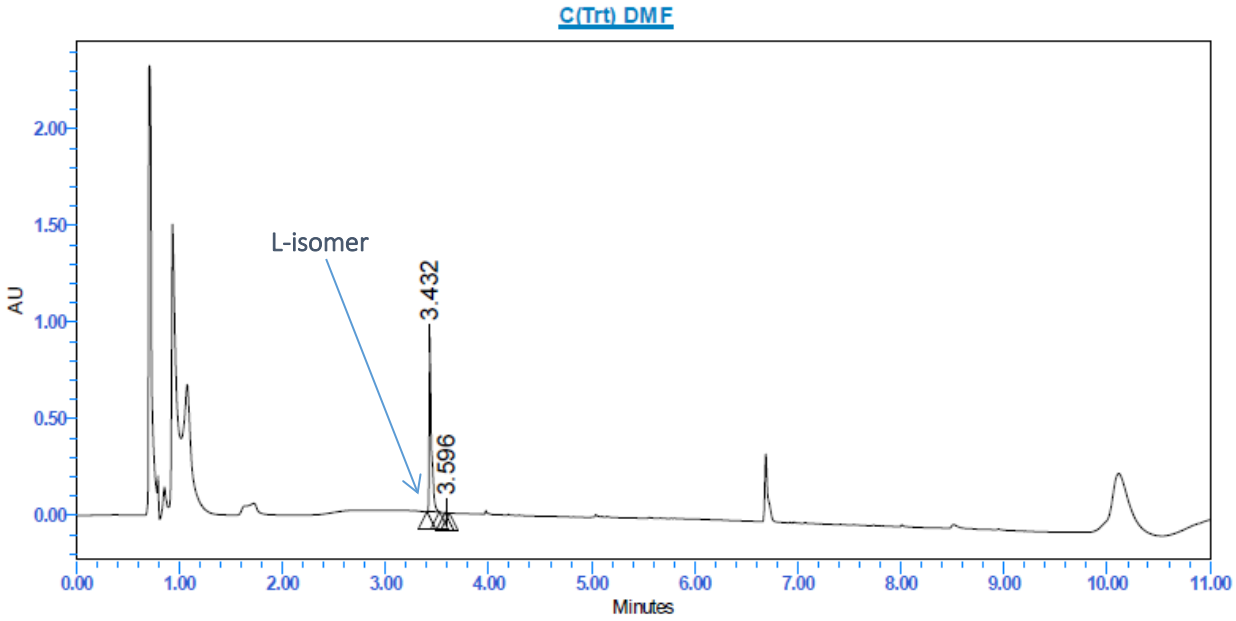
Amino acid racemisation in DMSO/DOL (1:9), His(Boc), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.044	3912162	2049530		56.45
2		3.152	3017698	1712864		43.55

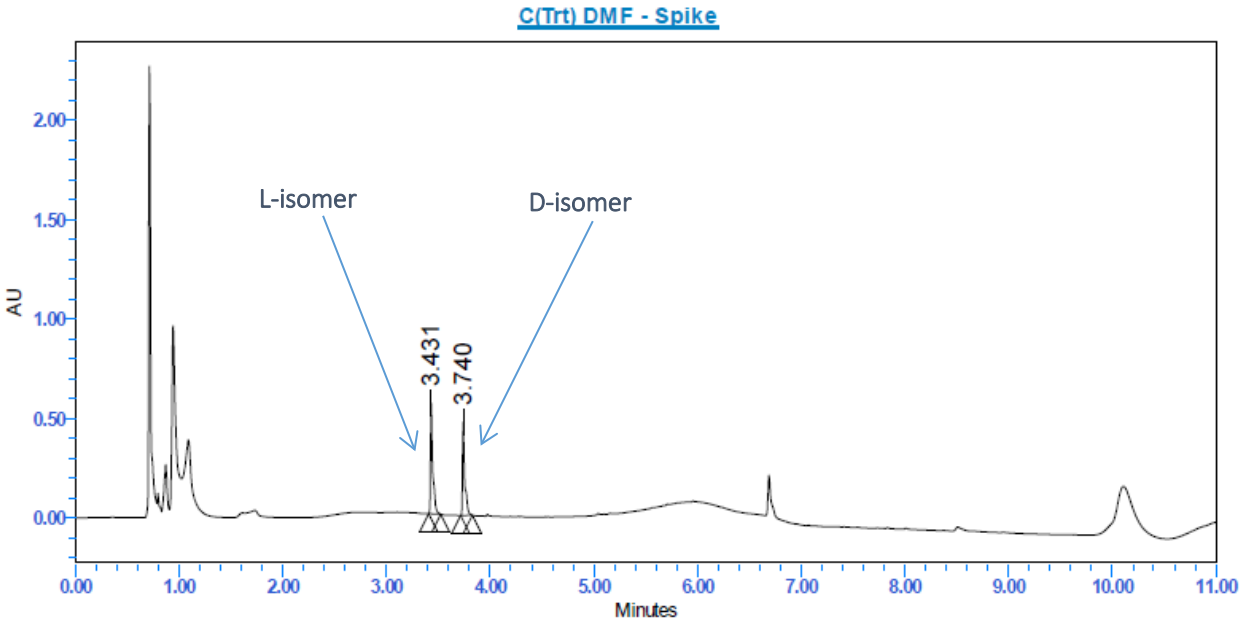
Amino acid racemisation in DMF, Cys(Trt), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.432	1325531	899227		99.89
2		3.596	1513	1775		0.11

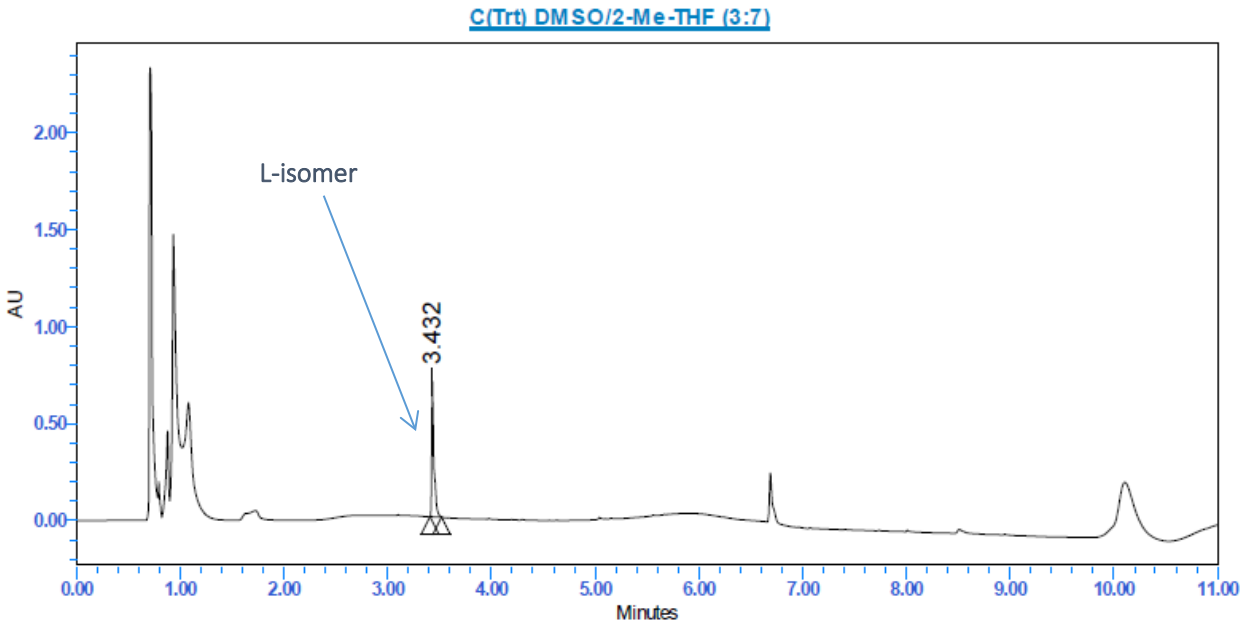
Amino acid racemisation in DMF, Cys(Trt), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.431	825878	557304		54.58
2		3.740	687381	469805		45.42

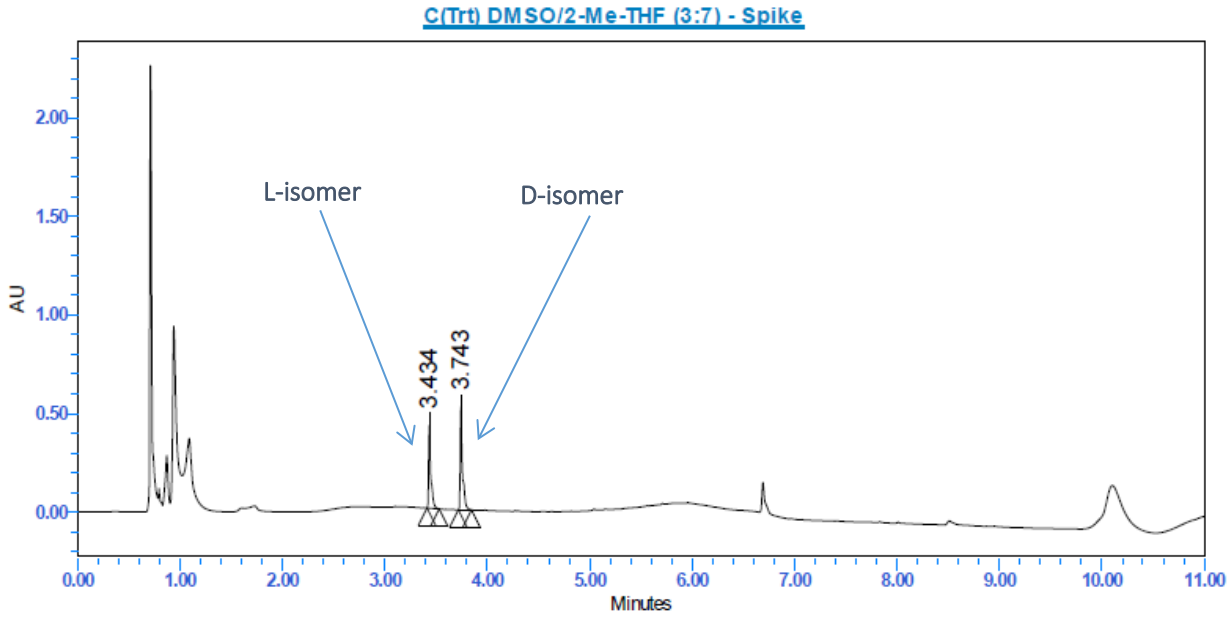
Amino acid racemisation in DMSO/2-Me-THF (3:7), Cys(Trt), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.432	1032232	697154		100.00

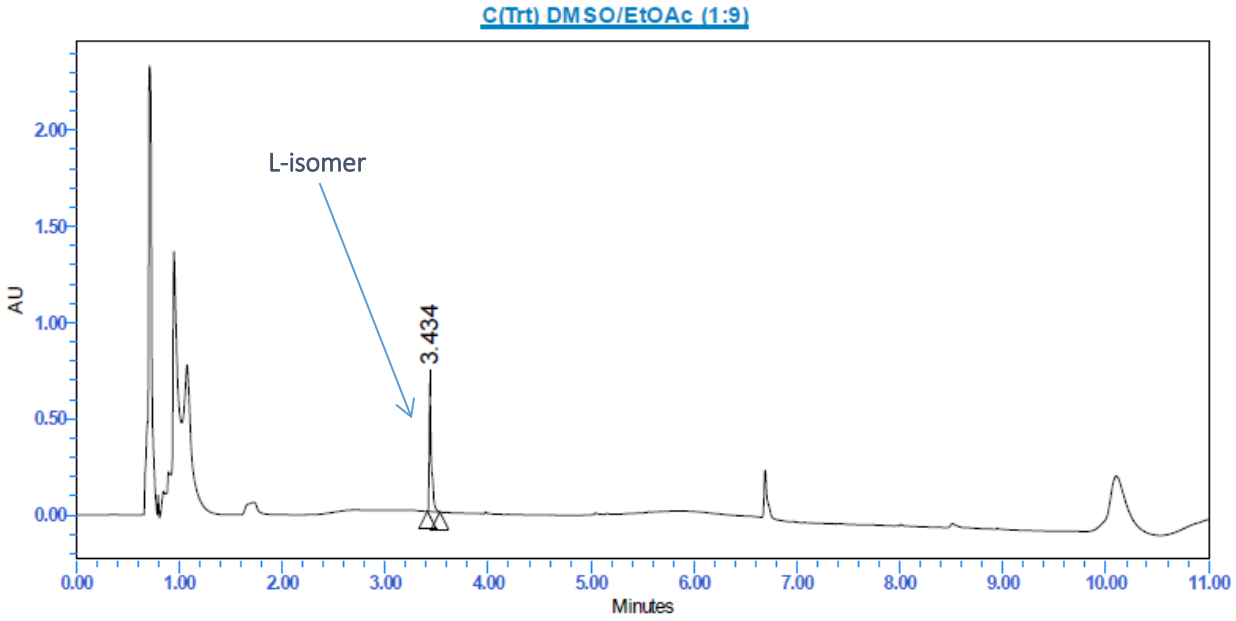
Amino acid racemisation in DMSO/2-Me-THF (3:7), Cys(Trt), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.434	623611	418888		44.64
2		3.743	773437	522982		55.36

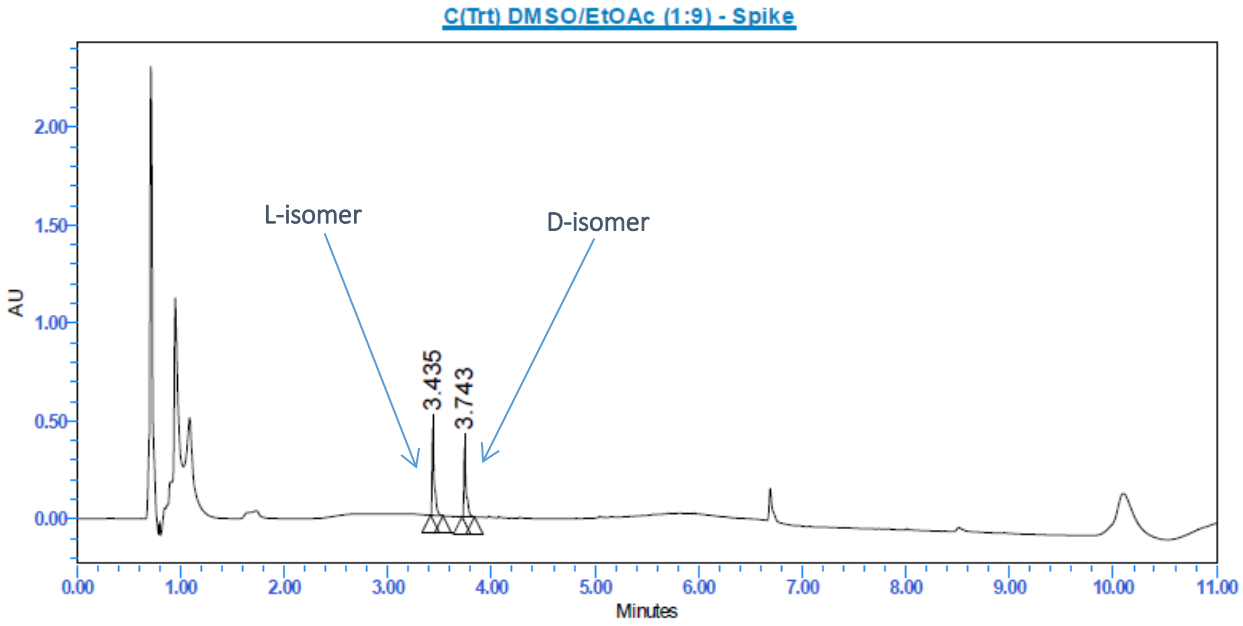
Amino acid racemisation in DMSO/EtOAc (1:9), Cys(Trt), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.434	952290	669622		100.00

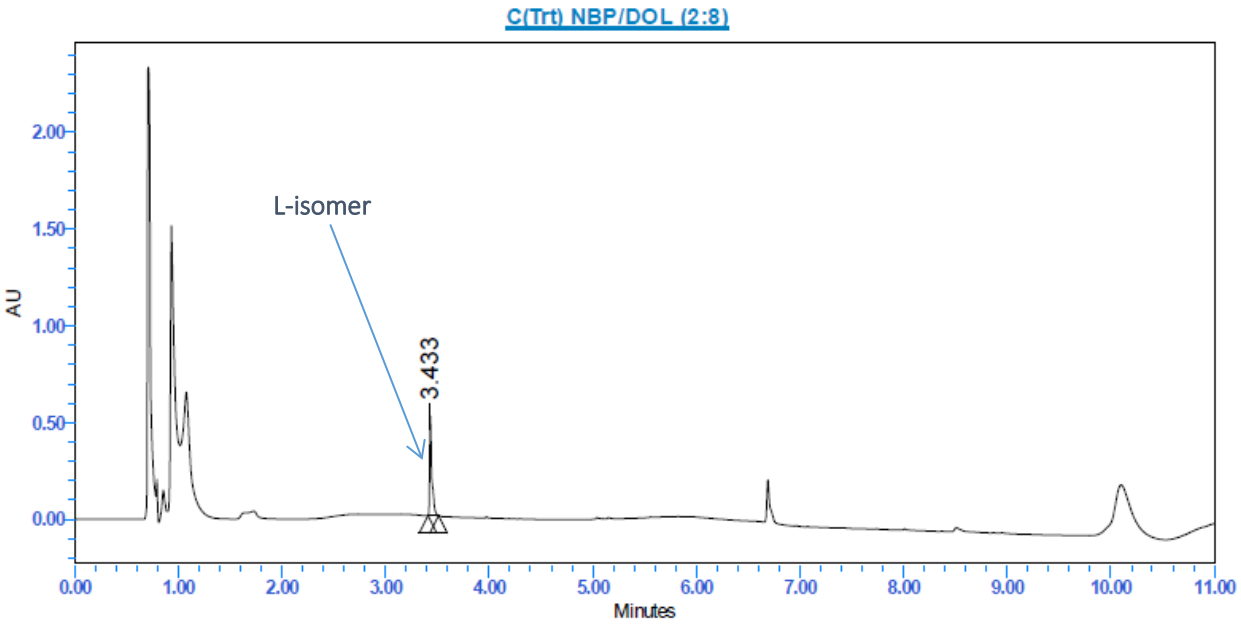
Amino acid racemisation in DMSO/EtOAc (1:9), Cys(Trt), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.435	638987	445283		55.33
2		3.743	515776	357363		44.67

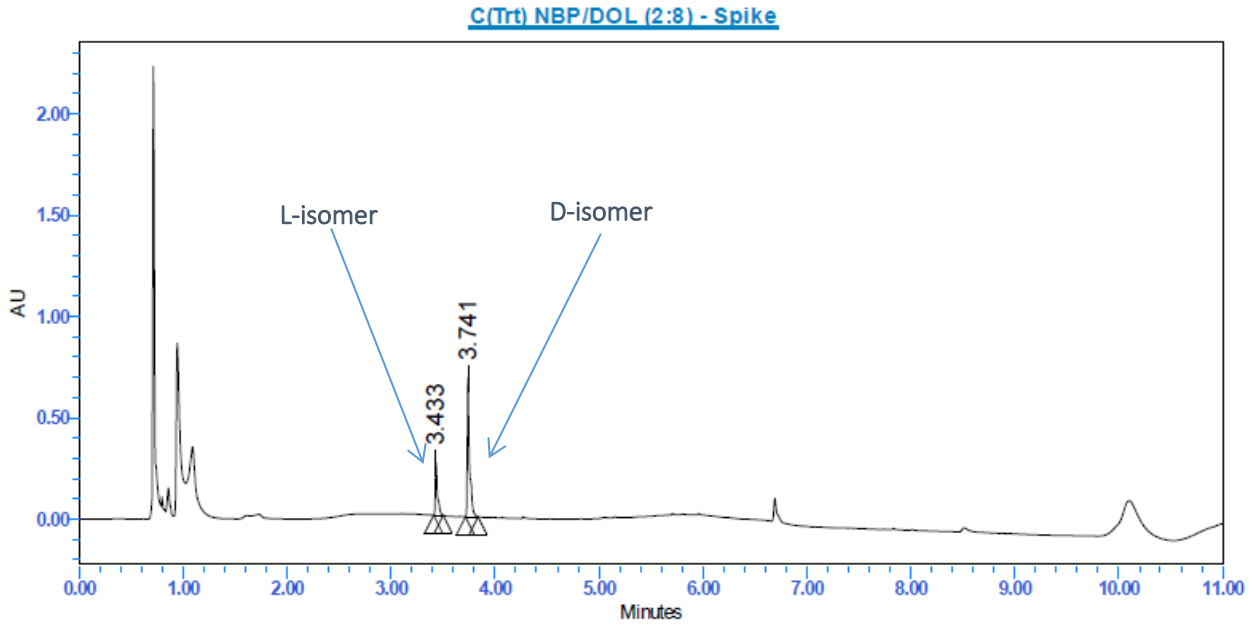
Amino acid racemisation in NBP/DOL (2:8), Cys(Trt), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.433	749660	514552		100.00

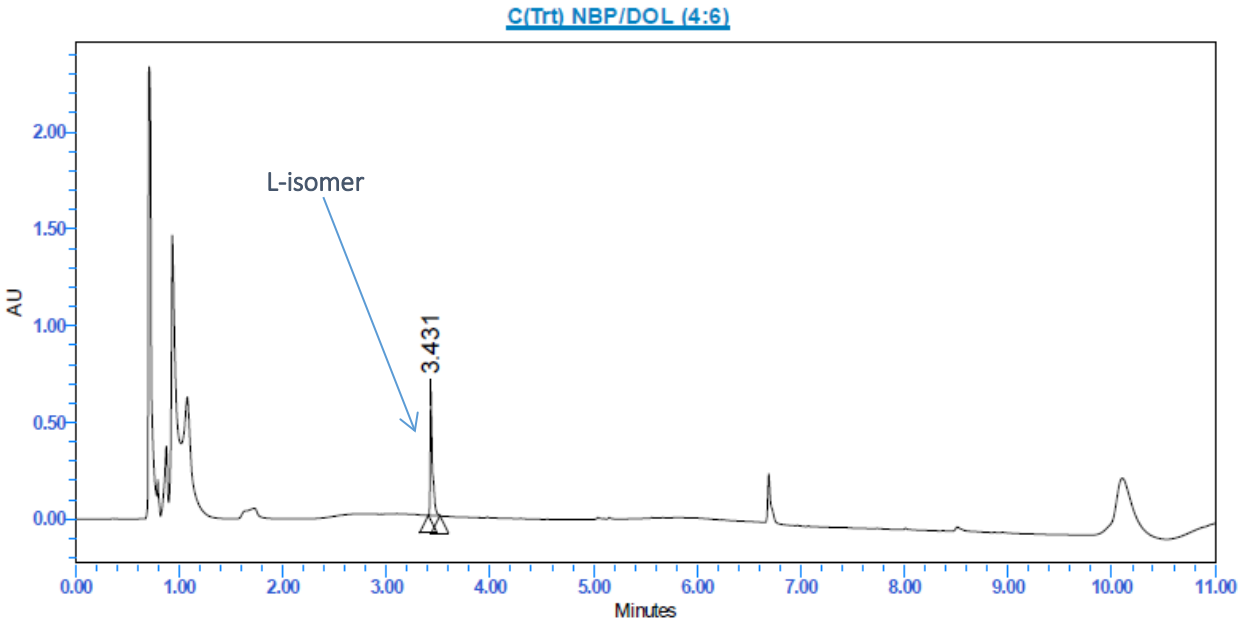
Amino acid racemisation in NBP/DOL (2:8), Cys(Trt), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.433	370069	258227		26.89
2		3.741	1006231	686781		73.11

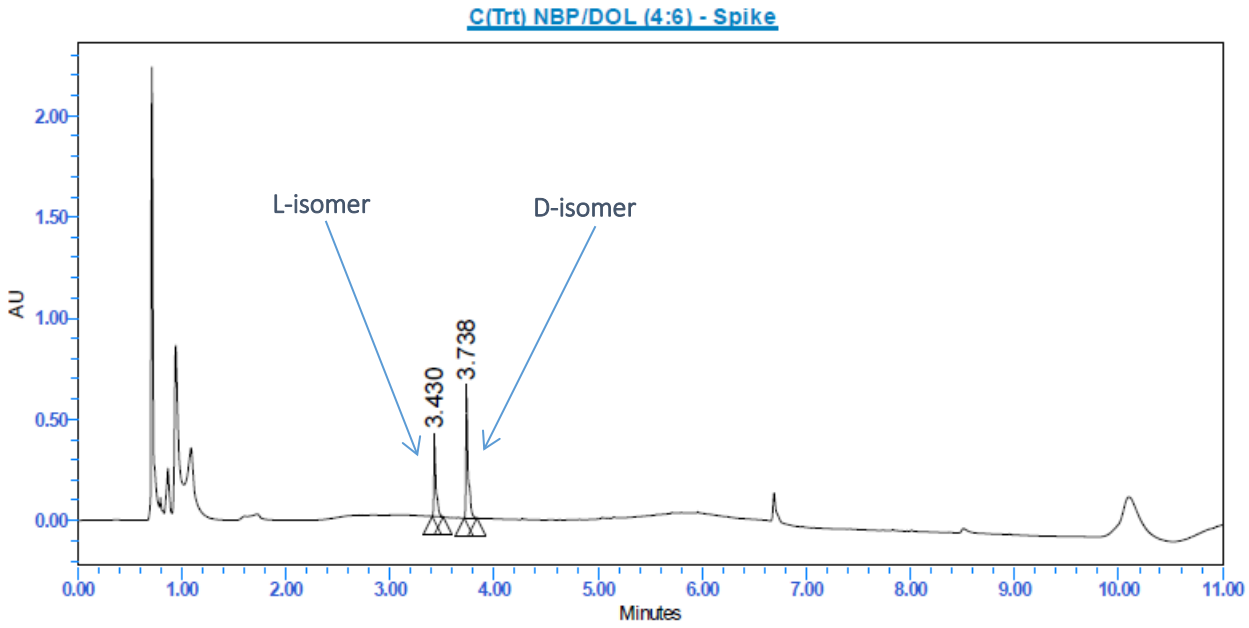
Amino acid racemisation in NBP/DOL (4:6), Cys(Trt), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.431	944433	639995		100.00

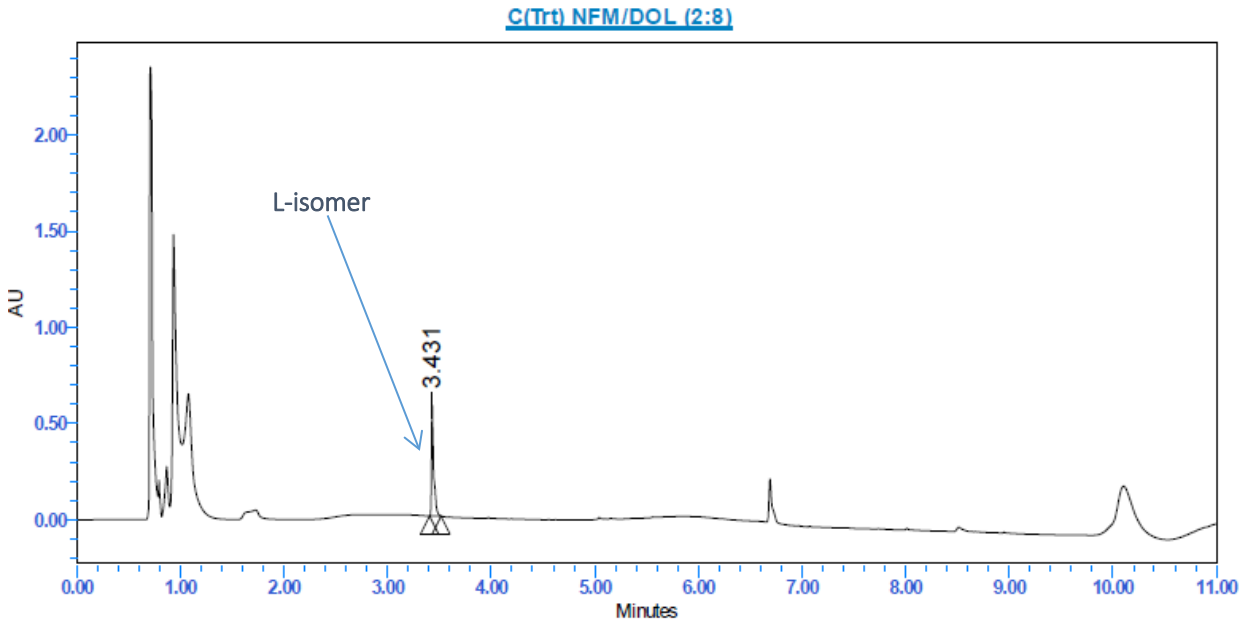
Amino acid racemisation in NBP/DOL (4:6), Cys(Trt), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.430	510609	347108		36.76
2		3.738	878542	596297		63.24

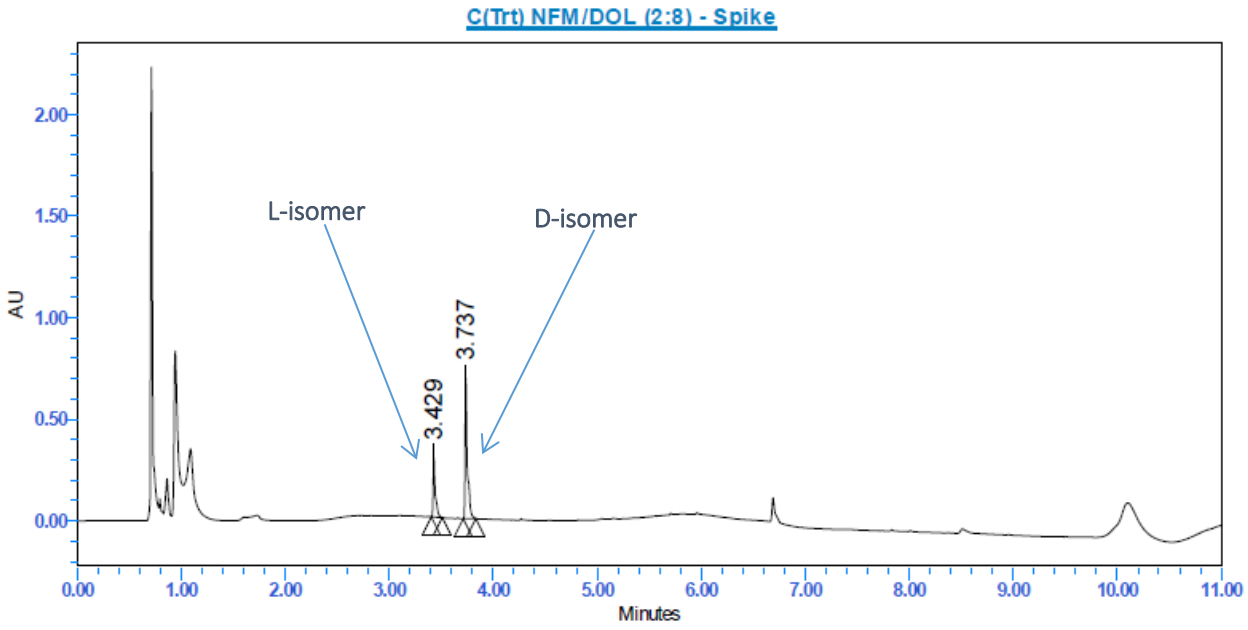
Amino acid racemisation in NFM/DOL (2:8), Cys(Trt), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.431	847399	576223		100.00

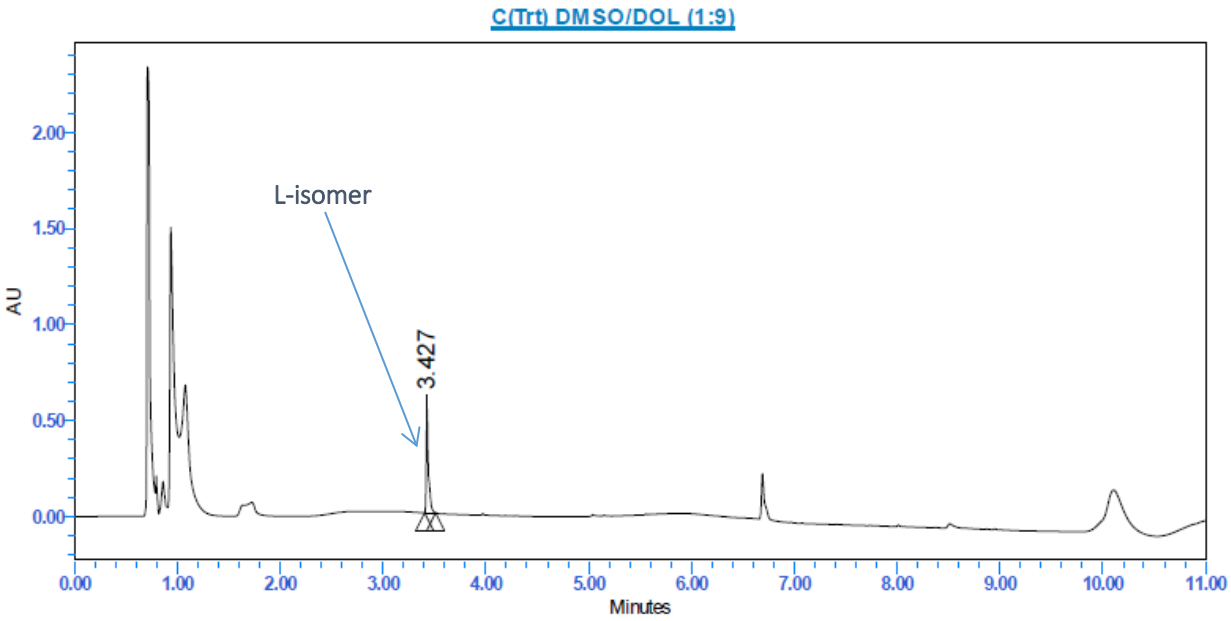
Amino acid racemisation in NFM/DOL (2:8), Cys(Trt), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.429	424406	292009		29.51
2		3.737	1013545	692351		70.49

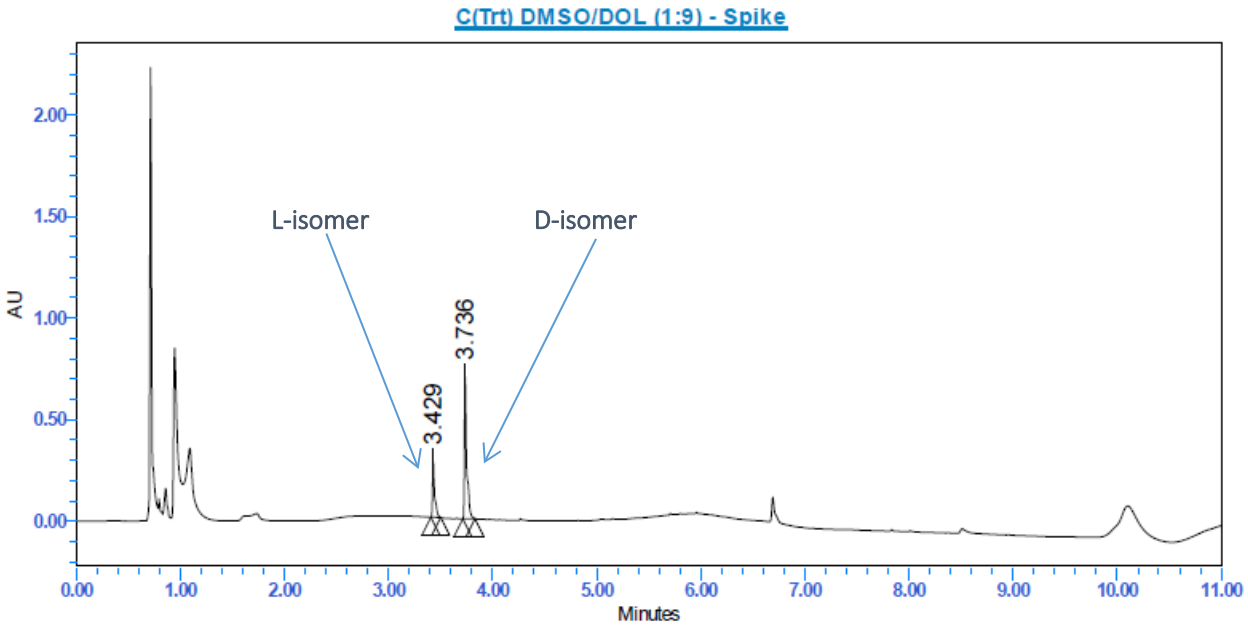
Amino acid racemisation in DMSO/DOL (1:9), Cys(Trt), no spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.427	800744	549075		100.00

Amino acid racemisation in DMSO/DOL (1:9), Cys(Trt), with spike



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		3.429	389764	271978		27.60
2		3.736	1022291	698934		72.40