Supporting Information

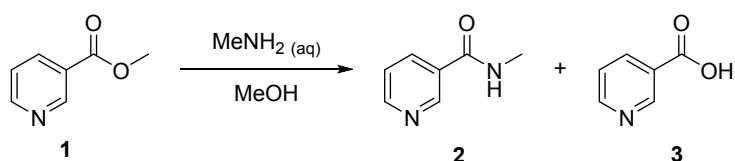## Online Quantitative Mass Spectrometry for the Rapid Adaptive Optimization of Automated Flow Reactors**

Nicholas Holmes, Geoffrey R. Akien, Robert J. D. Savage, Christian Stanetty, Ian R. Baxendale, Brian A. Taylor, Robert L. Woodward, Rebecca E. Meadows, A. John Blacker and Richard A. Bourne*

Contents

1 Reaction and Chemicals

*Scheme S1 - The reaction of methyl nicotinate **1** with aqueous methylamine to form the desired* N'-*methyl nicotinamide **2** and the impurity nicotinic acid **3***

Methyl nicotinate (99%, Alfa Aesar), methylamine (40% wt in water, Merck; 2 M in methanol, Alfa Aesar), methanol (HPLC grade, Sigma-Aldrich), niacin (99.5%, Acros) were used as starting materials and/or analytical standards. *N'*-methyl nicotinamide standard was synthesized and was determined as >99% by ^{1}H NMR. All commercial chemicals were used without further purification.

2 Equipment

2.1 Automated Reactor
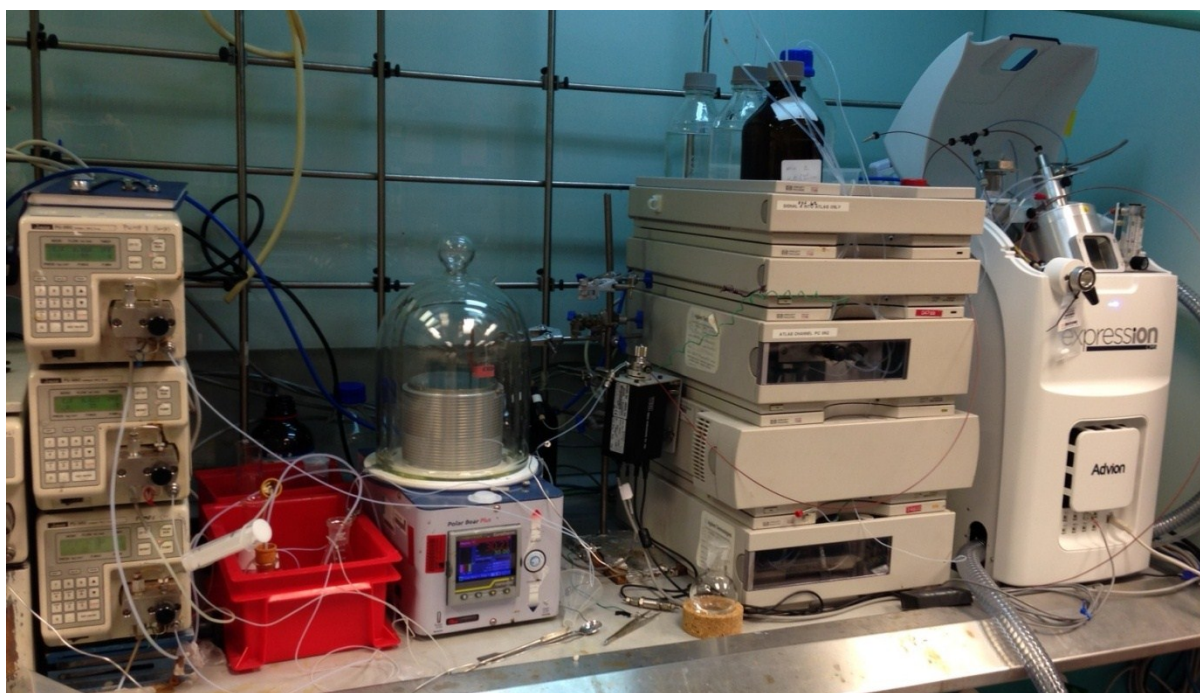
Figure S1 - Photo of automated flow reactor

Reagents were pumped using Jasco PU980 dual piston HPLC pumps and pump streams were mixed using Swagelok SS-100-3 tee-pieces. A 3 mL reactor was fitted to a Cambridge Reactor Design Polar Bear Flow Synthesizer. Sampling was achieved using a VICI Valco EUDA-Cl4W.06 sample loop with 0.06 µL injection volume. The reactor was maintained

under fixed back pressure using an Upchurch Scientific 250 psi back pressure regulator. Polyflon PTFE tubing (1/16" OD, 1/32" ID) was used throughout the reactor.
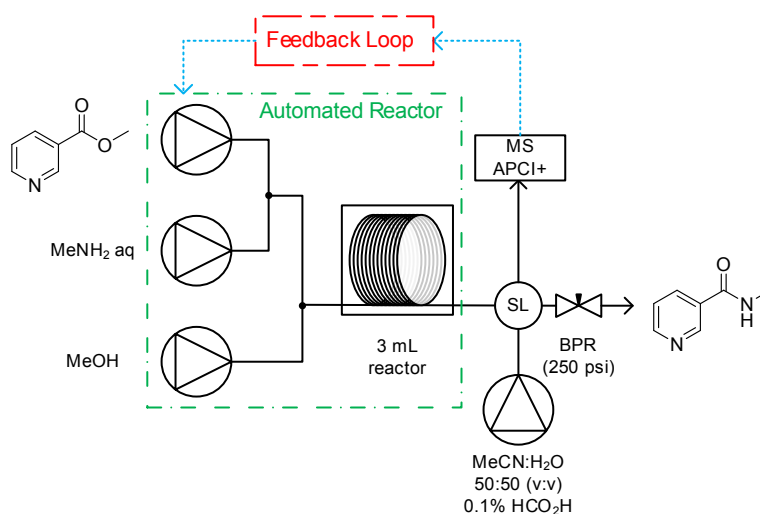

Figure S2 - Schematic for the automated reactor

2.2 MS Set-up

An Advion Expression CMS operating in positive APCI mode was used for collecting online and offline MS spectra. A mobile phase of 1:1 (v:v) H_2O:MeCN was used with 0.1% formic acid buffer and flow rate 0.3 mL min^{-1}. An Agilent G1311A quaternary pump was used to pump the mobile phase with separate feeds of H_2O (18.2 MΩ), MeCN and formic acid (10%, v:v). Agilent capillary tubing (0.17 mm ID) delivered mobile phase from the pump to the sample loop, peek tubing (red, 1/16" OD, 0.005" ID) from the sample loop to the MS.

The two ionization settings were compared using a stream of crude reaction material (Figure S3)
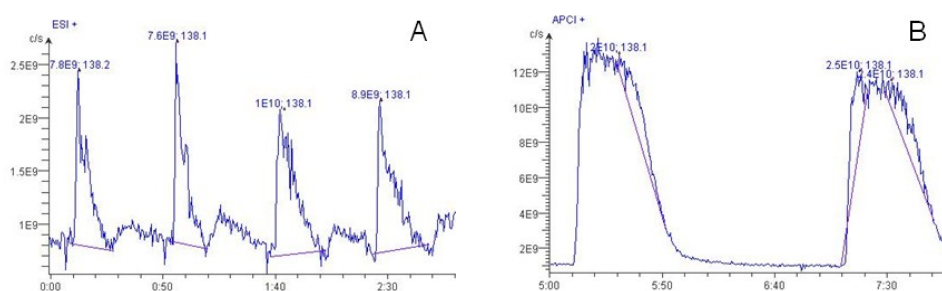

Figure S3 – Comparison of spectra generated in a 3 minute period between ESI+ (A) and APCI+ (B).

A 5:2 flow splitter was created by attaching 6.5 cm and 16.5 cm peek tubing (red) to a microvolume tee-piece, where the input was mobile phase from the sample loop. The shorter

tubing was a waste outlet, and the longer tube was the MS input. The change in back pressure between the different tube lengths was used to control the flow splitter. The mobile phase flow rate into the MS was calculated to be 0.12 mL/min (calculated based on the approximate pressure drop across the 2 tee-piece outlets). The total concentration of **1**-**3** is calculated to be 0.12 mmol L^{-1} when entering the APCI, according to the sample loop dilution and flow splitter. The actual analyte concentration is difficult to calculate as it depends on the alignment of the APCI to the chamber and the dispersion within the tube, neither of which were measured.
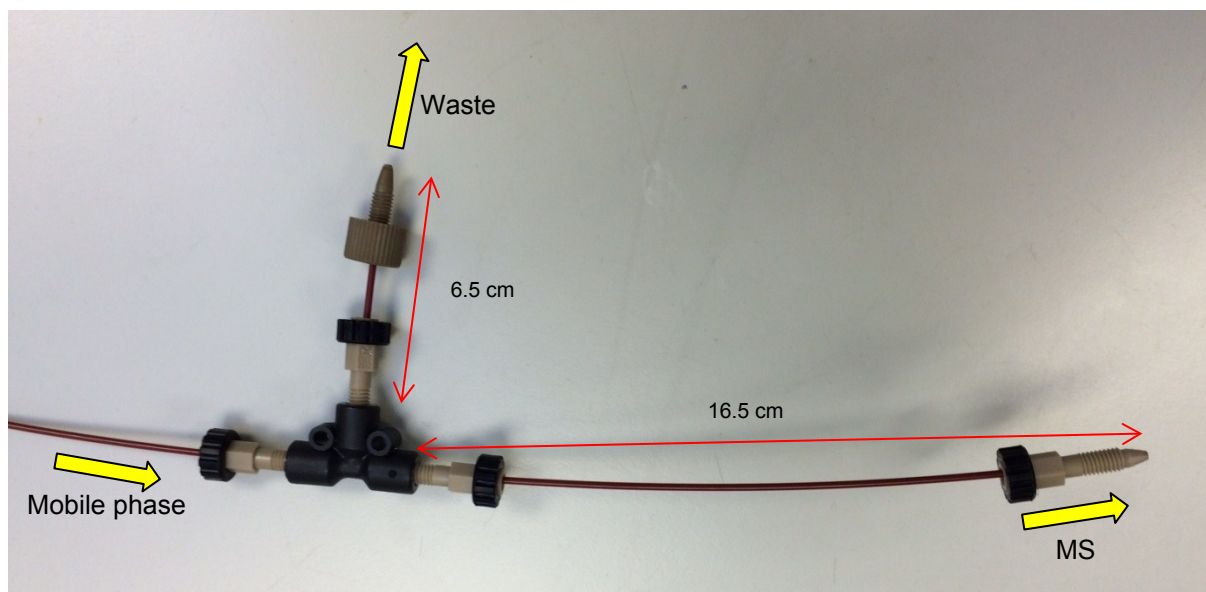

Figure S4 – Flow splitter used to reduce the mobile phase flow by ~2.5 times.

The MS ionization settings were: capillary temperature 200 °C, capillary voltage 150 V, source voltage offset 20 V, source voltage span 30 V, source gas temperature 350 °C.

2.3 Analytical

NMR analysis was carried out using a Bruker 300 MHz fourier transform machine. Chemical shifts are quoted as parts per million (ppm) with reference to an internal solvent peak of $SiMe_4$ (TMS). Peaks are quoted as s (singlet), d (doublet) and t (triplet) or multiples thereof (e.g. dd doublet of doublets, dt doublet of triplets etc.)

HPLC analysis was carried out using an Agilent 1100 fitted with a Sigma Ascentis Express column (5 cm x 4.7 mm, 2.7 µM particle size) and mobile phases: A H_2O (18.2 MΩ), B MeCN, C formic acid (10% in H_2O, v:v). The method used was 5% to 50% B 5 mins, 50% to 5% B 30 s, 5% B 1 min, flow rate 1.2 mL min^{-1}, column temperature 20 °C.

3 Experimental

3.1 Pump Reservoir Solutions

Solution reservoirs for the pumps were prepared by dissolving the methyl nicotinate (50 g, 36.5 mmol) in methanol (200 mL); and methylamine solution (40% wt aq, 200 mL, 5.15 mol) in distilled water (200 mL). Ester solution concentration = 1.46 mol L^{-1}, methylamine solution = 5.77 mol L^{-1}. The reactor was primed by pumping from the pump reservoirs at 1 mL min^{-1} until product was detected by MS.

3.2 *N'*-Methyl nicotinamide, 2

Methyl nicotinate (10.03 g, 73.2 mmol) was added in portions to a cooled solution (0 °C, ice bath) of methylamine in methanol (145 mL, 2M, 292 mmol). The reaction was allowed to warm to room temperature and stirred for 24 hours. The solvent was removed under vacuum to leave a white solid (9.23 g, 99%), mp 102-104 °C; δ_H: (300 MHz, $CDCl_3$, TMS) 8.96 (1H, dd, *J* 2.4 and 0.9, Ar-H), 8.71 (1H, dd, *J* 4.8 and 1.5, Ar-H), 8.12 (1H, dt, *J* 2.0 and 7.8, Ar-H), 7.38 (1H, ddd, *J* 7.8, 4.8 and 0.9, Ar-H), 6.36 (1H, br s, N-H), 3.04 (3H, d, *J* 4.8, NCH_3); MS (APCI+) 137 $[M+H]^+$, 178 $[M+MeCN+H]^+$. Analysis matches the literature[1]

3.3 Steady State Determination

Online MS was tested for its ability to be used as real-time analysis to check for steady state in the reactor. The reagent pumps were primed, and then **1** was pumped at 0.2 mL min^{-1} and $MeNH_2$ was pumped at 0.023 mL min^{-1} (2 mol eq) for a period of 30 minutes, during which a MS sample was taken at 60s intervals. After the 30 minute reagent pulse, methanol was pumped at 1 mL min^{-1} for 10 minutes. This was to clean the reactor and prevent accumulation in the MS. The process was repeated using 3 and 4 mol eq of $MeNH_2$ carrying out the three flow experiments sequentially (Figure S5).

The results were analyzed by internal normalization and linearity was observed when the standard deviation of the previous three points was less than or equal to 1.5%.

A steady state function was built to run after 1.1 residence times to test for steady state in the system.
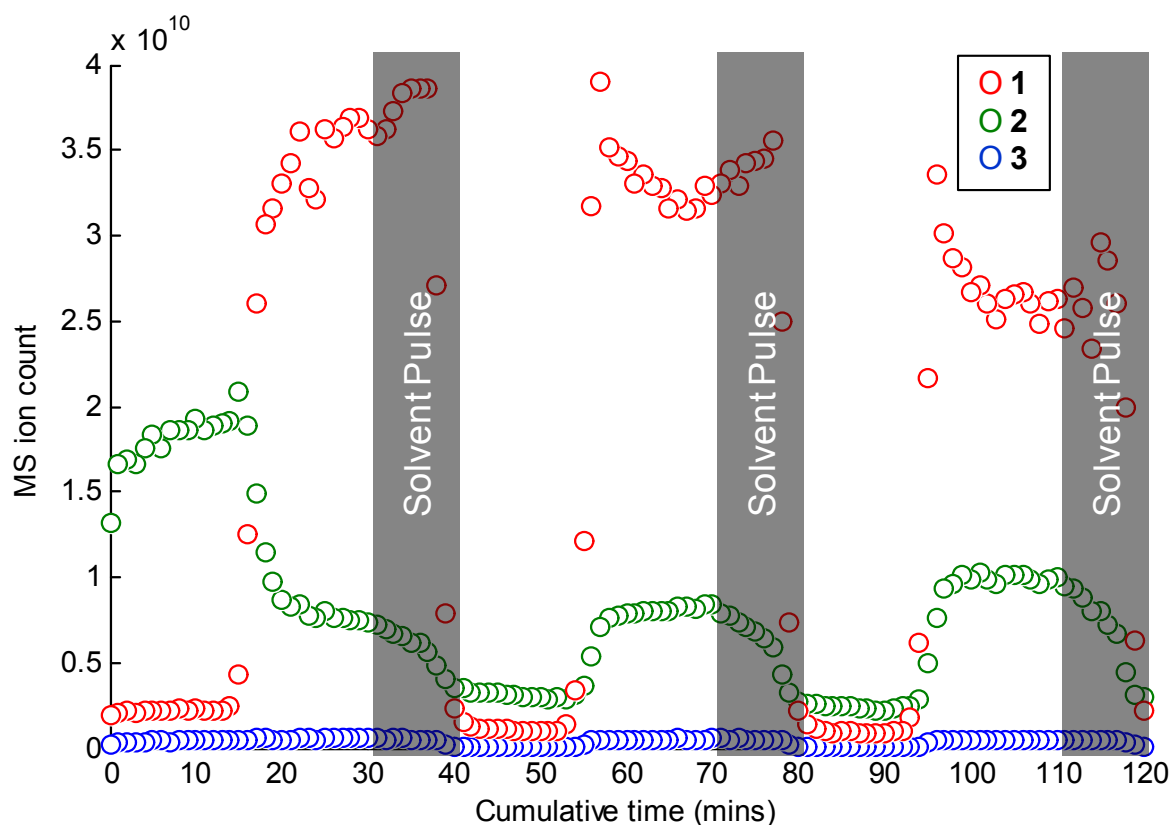
Figure S5 – Figure showing the reactions carried out to test the use of online MS as real-time analysis for determining steady state of a reactor
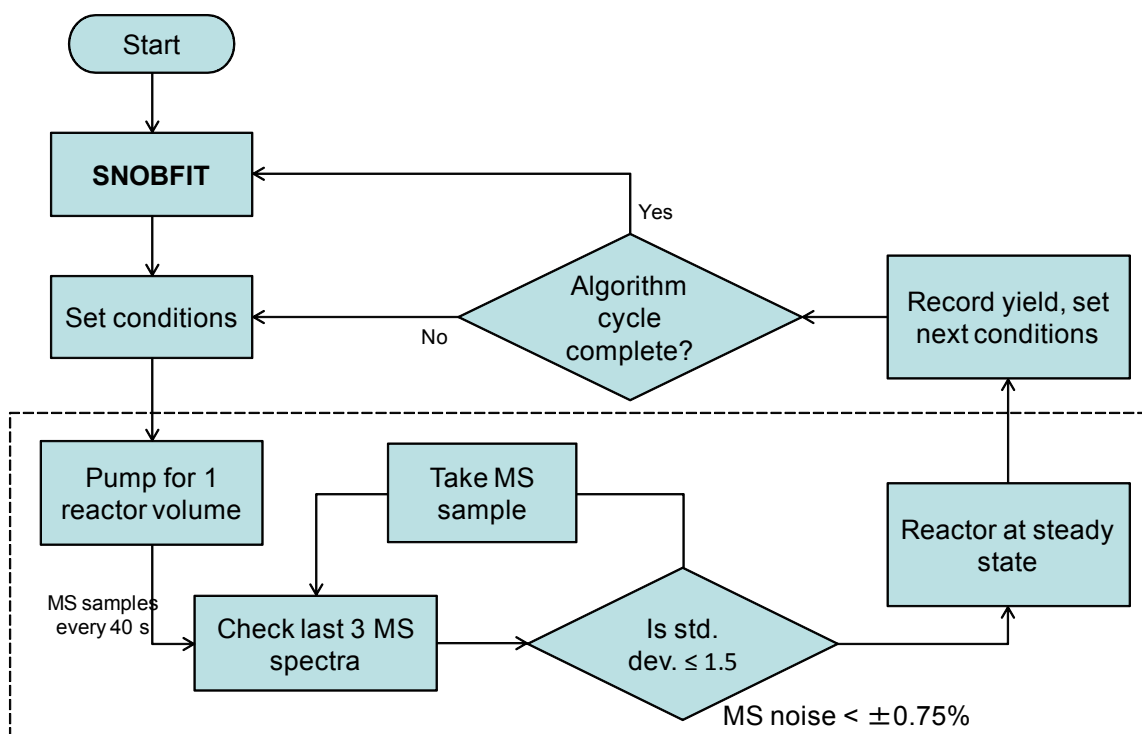

Figure S6 – Flow chart showing the self-optimization program cycle with steady state function

3.4 Isotope Calculations

There is an overlap in the peaks between **1** and **2** due to the [M+H]$^+$ adduct of **1** masking the [M+H+1]$^+$ isotope adduct of **2**. To correct this, the isotope abundance for each compound was predicted using ChemDraw (ver 13.0.0.3015) and verified experimentally.

The following calculations were used when calculating the MS response:

$$Ester = (P_{137} - (P_{138} \times 0.077)) \times 1.077$$

$$Amide = P_{138} \times 1.077$$

$$Acid = P_{124} \times 1.066$$
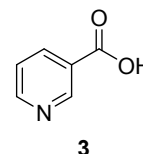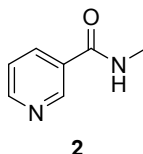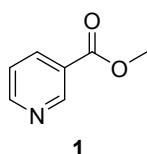
1	**2**	**3**
m/z: 137.05 (100.0%), 138.05 (7.7%)	m/z: 136.06 (100.0%), 137.07 (7.7%)	m/z: 123.03 (100.0%), 124.04 (6.6%)

Figure S7 - Isotope abundances for compounds **1** *to* **3**

3.5 Calibration

3.5.1 HPLC

The HPLC response was calibrated by preparing a single solution containing compounds **1**-**3** at 0.35 mol L^{-1} in DMSO. HPLC samples were taken at dilution factors of 1, 2, 4 and 8 (corresponding to concentrations of 0.35, 0.175, 0.0875, 0.0438 M) and the relative response factors were calculated by internal normalization. Existing calibration curves showed a linear response up to 1.4 M for **1** and **2** and 0.5 mol L^{-1} for **3**, which is in the same concentration range in which optimizations were carried out.

3.5.2 MS

A central composite faced (CCF) Design of Experiment (DoE) was constructed for the reaction shown in Scheme 1 and ran using both HPLC and MS as analysis.

Experiment	**1** / mL min^{-1}	MeNH$_2$ / mol eq	Temperature / °C
1	0.1	1	0
2	0.1	10	0
3	0.25	5.5	0

4	0.4	1	0
5	0.4	10	0
6	0.1	5.5	65
7	0.25	1	65
8	0.25	5.5	65
9	0.25	5.5	65
10	0.25	5.5	65
11	0.25	5.5	65
12	0.25	10	65
13	0.4	5.5	65
14	0.1	10	130
15	0.25	5.5	130
16	0.4	1	130
17	0.4	10	130
18	0.1	1	130

Table S1 - Summary of experiments ran in the CCF design of experiment

Experiment	HPLC Area			Percentage Yield / %		
	3	2	1	3	2	1
1	9.5	126.9	1480.8	0.5	5.7	93.8
2	18.8	548.4	0.0	4.0	96.0	0.0
3	24.5	525.1	247.3	3.3	58.1	38.6
4	5.5	67.9	1469.0	0.3	3.2	96.5
5	15.7	364.8	160.3	3.1	59.8	37.1
6	161.4	761.6	0.0	20.5	79.5	0.0
7	71.6	255.9	1220.4	4.2	12.4	83.4
8	148.2	694.5	12.7	20.2	77.8	2.0
9	151.3	701.4	13.2	20.4	77.5	2.1
10	136.8	659.2	5.1	20.0	79.1	0.9
11	157.5	722.8	28.0	20.1	75.7	4.1
12	94.5	459.1	0.0	20.1	79.9	0.0
13	136.7	652.7	54.1	18.6	72.9	8.5
14	199.6	346.7	0.0	41.3	58.7	0.0
15	361.6	524.2	0.0	45.7	54.3	0.0
16	235.2	245.7	1118.2	13.6	11.6	74.8
17	195.2	313.5	0.0	43.2	56.8	0.0

| 18 | 499.4 | 448.8 | 782.5 | 28.2 | 20.7 | 51.1 |

Table S2 - Results from HPLC CCF, showing absolute peak areas and calibrated internally normalized percentage yield

Experiment	**1** / %	**2** / %	**3** / %
1	1.2	3.7	95.5
2	1.1	95.9	2.8
3	1.5	70.6	27.8
4	1.2	6.8	92.5
5	1.5	67.7	30.8
6	4.8	93.4	1.5
7	2.0	16.1	82.3
8	5.3	90.2	4.3
9	5.6	90.2	4.0
10	5.6	90.2	4.0
11	6.0	89.6	4.1
12	5.3	92.7	1.8
13	5.1	85.6	9.1
14	13.2	84.2	2.4
15	14.6	83.7	1.3
16	4.8	17.0	78.5
17	13.9	83.4	2.5
18	1.2	2.0	97.3

Table S3 – Results from the MS CCF, showing internally normalised percentage yield, which is uncalibrated but including isotope correction calculation

The error in the measurement was calculated by using sum of squares, where R_i is the response of compound **i**:

$$Error = \sum_{i=1}^{3} \left(R_{i,HPLC} - R_{i,MS} \right)^2$$

A 'new' yield was calculated by multiplying the existing response by a response factor (initially set to 1). The error in the measurement between the 'new' MS yield and existing HPLC yield was minimized by changing the values of the 3 response factors. Optimum minimized values were calculated using the MS Excel solver add-in (GRG Non-Linear algorithm). A comparison between the calibrated LC and MS responses are shown in Table S4 and Figure S8.

Experiment	Percentage Yield (HPLC)/ %			Calibrated MS response / %		
	3	**2**	**1**	**3**	**2**	**1**
1	0.5	5.7	93.8	3.7	2.5	93.8
2	4.0	96.0	0.0	4.5	91.8	3.8
3	3.3	58.1	38.6	5.6	60.4	34.0
4	0.3	3.2	96.5	3.7	4.7	91.7
5	3.1	59.8	37.1	5.3	57.4	37.3
6	20.5	79.5	0.0	17.8	80.4	1.9
7	4.2	12.4	83.4	6.1	11.3	82.6
8	20.2	77.8	2.0	19.2	75.7	5.1
9	20.4	77.5	2.1	20.1	75.2	4.7
10	20.0	79.1	0.9	20.1	75.2	4.7
11	20.1	75.7	4.1	21.3	73.9	4.8
12	20.1	79.9	0.0	19.3	78.6	2.1
13	18.6	72.9	8.5	18.3	71.0	10.8
14	41.3	58.7	0.0	39.4	58.3	2.3
15	45.7	54.3	0.0	42.5	56.2	1.3
16	13.6	11.6	74.8	13.9	11.3	74.8
17	43.2	56.8	0.0	40.8	56.8	2.4
18	28.2	20.7	51.1	3.7	2.5	93.8

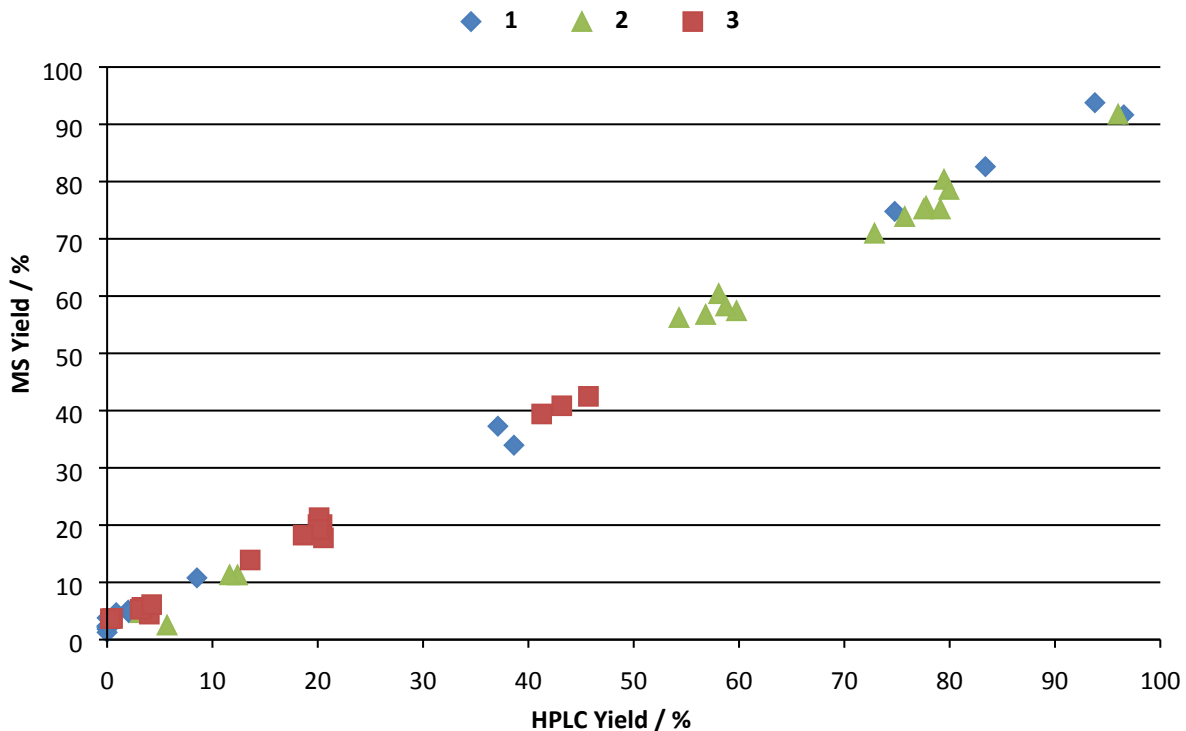*Table S4 - Comparison between calibrated MS responses and LC responses*

Figure S8 – Comparison between the calibrated responses by MS and HPLC for each individual compound
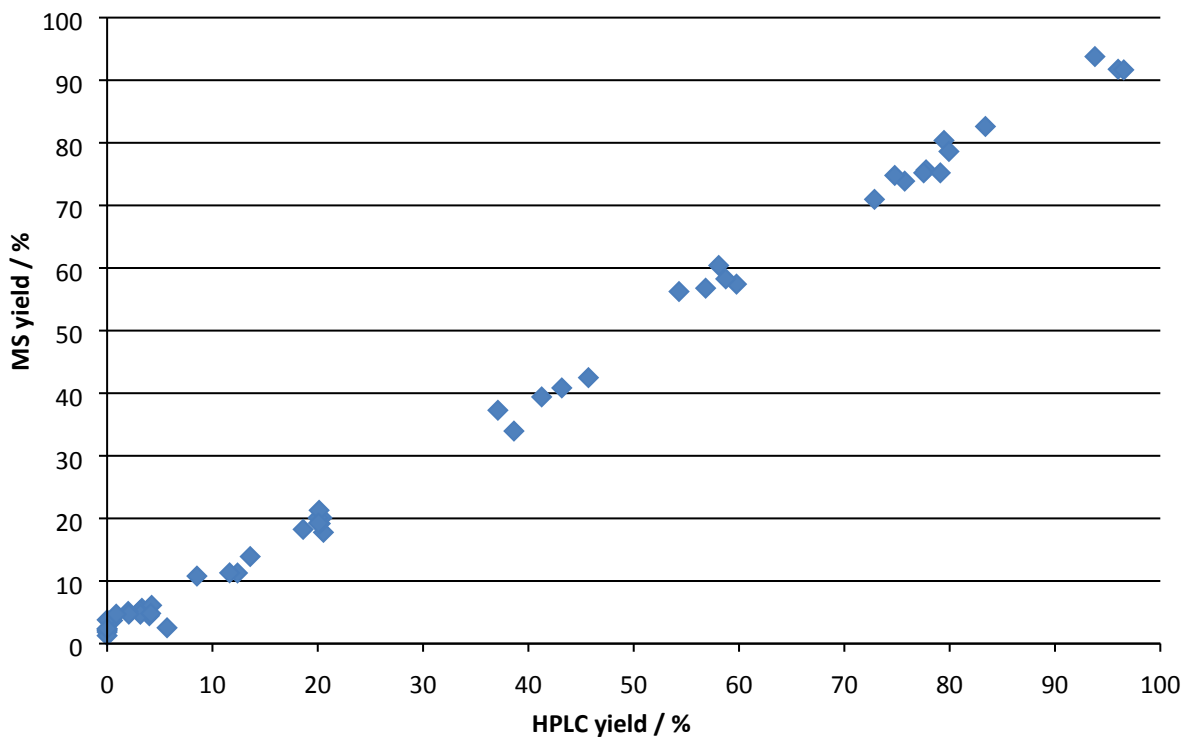

Figure S9 – Comparison between the calibrated responses by MS and HPLC for all combined compounds

3.6 Self-Optimization Results

Pump reservoir solutions were made according to the procedure in Section 3.1. An optimization program was written in MatLab that controlled the pump flow rates and reactor temperature; determined steady state; calculated a product yield; and controlled the inputs and outputs to and from the SNOBFIT algorithm. The reactor set-up was the same as shown in Figure S2, where pump 1 was primed with **1**, pump 2 with $MeNH_2$ and pump 3 with MeOH. Whilst the reactor was reaching temperature, pumps 1 and 2 were set to 0.020 mL/min and pump 3 to 0.500 mL/min. When the reactor was at temperature, the pumps were set to the flow rates calculated by the algorithm and pumped for 1.1 residence times. After 1.1 residence times, the steady state function was run with the MS sampling at 40 s intervals. When the reactor was at steady state, the yield was recorded and the optimization moved on to the next experiment (see Figure S6 for more details).

Limits	**1** flow / mL min^{-1}	$MeNH_2$ eq	Temperature / °C
Upper	0.100	1	0
Lower	0.400	10	130

Table S5 - Optimization limits used in the self-optimization and design of experiment

Limits	Pump 1 flow / mL min^{-1}	Pump 2 ratio^a	Pump 3 flow / mL min^{-1}	Temperature / °C
Upper	0.100	0.25	0.001	0
Lower	0.400	2.5	0.002	130

Table S6 – Actual optimization limit numbers that were given to the SNOBFIT algorithm. a – The flow rate of pump 2 was calculated by multiplying the number in the table by the flow rate of pump 1 (proportional to molar equivalents). b – Pump 3 needed to be included in the optimization to "clean" the reactor between experiments. The numbers given are the lowest limits possible for both the pump and algorithm program to run without problems. It is assumed that the minimal flow rates of pump 3 contribute an insignificant difference to the residence time and reactant concentrations.
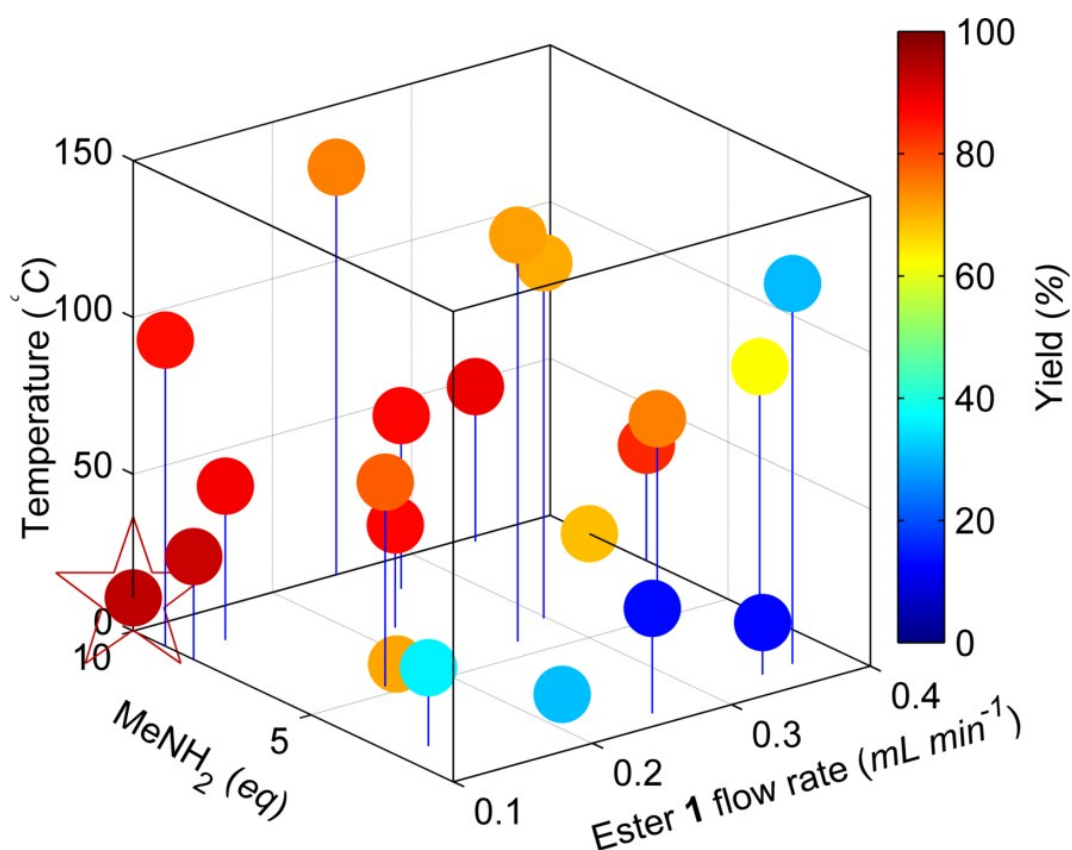
Figure S10 - 4-dimensional plot displaying the results of the self-optimization

Entry	1 flow		MeNH2 flow				MeOH	Temperature	Yield 2
	mL min⁻¹	mmol min⁻¹	ratio	mL min⁻¹	mmol min⁻¹	mol eq	mL min⁻¹	°C	%
1	0.187	0.271	1.503	0.281	1.620	6.0	0.001	0.2	70.8
2	0.264	0.383	1.407	0.372	2.141	5.6	0.002	129.6	71.8
3	0.256	0.372	0.372	0.095	0.550	1.5	0.002	33.4	12.8
4	0.344	0.499	1.201	0.414	2.384	4.8	0.002	65.5	74.7
5	0.343	0.497	0.443	0.152	0.875	1.8	0.002	16.6	12.6
6	0.341	0.494	2.452	0.836	4.816	9.7	0.001	49.4	88.9
7	0.392	0.568	0.950	0.372	2.145	3.8	0.001	81.5	62.2
8	0.100	0.145	2.286	0.229	1.317	9.1	0.001	97.6	86.3
9	0.209	0.303	0.554	0.116	0.667	2.2	0.001	8.1	30.7
10	0.123	0.178	0.647	0.080	0.458	2.6	0.001	25.0	35.9
11	0.298	0.432	1.540	0.459	2.643	6.1	0.001	113.4	70.7
12	0.367	0.532	0.469	0.172	0.991	1.9	0.001	121.3	30.6
13	0.400	0.580	2.225	0.890	5.126	8.8	0.001	0.0	68.4
14	0.222	0.322	1.851	0.411	2.367	7.4	0.002	32.7	87.0
15	0.400	0.580	1.827	0.731	4.209	7.3	0.002	36.8	83.5

16	0.246	0.357	2.500	0.615	3.542	9.9	0.002	130.0	74.9
17	0.100	0.145	2.500	0.250	1.440	9.9	0.001	10.6	93.4
18	0.100	0.145	2.068	0.207	1.191	8.2	0.002	32.8	92.3
19	0.133	0.193	2.184	0.290	1.673	8.7	0.002	49.1	88.0
20	0.262	0.380	2.195	0.575	3.313	8.7	0.002	55.2	87.1
21	0.161	0.233	1.319	0.212	1.223	5.2	0.002	65.1	78.4

Table S7 – Conditions and response from the self-optimization. Green shaded areas denote conditions generated by the SNOBFIT algorithm, yellow shaded area denotes response from MS

3.7 DoE Results

Pump reservoir solutions were made according to the procedure in Section 3.1. Conditions were generated using a custom written MatLab script based on the existing "ccdesign.m". The same optimization program that the self-optimization was run on was used for the DoE, but the call to the algorithm was removed and instead the pre-determined list of experiments was put in its place. The experiments and their responses are shown in Table S8.

Experiment	1 / mL min^{-1}	MeNH$_2$ / mol eq	Temperature / °C	3 / %	2 / %	1 / %
1	0.1	1	0	3.7	2.5	93.8
2	0.1	10	0	4.5	91.8	3.8
3	0.25	5.5	0	5.6	60.4	34.0
4	0.4	1	0	3.7	4.7	91.7
5	0.4	10	0	5.3	57.4	37.3
6	0.1	5.5	65	17.8	80.4	1.9
7	0.25	1	65	6.1	11.3	82.6
8	0.25	5.5	65	19.2	75.7	5.1
9	0.25	5.5	65	20.1	75.2	4.7
10	0.25	5.5	65	20.1	75.2	4.7
11	0.25	5.5	65	21.3	73.9	4.8
12	0.25	10	65	19.3	78.6	2.1
13	0.4	5.5	65	18.3	71.0	10.8
14	0.1	10	130	39.4	58.3	2.3
15	0.25	5.5	130	42.5	56.2	1.3
16	0.4	1	130	13.9	11.3	74.8

| 17 | 0.4 | 10 | 130 | 40.8 | 56.8 | 2.4 |
| 18 | 0.1 | 1 | 130 | 3.7 | 2.5 | 93.8 |

Table S8 – List of experiments and the responses of compounds 1-3 ran in the CCF DoE

The design was analyzed and response surface models were fitted using Umetrics Modde (ver 10.1) by including all square and interactions then removing terms with a p-value less than 0.05. The model for **3** (acid) was transformed logarithmically ($y = log_{10}x$) to improve the normality of its distribution. The following figures show a summary of the model displaying Figure S11 a) replicates plot, b) summary of fit, c) coefficients and d) residual normality plot for the model of amide **2**; Figure S12, summary of the fit; Figure S13, model coefficients; Figure S14, observed vs predicted plots for each compound model; and Figure S15, contour plot, using the predictor function of the model for amide **2**.
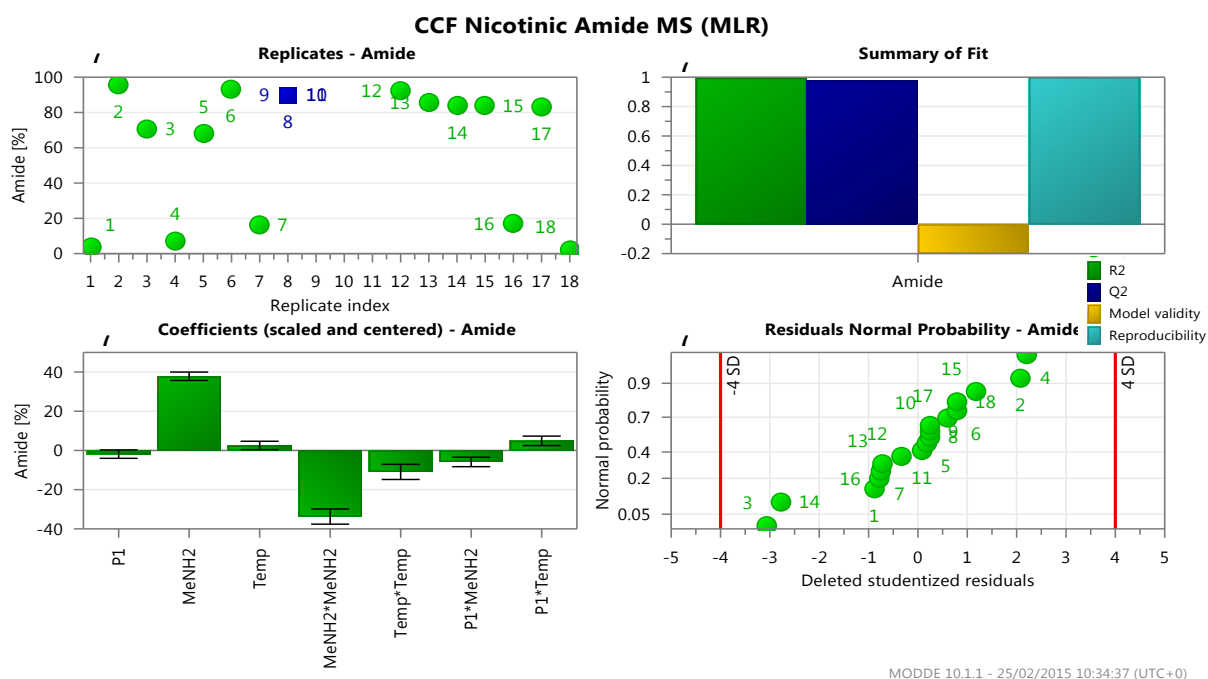

Figure S11 – Model summary for the model of 2: a) Replicates, shown in blue, highlight the reproducibility of the model; b) summary of fit, displaying R², which shows the accuracy of the fit, Q², which shows how accurately the model can predict, vailidity, which is low due to the high reproducibility (i.e. the model doesn't believe it is valid due to such high reproducibility); c) model coefficients and their weighting; d) normal probability plot, which a good model will fit a straight line with all points within the 4 SD (4 standard deviations) markers.
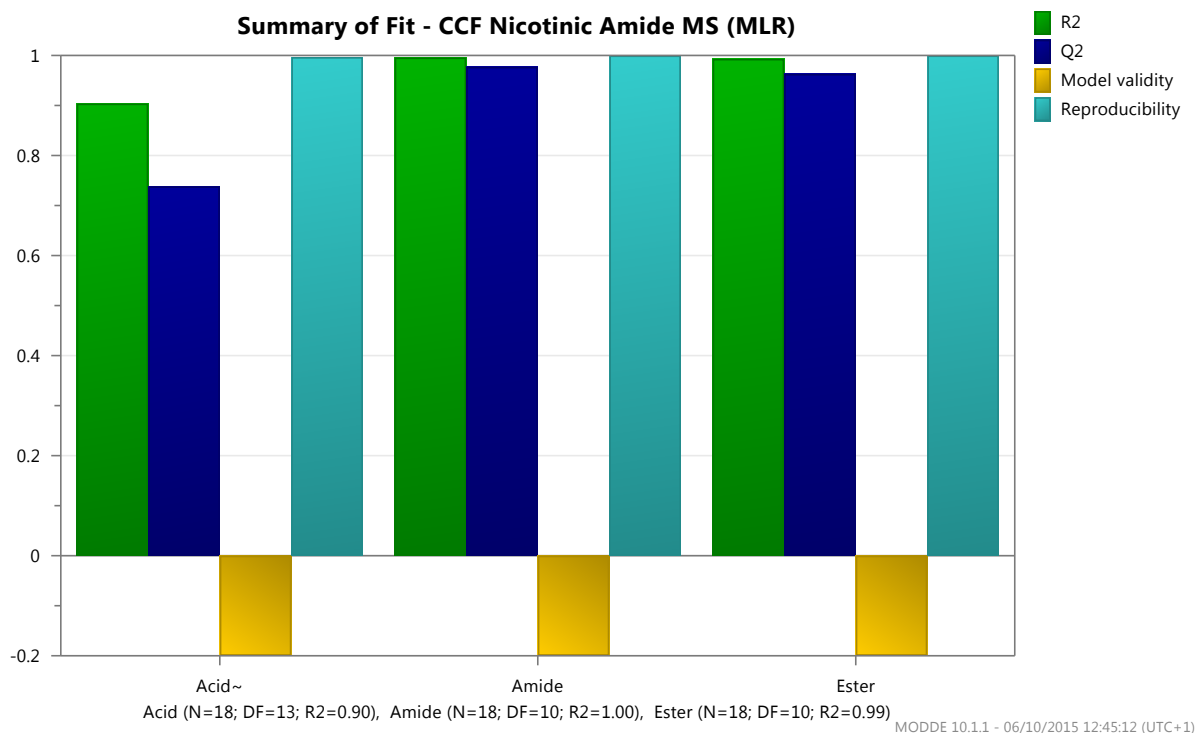
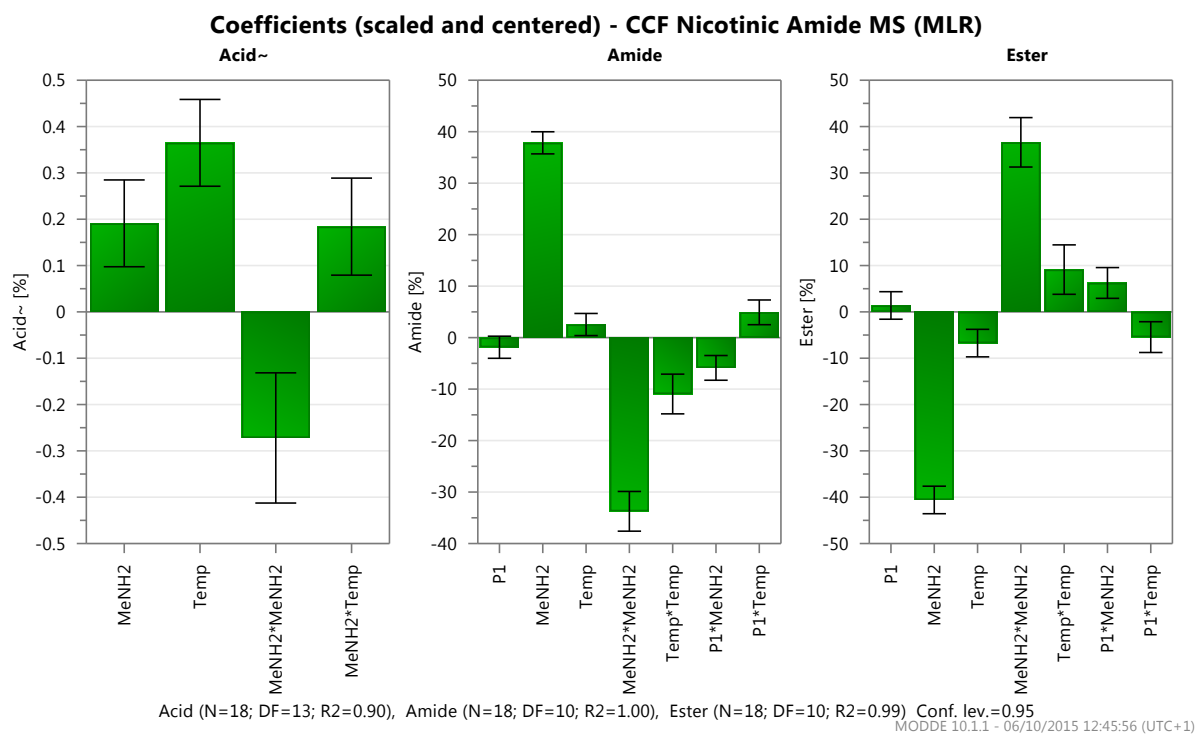Figure S12 – Summary of fit for the models of *1* (ester), *2* (amide) and *3* (acid).

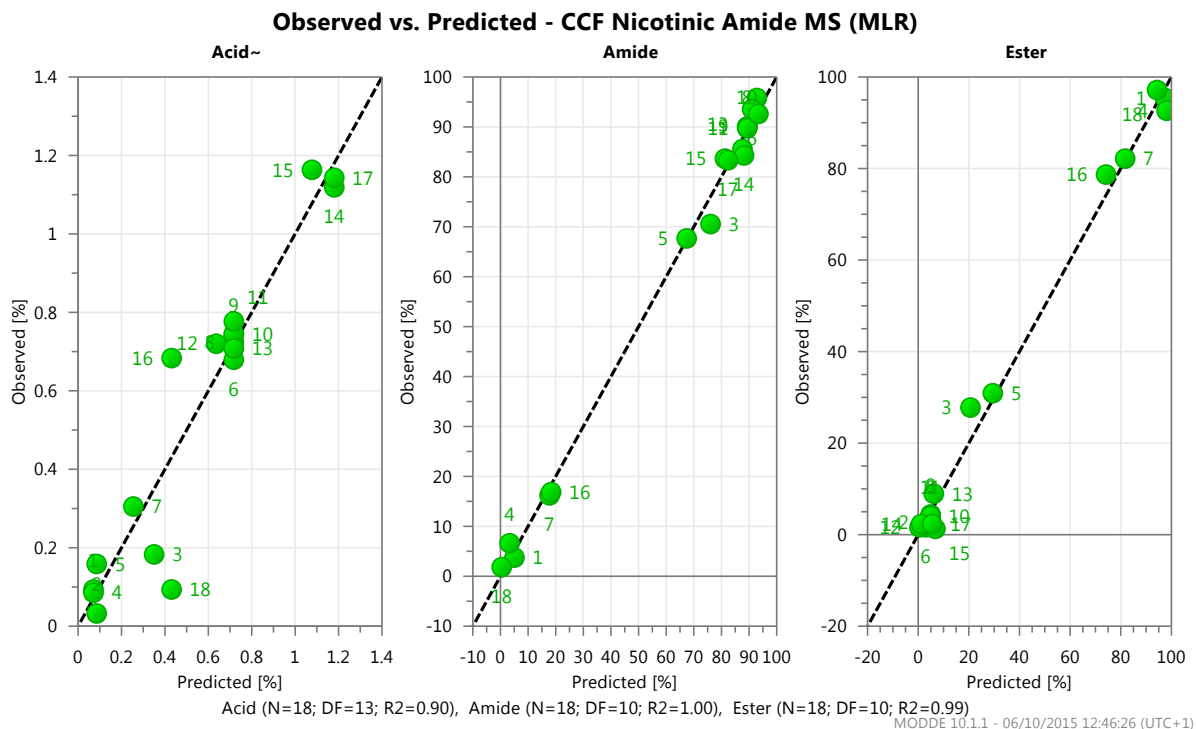Figure S13 – Coefficients of each model for compounds *1* (ester), *2* (amide) and *3* (acid).

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*Figure S14 – Plot of observed vs predicted of each model from the compounds **1** (ester), **2** (amide) and **3** (acid).*
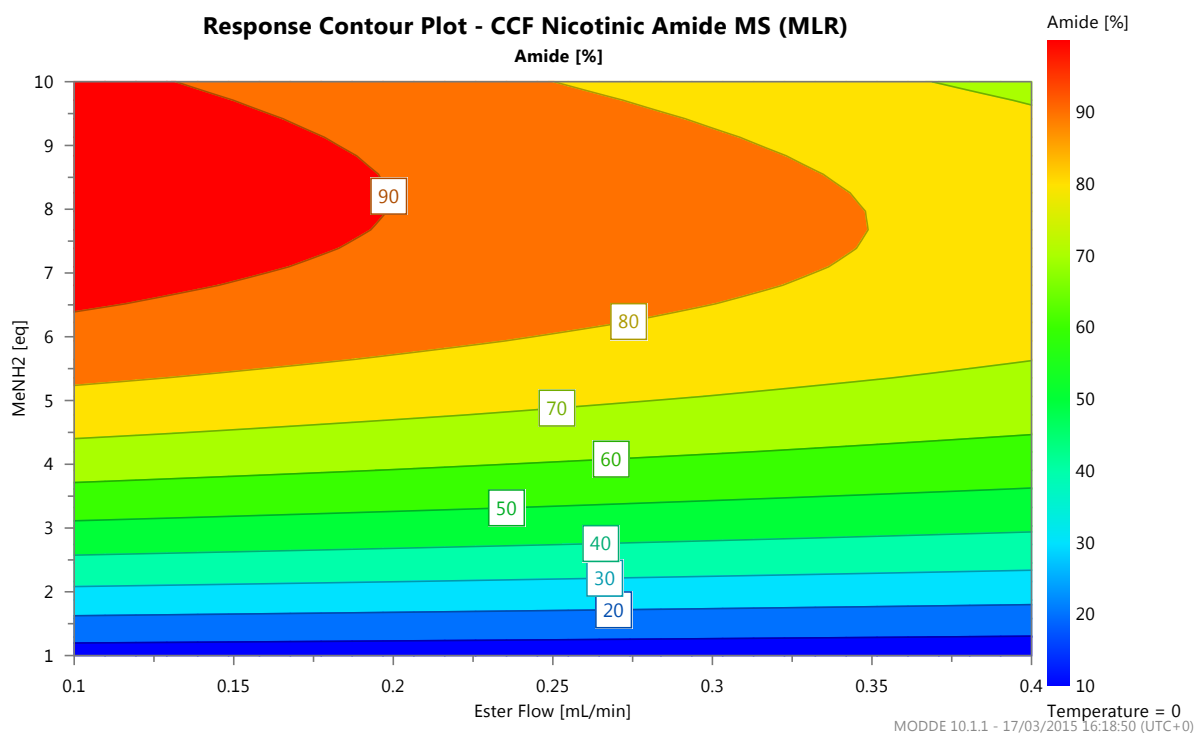

*Figure S15 – Contour plot at showing the effect of **1** flow rate (mL/min) and MeNH$_2$ eq on the yield of **2** at fixed temperature (0 °C).*

4 References

[1]   C. J. Pouchert, J. Behnke, in Aldrich Library of 13C and 1H FT NMR Spectra, Vol. 3, **1993**, p. 339B.