

# Lipase Immobilization Towards Improved Productivity on Kinetic Resolutions by Continuous-Flow Process

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## Supporting Information

## 1.1. Analysis of statistic data

### 1.1.1. Ethyl Acetate as Acyl donor.

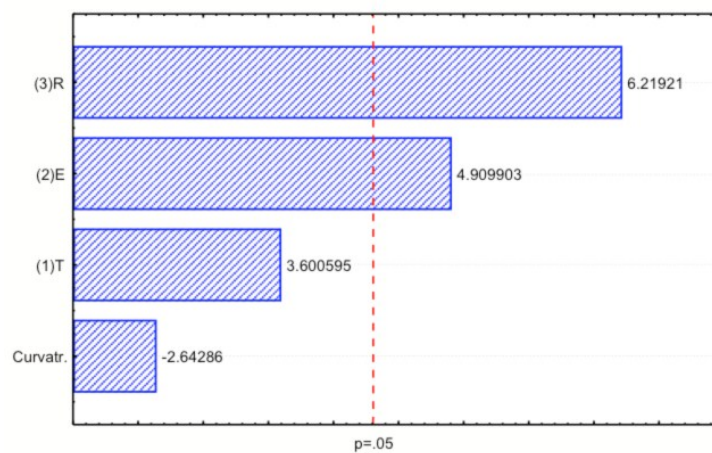
**Table S1 – 2<sup>3-1</sup> Fractional factorial experimental design with coded values for the reaction catalyzed by Novozyme 435 using ethyl acetate as acyl donor.**

Assay	Temperature (°C)	[E] (%)	Acylating agent/alcohol (mol/mol)	Conversion (%)	ee (%)
1	50(-1)	5(-1)	3/1(+1)	32	24
2	70(+1)	5(-1)	1/1(-1)	28	19
3	50(-1)	15(+1)	1/1(-1)	30	26
4	70(+1)	15(+1)	3/1(+1)	46	42
5	60(0)	10(0)	2/1(0)	32	24
6	60(0)	10(0)	2/1(0)	29	19
7	60(0)	10(0)	2/1(0)	31	17

**Table S2. Estimated effect for independent variables; R<sup>2</sup>=0,9764.**

Factor	Effect	p
Mean	33,75000	0,000512*
Curvatr.	-6,16667	0,118298
T	5,50000	0,069222
E	7,50000	0,039067*
R	9,50000	0,024893*

\*95% statistically significant



**Figure S1. Pareto chart**

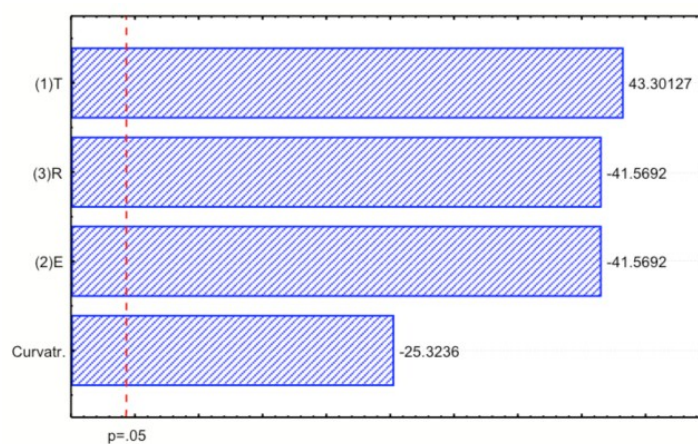
**Table S3 – 2<sup>3-1</sup> Fractional factorial experimental design with coded values for the reaction catalyzed by Amano AK Lipase using ethyl acetate as acyl donor.**

Assay	Temperature (°C)	[E] (%)	Acylating agent/alcohol (mol/mol)	Conversion (%)	ee (%)
8	50(-1)	5(-1)	3/1(+1)	7	>1
9	70(+1)	5(-1)	1/1(-1)	56	34
10	50(-1)	15(+1)	1/1(-1)	7	>1
11	70(+1)	15(+1)	3/1(+1)	7	>1
12	60(0)	10(0)	2/1(0)	8	3
13	60(0)	10(0)	2/1(0)	9	>1
14	60(0)	10(0)	2/1(0)	8	>1

**Table S4. Estimated effect for independent variables; R<sup>2</sup>=0.99967.**

Factor	Effect	p
Mean	19,5000	0,000219*
Curvatr.	-22,3333	0,001556*
T	25,0000	0,000533*
E	-24,0000	0,000578*
R	-24,0000	0,000578*

\*95% statistically significant



**Figure S2. Pareto chart**

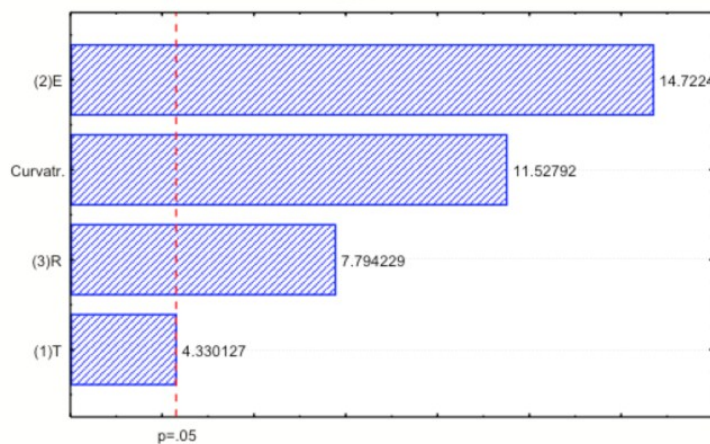
**Table S5 – 2<sup>3-1</sup> Fractional factorial experimental design with coded values for the reaction catalyzed by PS-C “Amano” I Lipase using ethyl acetate as acyl donor.**

Assay	Temperature (°C)	[E] (%)	Acylating agent/alcohol (mol/mol)	Conversion (%)	ee (%)
15	50(-1)	5(-1)	3/1(+1)	6	>1
16	70(+1)	5(-1)	1/1(-1)	4	>1
17	50(-1)	15(+1)	1/1(-1)	10	>1
18	70(+1)	15(+1)	3/1(+1)	19	12
19	60(0)	10(0)	2/1(0)	14	3
20	60(0)	10(0)	2/1(0)	14	4
21	60(0)	10(0)	2/1(0)	16	2

**Table S6. Estimated effect for independent variables; R<sup>2</sup>=0.99536.**

Factor	Effect	p
Mean	9,25000	0,000973*
Curvatr.	10,16667	0,007441*
T	2,50000	0,049414*
E	8,50000	0,004582*
R	4,50000	0,016065*

\*95% statistically significant



**Figure S3. Pareto chart**

### 1.1.2. Isopropenyl Acetate as Acyl donor.

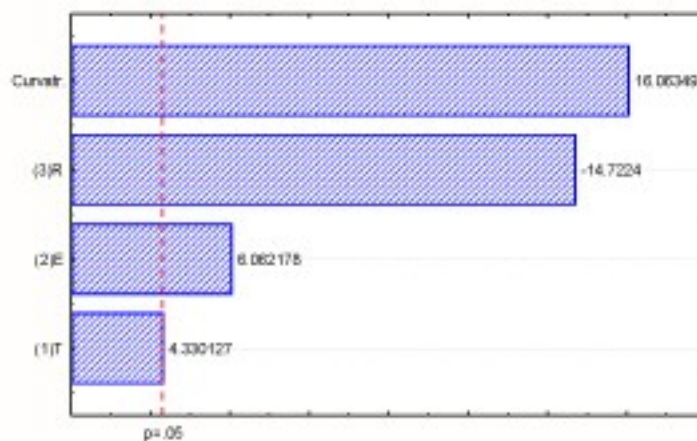
**Table S7 – 2<sup>3-1</sup> Fractional factorial experimental design with coded values for the reaction catalyzed by Novozyme 435 using isopropenyl acetate as acyl donor.**

Assay	Temperature (°C)	[E] (%)	Acylating agent/alcohol (mol/mol)	Conversion (%)	ee (%)
22	50(-1)	5(-1)	3/1(+1)	38	43
23	70(+1)	5(-1)	1/1(-1)	49	54
24	50(-1)	15(+1)	1/1(-1)	50	94
25	70(+1)	15(+1)	3/1(+1)	44	78
26	60(0)	10(0)	2/1(0)	52	66
27	60(0)	10(0)	2/1(0)	52	42
28	60(0)	10(0)	2/1(0)	53	63

**Table S8. Estimated effect for independent variables; R<sup>2</sup>=0,99624.**

Factor	Effect	p
Mean	45,2500	0,000041*
Curvatr.	14,16667	0,003853*
T	2,5000	0,049414*
E	3,5000	0,026148*
R	-8,5000	0,004582*

\*95% statistically significant



**Figure S4. Pareto chart**

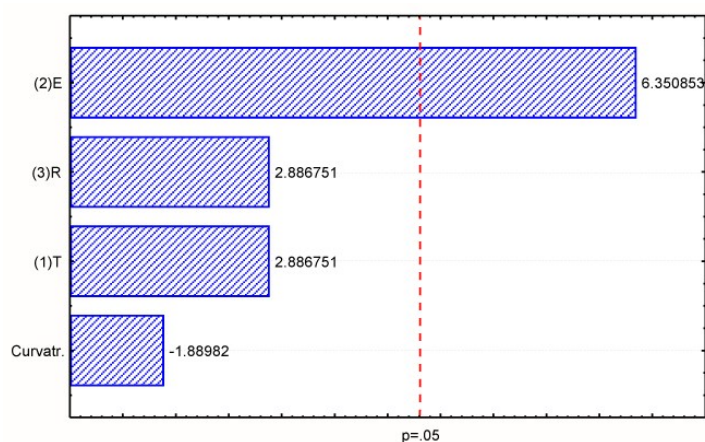
**Table S9 – 2<sup>3-1</sup> Fractional factorial experimental design with coded values for the reaction catalyzed by Amano AK Lipase using isopropenyl acetate as acyl donor.**

Assay	Temperature (°C)	[E] (%)	Acylating agent/alcohol (mol/mol)	Conversion (%)	ee (%)
29	50(-1)	5(-1)	3/1(+1)	9	>1
30	70(+1)	5(-1)	1/1(-1)	9	>1
31	50(-1)	15(+1)	1/1(-1)	15	6
32	70(+1)	15(+1)	3/1(+1)	25	22
33	60(0)	10(0)	2/1(0)	11	7
34	60(0)	10(0)	2/1(0)	14	10
35	60(0)	10(0)	2/1(0)	11	3

**Table S10. Estimated effect for independent variables; R<sup>2</sup>=0,96804.**

Factor	Effect	p
Mean	14,50000	0,003548*
Curvatr.	-5,00000	0,199359
T	5,00000	0,101973
E	11,00000	0,023908*
R	5,00000	0,101973

\*95% statistically significant



**Figure S5. Pareto chart**

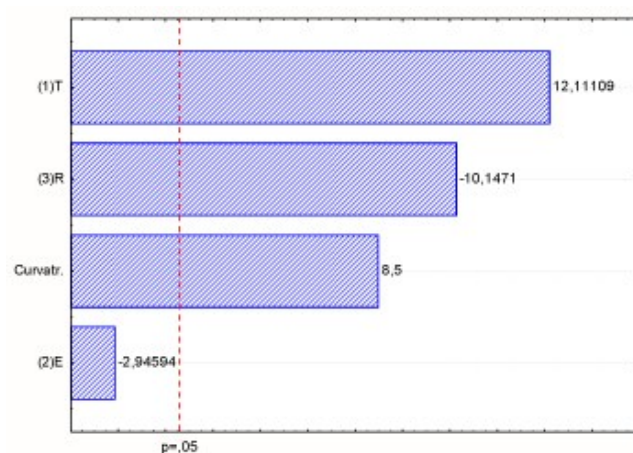
**Table S11 – 2<sup>3-1</sup> Fractional factorial experimental design with coded values for the reaction catalyzed by Lipase PS-C “Amano” I Lipase using isopropenyl acetate as acyl donor.**

Assay	Temperature (°C)	[E] (%)	Acylating agent/alcohol (mol/mol)	Conversion (%)	ee (%)
36	50(-1)	5(-1)	3/1(+1)	18	8
37	70(+1)	5(-1)	1/1(-1)	52	46
38	50(-1)	15(+1)	1/1(-1)	29	21
39	70(+1)	15(+1)	3/1(+1)	32	10
40	60(0)	10(0)	2/1(0)	41	32
41	60(0)	10(0)	2/1(0)	44	40
42	60(0)	10(0)	2/1(0)	43	23

**Table S12. Estimated effect for independent variables; R<sup>2</sup>=0,96804.**

Factor	Effect	p
Mean	32,7500	0,000543*
Curvatr.	19,8333	0,013560*
T	18,5000	0,006749*
E	-4,5000	0,098496
R	-15,5000	0,009573*

\*95% statistically significant



**Figure S6. Pareto chart**

### 1.1.3. Vinyl Acetate as Acyl donor.

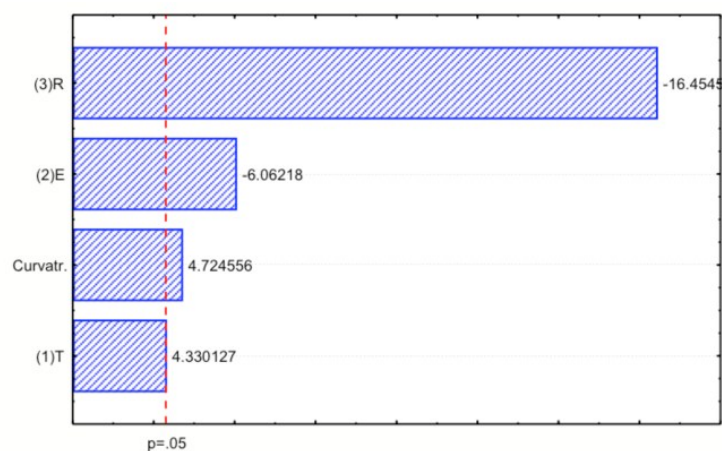
**Table S13 – 2<sup>3-1</sup> Fractional factorial experimental design with coded values for the reaction catalyzed by Novozyme 435 using vinyl acetate as acyl donor.**

Assay	Temperature (°C)	[E] (%)	Acylating agent/alcohol (mol/mol)	Conversion (%)	ee (%)
43	50(-1)	5(-1)	3/1(+1)	45	80
44	70(+1)	5(-1)	1/1(-1)	57	41
45	50(-1)	15(+1)	1/1(-1)	51	96
46	70(+1)	15(+1)	3/1(+1)	44	93
47	60(0)	10(0)	2/1(0)	51	70
48	60(0)	10(0)	2/1(0)	51	78
49	60(0)	10(0)	2/1(0)	52	78

**Table S14. Estimated effect for independent variables; R<sup>2</sup>=0,9943.**

Factor	Effect	p
Mean	49,25000	0,000034*
Curvatr.	4,16667	0,041998*
T	2,50000	0,049414*
E	-3,50000	0,026148*
R	-9,50000	0,003673*

\*95% statistically significant



**Figure S7. Pareto chart**



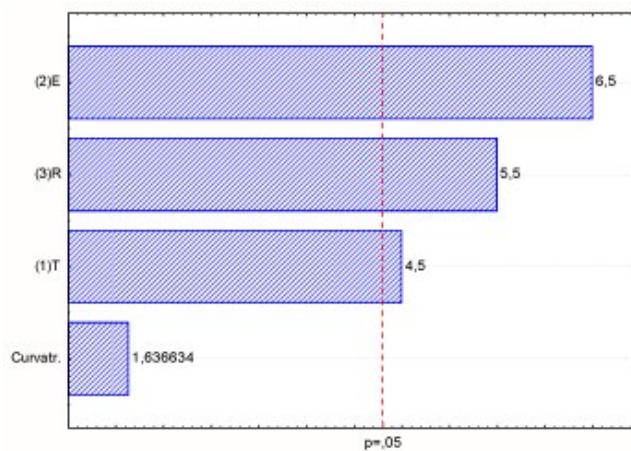
**Table S15 – 2<sup>3-1</sup> Fractional factorial experimental design with coded values for the reaction catalyzed by Amano AK Lipase using vinyl acetate as acyl donor.**

Assay	Temperature (°C)	[E] (%)	Acylating agent/alcohol (mol/mol)	Conversion (%)	ee (%)
50	50(-1)	5(-1)	3/1(+1)	48	93
51	70(+1)	5(-1)	1/1(-1)	47	39
52	50(-1)	15(+1)	1/1(-1)	49	96
53	70(+1)	15(+1)	3/1(+1)	59	87
54	60(0)	10(0)	2/1(0)	54	66
55	60(0)	10(0)	2/1(0)	52	59
56	60(0)	10(0)	2/1(0)	51	63

**Table S16. Estimated effect for independent variables; R<sup>2</sup>=0,97947.**

Factor	Effect	p
Mean	50,75000	0,000097*
Curvatr.	2,50000	0,243350
T	4,50000	0,046002*
E	6,50000	0,022860*
R	5,50000	0,031504*

\*95% statistically significant



**Figure S8. Pareto chart**

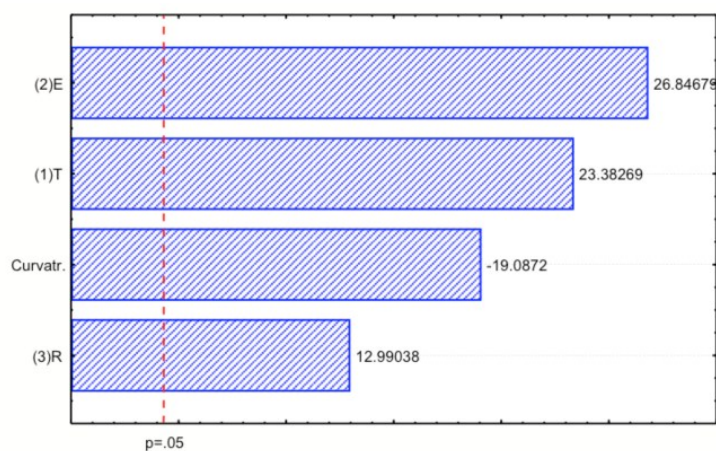
**Table S17 – 2<sup>3-1</sup> Fractional factorial experimental design with coded values for the reaction catalyzed by Lipase PS-C “Amano” I Lipase using vinyl acetate as acyl donor.**

Assay	Temperature (°C)	[E] (%)	Acylating agent/alcohol (mol/mol)	Conversion (%)	ee (%)
57	50(-1)	5(-1)	3/1(+1)	35	40
58	70(+1)	5(-1)	1/1(-1)	42	34
59	50(-1)	15(+1)	1/1(-1)	43	66
60	70(+1)	15(+1)	3/1(+1)	64	88
61	60(0)	10(0)	2/1(0)	37	45
62	60(0)	10(0)	2/1(0)	37	45
63	60(0)	10(0)	2/1(0)	38	46

**Table S18. Estimated effect for independent variables; R<sup>2</sup>=0,99889.**

Factor	Effect	p
Mean	45,7500	0,000040*
Curvatr.	-16,8333	0,002734*
T	13,5000	0,001824*
E	15,5000	0,001385*
R	7,5000	0,005874*

\*95% statistically significant



**Figure S9. Pareto chart**

## 1.2. GC-FID Analysis

### 1.2.1. Chromatograms

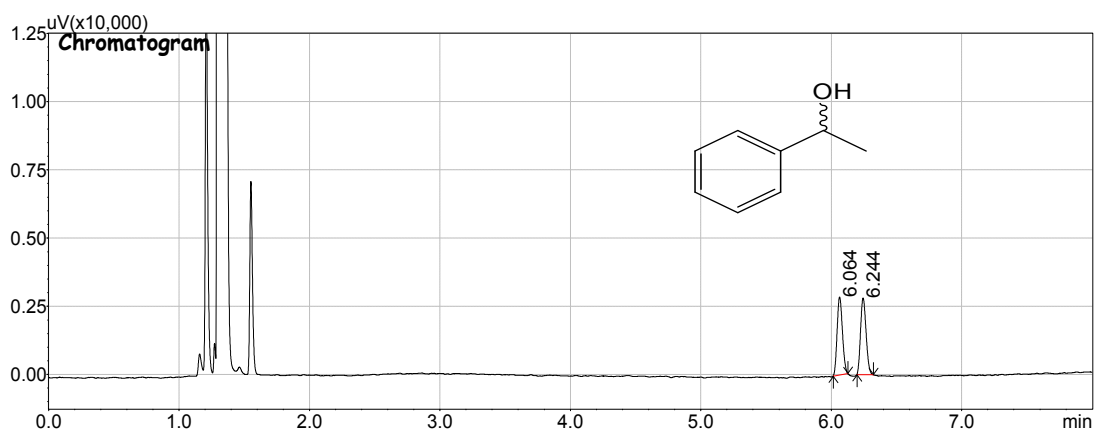


Figure S10. GC-FID of (*RS*)-1-Phenylethanol. Experimental conditions: *rac*-1-Phenylethanol (1mmol, 122mg), in cyclohexane (3 mL).

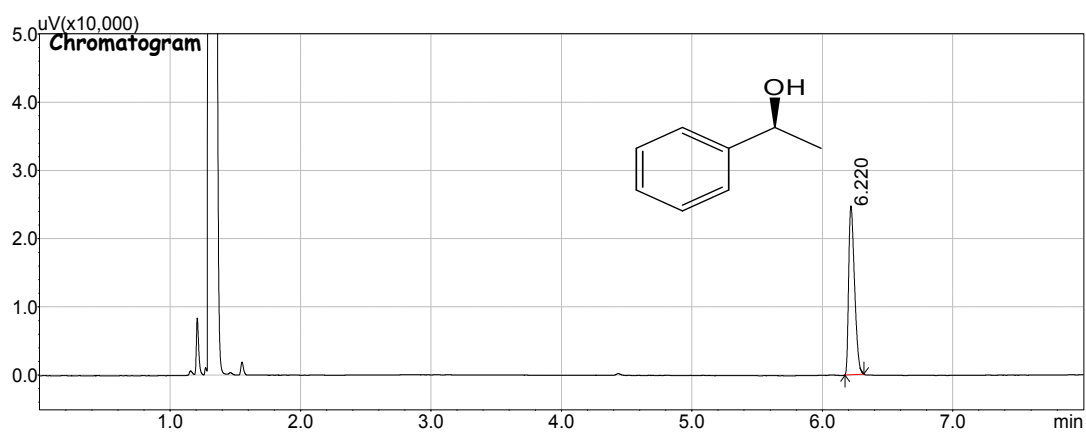


Figure S11. GC-FID of (*S*)-1-Phenylethanol. Experimental conditions: *rac*-1-Phenylethanol (1mmol, 122mg), in cyclohexane (3 mL).

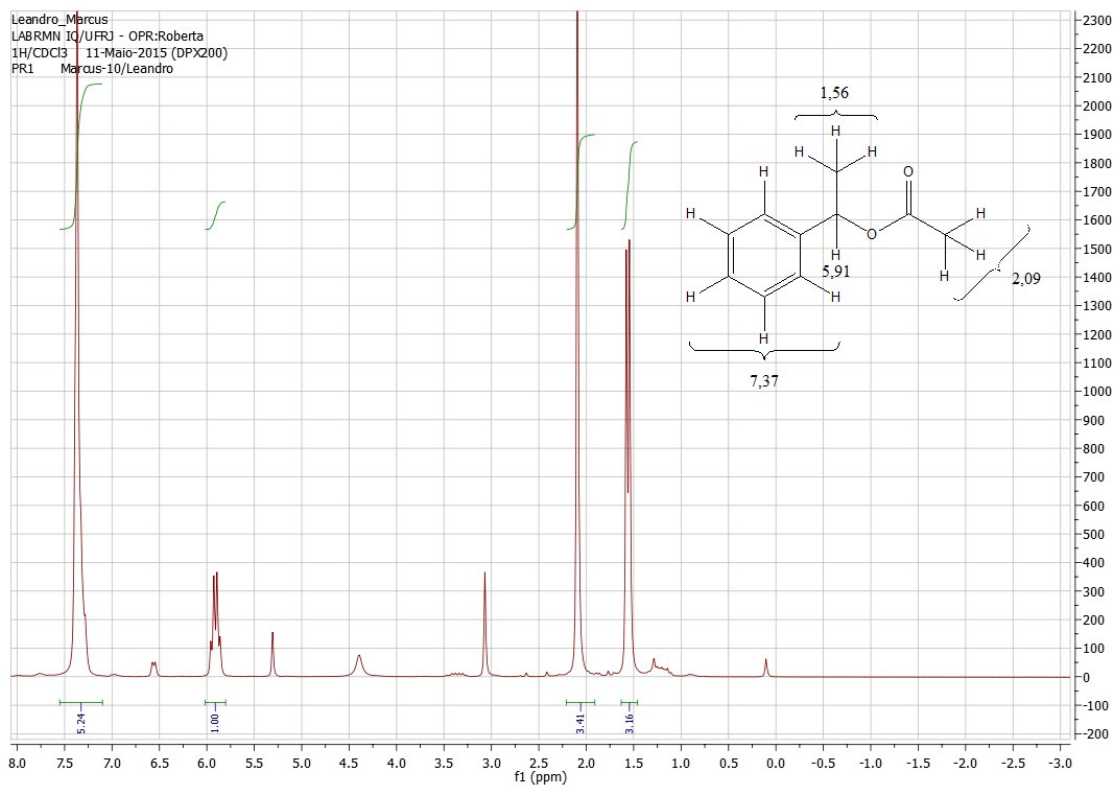


Figure S12:  $^1\text{H}$  RMN spectra of product of chemistry acetylation of (*RS*)-1-Phenylethanol

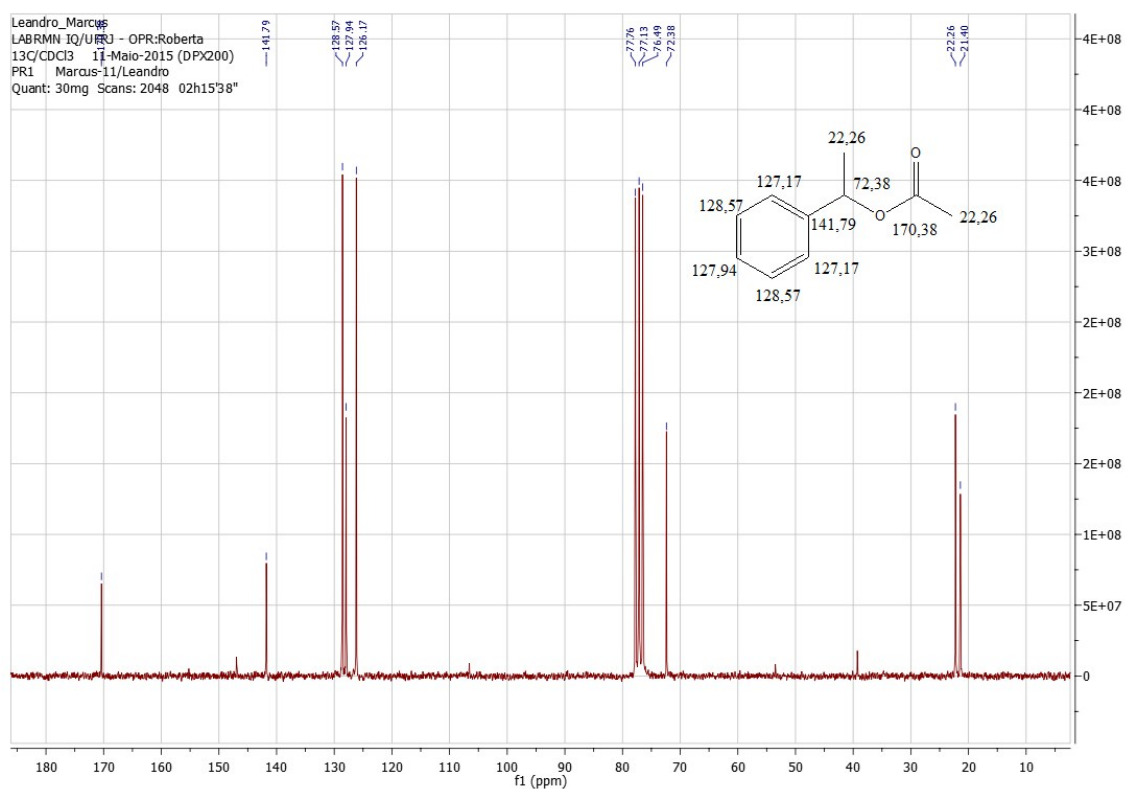


Figure S13:  $^{13}\text{C}$  RMN spectra of product of chemistry acetylation of (*RS*)-1-Phenylethanol

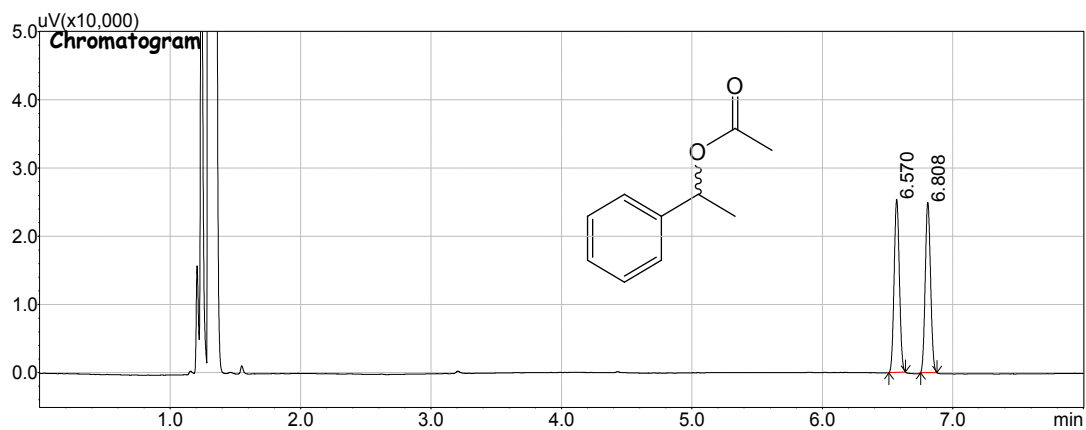


Figure S14. GC-FID of (*RS*)-1-Phenylethyl acetate.

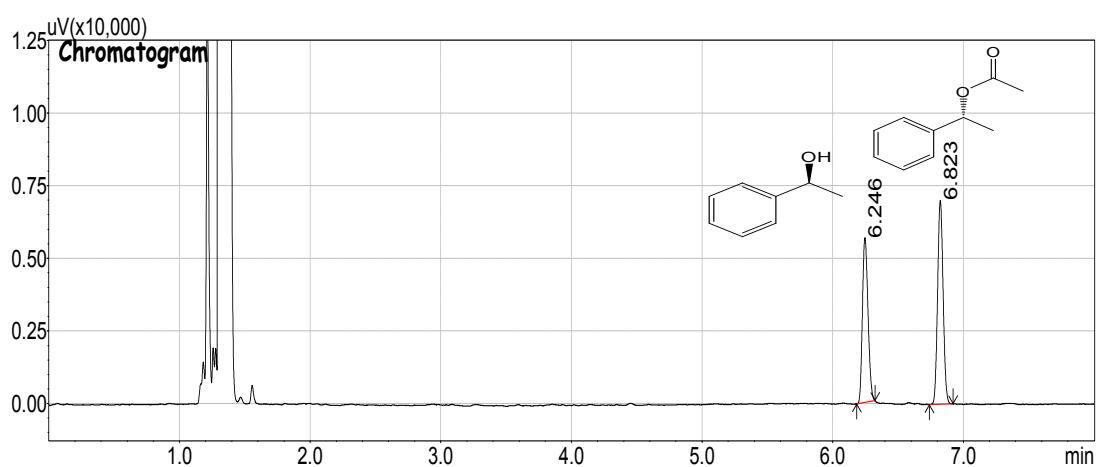


Figure S15. GC-FID of enzymatic acetylation of (*RS*)-1-Phenylethanol with Novozymes 435 for 24 hours. Experimental conditions: *rac*-1-Phenylethanol (1mmol, 122mg) 15% (w/w) of biocatalist, in cyclohexane (3 mL).

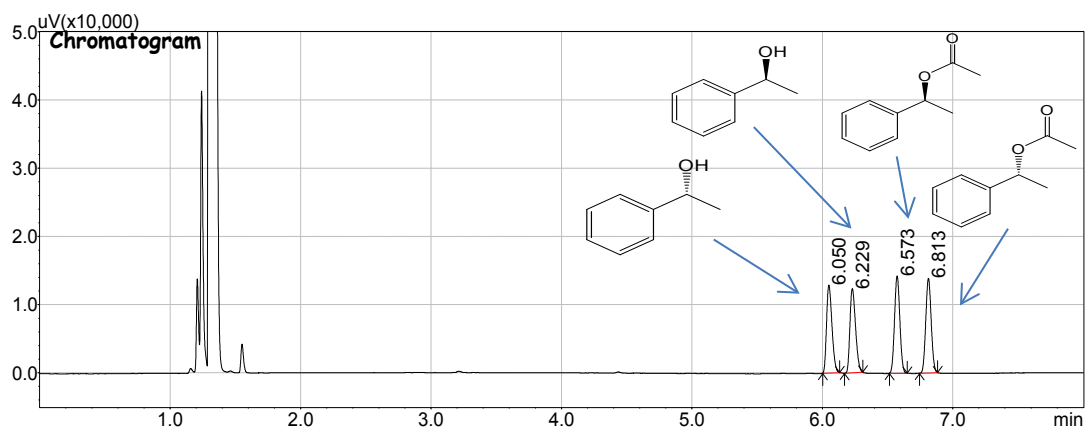


Figure S16: GC-FID of (*RS*)-1-Phenylethyl acetate and (*RS*)-1-Phenylethanol

## 2. Kinetic Resolution Experiments

### 2.1 – Chromatograms of Kinetic Resolution in continuous flow reactor

All reactions were conducted at 50°C, with 0.33M of 1-phenylethanol and 0.33M of vinyl acetate.

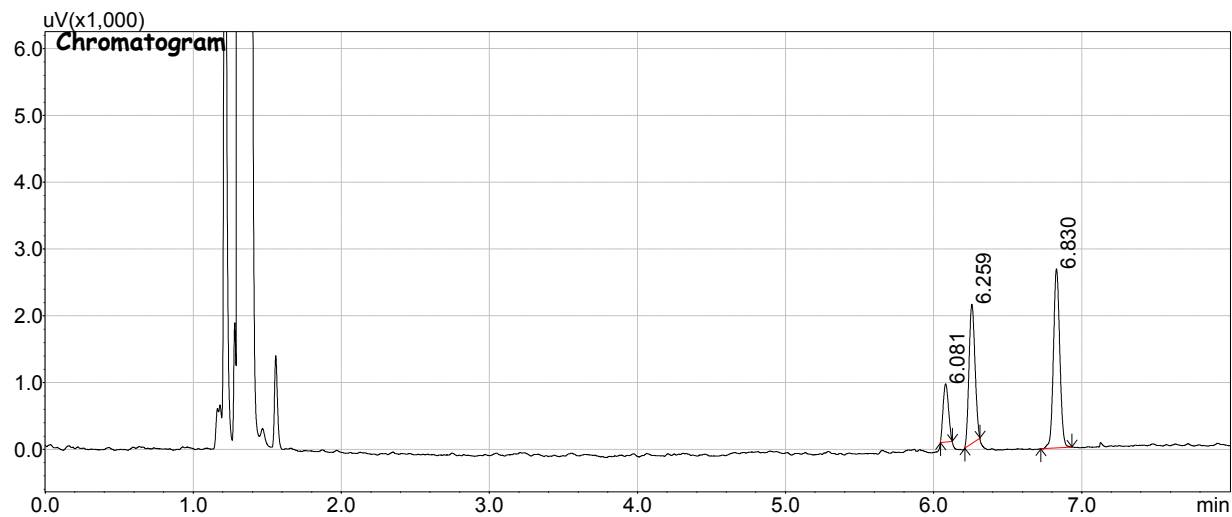


Figure S17: Novozymes 435 with 7 minutes of residence time.

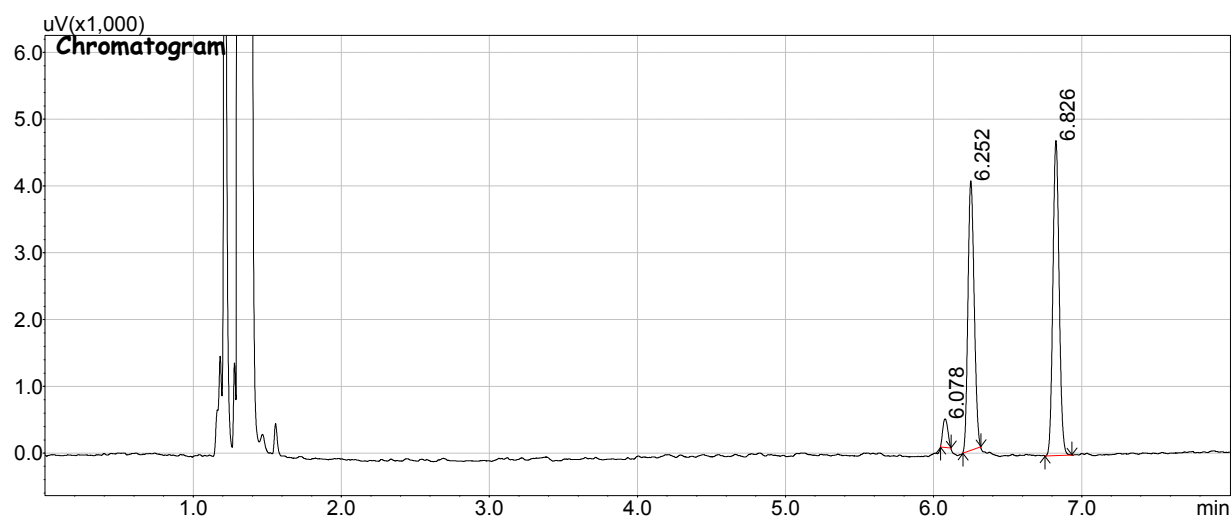


Figure S18: Novozymes 435 with 15 minutes of residence time.

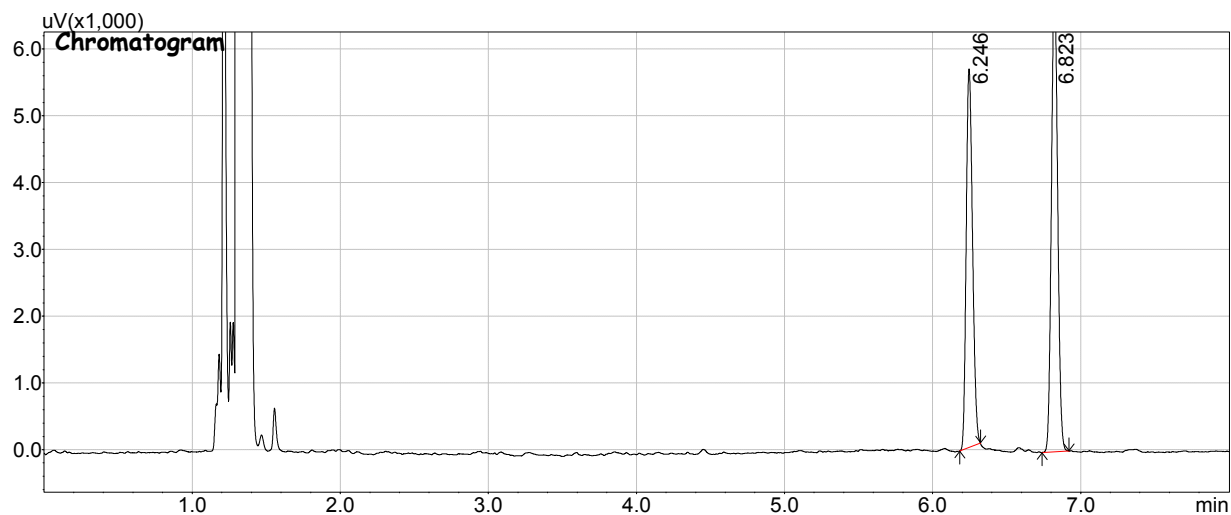


Figure S19: Novozymes 435 with 20 minutes of residence time.

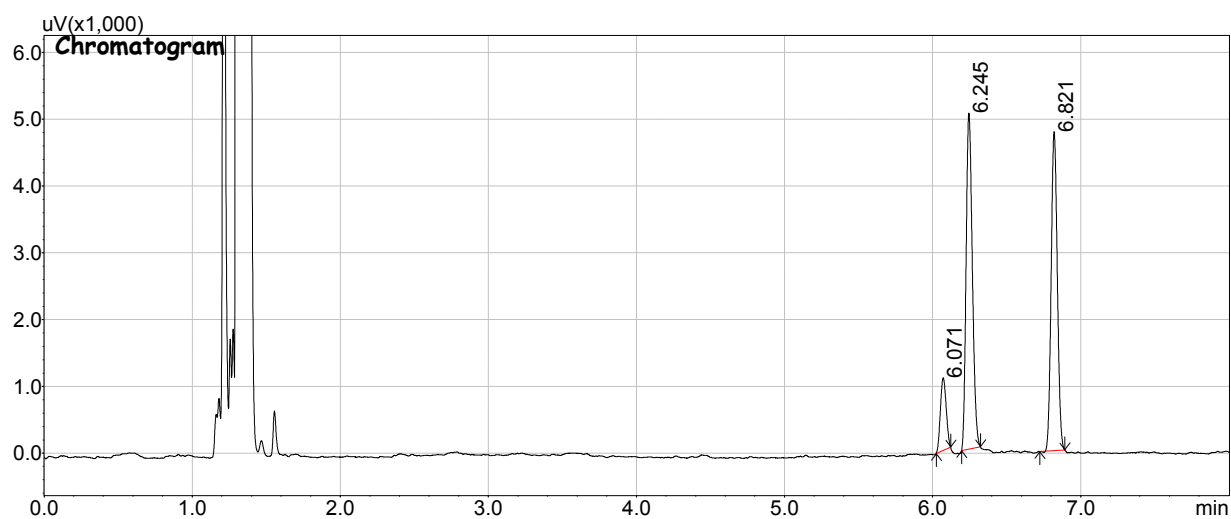


Figure S20: Accurel MP-1000 with 7 minutes of residence time.

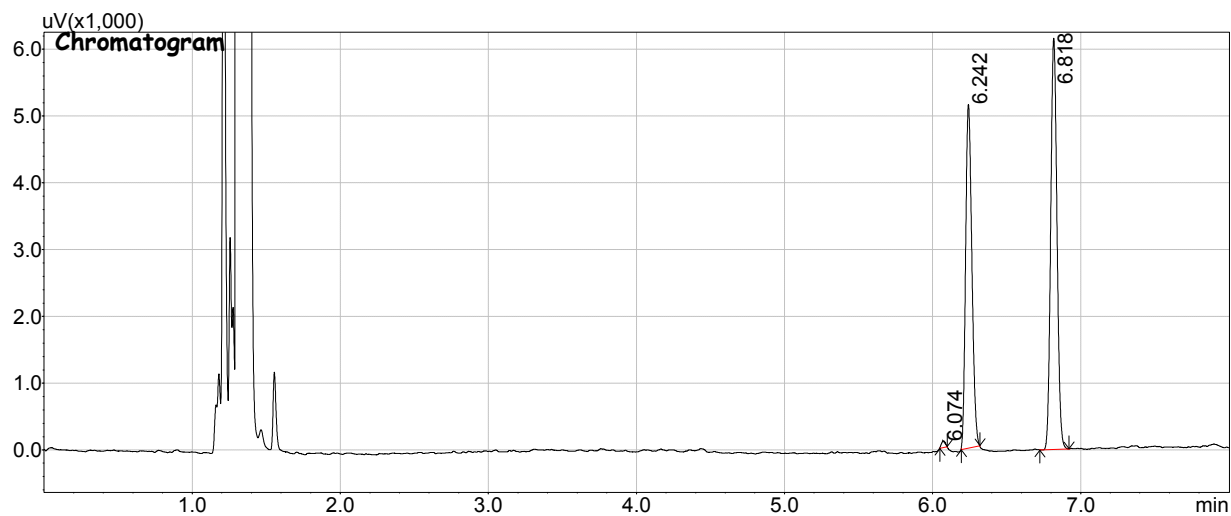


Figure S21: Accurel MP-1000 with 15 minutes of residence time.

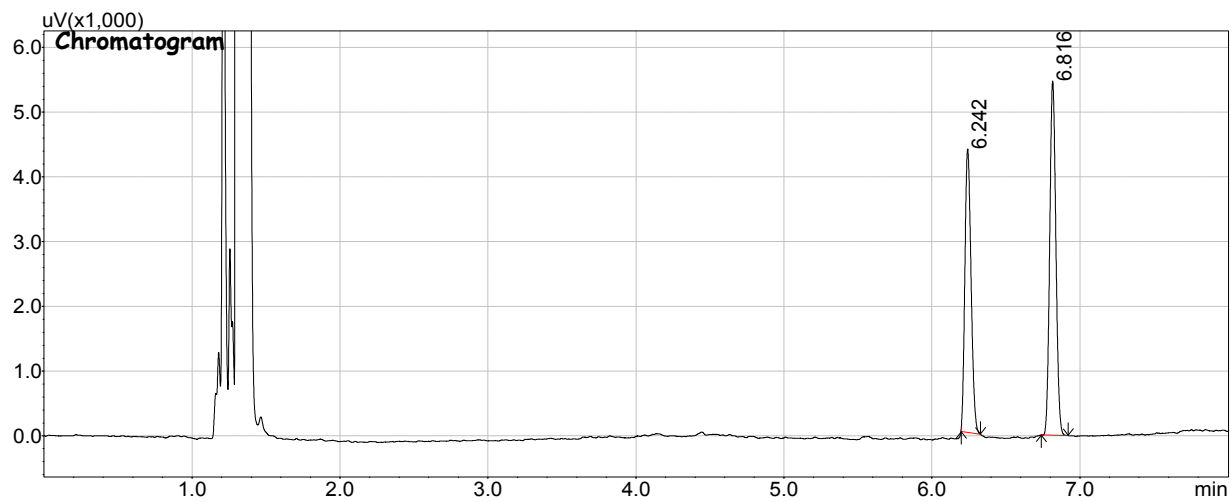


Figure S22: Accurel MP-1000 with 20 minutes of residence time.

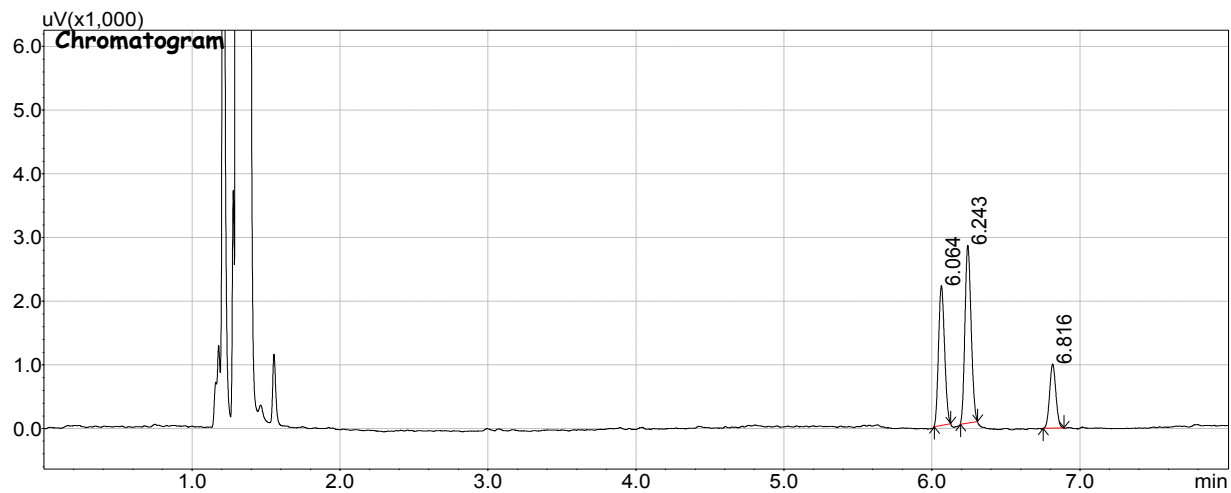


Figure S23: IB-S861 with 7 minutes of residence time.

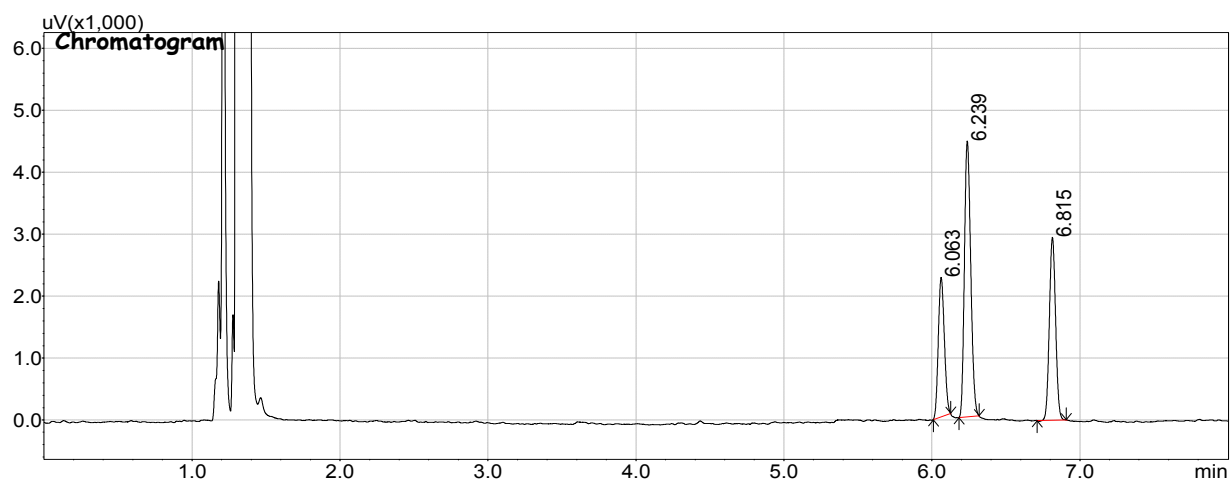


Figure S24: IB-S861 with 15 minutes of residence time.



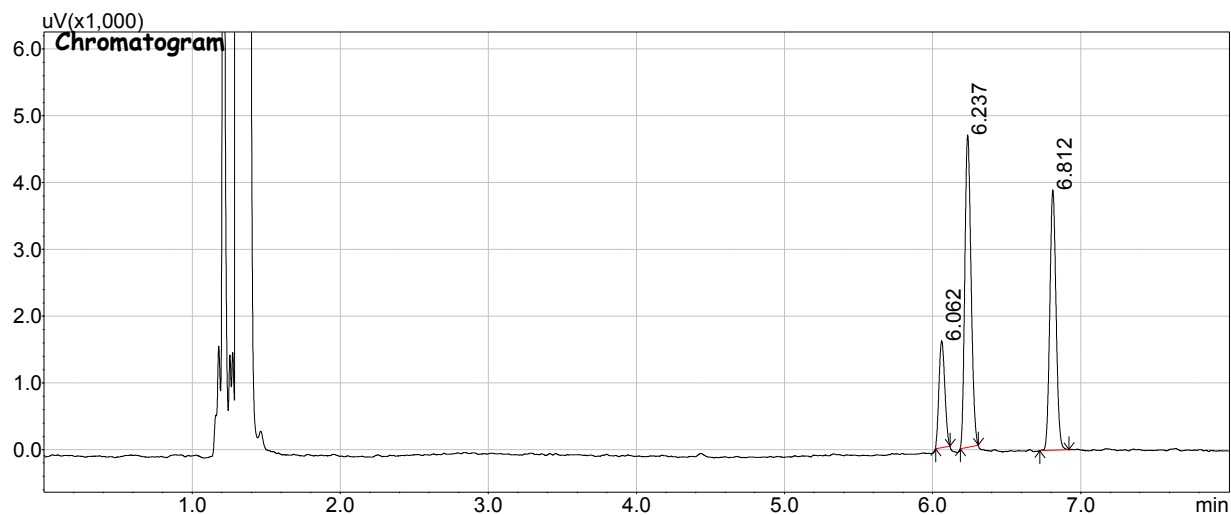


Figure S25: IB-S861 with 20 minutes of residence time

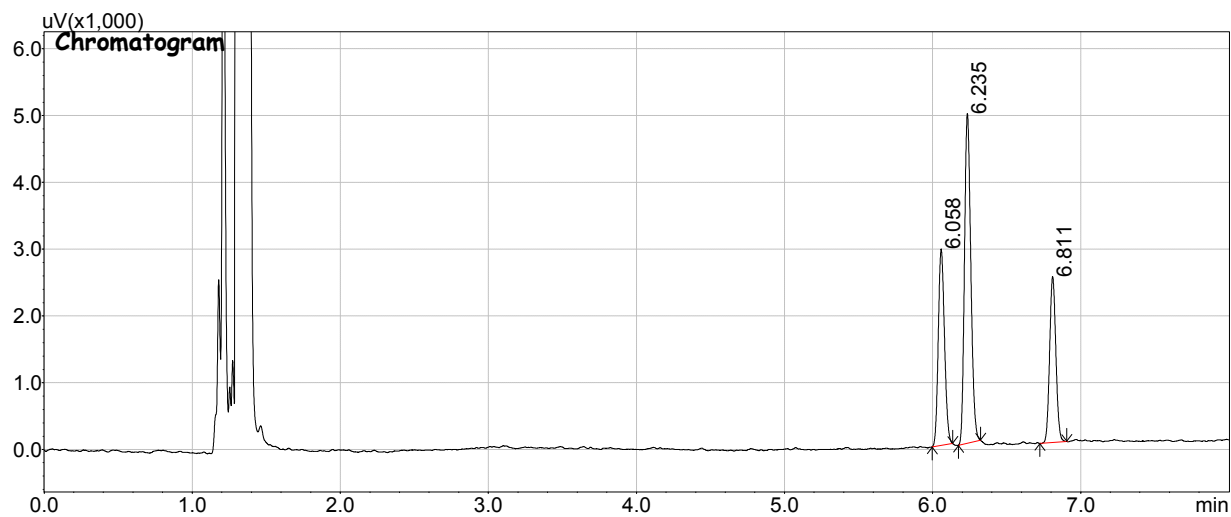


Figure S26: IB-EC1 with 7 minutes of residence time

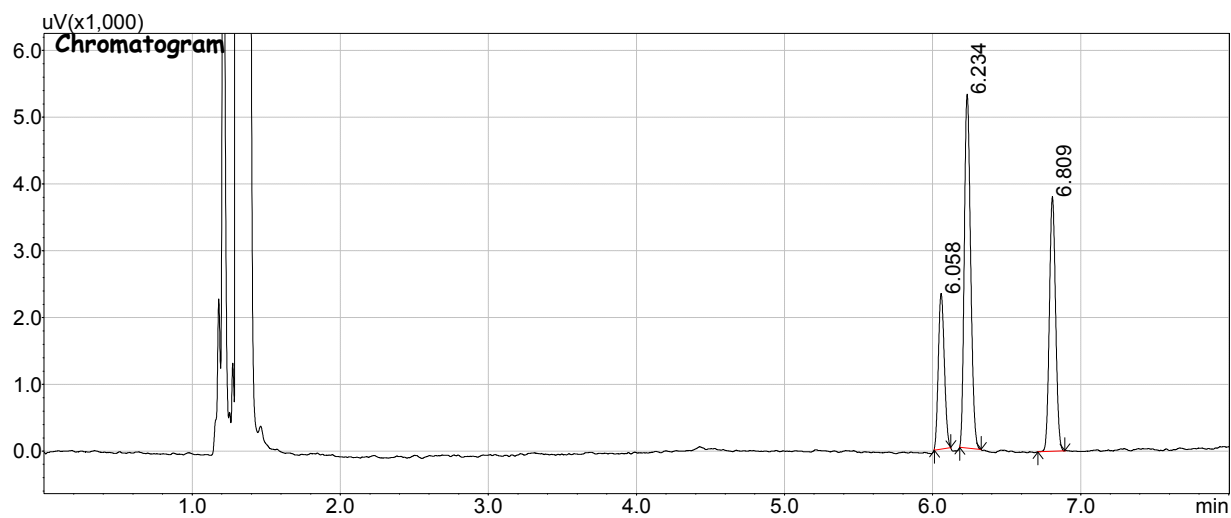


Figure S27: IB-EC1 with 15 minutes of residence time

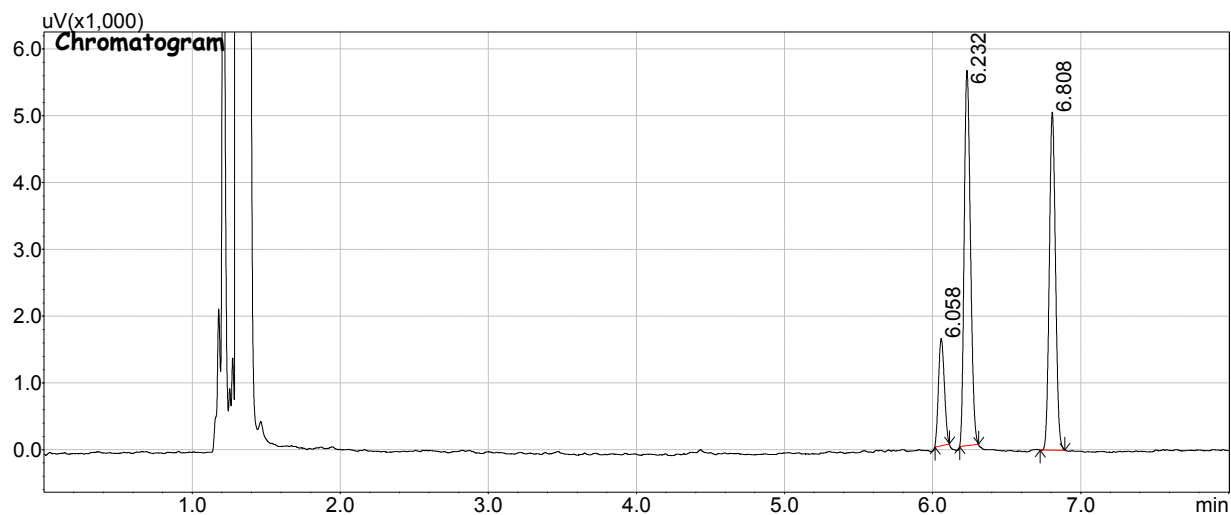


Figure S28: IB-EC1 with 20 minutes of residence time

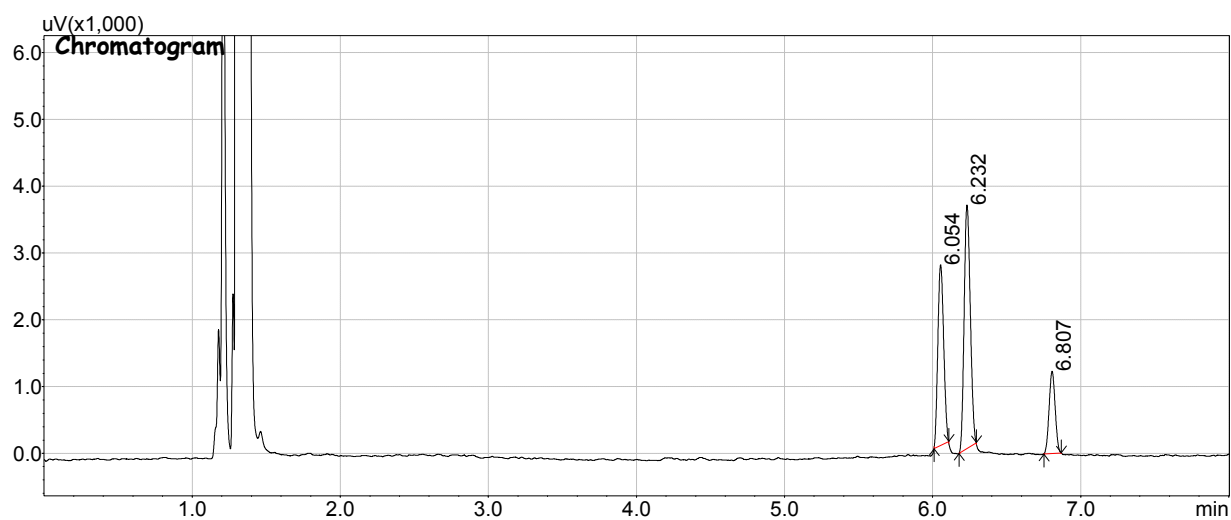


Figure S29: ECR1091F with 7 minutes of residence time.

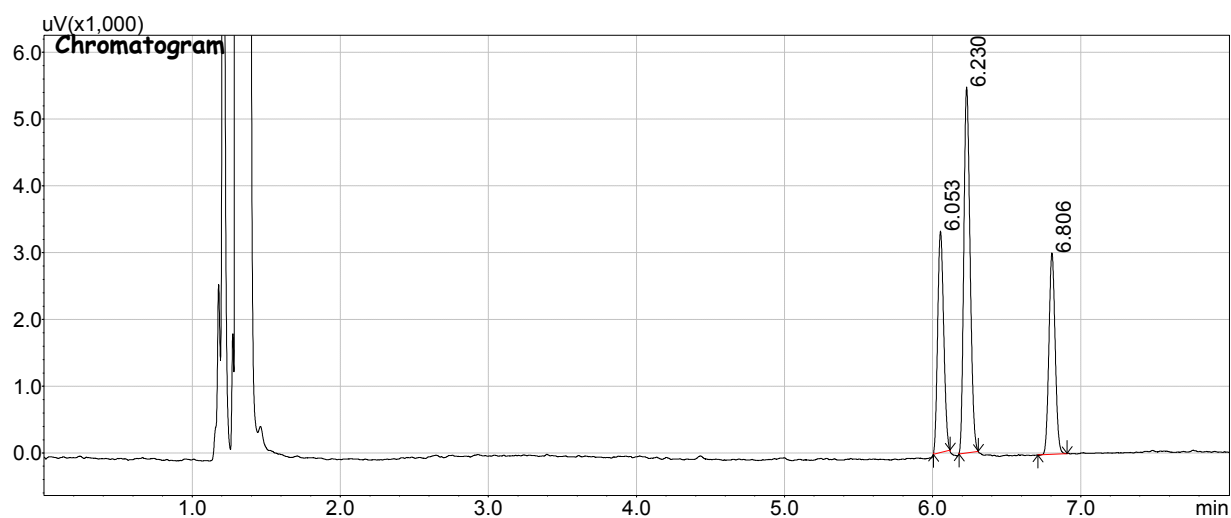


Figure S30: ECR1091F with 15 minutes of residence time.

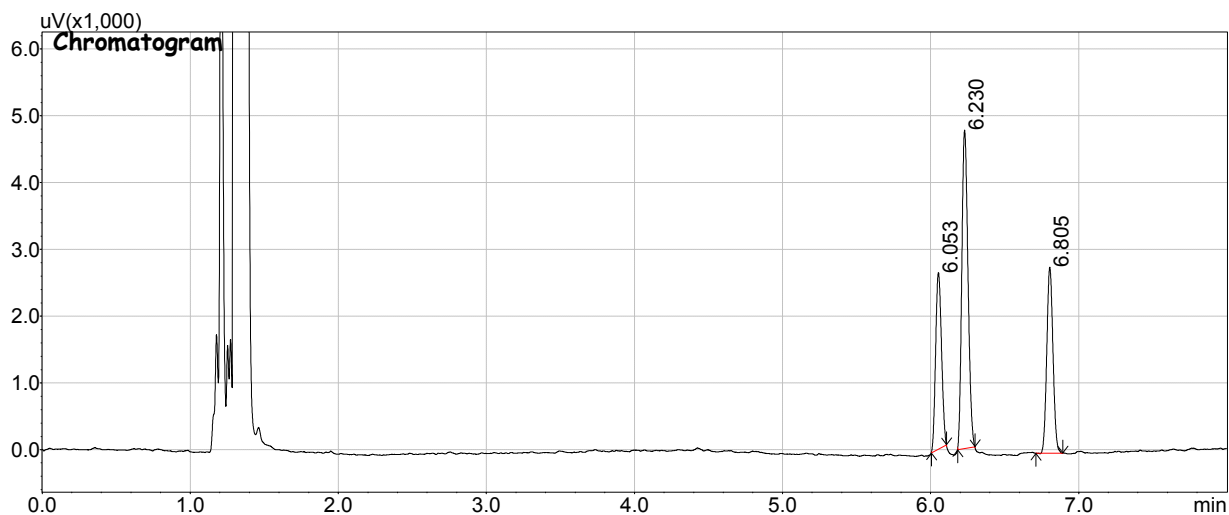


Figure S31: ECR1091F with 20 minutes of residence time

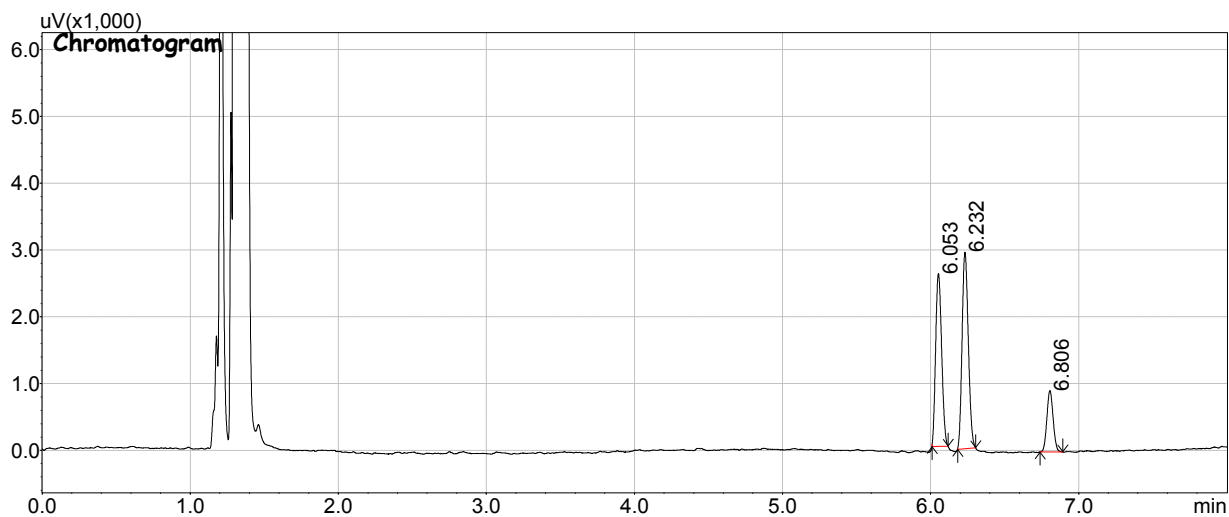


Figure S32: ECR8806F with 7 minutes of residence time.

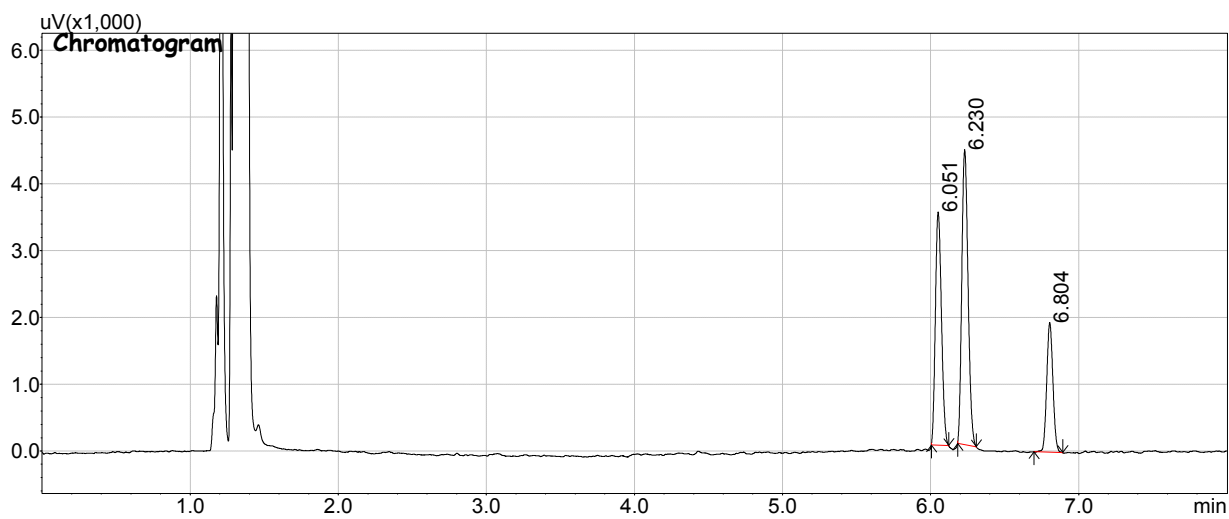


Figure S33: ECR8806F with 15 minutes of residence time.

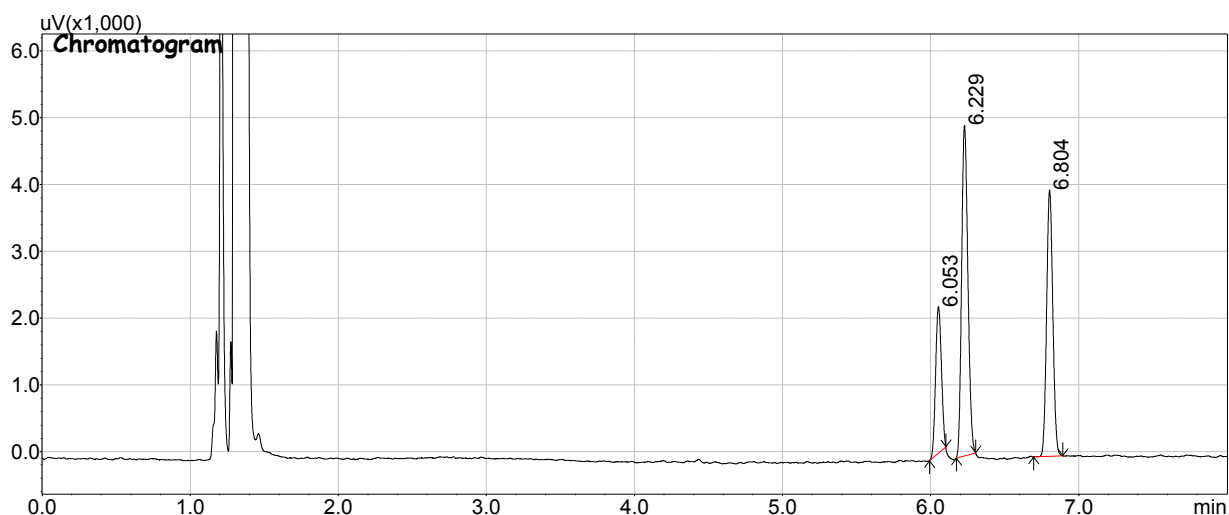


Figure S34: ECR8806F with 20 minutes of residence time.

### 2.1.2 - Chromatogram for Kinetic Resolution using Lipase B from *Candida antarctica* immobilized on Accurel MP-1000 in batch and continuous flow conditions.

- **Batch reactor**

All reactions under batch conditions occurred at 50°C, with 0.33M of 1-phenylethanol, 0.33M of vinyl acetate and 15% of biocatalyst in 3 mL of cyclohexane for 2 hours.

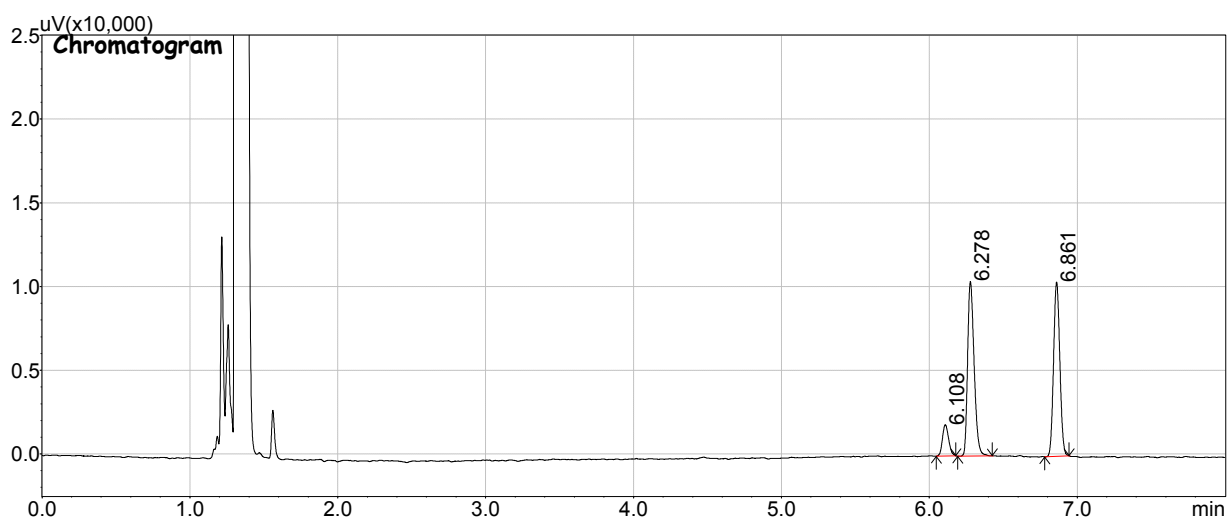


Figure S35: 1° cycle in batch.

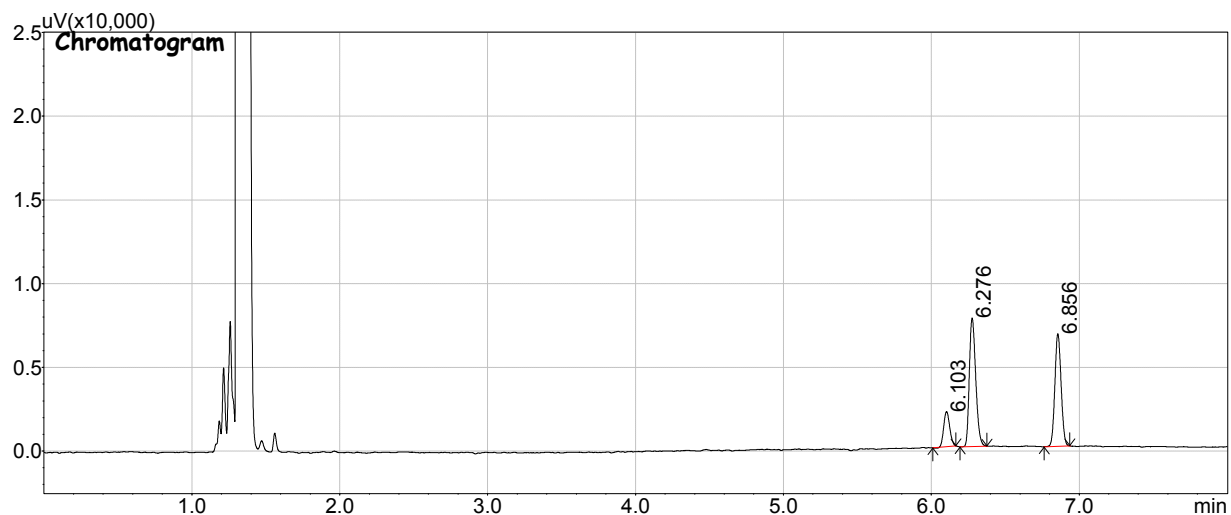


Figure S36: 2° cycle in batch.

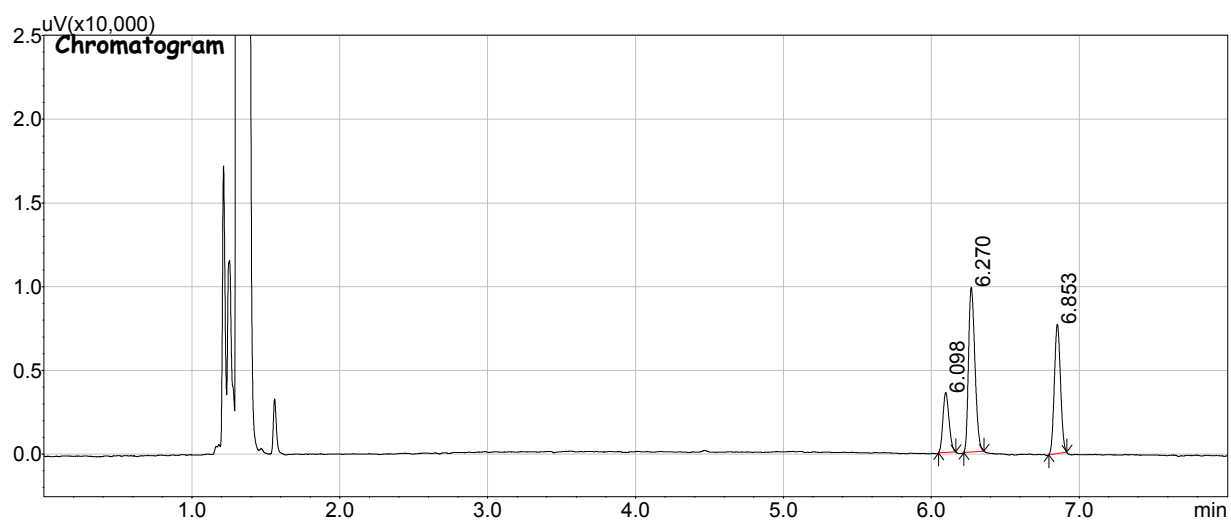


Figure S37: 3° cycle in batch

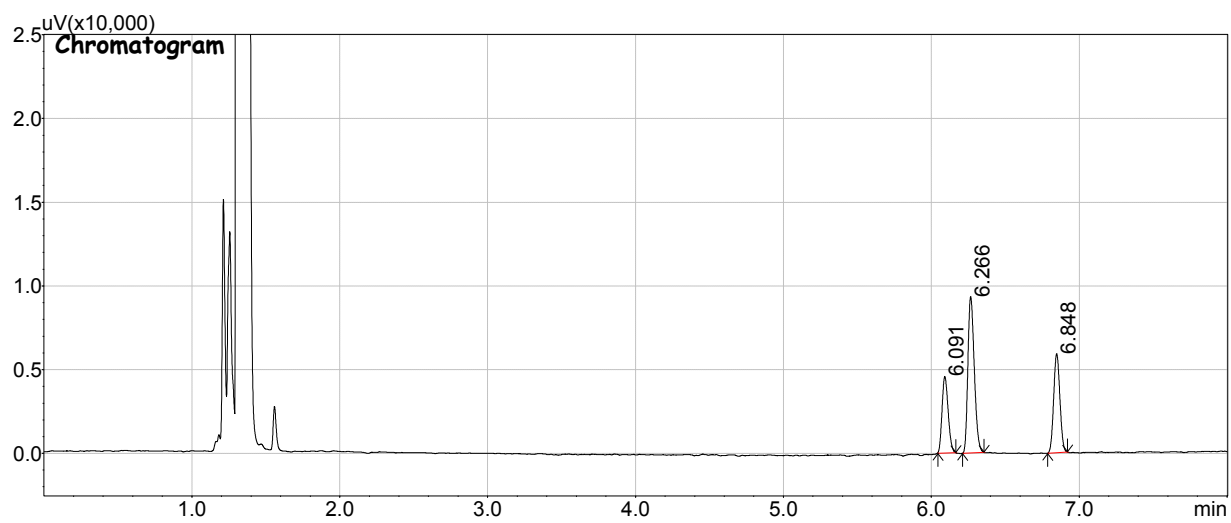


Figure S38: 4° cycle in batch

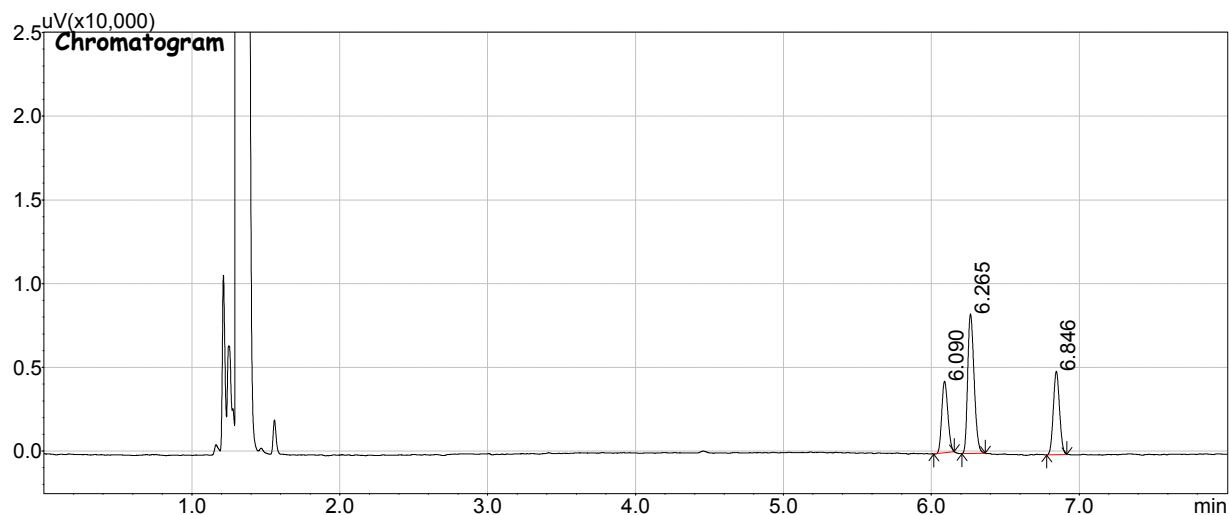


Figure S39: 5<sup>o</sup> cycle in batch

- **Continuous flow reactor**

All reactions under continuous flow conditions occurred 50°C, with 0.33M of 1-phenylethanol, 0.33M of vinyl acetate and 15 minutes of residence time.

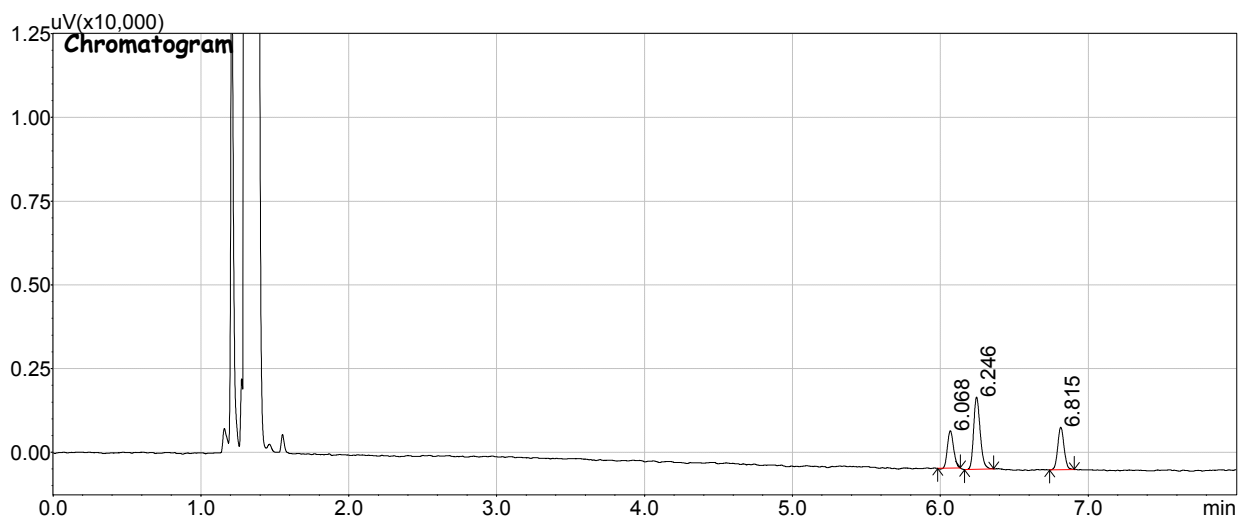


Figure S40: 1<sup>o</sup> cycle in continuous flow

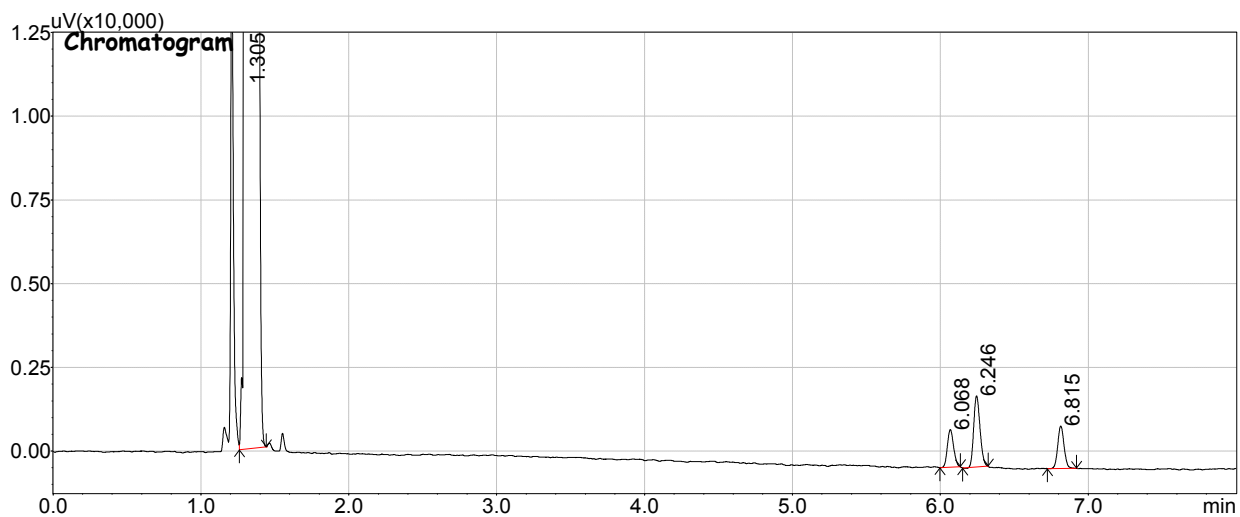


Figure S41: 2° cycle in continuous flow

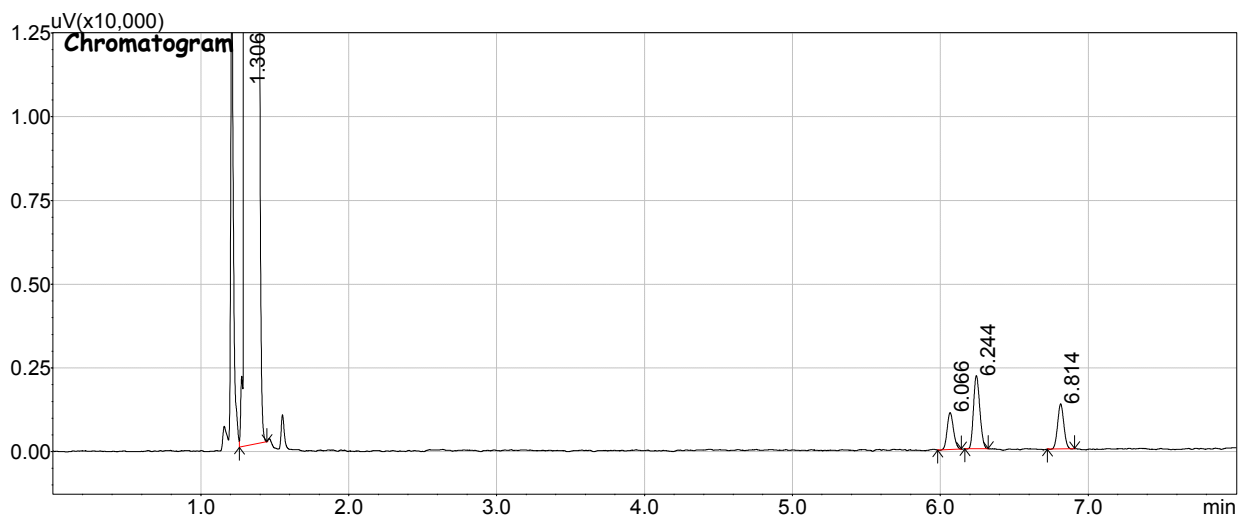


Figure S42: 3° cycle in continuous flow

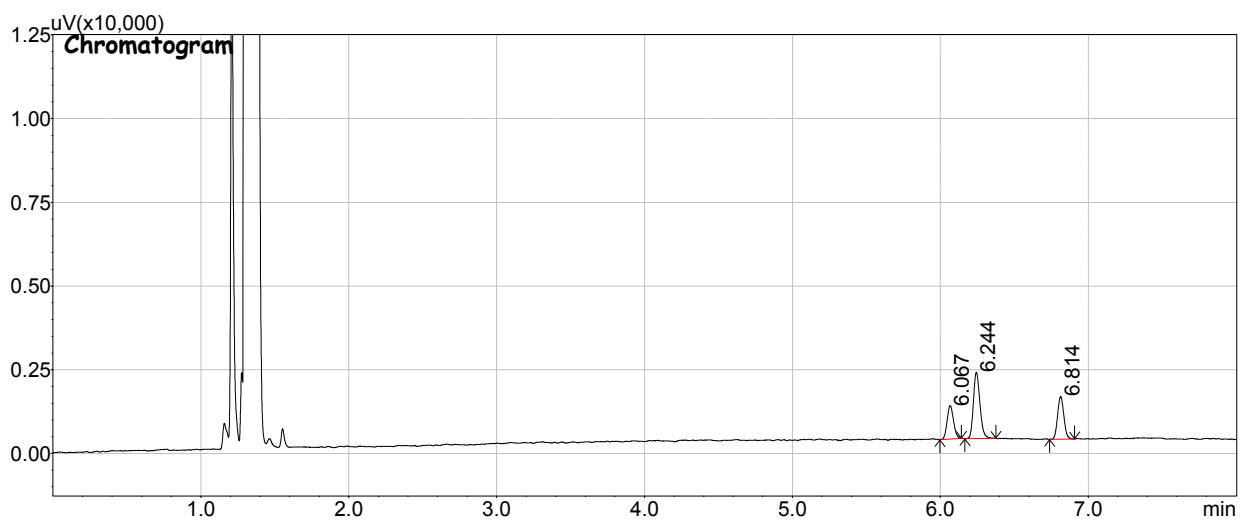


Figure S43: 4° cycle in continuous flow

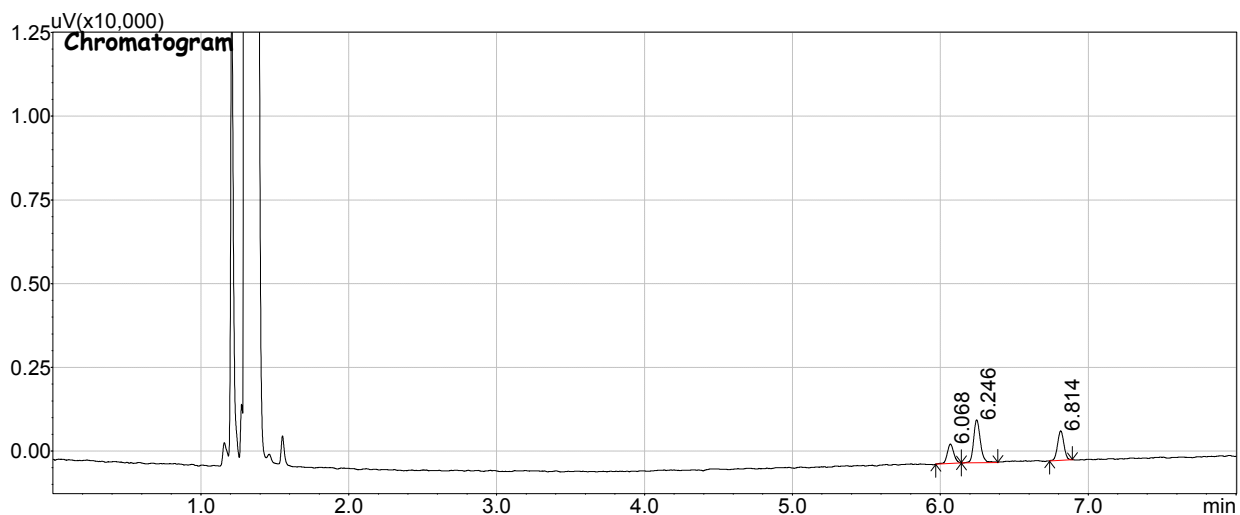


Figure S44: 5° cycle in continuous flow

### 3. Scanning Electronic Microscopy (SEM)

For all samples experimental conditions was: 1mg of sample metallized by gold under vacuum, and analysed in a Zeiss EVO® 50H microscopy

- **Accurel Support**

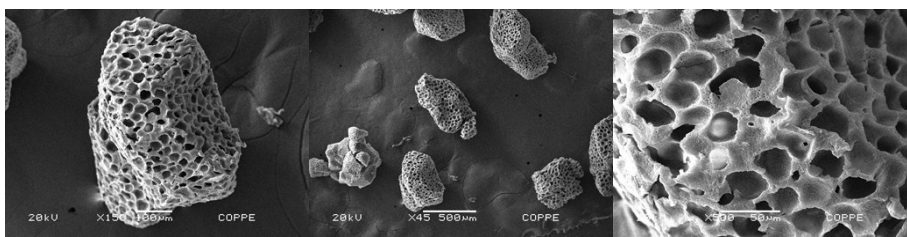


Figura S45 : Accurel Support in amplified x50, x150 e x500

- **Accurel Biocatalyst**

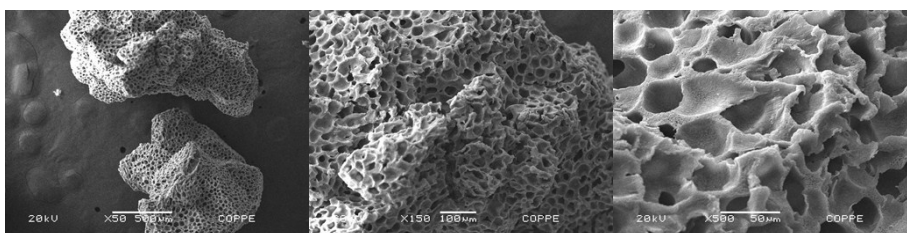


Figura S46 : Accurel Biocatalyst in amplified x50, x150 e x500



- **IB-S861 Support**

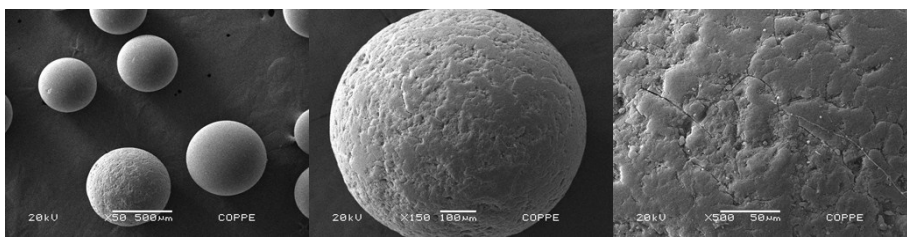


Figura S47 : IB-S861 Support in amplified x50, x150 e x500

- **IB-S861 Biocatalyst**

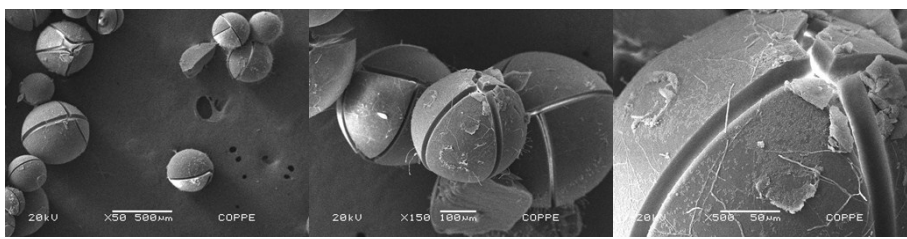


Figura S48 : IB-S861 Biocatalyst in amplified x50, x150 e x500

- **IB-EC1 Support**

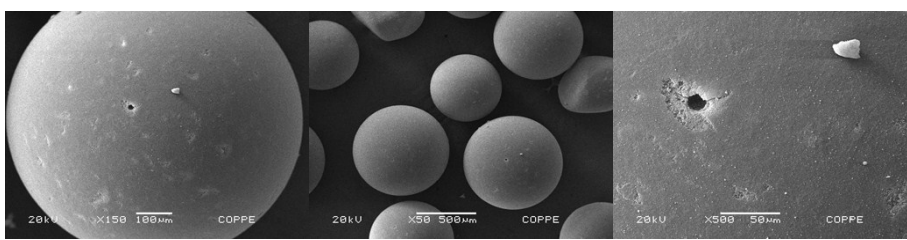


Figura S49 : IB-EC1 Support in amplified x50, x150 e x500

- **IB-EC1 Biocatalyst**

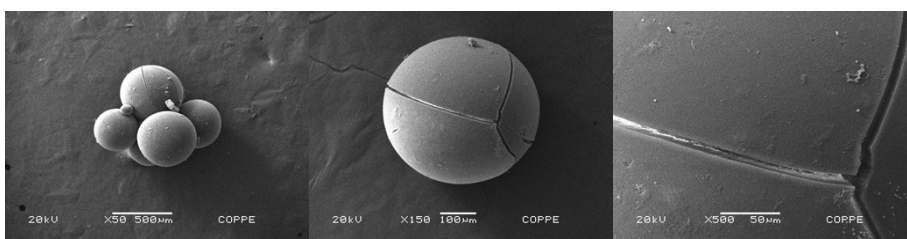


Figura S50 : IB-EC1 Biocatalyst in amplified x50, x150 e x500

- **ECR1091F Support**

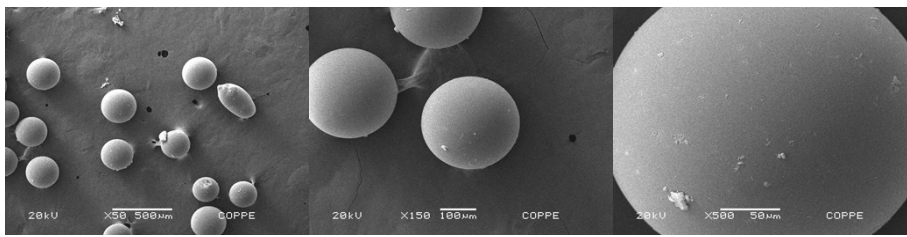


Figura S51 : ECR1091 Support in amplified x50, x150 e x500

- **ECR1091F Biocatalyst**

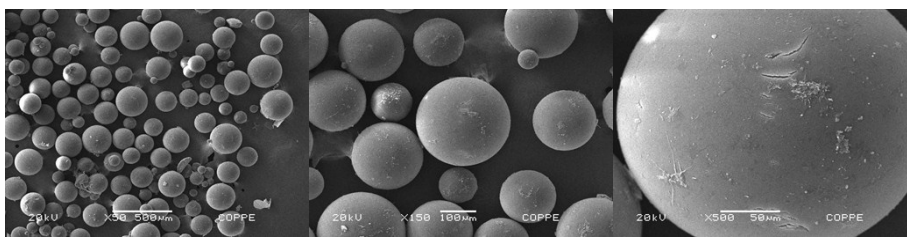


Figura S52 : ECR1091 Biocatalyst in amplified x50, x150 e x500

- **ECR8806F Support**

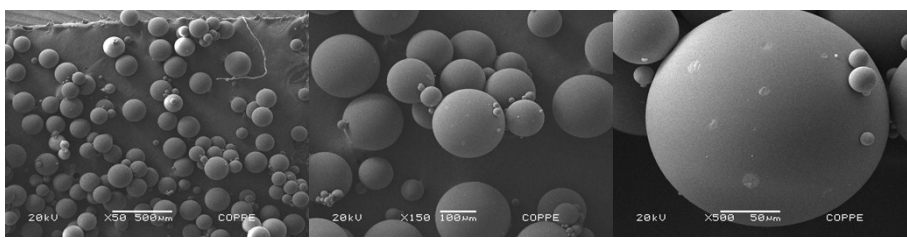


Figura S53 : ECR8806 Support in amplified x50, x150 e x500

- **ECR8806F Biocatalyst**

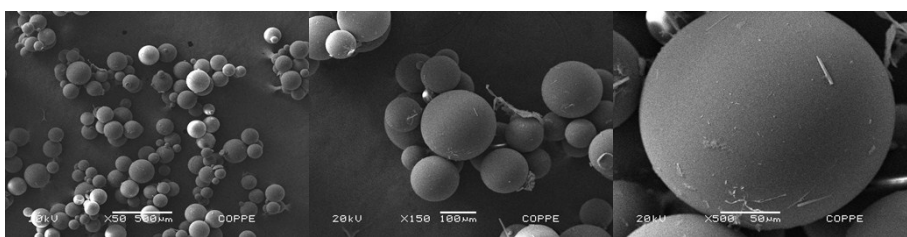


Figura S54 : ECR8806 Biocatalyst in amplified x50, x150 e x500

## 4. IR-RT Spectra

All IR-RT spectra were obtained using the methodologies described in Materials and Methods

- **Support and Biocatalyst of Accurel**

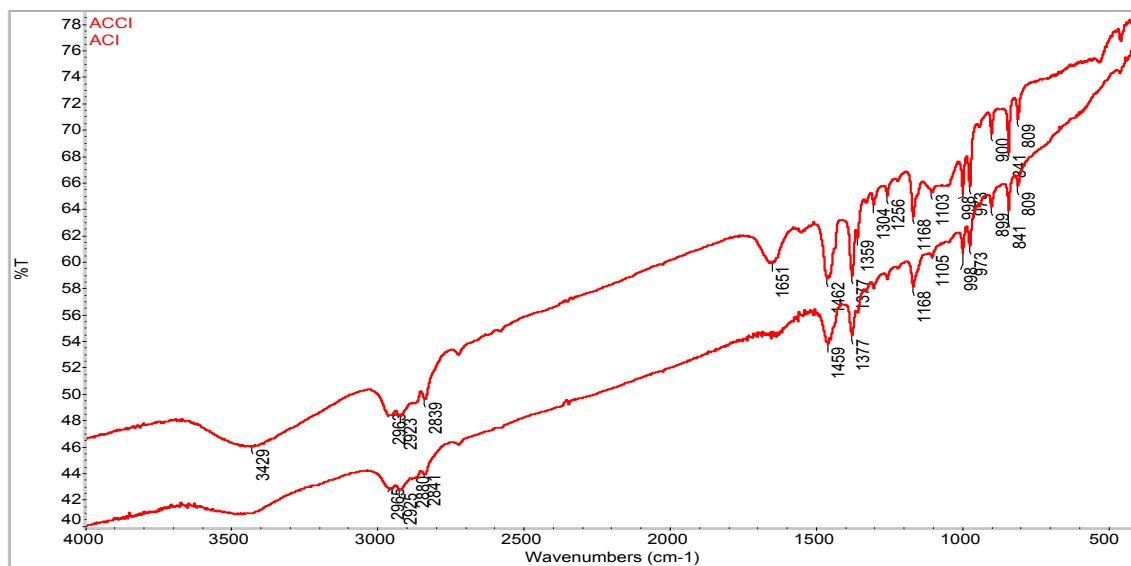


Figura S55: Support Infrared comparison (lower curve) and the biocatalyst (upper curve) of Accurel

- **Support and Biocatalyst of ECR1091F**

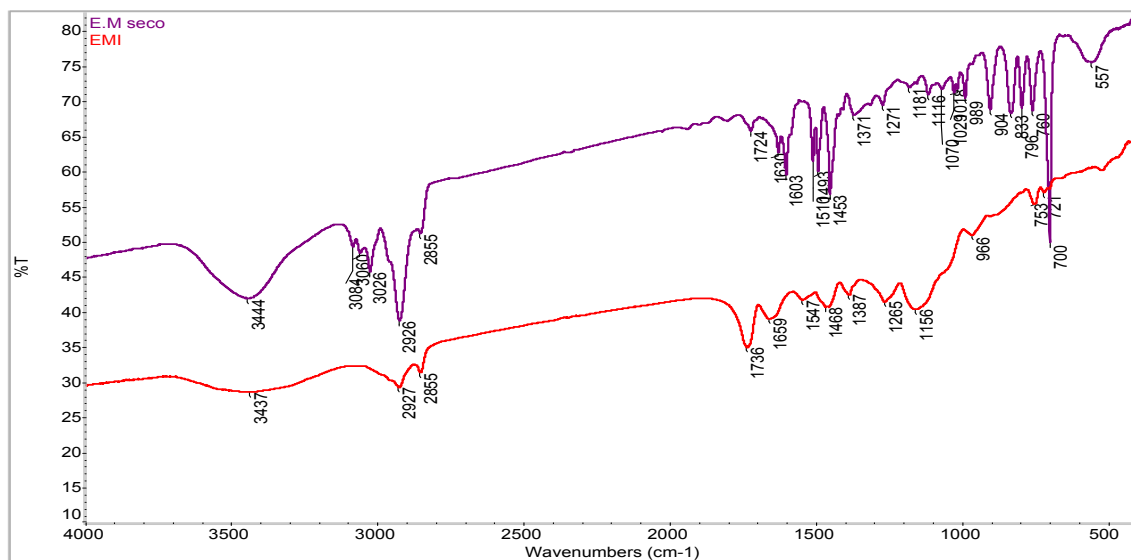


Figura S56: Support Infrared comparison (upper curve) and the biocatalyst (lower curve) of ECR1091F

• **Support and Biocatalyst of ECR8806F**

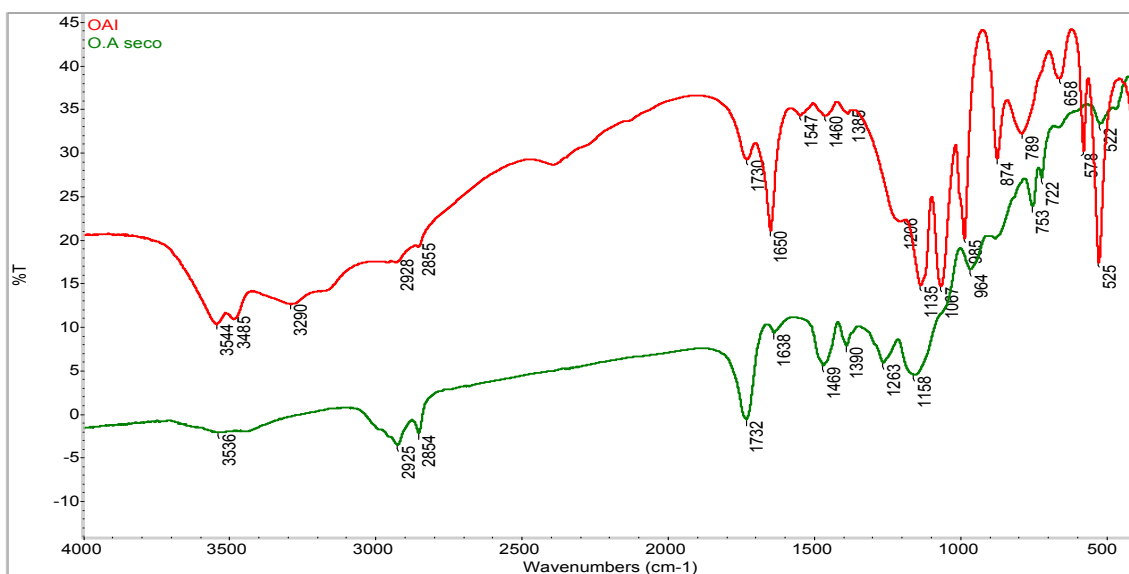


Figura S57: Support Infrared comparison (lower curve) and the biocatalyst (upper curve) ECR8806

• **Support and Biocatalyst of IB-S861**

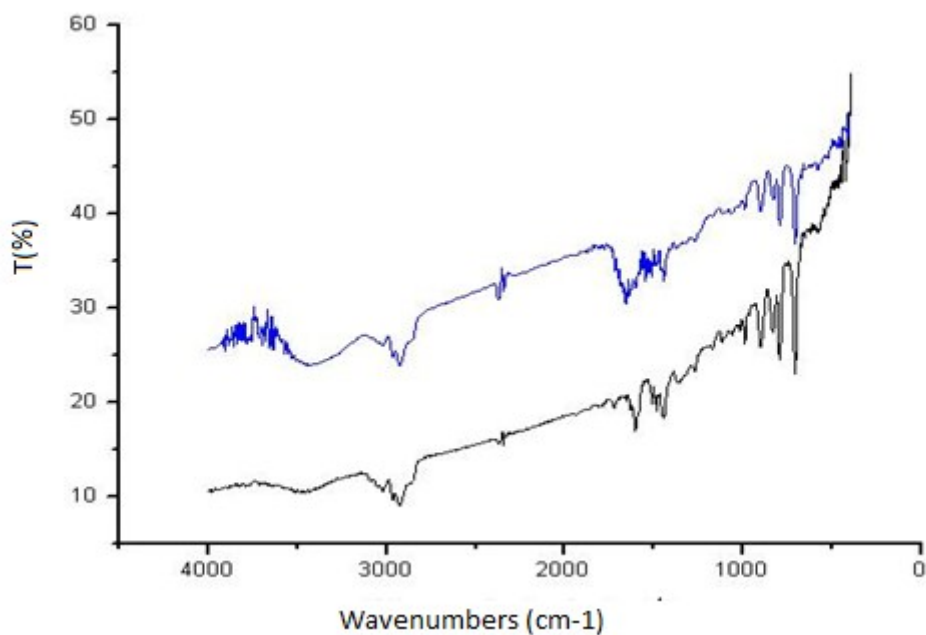


Figura S58: Support Infrared comparison (lower curve) and the biocatalyst (upper curve) of IB-S861

- **Support and Biocatalyst of IB-EC1**

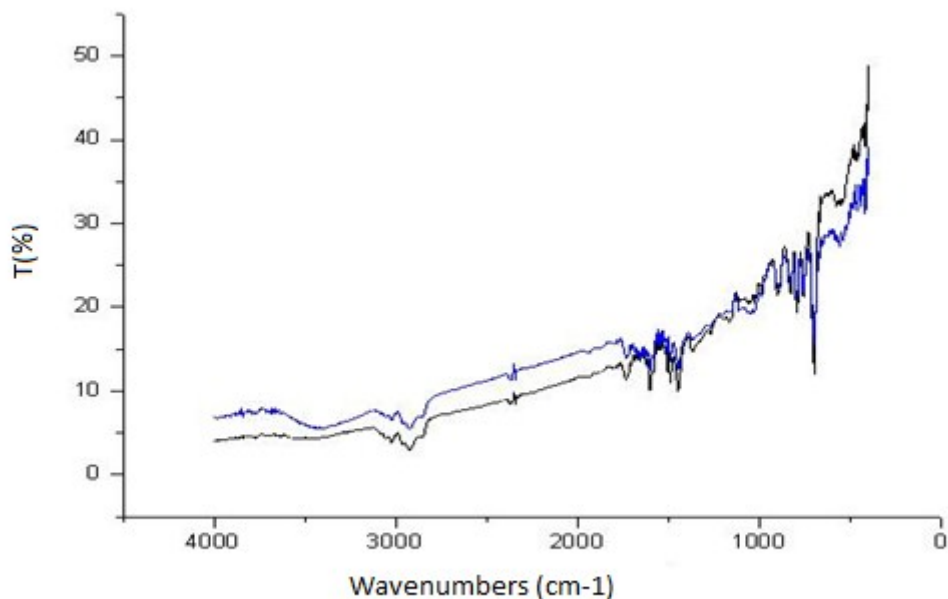


Figura S59: Support Infrared comparison (lower curve) and the biocatalyst (upper curve) of IB-EC1

### 5. Thermo gravimetric analysis

All Thermo gravimetric analysis obtained using the methodologies described in Materials and Methods

- **TG comparison Support/biocatalyst ECR8806F**

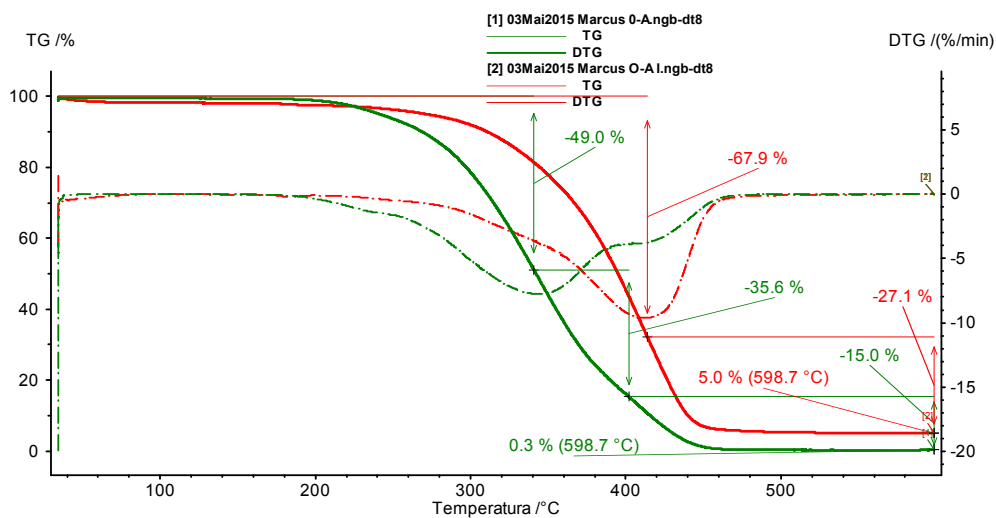


Figura S60: TG spectrum comparison support/biocatalyst ECR8806F

- **TG comparison Support / biocatalyst ECR1091F**

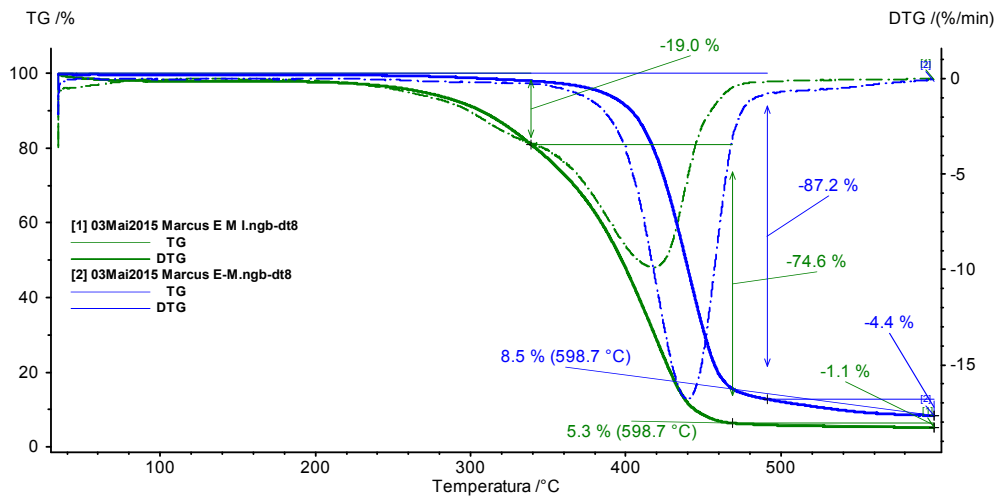


Figura S61: TG spectrum comparison support/biocatalyst ECR1091F

- **TG comparison Support / biocatalyst IB-S861**

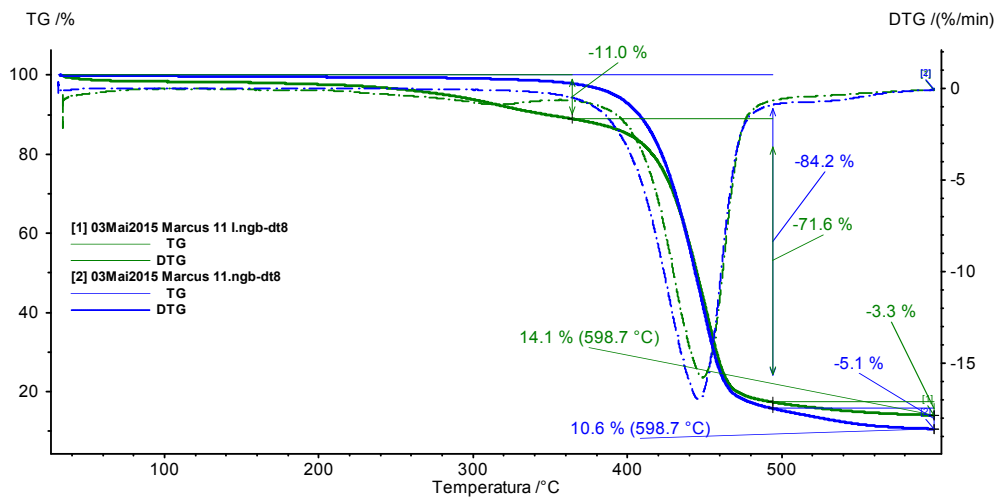


Figura S62: TG spectrum comparison support/biocatalyst IB-S861

- **TG comparison Support / biocatalyst IB-150A**

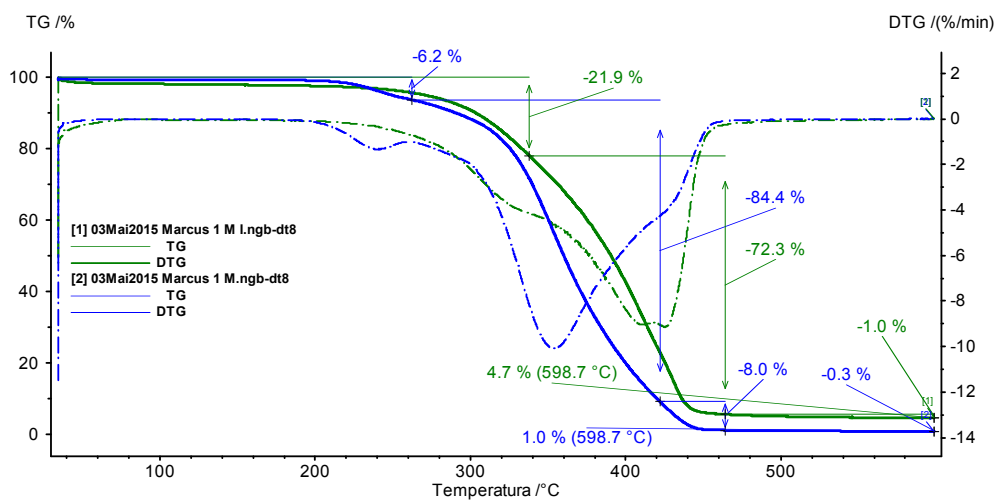


Figura S63: TG spectrum comparison support/biocatalyst IB-150A

- **TG comparison Support / biocatalyst IB-EC1**

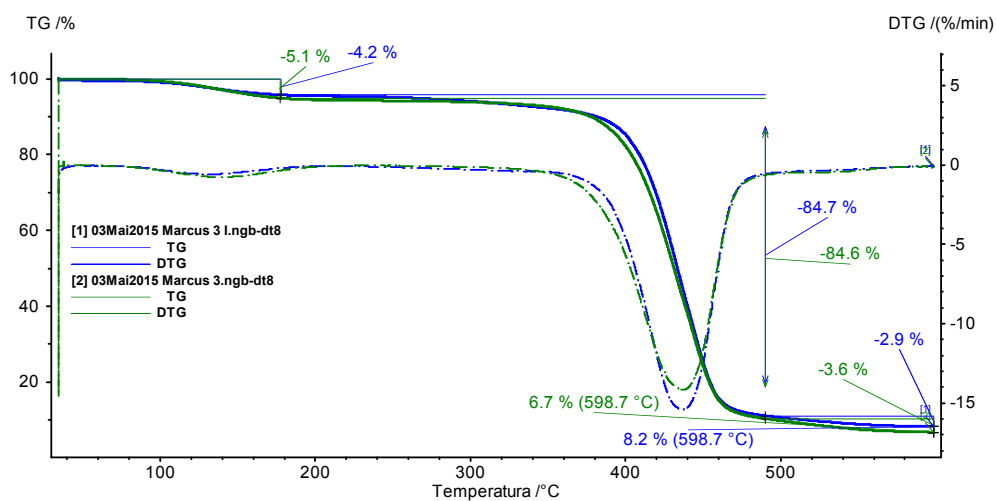


Figura S64: TG spectrum comparison support/biocatalyst IB-EC1

- **TG comparison Support / biocatalyst Accurel**

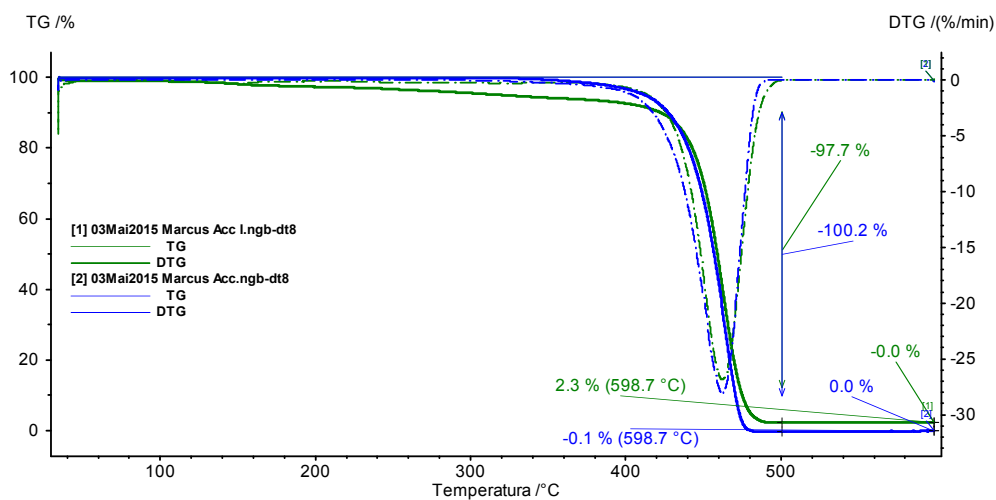


Figura S65: TG spectrum comparison support/biocatalyst Accurel

## 6. Commercial support characteristics

Support	Characteristic
Accurel MP1000	Polypropylene, particle size >1500 $\mu$ m
IB-EC1	Polyacrylic, carboxylic ester. Water content < 5%
IB-S861	Polystyrene, aromatic. Water content 35 - 70%
ECR1091F	Macroporous styrene, surface area >450m <sup>2</sup> /g, pore diameter 950-1200 (Å), particle size 150-300 mm
ECR8806F	Octadecyl methacrylate, surface area >50m <sup>2</sup> /g, pore diameter 500-700 (Å), particle size 150-300 mm