Standardless, Automated Determination of Chlorine-35 by ^{35}Cl Nuclear Magnetic Resonance

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We present an example of a robust, fully automated, walk-up method to quantify chloride concentration in sample solutions using ^{35}Cl nuclear magnetic resonance (NMR). Minimal user input is required, no standards are acquired at the time of analysis and the submission, acquisition, processing and production of results are seamlessly integrated within existing NMR automation software.

The method demonstrated good linearity with $R^2 = 0.999$ over three orders of magnitude of analyte concentration, results that are highly independent of analyte functionality, and the stability of instrument response was such that analyses of additional standards were not required for a period of several months. At a nominal sample concentration of 10 mg/ml in D_2O at 400 MHz, a detection limit and a quantitation limit of 0.1 and 0.5% w/w, respectively, was achieved in a 1 hour run time.

Robust methodology was achieved by applying a rigorous approach to method development and validation to determine and evaluate fully the time- and sample-dependent factors that affect quantitation in this experiment.

***Keywords**: NMR, 35Cl, chloride, quantitation, walk-up, automation, pharmaceutical analysis*

Introduction

The maxim that “nuclear magnetic resonance (NMR) is inherently quantitative” has become a central dogma of NMR spectroscopy in literature and presentations. Although correct, it may be more accurate to state that the *phenomenon* of NMR is inherently quantitative, as it is not necessarily true that any given NMR spectrum of a sample as acquired under a specific set of experimental conditions will yield quantitative results that are accurate and precise (Freeman 1987; Claridge 1999). To develop and validate a method capable of yielding acceptably quantitative data, two approaches can be considered.

In the first approach, only the basic parameters affecting quantitation are determined during method development (*e.g.* T_1, T_2). Conditions are then either set to ensure a quantitative response directly from the signal integrals, or are optimised for time efficiency and any expected decrease in response is calculated using the measured relaxation parameters and experiment delays. All other instrument-, sample- and time-dependent factors are taken into account by performing additional validation and calibration at the time of analysis through spiking known quantities of internal standards into the sample, or acquiring spectra of external standards immediately pre- and post-sample analysis. Alternatively, an artificial reference peak can be induced in the spectrum using Electronic Reference To access In Vivo Concentrations - ERETIC (Akoka, Barantin, and Trierweiler 1999), or QUANTification by Artificial Signal - QUANTAS (Farrant et al. 2010). Quantitation is then achieved by comparing the relative integrals of the analyte and artificial reference peaks, in a process analagous to using an internal standard. Another way to determine instrument and sample variation at the time of analysis is to apply the principles of reciprocity (Hoult and Richards 1976) by measuring the pulse width for each sample and comparing the values to those

acquired on a previous reference. This has been demonstrated by application of PULse Length based CONcentration determination - PULCON (Wider and Dreier 2006).

In the second approach, rather than carrying out continual validation and/or calibration at the time of analysis for each sample, the factors affecting signal response are considered and explored and then a region over which the method can operate without further validation is defined. In this way, a method can be developed and validated such that it is robust, accurate and precise, but does not require additional experimentation at the time of analysis.

For either approach the final time-saving component in developing a completely automated method is the creation of walk-up automation software that provides a simple interface requiring minimal user interaction. Although more complex NMR data can require many hours of manual processing, for routine processes and calculations it can generally be assumed that if a task or series of tasks can be performed by an analytical expert using a computer, then it can be automated. This may require either the writing of entirely new pieces of software or, more frequently, the coding of custom programmes to enable more flexible access to the capabilities within existing automation procedures.

For most analysts, NMR would not be considered, by first intent, for routine chloride analysis. Quantification of chloride ions would usually be performed by Ion Chromatography (IC) (Christian 2004). IC is a highly industrialised technique that can be hyphenated to advanced automation systems capable of weighing, dissolving and injecting samples into the instrument, processing data and reporting results. Run times can also be short (< 10 min per chromatogram) for basic separation of anions.

Whilst IC is a common technique it may not, however, be available in all analytical laboratories and, if present, may not be set up as an automated walk-up system. If not automated, an IC system would require human intervention in the form of analytical experts to prepare samples, queue them on an autosampler, integrate the chromatographic data and report the results back. Regardless of automation, standards and diluent blanks would be need to be prepared and analysed before and after analysis of genuine samples, increasing the overall analysis time. As with all liquid chromatography techniques, IC also needs a constant flow of mobile phase to operate, requiring further time on the part of the analytical expert to prepare solutions, flush columns and then dispose of waste.

High-field NMR spectrometers are now a standard feature of many chemical laboratories and are ideally suited for automation and walk-up analysis. With the exception of scheduled routine maintenance and housekeeping (*e.g.* regular magnet fills of liquid nitrogen and helium, emptying of the autosampler and checking overall instrument performance), the systems require minimal or no daily intervention.

The proceeding work gives an example of extensive method development applied to the quantification of chloride in solutions containing other organic or inorganic analytes by ^{35}Cl NMR. The work demonstrates how the seamless integration of simple programming code into established automation software can allow the handling of sample preparation information, data processing, quantification and reporting of data within a single piece of software. There are relatively few examples in the literature of using ^{35}Cl quantitatively in this way. In one reported example (Lim and Lee 2006), quantitation was achieved by loading the sample into a 5 mm NMR tube into which was co-axially placed a sealed 3 mm NMR tube containing a solution of tetraethylammonium chloride as an internal standard. While this work demonstrates the general principle, they are no examples of extensive validation or

automation for this particular application. It should, however, be noted that there are examples of extensive validation of ^{1}H quantitative methods conducted across multiple laboratories, and considering many aspects of uncertainty (Malz and Jancke, 2005).

The factors considered likely to affect signal response and the final results were investigated, many of which are in line with general guidance for method validation (ICH Q2(R1) 1996) and some that are specific to NMR spectroscopy, namely: linearity, detection and quantitation limits, repeatability, coil loading, accuracy (including sample matrix effects), and processing.

Experimental

All data were acquired on a 400 MHz Bruker Avance-III NanoBay NMR spectrometer equipped with a BCU-05 temperature controller using a 5 mm probe with a broad-band channel tunable to resonant frequencies in the range ^{31}P to ^{15}N (also including ^{19}F), automatic tuning and matching, z-gradients and a BACS-60 auto-sampler. The spectrometer was controlled by Bruker Topspin version 3.0, running under Microsoft Windows 7 and using software module IconNMR for automated spectrometer control. Data were typically acquired over a frequency width of 11792 Hz into 4096 data points using a pulse and acquire sequence with a 90° pulse calibrated at 13.4 µs at 100 W, an acquisition time of 174 ms, a sample temperature of 300 K and a dead time of 17 µs to minimise ring-down artefacts at the start of the free induction decay. The ^{35}Cl T_1 of a solution of NaCl in D_2O at a concentration of 10 mg/ml Cl^- was measured to be 32 ms (comparable to a calculated literature value of 40 ms in water at infinite dilution (Hertz 1973)). As the acquisition time is > $5 \times T_1$, an arbitrarily short relaxation delay of 1 ms was set. Data were acquired using 6144 transients per experiment, requiring a total of approximately 21 minutes of instrument time. Data were Fourier transformed with 30 Hz exponential line broadening function and the free induction decay was zero filled with a factor of 4. All data processing was handled *via* a

custom written programme running under Topspin version 3.0. The programme is available from the author on request.

Linearity

Solutions of sodium chloride in D_2O were prepared with chloride concentrations of 0.01, 0.02, 0.06, 0.1, 0.2, 0.4, 0.6, 1.0, 1.5, 2.0, 2.5, 5.0 and 10.0 mg/ml.

Accuracy

Solutions were prepared by dissolving 20 compounds, representing a range of common organic compound functionalities and salts, in a stock solution of sodium chloride in acetonitrile/D_2O 50/50 v/v with a nominal concentration of organic compound of 10 mg/ml and a nominal chloride content of 2.0% w/w. Further solutions of inorganic chlorides were prepared containing different counter-ions. Solutions of magnesium chloride, potassium chloride, lithium chloride, ammonium chloride, calcium chloride and caesium chloride were prepared in acetonitrile/D_2O 50/50 v/v such that a nominal concentration of 2.0% w/w of chloride was obtained for each.

Repeatability/instrument stability

A solution of sodium chloride in D_2O/acetonitrile 50/50 v/v with chloride concentration of 0.2 mg/ml (equivalent to 2.0% w/w with respect to a nominal sample concentration of 10 mg/ml) was analysed periodically over a period of 24 hours (24 spectra in total), then over a period of 7 days (54 spectra in total) and finally over a period of 10 weeks (139 spectra in total).

A sub-set of the linearity experiments were repeated approximately 3 years after the original analyses. Solutions of sodium chloride in D_2O were prepared with chloride concentrations of 0.1, 0.2, 0.5, 1.0, 2.5, 5.0 and 10.0 mg/ml.

Results and discussion

Linearity

Chloride signal (area units) measured as a function of chloride concentration (mg/ml) in the range 0.01 mg/ml to 10 mg/ml showed a linear response fit with a correlation coefficient > 0.999 and an equation of $y = 2.9307\times10^8 x + 3.7840\times10^6$. Linearity was demonstrated across a range representing 0.1 – 100% w/w compared with a nominal concentration of organic analyte of 10 mg/ml. These data were comparable to linearity achieved previously (Lim and Lee 2006) over the range 0.02 mg/ml to 5 mg/ml with a correlation coefficient > 0.999 on a 700 MHz NMR spectrometer.

The response of the receiver across a range of gain values from 0.25 to 179 was also assessed and found to be acceptably linear with $R^2 = 0.999$. The regression curve was then used in the processing programme to determine the chloride content from the absolute peak area, post Fourier transformation and phase correction (*vide infra*).

Signal to noise

The minimum signal-to-noise ratios acceptable to determine limit od detection (LoD) and limit of quantitation (LoQ) are commonly quoted as 3:1 and 10:1 respectively. However, these values are often taken from guidance intended for chromatographic data and by measuring peak-to-peak noise using a calculation of signal-to-noise$_{p\text{-}p}$ = 2H/h where H is the

peak height from the zero point of the baseline and h is the height of the peak-to-peak noise. Topspin, as used to process these data, determines noise based on a root-mean-squared calculation and determines signal-to-noise$_{\text{sino real(RMS)}}$ = H/2h. Applying a conversion of $h_{\text{peak-to-peak}} = 5 \times h_{RMS}$ then this equates to minimum detection and quantitation limits using the "sino real" command of 3.75:1 and 12.5:1 respectively.

Under the experimental conditions used, the 0.01 mg/ml chloride standard yielded an NMR response with signal-to-noise$_{\text{sino real}}$ = 3.92:1. This is greater than the required value of 3.75:1 and so is acceptable for the detection limit. The 0.06 mg/ml chloride standard yielded an NMR response with signal-to-noise$_{\text{sino real}}$ = 16.04:1. This is above the limit of 12.5:1, and so is acceptable for the quantitation limit. It is possible to estimate the percentage chloride content that a peak at 12.5:1 would represent and thus estimate the theoretical quantitation limit:

$$\textbf{Estimated quantitation limit} = \frac{\mathbf{12.5}}{\mathbf{16.04}} \times \mathbf{0.06}\% \quad \textbf{(1)}$$

$$= \mathbf{0.0468\ mg/ml\ chloride}$$

Thus, if a sample of nominal concentration 10 mg/ml was analysed using the conditions described for the current method, then detection and quantitation limits for chloride content would be 0.1 and 0.5% w/w respectively. This will hold true for any solutions giving rise to a chloride response with the same half-height linewidth. As the actual samples are more likely to be dissolved in acetonitrile-d_3/D_2O 50/50 v/v (*vide infra*), the signal-to-noise ratio and detection and quantitation limits will be calculated for each spectrum and used as limits against which to report data within the processing programme. This step improves the robustness of the method by taking into account the quality of each spectrum at the time of

analysis and will avoid reporting data against an inappropriate detection and quantitation limit if there is excessive noise in a sample spectrum due to unforeseen instrument problems.

The detection and quantitation limits shown above were obtained with run times of approximately 20 minutes, the rationale being that longer run times for data acquired during working hours on a walk-up analytical instrument would be impractical for reasons of maintaining sample throughput. Longer run times submitted as “overnight experiments” would then achieve lower detection and quantitation limits, and could be used on samples with limited availability or solubility. Sensitivity can also be increased in IC methods by increasing the injection volume. However, NMR offers the additional advantage that whilst there will be a maximum injection volume for a given IC method, there is theoretically no upper limit to the number of transients that can be acquired in an NMR experiment.

Repeatability

Replicates over the initial 24 hour time period returned an average result of 1.57 ± 0.06% w/w chloride for a total of 25 results, where the error is quoted as ± 1 standard deviation. Whilst this level of variability would be considered acceptable for a quantitative method, linear regression analysis of the data showed possible evidence of a decreasing trend in response over time with $R^2 = 0.3922$.

There was no evidence of precipitation in the sample, the NMR tube was sealed with parafilm to prevent evaporation, and insolubility would not be an expected issue for an aqueous solution of sodium chloride at the concentration prepared here.

The presence of a continually decreasing response is a major issue where the aim of a method is to quantify based on an absolute response over a period of time. For this reason a spectrum was acquired on the same sample once every day for a further seven days, and then once every hour for a period of 24 hours.

Error bars of ±1 standard deviation for the two 24 hour periods overlap with each other and so although there is a drop in the average response over the 1 week period, the change is not significant compared to the degree of variability of response over 1 day. In order to definitively establish and quantify the trend, data were acquired over a period of approximately 10 weeks. The data were typically acquired in blocks of at least 12 experiments per 24 hour period during this time. Less of a trend was apparent over this 10 week duration compared to the data acquired every hour over a 24 hour period. This fact is confirmed by taking a plot of the average values for each 24 hour time period (Figure 1). There is variation in the average values for a given day, but no overall trend over time as there is overlap between error bars for all days. Furthermore, the standard deviation of the averaged values across the days is 2.03%, whereas the standard deviations for the values for each day range from 2.0% to 3.8%. This shows that there is more intra-day variation than inter-day variation in the data. As shown in Figure 1, the spectrometer was turned off for a period of approximately 24 hours and no significant differences were observed in the data acquired before and after this time.

To further exemplify the effect of the level of variation upon an actual measurement, the day with the greatest level of variation in results would yield a chloride content of 1.50 ± 0.08% w/w, equating to 5.0% relative standard deviation. This is an acceptable level of variation for a typical analytical method and will be considered to be the uncertainty in measurement for

this method. For comparison, the average of all data over this period yielded a chloride content of 1.58% w/w.

It is noted that, as all data discussed here were acquired with the same NMR tube and probe tuning and matching were not adjusted between consecutive data accumulations on the same day, it follows that the sources of the observed variations must lie in either the instrument response or the data processing. It should also be noted that day-to-day variability is usually assessed as part of intermediate precision. This should include instrument-to-instrument and analyst-to-analyst variability. In this instance, only one spectrometer was available to test the method on, and the automated processing was such that the only analyst variation that would be encountered here would be due to sample preparation.

The method, and therefore by inference the response of the spectrometer, was shown to be robust over a period of at least 10 weeks without the need for further acquisition of standards. This included 24 hours of complete spectrometer down-time during the 10 week period.

The repeat linearity experiments after 3 years yielded a regression equation that showed a change in instrument response. This change was significant enough to require the regression parameters to be updated in the experiment at this time. Although the aim of this work is to demonstrate that modern NMR spectrometers are capable of quantitation by absolute response without the need for standards, it is advisable to carry out regular performance checks for any analytical method in use over time. For example, a system suitability check, in the form of acquisition and recording of data on a standard sample on

the day of analysis, would confirm the overall performance of the instrument and monitor any progressive changes, such as drift in amplifier response that may require regression data to be re-acquired.

Additional replicate analysis was also performed with a total of 7 batches of NMR tubes across 3 manufacturers. It was found that there was no significant variation between batches of the tubes from the manufacturer typically used for this analysis, but a difference in response was observed with tubes from different manufacturers. This shows that for a method that relies on absolute response, care must be taken to use NMR tubes of the same manufacturer and specification for both validation and subsequent analyses.

Coil loading

Here we consider the application of the principle of reciprocity to NMR spectroscopy (Hoult and Richards 1976): “RF susceptibility and conductivity effects which influence the coupling of the precessing nuclear magnetization to the coil will have the same effect on the radiofrequency field (produced by the same coil at the same frequency) that causes the excitation of the same parcel of nuclei.” (Burton, Quilliam, and Walter 2005). Put another way, anything affecting energy transfer between the nuclear spins within a sample and the coil within the probe will affect that transfer equally in both directions. The efficiency of this transfer is characterised by the quality factor which is proportional to signal intensity. For this reason, the spectrometer response may be affected not only by the presence of other species in the sample matrix, but also the concentration of the analyte itself.

If, for example, increased salt concentration leads to a lowering of the quality factor, then more energy will be required by the amplifier to achieve a true 90° pulse. From the other

perspective, a change in quality factor with the same pulse power and width will lead to a decrease in the effective flip angle in the sample and a reduced response. For this reason, methods have previously been devised to account for these differences by measuring the 90° pulse width for each sample and then using the difference between this value and the 90° pulse width to determine the increase or drop in response. In this way, a quantitative response could be calculated for each sample.

The extent of the effect was investigated with the selected analytes to determine if pulse calibration was required as part of the method. This was investigated indirectly, by observing the difference in the tuning/matching curve, and directly, by measuring the change in p_{90} for different samples.

The tuning and matching curve was recorded for all samples used in the linearity measurements and no significant differences were observed over this range. Although this does not take into account any differences from the presence of organic molecules, it is expected that the inorganic ions of Na^+ and Cl^- will have the greatest effect on the quality factor. We can conclude that the effect on tuning and matching, and hence the quality factor, is negligible for the concentration range used in this work.

The ^{35}Cl p_{360} was determined for a series of samples with varying chloride concentration, and the p_{90} subsequently calculated as $p_{360}/4$ (Figure 2).

A non-linear relationship is observed between pulse width and chloride concentration, with the lower concentrations having shorter pulse widths as the quality factor increases. The

overall change in p_{90} over this range is no more than 0.2 μs. As the flip angle deviates from p_{90} by θ, so the intensity of the signal decreases from the maximum value by the factor cos θ (Burton, Quilliam, and Walter 2005). For a change of ± 1 μs this equates to a decrease in the signal of 2.51% relative to the maximum. For a sample containing 1.58% w/w chloride (*i.e.* the average value from the repeatability experiments), a decrease of 0.04% w/w would result. This is less than the error of ± 0.08% w/w obtained from repeat analysis of the same sample.

Due to low signal-to-noise ratio, it was not possible to accurately measure p_{90} for samples of lower concentration than 1 mg/ml chloride. However, no significant changes in the tuning and matching curve were observed at these concentrations and a linear response has already been established down to a chloride concentration of 0.1% w/w.

For the work discussed here in aqueous solution, it has been shown that the method is robust with respect to the effect of chloride ion concentration upon the quality factor for this probe. This further supports the proposition that subsequent analyses do not require standards of matching chloride concentration to be run at the time of data acquisition, or that an approach such as PULCON is required to correct for the effects of coil loading.

Accuracy

The recovery results for the samples prepared containing *ca.* 2% w/w chloride are presented in Table 1. The theoretical chloride content is calculated from the weight of the analyte, dilution volume and quantity of chloride spiked in, and the experimentally measured chloride content is the result as determined by the automated processing method. Recovery is then calculated as the percentage by which the experimentally measured value differs from the theoretical value.

Only one value falls just outside the range of 90-110% recovery, namely butylhydroxy toluene, although it should be noted that only single preparations were made of each sample.

Analysis of the solutions of different inorganic chlorides yielded recovery values 90-110% of the theoretical chloride content (based on the formula for each compound) for all samples.

For the purposes of the method, we have demonstrated that the method is robust with respect to sample matrix, for the presence of a variety of common chemical functionalities and different chloride counter-ions. The effect from chlorides containing paramagnetic metals was not explored due to the hygroscopic character of the available metal chlorides, hence this aspect should be considered and investigated before the method is applied to such samples.

Automation

The ultimate aim of this work was to develop a fully automated ^{35}Cl NMR quantitative chloride experiment for a walk-up NMR spectrometer. To achieve this, the method not only had to be developed with respect to experimental conditions and parameters, but also had to be capable of data acquisition, processing and reporting with minimal user interaction required.

For most experiments run in a high throughput, automation setting, the user is usually only required to input a dataset name, the solvent, the type of experiment required and some title information such as batch and/or sample preparation details. For the automated ^{35}Cl experiment, however, the user is also required to input sample weight and solution volume.

The calculation to produce the result would then need to include other information from acquisition parameters, such as receiver gain and number of scans, and also the regression parameters determined from the linearity experiments. Finally, a result would be reported back to the user in the correct format. A processing programme was therefore written to handle the different user inputs, process the data and calculate and report the result.

The Topspin user acquisition parameters (USERA1 and USERA2) allow users to input the sample weight and volumes directly into the walk-up IconNMR software. Post-acquisition, the programme then Fourier transforms the data and performs phase correction and baseline correction. The main peak is integrated and the final chloride result calculated, added to the title of the experiment and the spectrum, including title, is printed to a PDF file. The only user intervention required is the sample preparation and entering the initial weight and volumes into IconNMR. Two specific aspects of the processing programme are discussed in more detail below.

Determining optimal integration region

During the development of the processing package, it was found that the peak width of the chloride resonance varied considerably depending on the other species present in solution. For example, solutions of NaCl in D_2O gave half-height linewidths of *ca.* 20 Hz, whereas for some analytes dissolved in D_2O/Acetonitrile 50/50 v/v, this would rise to as much as 80 Hz. Setting an arbitrarily broad region for integration increases the variability of the results by increasing the noise region included in the integral for more narrow resonances.

To resolve this issue, an additional sub-routine was written into the programme to determine the approximate linewidth of the chloride resonance. This function directly accesses the binary file that contains the real FT data as produced by Topspin to determine values of individual data points.

In this function, the absolute intensity of the highest peak (apex) is first read in from the binary file. Then, the next two data points to the right of the apex (*i.e.* at lower ppm values) are read in. If both of these are greater than half of the apex intensity, then the process is repeated for each subsequent data point, moving stepwise down the right hand side of the peak (Figure 3Figure). When point 2 is less than the half-height intensity, the programme determines whether peak 1 or 2 are closest to the half-height and then stores the index of this point. The programme moves the pointer back to the apex and repeats the process moving stepwise down the left hand side of the peak, stopping again when the point nearest to half height intensity is found.

By subtracting the index value from the half-height point on each side of the peak, the approximate peak width can be determined in units of data points. Finally, multiplying this number by the spectral width in Hz and dividing by the number of points in the real spectrum yields the approximate peak width in Hz.

The integral region is then set such that minimum noise is included in the integration calculation. Note that integration is achieved not by using the built-in functions in Topspin but by a similar process of reading in the intensity of the data points over the region of interest and adding these values to produce a total absolute area.

Automatic phase correction

In the ^{35}Cl NMR spectra discussed here, with the current experimental set up, the zero order phase correction required to produce a resonance in the FT NMR spectrum of pure absorption phase is close to 180°. However, slight changes in the relative phase difference between the pulse and the receiver from one sample to the next require the zero order phase correction to be adjusted for each spectrum.

This can be done manually, which can be highly subjective, or by use of automated phase correction routines. The automated routines available in Bruker Topspin were not capable of achieving a consistent absorption phase peak in this case. Custom routines have been developed that achieve robust phase correction of ^{1}H spectra through a combination of baseline correction, area minimisation and negative area penalisation (Brouwer 2009). However, the ^{35}Cl spectra acquired here contain a single peak and the baseline only needs to be considered over a narrow region around this peak. For this reason, a more simplified approach proved acceptable and a custom routine was written into the processing programme.

A basic measure of ideal peak shape is the symmetry of the peak. If an imaginary line is drawn down the peak from the apex, and the distance from half way up the line to the edge of the peak on each side is taken, then the difference between these two distances will be at a minimum when the peak is symmetrical.

This approach has the advantage that it can be completely controlled and run *via* a processing programme that directly manipulates the sample spectrum and then determines

the required values by accessing the binary data. Figure 4Figure shows a plot of results as acquired by a subsequent modification of the processing programme.

The asymmetry factor is calculated by the absolute difference between the apex line at half height and each edge of the peak.

Conclusions

We have shown here that specific, robust, and quantitative NMR methodology can be developed by rigorously investigating the factors effecting signal response. This has been conducted informally in this work, but it could take the form of a comprehensive risk assessment prior to method development. We have also shown that it is possible to quantify species without the need for standards at the time of analysis, as long as the receiver/transmitter coil response can be demonstrated to be sufficiently independent of sample matrix and analyte loading.

Finally, it was demonstrated that when existing processing macros are unable to produce the required data quality, custom programmes can be embedded into automation software to ensure a seamless walk-up user experience from submission to results.

In summary, we have shown in this example that existing NMR spectrometers can be used flexibly to allow synthetic chemists to save considerable time and effort in acquiring accurate and sensitive data on a common analyte. The time expended in the development and validation of this, and similar, methods is readily gained back from many chemists being able to access new and powerful walk-up tools to more rapidly expidite their work.

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Table 1. Recovery values for analyte solutions in acetonitrile-d_3/D_2O containing chloride

Analyte	Theoretical chloride content % w/w	Experimentally measured chloride content % w/w	% Recovery
Acesulfame potassium	1.93	1.83	94.8
Quinine	1.93	1.87	97.0
Succinimide	1.80	1.78	99.0
Paracetamol	1.91	1.89	99.1
Benzoic acid	1.81	1.73	95.5
Succinic acid	1.83	1.80	98.4
Butylhydroxytoluene	1.84	1.65	89.7
Ascorbic acid	2.13	2.09	98.3
Vanillin	1.83	1.87	102.3
2-Hydroxy-5-methylbenzaldehyde	1.95	1.84	94.6
Benzophenone	1.88	1.82	96.8
3-Methyl-4-nitrobenzoic acid	1.87	1.89	101.1
Benzenesulphonic acid	1.94	1.92	98.8
Glucose	1.94	1.86	96.0
Benzeneboronic acid	1.88	1.71	90.9
Glycine	1.90	1.81	95.5
Sodium dodecyl sulphate	1.84	1.78	96.7
Potassium carbonate	1.92	1.79	93.4
Di-Sodium hydrogen phosphate	1.90	1.83	96.5
Sodium acetate	1.90	1.85	97.3

Figure 1. Change in chloride response over a period of 10 weeks, error bars show ± 1 standard deviation, $y = 6.18\times10^{-2}x + 9.5435\times10^{1}$, $R^2 = 0.6551$

Figure 2. Effect of chloride concentration on ^{35}Cl pulse width

Figure 3. Schematic showing the principles of the function to measure half-height linewidth

Figure 4. Plot of asymmetry factor as a function of zero order phase correction, as determined by the processing programme

Fig. 1

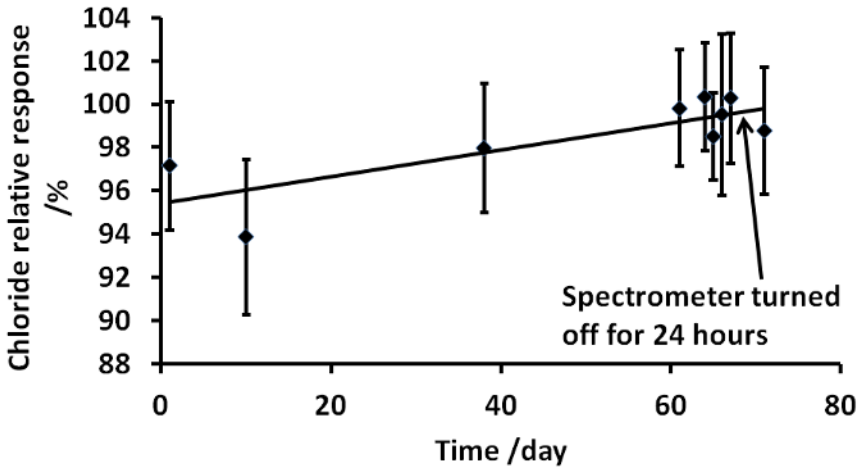

Chloride relative response /%
104
102
100
98
96
94
92
90
88
0
20
40
60
80
Time /day
Spectrometer turned off for 24 hours

Fig. 2

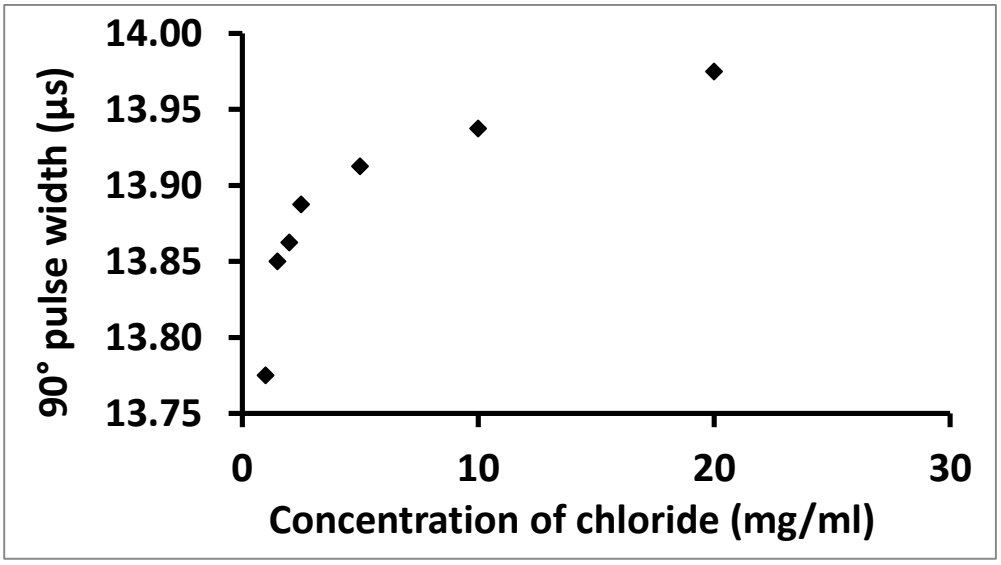

14.00
13.95
13.90
13.85
13.80
13.75
90° pulse width (µs)
0
10
20
30
Concentration of chloride (mg/ml)

Fig. 3

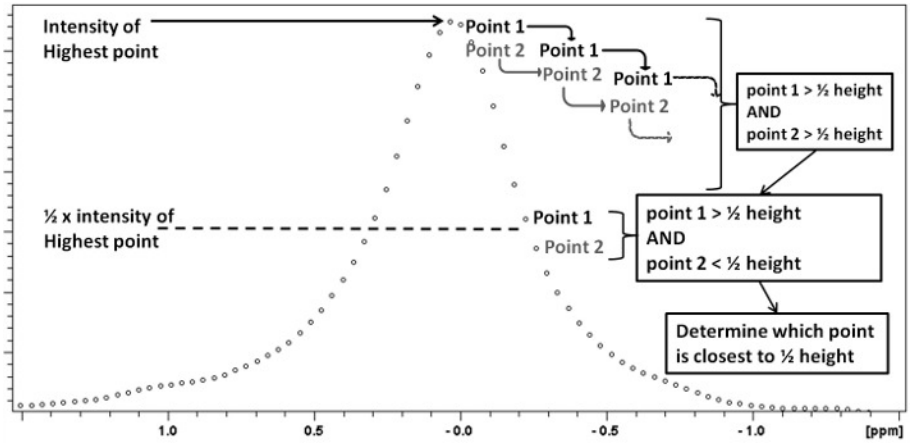
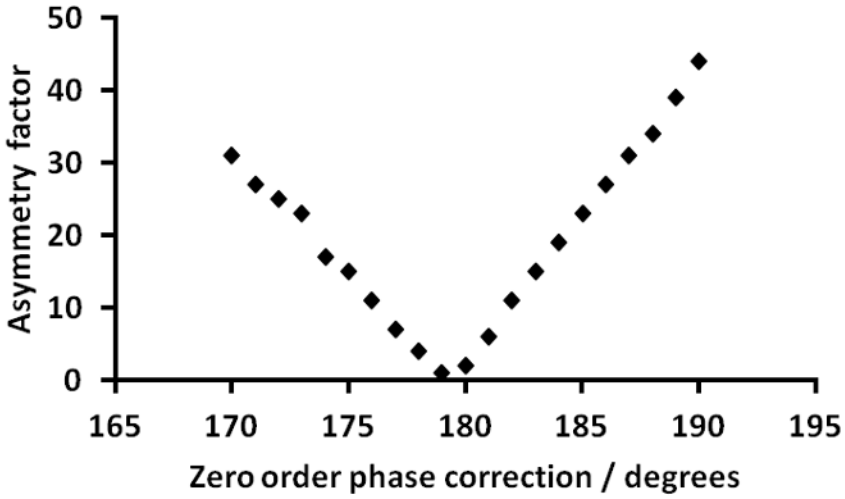

Intensity of
Highest point
Point 1
Point 2
Point 1
Point 2
Point 1
Point 2
point 1 > ½ height
AND
point 2 > ½ height
½ x intensity of
Highest point
Point 1
Point 2
point 1 > ½ height
AND
point 2 < ½ height
Determine which point
is closest to ½ height
1.0
0.5
-0.0
-0.5
-1.0
[ppm]

Fig. 4


Asymmetry factor
50
40
30
20
10
0
165
170
175
180
185
190
195
Zero order phase correction / degrees