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Coupling and Optimisation of On-Line Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry for Process Monitoring to cover the broad range of process concentration

Alexander Blanazs, Tony W. T. Bristow*, Steven R. Coombes, Tom Corry[$], Mike Nunn and Andrew D. Ray

Pharmaceutical Technology and Development, AstraZeneca, Silk Road Business Park, Charter Way, Macclesfield, Cheshire, SK10 2NA.

*Corresponding author

[$]Current Address - School of Chemistry, The University of Manchester, Oxford Road, Manchester, M13 9PL, United Kingdom.

Abstract

Real time on-line monitoring of chemical processes can be carried out by a number of analytical techniques, including optical & vibrational spectroscopies, Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS). As each technique has unique advantages and challenges, combinations are an attractive option.

The combination of a 500 MHz ^{1}H NMR and a small footprint mass spectrometer to monitor a batch reaction at process concentration was investigated. The mass spectrometer was coupled into the flow path of an on-line reaction monitoring NMR. Reaction mixture was pumped from a 100 ml vessel to an NMR flow tube before returning to the vessel. Small aliquots were diverted into a sampling make-up flow using an active flow splitter and passed to the mass spectrometer.

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Advantages of the combination were observed. ^{1}H NMR was ideal for quantitation of high level components, whereas MS showed a greater capability for detecting those at low level. In preliminary experiments MS produced a limited linear relationship with concentration (0.02 % to 2 % relative concentration, 0.01 mg/mL – 1.25 mg/mL), due to signal saturation at the higher concentrations. NMR was unable to detect components below 0.1 % relative to concentration maximum. Optimisation of sample transfer to the MS extended the linearity to 10 % relative to the concentration maximum. Therefore, the combination of on-line NMR and MS allows both qualitative and quantitative analysis of reaction components over the full process range. The application of the combination was demonstrated by monitoring a batch chemical reaction and this is described.

Introduction

Direct on-line analysis of a chemical process is advantageous as this allows the progress to be evaluated in real time, the point of completion to be established and impurity formation to be understood.

Many analytical techniques have been employed and evaluated to monitor both batch and continuous chemical processes. Examples include in-line UV absorption, infra-red (IR) and Raman spectroscopies that employ in-line flow cells. [1-5] However it is not always possible to identify intermediates and impurities from the IR data and IR may also lack sensitivity to detect low level impurities. On-line reaction monitoring using high performance liquid chromatography (HPLC) is another useful approach which has been demonstrated with the application of a microflow HPLC for on-line monitoring of batch and continuous flow reactors. [6,7]

High-field NMR is an attractive option to monitor a process from both a qualitative and quantitative perspective and its application has been demonstrated for a pharmaceutical process.[8] Coupling a high-field NMR spectrometer to a batch reaction is a considerable challenge due to the size and requirements of a high-field spectrometer, along with issues such as co-location. However, a number of options are available including dedicated flow probes designed for on-flow applications or flow tube type solutions enabling the use of standard 5 mm probes. Khajeh and co-workers have proposed an NMR flow-cell comprising a standard 5 mm NMR tube for continuous process monitoring.[9] The flow-cell allows

solution to be pumped continuously through the NMR tube and then returned to the process vessel. The concentric design of the flow-cell minimises changes in magnetic field homogeneity as the cylindrical symmetry is maintained. Commercial systems are also available;[10] these benefit from the ability to acquire accurate kinetic data on-flow in contrast to "in-tube" reactions as demonstrated by Foley et al.[11] In the same report, Foley et al also presented evidence for the effect of mixing on the rate of reaction determined by NMR, with the rate determined highly dependent on the NMR monitoring method used.[11]

A double chamber NMR tube has also been investigated by Mix and co-workers for monitoring reactions conducted in a NMR tube.[12] The study concludes that a 3 mm glass tube can be placed inside a 5 mm glass NMR tube with a small piece of silicone sealant, this seal then creates two compartments inside the NMR tube. On removal of the inner tube inside the NMR magnet using a rod, the reactants mix initiating the reaction. An outer casing approach is suggested by Lindon and co-workers.[13] In a publication by Dalitz, the use of non-deuterated solvents and the effect of flow rate on sample through the NMR flow-cell was discussed.[14] In an alternative pharmaceutical application, Coombes et al demonstrated quantification of three active pharmaceutical ingredients simultaneously in an on-line dissolution experiment, with an external standard approach used for calibration.[15] Imine/aminal formation in a range of different solvents and catalysed esterification reactions are other examples where synthetic reactions have been monitored quantitatively using high-field NMR. [16, 17] Despite the challenges of coupling a high-field spectrometer to a reaction vessel for on-line monitoring, greater selectivity over IR and UV spectroscopies simplifies calibration and data analysis. Small footprint low-field NMR is also an option for process monitoring due to the reduced cost and increased flexibility, and their use has also been demonstrated. [18, 19]

Mass spectrometry (MS) has also been employed to monitor reactions in real time. MS can have an advantage over other analytical techniques in that the specificity of the m/z of an ion can be employed to confirm the formation of a proposed product and characterise impurities and the presence of residual reactants. A key challenge issue for on-line MS is the transfer of a sample from the process that is initially unsuitable for MS. This is due to issues of high process concentration and possibly a reaction solvent that is not conducive to

efficient ionisation of the components it contains. The sample must be representative of the reaction whilst at the same time being compatible with the MS system (for example the ionisation technique). A number of examples of the application of MS to on-line monitoring can be found in the review by Fabris and this demonstrates how such considerations can be overcome.[20]

Del Orco and colleagues continuously monitored a reaction by sampling a small volume of the reaction liquor into a flow of carrier solution which was eventually transferred to the bench-top mass spectrometer.[21] Zhu et al and McCullough et al independently demonstrated the application of extractive electrospray ionisation (EESI) to monitor a batch process.[22,23] In order to obtain greater specificity for real time reaction monitoring Harry et al and Roscioli et al have independently demonstrated ion-mobility mass spectrometry (IM-MS) for pharmaceutical process understanding.[24, 25] Chen and Lin have recently reviewed the monitoring of chemical transformations by MS using a variety of ion sources for direct analysis and focussing on the range of ambient ionisation techniques.[26]

Miniaturised, lightweight and portable atmospheric pressure ionisation (API) mass spectrometers further facilitate on-line MS for process analysis.[27] which allows the system to be positioned with any process under investigation. The potential of such devices has been demonstrated in two recent publications. In the first, the device was coupled to a Uniqsis Flow Syn continous flow chemistry reactor.[28] In the second Bristow and co-workers monitored the progress of a continuous flow Hofmann rearrangement reaction.[29] In this study reactent dilution was achieved by transfer of small aliquots of sample solution into a sampling make-up flow, followed by electrospray ionisation. The setup was able to detect at least seven compounds in the reaction mixture when used on-line. It was identified in this study that optimisation of make-up flow composition and sample dilution factor is important in understanding reaction optimisation. This is analogous to the observations on mixing recently reported by Foley et al for on-line NMR experiments.[11]

Demonstrating the broader applicability of on-line MS to monitor pharmaceutical processes, Ray and co-workers reported the on-line analysis of a tablet dissolution process. [30] Individual dissolution profiles were obtained in real time for a tablet containing three active pharmaceutical ingredients and lactose. Such detailed information on release is important for design and development of pharmaceutical formulations.

As described many individual techniques have been applied to process monitoring. They have their own unique advantages, some limitations and therefore on-line combinations are of interest. One potentially powerful option is the combination of NMR and a mass spectrometer. The quantitative and qualitative power of NMR and the inherent sensitivity of the MS, would provide an analytical system that would allow the typically wide concentration range of the components of a chemical process to be studied in a single experiment. This is technically challenging. However, the combination of LC-NMR-MS has been demonstrated previously and illustrates the potential. In 2003 Corcoran and Spraul published a review outlining the application of LC-NMR-MS in drug discovery. [*31*] The review describes solutions to the challenge of coupling the three techniques and a range of applications including synthesis of natural products, combinatorial chemistry and drug metabolism and pharmokinetics (DMPK). There are a number of individual applications of the approach described in the literature. Examples of specific pharamaceutical applications have been described in separate publications by Shockor, Hansen and their co-workers. [32, 33] In the former report the detection and characterisation of xenobiotics and metabolites in human urine is described. In the latter study, LC-NMR-MS was applied to the structural characterisation of the constituents of an extract from a plant natural product.

In this study on-line NMR-MS to monitor a batch chemical process at typical process concentrations up to 71.0 mg/mL has been investigated. In this experiment the multi-component sample mixture is transferred continually to the NMR and MS for analysis. This approach differs from LC-NMR-MS where stop-flow experiments and LC pre-separation delivers the individual components to the NMR and MS. Also, sample preparation and chromatographic separation results in concentrations of a more appropriate level for the two techniques.

For on-line NMR-MS, the optimisation of the instrumental combination to cover a broad dynamic range of reactant and product concentration (for example 0.01 mg/mL – 62.7 mg/mL, typical of process concentrations) is described. Individually, the instruments are unable to monitor this entire concentration range. NMR suffered poor signal to noise at the lower level of concentrations investigated and MS suffered from non-linearity of response at high sample concentrations due to MS signal (detector) saturation. Following this optimisation study, the system was applied to monitor a batch chemical process and this is also described.

Experimental

Materials: All materials, unless specified, were sourced from Sigma-Aldrich Company Ltd. (Gillingham, United Kingdom) at the highest available purity. Ultrapure water (18.2 MΩ) was obtained from a MilliQ unit (Watford, Hertfordshire). Metmercazole and Pyrmetazole, the product of the reaction studied were available at AstraZeneca. All of these materials were fully characterised by proton NMR and LC-MS prior to use. Pyrmethyl chloride hydrochloride was also synthesised from pyrmethyl alcohol (from AstraZeneca) as required.

Equipment setup: An EasyMax 102 Advanced Synthesis Workstation (Mettler-Toledo Ltd., 64 Boston Road, Beaumont Leys, Leicester, LE4 1AW) with a 100 mL reaction vessel was used for all reactions and was coupled on-line with a Bruker Avance 500 MHz spectrometer (Bruker Biospin, Rheinstetten, Germany). Both conventional ^{1}H experiments and solvent suppression ^{1}H experiments were employed (Bruker noesy1D pre-set solvent suppression pulse program noesygppr1d). PEEK tubing with a standard stainless steel HPLC mobile phase inlet filter was inserted into the reaction vessel and was connected to an Ismatec (IDEX Health & Science GmbH, Wertheim, Germany) IP 65 rotary piston pump. Stainless steel HPLC tubing (0.5 mm ID) tubing was also fed from the pump out of the fume cupboard housing the reactor. PEEK tubing was connected to the stainless steel tubing and attached to the flow-cell of the NMR spectrometer. This contained a conventional 5 mm NMR tube with a leak sensor.[15] PEEK tubing was attached to the exit port of the flow-cell and fed through the heated transfer lines back into the reaction vessel completing a circular sample path and has been described previously.[15] All tubing external to the reaction vessel and

NMR magnet bore was insulated with high density polyethylene foam which ran alongside the heated transfer lines from a Julabo oil bath to manintain temperature control. A thermostatic heater/chiller was used to pump oil through tubing placed in parallel with the reaction flow and inside the insulating foam. This provided temperature control from the reaction vessel to the NMR.

To complete the on-line NMR-MS experimental configuration, a Waters Acquity QDa mass spectrometer (Waters Limited, Elstree, England) was coupled into the flow path described above. The QDa is a small footprint mass spectrometer (dimensions: 35.3 × 20 × 75cm) that is transportable because of its low weight (approx. 30 kg) and dimensions. To transfer samples to the mass spectrometer, the sample return flow was connected into a Rheodyne 100-000 MRA (IDEX Health & Science, Wertheim-Mondfeld, Germany). The MRA samples small aliquots of solution from the process flow, providing a 1 in 1000 dilution (in our typical set-up) and passes these into a sampling make-up flow (0.1 % formic acid (v/v) in 60:40 (v/v) acetonitrile:water) which is required for positive ion electrospray ionisation. Reaction mixture not sampled exits the MRA by a third port and is returned to the reaction vessel and this has been described previously.[29, 30] The electrospray sampling make-up flow was delivered to the MRA by a binary HPLC pump (Waters Limited, Elstree, England) at a typical flow rate of 1 mL/min. The sample flow entering the MRA was fitted with a KrudKatcher Classic 0.5 µm stainless steel filter (Phenomenex, Macclesfield, England) to prevent any particulates from damaging/blocking the MRA.

Samples were analysed by positive ion electrospray ionisation and for all experiments the mass spectrometer was operated in scan mode typically in the range 100-1000 m/z with a scan time of 0.5 seconds. Other key MS parameters were source temperature of 100 °C, a probe temperature of 300 °C, a capillary voltage of 1.5 kV and a cone voltage 25 V.
Nebuliser nitrogen gas flow rate was regulated at 6.75 ±0.25 bar (98 ±4 psi) outlet pressure

Experimental (i) - The assessment of the MS and NMR linearity

Pyrmethyl chloride hydrochloride solutions were prepared at a range of concentrations from 0.02% to 120% of the typical reaction concentration equivalent to 0.01 mg/mL and 62.7 mg/mL. Pyrmetazole solutions were prepared at a range of concentrations from 0.02% to 100% of the reaction concentration equivalent to 0.02 mg/mL and 71.0 mg/mL. These solutions (each calibration solution introduced separately and sequentially) were analysed to assess a number of key experimental parameters for the combination of NMR and MS, with solution concentrations confirmed by HPLC. The solutions were intended to mimic reaction conditions allowing MS and NMR linearity to be assessed. The NMR-MS reaction monitoring system was employed as described with individual solutions pumped through the NMR flow-cell, into the active splitter (to provide a 1000:1 dilution for MS) and back into the reaction vessel. The pump flow rate was set to 1.4 mL/min to prevent high back-pressure resulting in NMR flow-cell leakage. A time delay of 8 minutes was observed from the solution being provided to the pump, to a signal intensity plateau on the mass spectrometer. For this reason, all sample solutions analysed in this experiment were circulated for around 10 minutes before NMR acquisition and MS analysis. The time window used for averaging the MS data was approximately 4 minutes post reaching maxima. In these experiments all solutions were analysed from low concentration to high concentration in order to minimise carryover and system contamination.

Experimental (ii) - On-line reaction monitoring

The nucleophilic substitution reaction to form pyrmetazole and monitored by NMR-MS is shown in Scheme 1. Pyrmethyl chloride (5.39 g) was charged to the reactor and dissolved in methanol (60 mL). The pump transferring reaction mixture from the reactor to the NMR and MS was set to a flow-rate of 0.65 mL/min. A suspension of metmercazole (4.49 g) in methanol (30 mL) was then added. The solution was heated to reflux and the jacket temperature maintained at 70 ˚C with a condenser in place.

The temperature in the NMR was set to 313K to match the temperature of the incoming reaction liquor. It should be noted that the heated transfer lines from the reaction vessel only insulate the stainless steel transfer tubing to the top of the NMR magnet. At this point it is connected to an "un-insulated" section of PEEK tubing that runs down the upper barrel of the magnet, resulting in an approximate temperature drop of 25K.

A sample containing the two starting materials and product was also analysed off-line by NMR in non-deuterated methanol in order to identify suitable NMR signals for reaction monitoring (Figure 1).

Results and Discussion

1. Assessment of the relative linearity of on-line NMR and MS using Pyrmethyl Chloride Hydrochloride

Figure 2 shows the change in response for both NMR and MS on-line as a function of the concentration of the pyrmethyl chloride hydrochloride solutions. This was in the range 0.02% to 120% of the reaction concentration, equivalent to 0.01 mg/mL and 62.7 mg/mL. The NMR integrals recorded show a very strong linear correlation with solution concentration (Figure 2a) however, the MS ion abundance shows a significant deviation from linearity beyond 2% (1.25 mg/mL) which is attributed to MS signal (detector) saturation (Figure 2b). This is more clearly illustrated in Figure 2c where MS ion abundance is plotted as a function of concentration in the range 0.01% to 10% (6.0 mg/mL) of the reaction concentration.

It was also important to evaluate the effect of other known reaction components on MS response. Therefore, a second set of pyrmethyl chloride hydrochloride solutions were prepared in the presence of pyrmetazole in the range 0.01% to 10% of the reaction concentration. The pyrmetazole concentration was varied to qualitatively mimic the reaction composition. The solution with 12 mg/mL pyrmetazole is out of this trend to ensure that the linearity of the pyrmetazole concentration range wasn't artificially creating the apparent pyrmethyl chloride linearity. If this were the case this particular solution would

have given a large signal in the residuals plot created with the MiniTab statistical analysis (not shown).

Figure 3 shows extended linearity over a wider concentration range in the presence of pyrmetazole, which is likely to result from ion suppression reducing the overall ion formation from pyrmethyl chloride hydrochloride. For example, the MS ion abundance was 5.5×10^7 at 6 mg/mL in the absence of pyrmetazole (Figure 2c) and was 3.0×10^7 in the presence of pyrmetazole (Figure 3). However, it is also important to note that the lowest solution concentration was still detected maintaining the required MS sensitivity to study this reaction system.

In Bruins key publication on the mechanisms of electrospray, it is concluded that although electrospray is a quantitative ion source for mass spectrometry, the signal saturates at around 10 µmol dm^{-3} due to the surface of the droplet emitting ions being full.[34] For pyrmethyl chloride, a concentration of 10 µmol dm^{-3} corresponds to a reaction concentration of 2.22 mg/mL prior to MRA dilution. This corresponds to around 4 % of the maximum reaction concentration of pyrmethyl chloride. Therefore, the limited MS linearity at the concentrations examined here is explainable.

One can see in Figure 3 that the mass spectrometer response begins to deviate from linearity at concentrations greater than 3 mg/mL (5 % of the reaction maximum concentration) compared to 1.25 mg/mL in the absence of the matrix. These data therefore demonstrate that pyrmethyl chloride hydrochloride response at levels between 0.01 % and 5 % of the maximum reaction concentration show a linear response with concentration. These experiments were also repeated and verified using pyrmetazole in the range of concentrations 0.02% to 100% of the reaction concentration equivalent 0.02 and 71.0 mg/mL, with equivalent results for linearity for MS and NMR.

2. Assessment of the relative LOD and LOQ for on-line NMR and MS using Pyrmethyl Chloride Hydrochloride

To further illustrate the advantage of coupling the two techniques an assessment of LOD/LOQ for on-line NMR-MS was carried out. Though it is clear that NMR has an advantage over MS in terms of the linear dynamic range over this broad process concentration range, MS out performs NMR in terms of overall sensitivity and it's ability to detect low level reaction components. Figure 4 shows a waterfall plot for the ^{1}H NMR data of pyrmethyl chloride hydrochloride at a range of concentrations (0.01 mg/mL to 6 mg/mL). A signal to noise ratio of 17:1 was observed for the sample solution at 0.5 mg/mL and 2:1 for the sample solution at 0.06 mg/mL (signal to noise values were determined after 8 scans with a 3s recycle time and were measured between 8.8 - 8.4 ppm, with 1 ppm of noise (10 ppm – 9 ppm)). Below this concentration the signal cannot be distinguished from noise. By contrast the m/z 186 ion of pyrmethyl chloride hydrochloride was clearly detected at the lowest concentration, illustrating on-line MS sensitivity of at least 5 times greater than NMR with this experimental configuration. By MS, the strong correlation between low-level solution concentration and signal intensity demonstrates that good S:N can be achieved at low concentrations of pyrmethyl chloride hydrochloride.

Following this initial optimisation and development of the understanding of the capability, the combined system was capable of monitoring the process reaction in the concentration range 0.02% - 100%. However, as the MS linear dynamic range was limited to approximately 3 mg/mL (2%) at this stage (Figure 3), further optimisation of the MS experiment was undertaken to improve this measurement capability.

3. Extending the linearity of on-line MS

To extend the linear dynamic range of the mass spectrometry experiment, a number of parameters were further optimised whilst analysing pyrmethyl chloride hydrochloride solutions (0.01% to 10% relative reaction concentrations) in the presence of of pyrmetazole. The active splitter sample dilution parameter was further increased to 4000:1 and 6666:1 (from the initial 1000:1) using a sampling make-up flow of 2 mL/min. Such a high flow rate is unsuitable for direct coupling to the electrospray ion source of the QDa and was therefore combined with a post active splitter (pre-MS) flow split of approximately 1.65 : 0.35 (waste : MS). This optimised system resulted in the extension of the MS linear dynamic range for

pyrmethyl chloride hydrochloride to 6 mg/mL (10% relative reaction concentration) due to this significantly increased dilution step (Figure 5), whilst at the same time maintaining the ability to detect the lowest concentration solution (0.01% relative reaction concentration).

4. On-line NMR-MS Reaction Monitoring of a Nucleophillic Substitution Reaction.

Using the optimised NMR-MS reaction monitoring configuration, the nucleophilic substitution reaction to produce pyrmetazole was studied (Scheme 1). Both NMR and MS spectra generated online during reaction monitoring (Figure 6) were found to be equivalent to standard spectra supporting peak assignment. Although the order of the NMR signals around 7 ppm are slightly different when measured on-line, assignment could be made based on line shape and multiplicity. These differences are likely to be due to concentration, pH and temperature differences as the reference NMR spectra were acquired at 300 K, whereas during on-line analysis the acquisition temperature was set to 313 K. The temperature in the NMR was set to 313 K to match the temperature of the incoming reaction liquor. It should noted that shimming at 313 K was not a major issue. The half-height line width (for the 0.5 mg/mL solution) of the signal at 8.65ppm was 6.5Hz. Whilst this is broader than would have been liked, the signal of interest is well resolved from all other signals and the drawing of an accurate integral was straight forward.

On-line monitoring of the reaction by MS and NMR generated the reaction profiles shown in Figure 7. The quantitative reaction profiles (Figure 7a) were produced by integrating the peaks of interest δ_H 8.7 and 8.5 on waterfall plot (Figure 8). It is interesting to note here that the reaction concentrations begin to plateau much sooner by MS than by NMR, however the explanation for this is not clear at this time.

Conclusion

The on-line combination of NMR and MS has been shown to have clear advantages for reaction monitoring over the individual techniques. Using a flow-tube, NMR can provide structural and quantitative kinetic information for a reaction. MS has shown to be highly advantageous for structure elucidation and has a clear sensitivity advantage when detecting low level components and impurities in the reaction.

In this study, linearity of NMR response for both pyrmethyl chloride hydrochloride and pyrmetazole at concentrations between 0.02 % – 120 % and 0.02 % – 100 % respectively has been demonstrated. Linearity of MS response was initially demonstrated to be limited for pyrmethyl chloride hydrochloride to concentrations between 0.01 % and 2 % of maximum process concentration. Further optimisation extended this range to 10 % or 6 mg/mL. The limited linear dynamic range of MS at high concentrations and the poorer sensitivity on NMR for low level components was overcome by combining the two techniques in tandem on-line. Finally, the combination was demonstrated successfully by direct monitoring of a nucleophilic substitution reaction.

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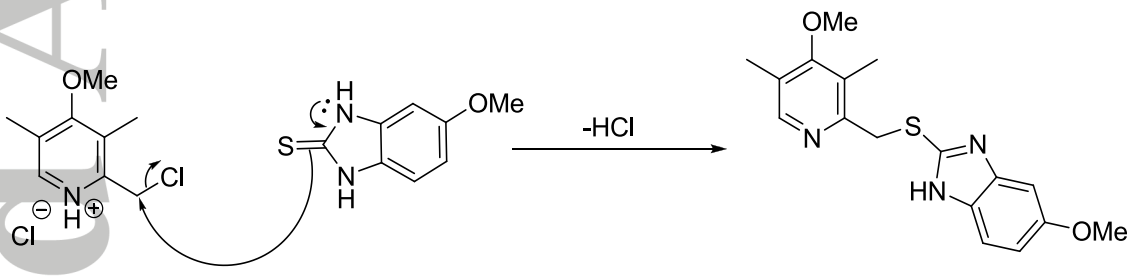


Scheme 1. The nucleophillic substitution reaction used to form pyrmetazole. Compound names (left to right) are pyrmethyl chloride hydrochloride, metmercazole and pyrmetazole.

Table 1. The solution concentrations used to determine the effect of additional reaction components on the MS response for Pyrmethyl chloride hydrochloride.

Concentration of Pyrmethyl chloride hydrochloride (% of reaction maximum)	Concentration of pyrmetazole (mg/mL)	m/z 186 abundance ($\times 10^7$) in the absence of sample matrix	m/z 186 abundance ($\times 10^7$) in the presence of sample matrix
0.01	12	0.240	0.0217
0.05	24	0.703	0.0281
0.2	21	1.13	0.122
0.5	18	2.87	0.251
1	15	3.36	0.588
2	12	4.63	1.20
5	9	4.99	2.33
10	6	5.83	3.43

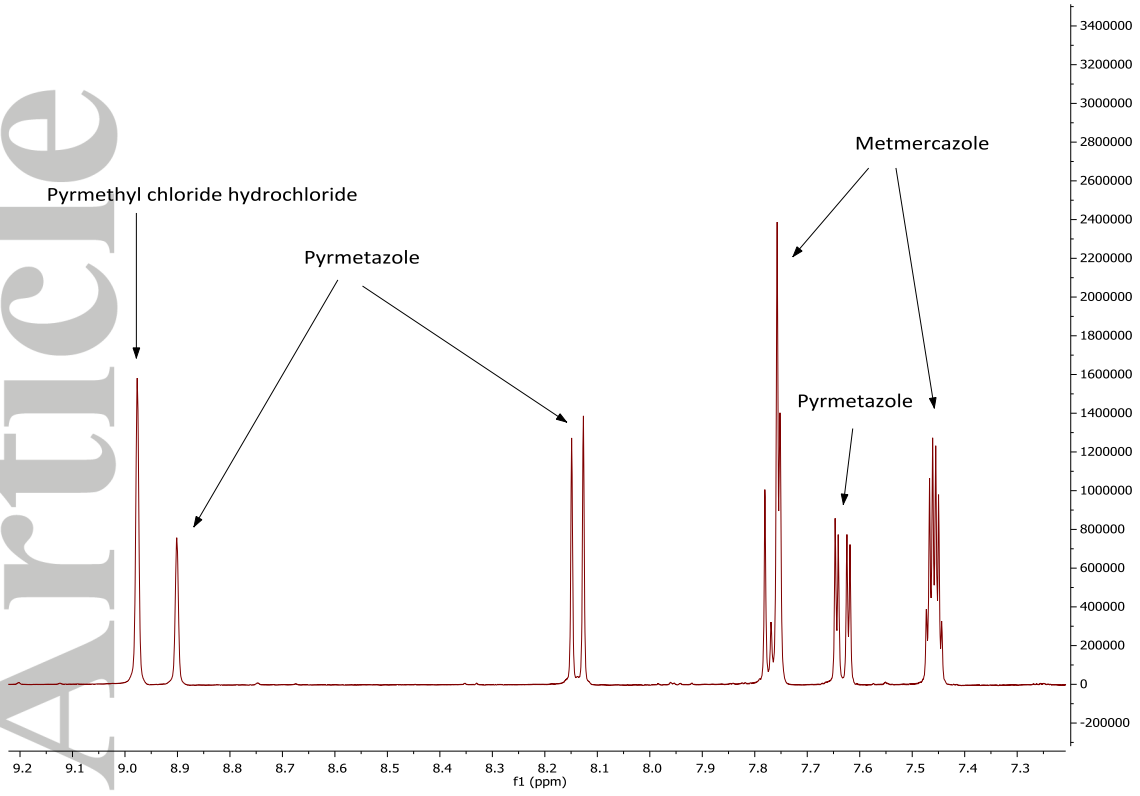
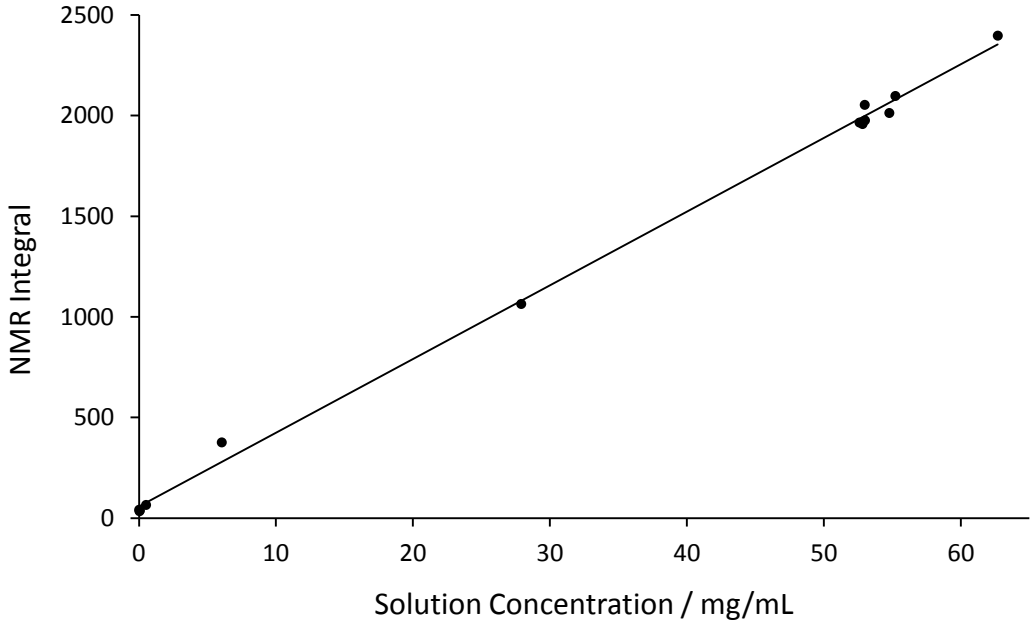
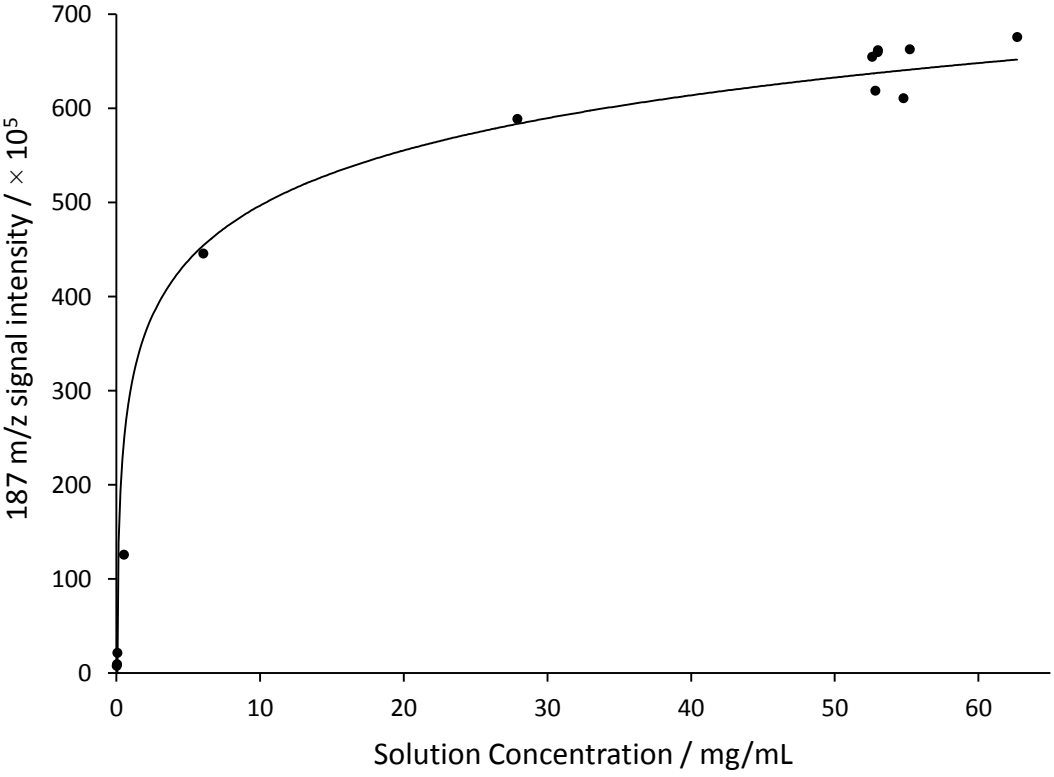


Figure 1. NMR signals selected for reaction monitoring.

(a)



(b)



(c)

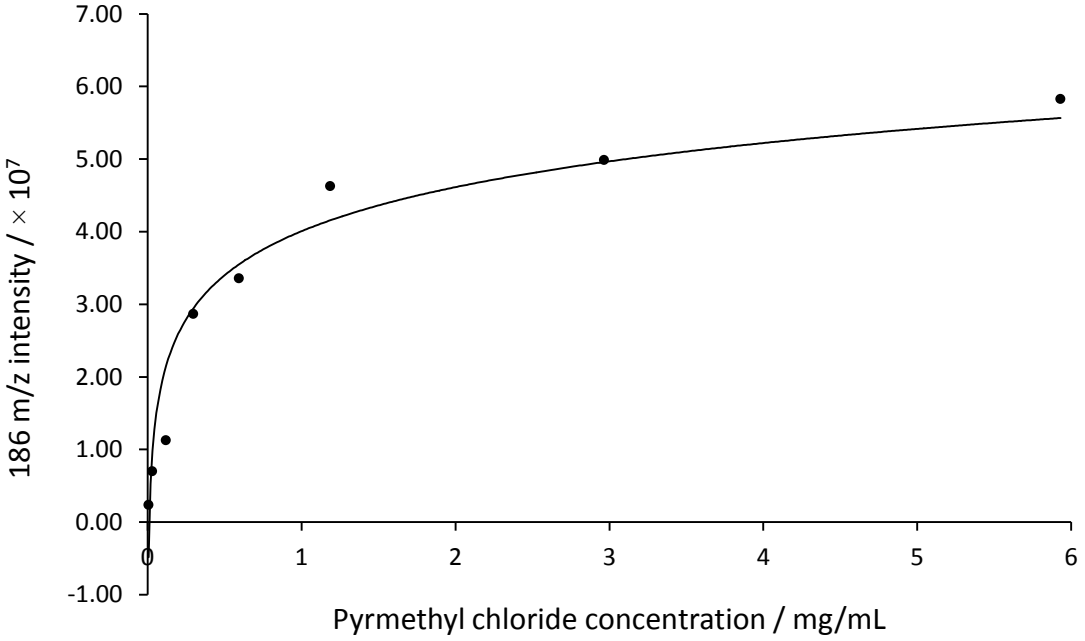


Figure 2. Evaluation of the linearity of the NMR and MS over the process concentration range (a) NMR linearity plot for pyrmethyl chloride hydrochloride, (b) MS linearity plot for pyrmethyl chloride hydrochloride and (c) MS low-level linearity assessment of pyrmethyl chloride hydrochloride in the absence of pyrmetazole.

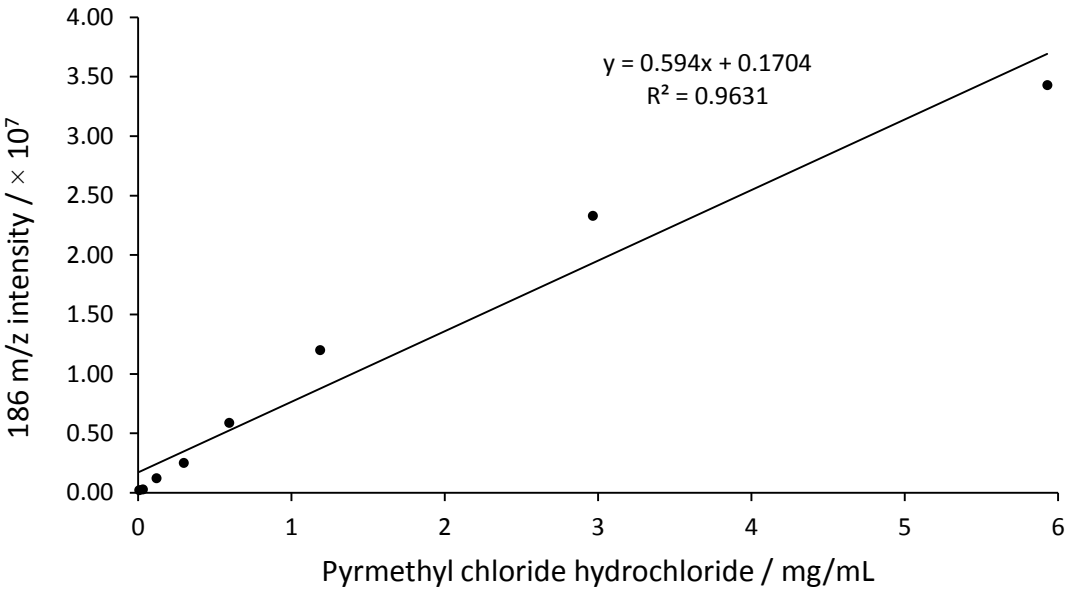


Figure 3. MS low-level linearity assessment of pyrmethyl chloride hydrochloride in the presence of varying levels of pyrmetazole.

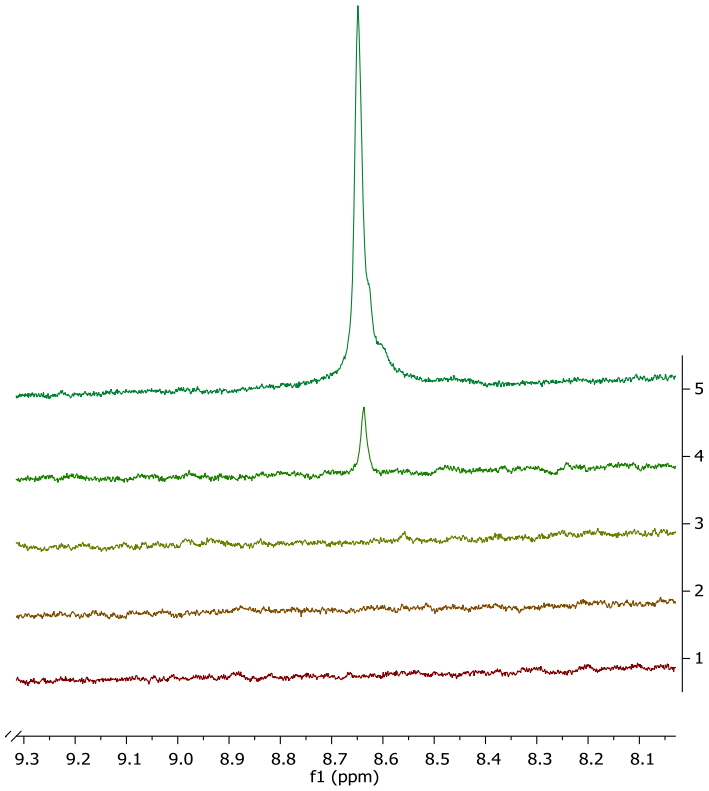


Figure 4. Waterfall plot of pyrmethyl chloride hydrochloride relevant ^{1}H signal corresponding to solution concentrations of 0.01, 0.03, 0.06, 0.5 and 6 mg/mL (1-5 respectively).

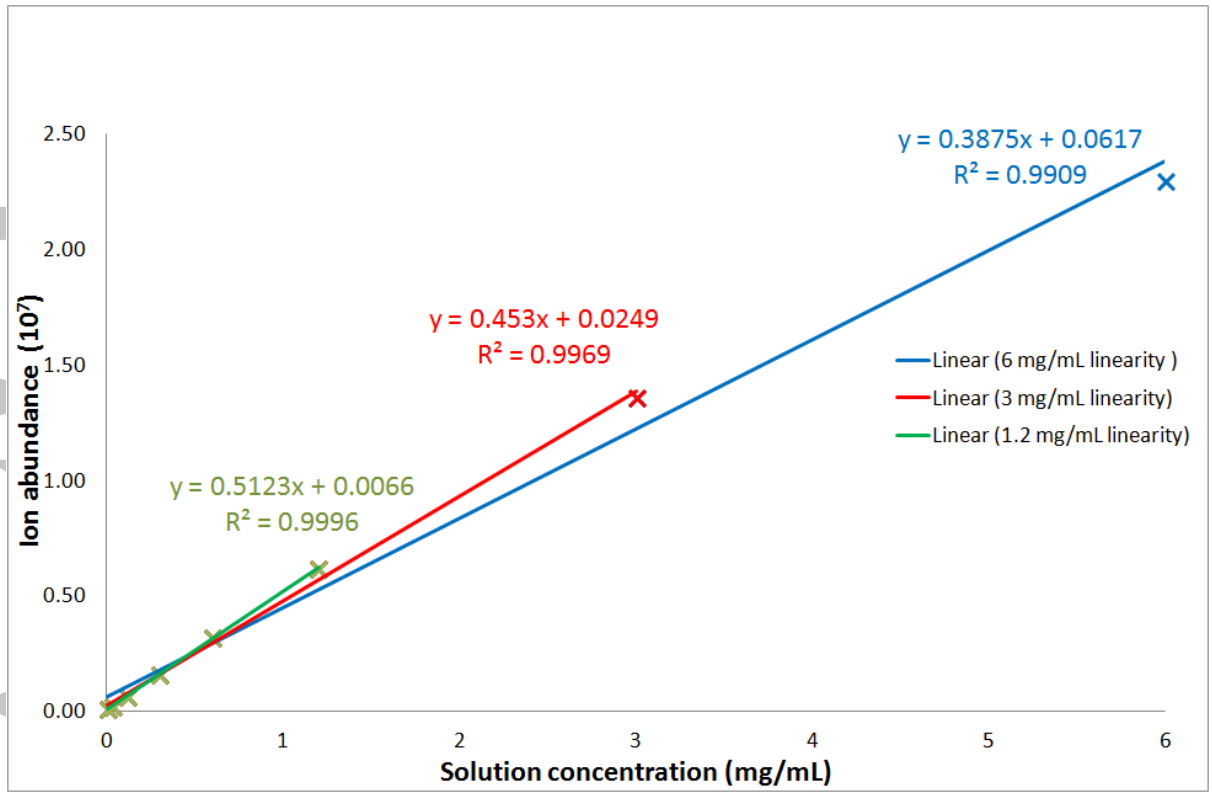


Figure 5: Extension of MS linear dynamic range at process concentrations - plot of ion abundance (10^7) as a function of the calibration standards (mg/mL) of pyrmethyl chloride hydrochloride in the presence of sample matrix and with the addition of a pre-MS flow split.

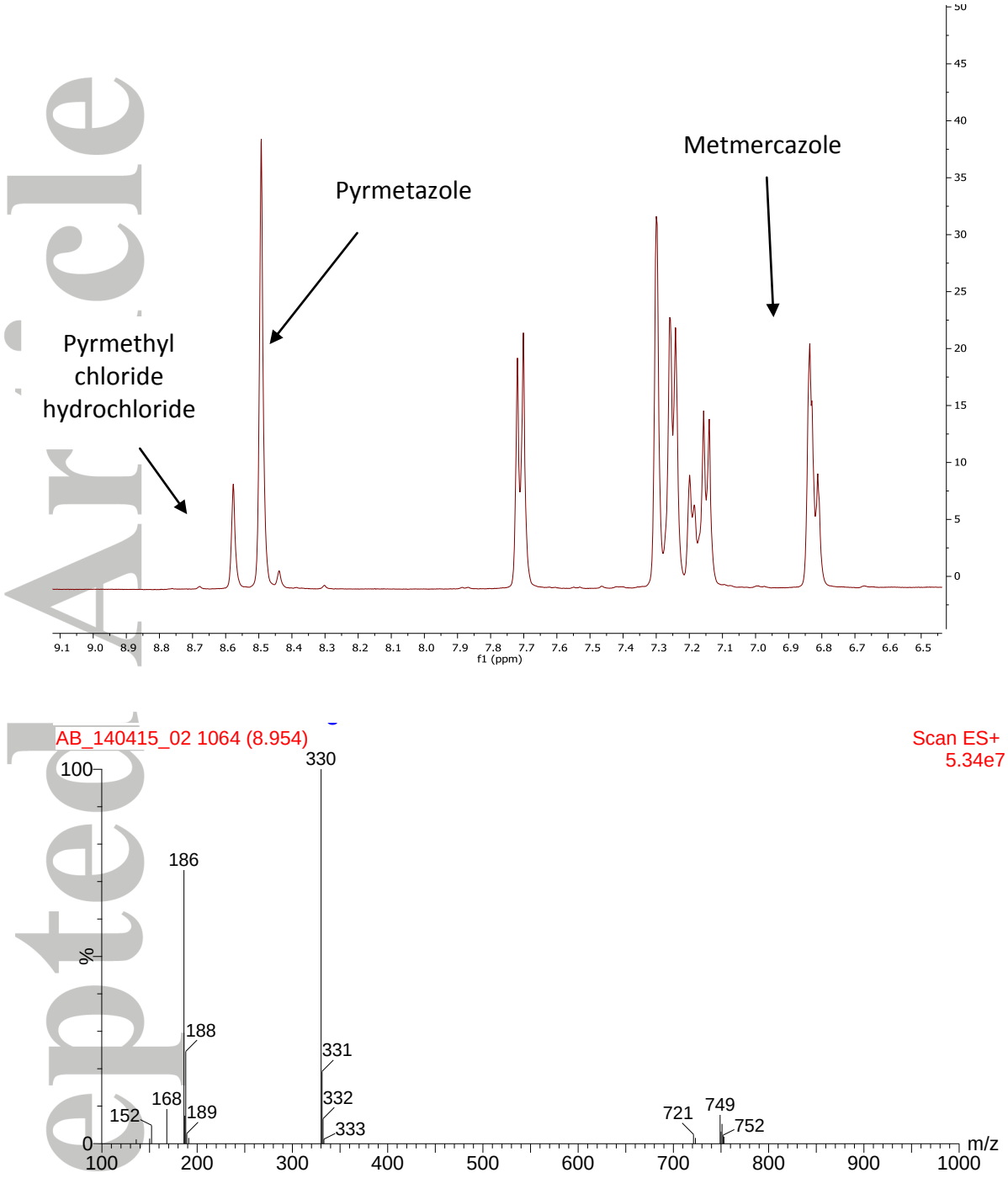


Figure 6. ^{1}H NMR and MS spectra acquired on-line during reaction monitoring.

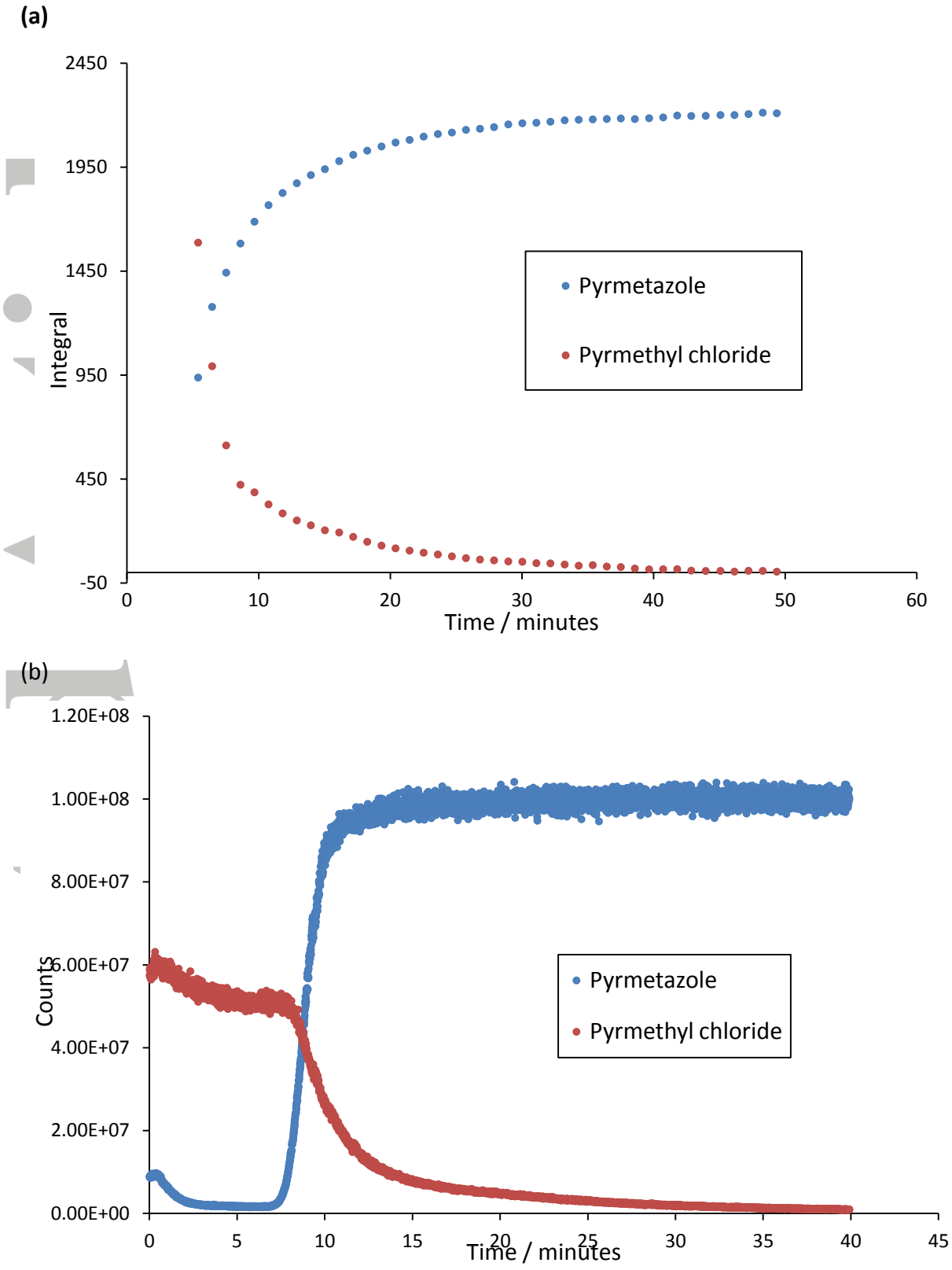


Figure 7. Reaction profiles generated using (a) NMR integrals and (b) the ions at m/z 186 and 330 m/z corresponding to starting material and product respectfully for the nucleophillic substitution reaction.

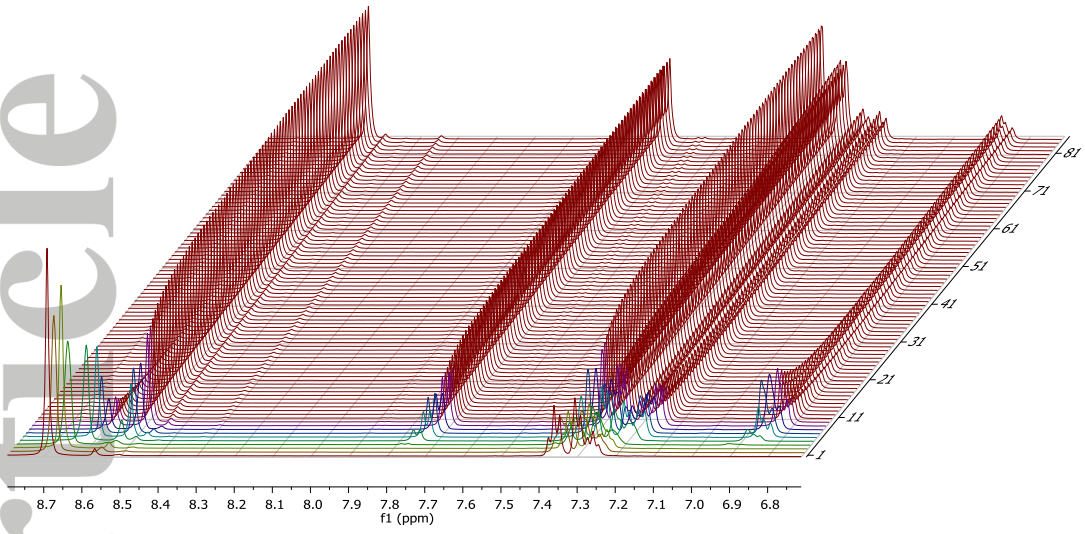


Figure 8. NMR waterfall plot from the reaction monitoring acquisition (y axis = intensity and z axis = acquisition number).