



# **Content determination of explosive precursors and narcotic salts using $^{35}\text{Cl}$ -nuclear magnetic resonance**

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## Abstract

Explosive precursors and narcotic salts are chemicals contributing to an undesirable development of the Swedish society, both in terms of criminal activities and harm to the environment. Reducing the illegal use of these chemicals is important in the work towards a safer society. National Forensic Centre (NFC) is the state agency responsible for forensic investigations for the Swedish Police Authority. The Drug Analysis and Chemistry-Technology section at NFC were both in need for an accurate quantification method to determine the content of Cl in narcotic salts and explosive precursors. Nuclear magnetic resonance (NMR) spectroscopy was assessed to be suitable since a recently published article had shown applicability of  $^{35}\text{Cl}$  NMR on narcotic salts. The aim of the method was to find the most appropriate parameter settings for the compounds of interest, including operating frequency,  $90^\circ$  pulse length, number of scans, relaxation time, and relaxation delay. To ensure a reliable and accurate method, the following validation parameters were studied; linearity, limit of detection (LOD), limit of quantification (LOQ), intermediate precision, trueness, repeatability, and ruggedness. Dimethyl sulfoxide ( $d_6$ -DMSO) was chosen as the preferred solvent for the Drug Analysis section since it is a common solvent for their  $^1\text{H}$ -NMR analysis. For explosive precursors results showed advantages of using deuterium oxide ( $\text{D}_2\text{O}$ ) as solvent, considering accuracy, solubility and shorter analysis time.

Concluding, the chosen criteria of signal-to-noise (S/N) ratio  $>6$  resulted in an LOQ of around 0.15g/L, though this was dependent upon the number of scans utilized. Successful pulse length experiments determined exact  $90^\circ$  pulse lengths for each sample and solvent combination. The longitudinal relaxation time  $T_1$  was also successfully determined, and since it was multiplied with five to ensure complete relaxation to stable state the relaxation delay  $D_1$  was assumed as an insignificant parameter for the determination of chloride. Quantification was based upon the pulse-length based concentration determination (PULCON) using an external standard. The ruggedness can be studied additionally by another experienced operator (since trueness was strongly dependent upon the preparation of the external standard solution). The method displayed good linearity over the mass range normally utilized in such quantifications. The conclusion drawn in the thesis is that the method shows great promise but additional analyzes are still required before implementation at NFC.

**Keywords:** Nuclear magnetic resonance (NMR), explosive precursors, narcotic salts, method validation.

## Table of Content

<b><u>1.</u></b>	<b>Introduction .....</b>	<b>1</b>
<b>1.1.</b>	<b>Aim.....</b>	<b>2</b>
1.1.1.	Specific objectives.....	2
<b><u>2.</u></b>	<b>Background .....</b>	<b>3</b>
<b>2.1.</b>	<b>Explosive precursors .....</b>	<b>3</b>
<b>2.2.</b>	<b>Nuclear magnetic resonance spectroscopy – Properties of method.....</b>	<b>4</b>
<b>2.2.1.</b>	<b>Measurement of spectra.....</b>	<b>5</b>
<b>2.2.2.</b>	<b>Instrumental parameters .....</b>	<b>5</b>
<b>2.3.</b>	<b>Quantification – Pulse-length based concentration determination (PULCON). 7</b>	<b>7</b>
<b>2.4.</b>	<b>Validation and verification .....</b>	<b>8</b>
<b>2.5.</b>	<b>Previous studies .....</b>	<b>8</b>
<b><u>3.</u></b>	<b>Method .....</b>	<b>9</b>
<b>3.1.</b>	<b>Materials .....</b>	<b>9</b>
<b>3.2.</b>	<b>Sample preparation .....</b>	<b>10</b>
<b>3.3.</b>	<b>Procedure .....</b>	<b>10</b>
3.3.1.	Calibration of working window and choice of solvent .....	10
3.3.2.	Determination of 90° pulse – P <sub>1</sub> experiment.....	12
3.3.3.	Linearity and limit of detection and quantification - Number of scans .....	12
3.3.4.	Relaxation time - T <sub>1</sub> determination .....	13
3.3.5.	Repeatability.....	14
3.3.6.	Intermediate precision and ruggedness .....	15
3.3.7.	Trueness .....	16
<b><u>4.</u></b>	<b>Results and discussion .....</b>	<b>17</b>
<b>4.1.</b>	<b>Choice of solvent and working window .....</b>	<b>17</b>
<b>4.2.</b>	<b>Determination of 90° pulse length - P<sub>1</sub> optimization .....</b>	<b>20</b>
<b>4.3.</b>	<b>Relaxation time - T<sub>1</sub> determination.....</b>	<b>22</b>
<b>4.4.</b>	<b>Validation .....</b>	<b>23</b>
4.4.1.	Linearity and limit of detection/quantification - Number of scans .....	23
4.4.2.	Repeatability.....	26
4.4.3.	Intermediate precision and ruggedness .....	26
4.4.4.	Trueness .....	30
<b>4.5.</b>	<b>Summary .....</b>	<b>33</b>
4.5.1.	Final parameters for analysis.....	33
4.5.2.	Future analyzes.....	33
4.5.3.	Conclusion.....	34
<b><u>5.</u></b>	<b>References .....</b>	<b>35</b>
<b><u>6.</u></b>	<b>Appendix .....</b>	<b>36</b>

## **Abbreviations**

ATR-FTIR - Attenuated total reflection Fourier transform infrared

CNS – central nervous system

CT section - Chemistry & Technology Section at National forensic centre

DA section - Drug Analysis Section at National forensic centre

ISO - International Organization for Standardization

LOD – limit of detection

LOQ – limit of quantification

NFC – National forensic centre

NMR - Nuclear magnetic resonance

PULCON – Pulse length-based concentration determination

S/N – Signal-to-Noise

qNMR – Quantitative NMR

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## 1. Introduction

Explosives are both dangerous and toxic and can cause death and harm to humans and wildlife when used inappropriately. Explosives are commonly used in pyrotechnics, including black powder and fireworks. They contaminate the soil and water they encounter and cause health effects on human beings. (1) During the last years in Sweden, the criminal use of explosives is increasing at an alarming rate. Therefore the ability to identify and quantify chemicals that can be used in the making of explosives, i.e. explosive precursors, is essential in the work to counter gang crime. (2) The use of narcotics is also a common problem in Sweden, not only causing problems for the users but also for society. Severe health effects both from short and long-time use, and the high risk of addiction are only some of the negative effects. A major part of both the Swedish drug and explosive market are also governed by the criminal networks. Hence, getting the Swedish population to reduce the use of narcotics and explosives are important in the police's work towards a better society. (3)

National forensic centre (NFC) is a state agency that coordinates the Swedish police authority's forensic operations. The department primarily conducts evidence analyses for criminal investigations, together with research and development within forensic areas. (4) The centre consists of several sections, with separate purposes and orientations. Two of them are the Chemistry & Technology (CT) and the Drug Analysis (DA) Sections. The basis of this project is a collaboration between these two sections. Both are in need of a method for the quantitative determination of chlorine in certain chemical substances. At the DA section, counter ions to narcotics in salt form are today assessed with simple non-quantitative precipitation tests. At the CT section the determination of explosive precursors is a recurring issue. It was therefore desirable to develop a simple and accurate quantification method suitable for both narcotic salts and explosive precursors. NMR was assessed to be suitable for this project, since it has been well established as a routine method at NFC for the quantitative determination of chemical substances containing other NMR spin-active nuclei, such as protons. Recently, Guillou and Schönberger (5) showed that quantitative NMR can also be applied to the quadrupolar atomic nuclei  $^{35}\text{Cl}$ . Using an external standard, the method can determine both identity and concentration.

## 1.1. Aim

The aim of this thesis was to develop and optimize an analysis technique for content determination of substances related to explosives and narcotics to improve and expand the analysis portfolio at NFC. The analysis technique, using  $^{35}\text{Cl}$ -NMR, should be optimized through the establishment of suitable experimental parameters and then validated to ensure a specific and reliable method.

### 1.1.1. Specific objectives

Specific objectives of the study were the optimization of the following parameters:

- Working spectral window
- $90^\circ$  pulse length
- Number of scans
- $T_1$  Relaxation time
- $D_1$  Relaxation delay

Further objectives were to validate the optimized method by assessing the following parameters:

- Intermediate precision
- Linearity
- Limit of detection (LOD)
- Limit of quantification (LOQ)
- Repeatability
- Ruggedness
- Trueness

## **2. Background**

### **2.1. Explosive precursors**

Explosives constitutes complex crime investigations. To discourage the manufacture of home-made explosives, the United Nations have developed certain regulations. The restriction of chemical products handled by private individuals is mentioned in Regulation (EU) 2019/1148 (6). This law enables authorities to control the transfer of explosive precursors, the starting materials for synthesizing explosives. In the legislation, certain compounds over a specific concentration are either totally illegal or require a certain permission for a person to possess. In the forensic investigations of such materials it is therefore important to be able to quantify the concentration of the precursor in order to identify if the starting material used in the production of explosives are of an illegally high concentration or not. (7)

The definition of explosives also includes pyrotechnic articles, which are comprised of a mixture of substances working as oxidizing agent and fuel respectively. These complicate criminal investigations since there are both legal and illegal pyrotechnical products available. Fireworks and emergency flares are examples of legal products on the market, even though they are limited to a certain mass without requiring permission. Many of the precursors to these explosives function as oxidizing agents. (8) In this thesis a method for quantification of such oxidizing agents containing chlorine will be described.

### **2.2. Narcotic salts**

Narcotics are subject to strict legal control in Sweden by several laws, including the Narcotic Penal Code (1968:64) (9). Some narcotics are converted to the hydrochloric salt form in order to increase the dissolution and absorption into stomach and blood stream, i.e. increase effectiveness. It also facilitates the administrative intake and natural stability. (10)

Cocaine hydrochloride is an example of a compound in salt form classed as a narcotic drug. It stimulates the central nervous system (CNS) and inhibits the re-uptake of dopamine, serotonin, and norepinephrine. This stimulates CNS into euphoria, but also an inhibition of nerve impulses which results in reversible loss of sensation. (11)

### 2.3. Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is both a qualitative and quantitative method, commonly used for elucidation of molecular structure. The basis of the method is measuring the interaction of a radio-frequency electromagnetic field with an external magnetic field. The spectrum produced from the NMR technique provides unique opportunities in molecular structure analysis. The electromagnetic radiation, consists of an electric and a magnetic field, oscillating in a sinusoidal motion and traveling at a fixed velocity. This property affects the behavior of photons, which are radiation particles with specific energy proportional to their frequency. If two photons receive the same frequency and amplitude it means that they possess the same amount of energy. In NMR, a photon gets absorbed by a nucleus within a sample and the energy gets transferred to the nucleus, causing it to be in excited state. When the nucleus then re-emits the photon, in order to get back to its stable ground state, the excess energy gets released. (12)

Another important property of the atomic nuclei utilized in NMR is nuclear spin. Atoms with even mass numbers are not spin active and can therefore not be analyzed in NMR. For an atom to be spin active, the number of neutrons and protons has to be odd. Spin active nuclei in the presence of a strong magnetic field can reside in a number of specific nuclear spin states, which is utilized in NMR analysis. (13) A nuclear spin of  $\frac{1}{2}$ , which  $^1\text{H}$  and  $^{13}\text{C}$  have, leads to two orientations of magnetic spins in a strong magnetic field. They give rise to spherical and homogenous magnetic and electric fields, due to a symmetrical distribution of spinning charge. This non-complicated behavior facilitates an easier NMR analysis, and  $\frac{1}{2}$  spin nuclei are therefore most commonly studied. Atomic nuclei with nuclear spin  $>\frac{1}{2}$  are quadrupolar and possess fields which are non-symmetrical, due to a non-spherical distribution of spinning charge.  $^{35}\text{Cl}$  has a nuclear spin of  $\frac{3}{2}$ , which makes it complicated, but not impossible, to analyze by NMR. (12)

NMR instruments become more sensitive the higher the magnetic field strength they possess. This leads to larger separation between the energies of the spin states and thus greater differences between the populations of atoms in the excited state versus the emitted state. (6) The  $^{35}\text{Cl}$  nuclei give signal in the region of field strength 39.204 Tesla in a 400 MHz NMR instrument. (14)



### 2.3.1. Measurement of spectra

The modern NMR method uses a pulse-mode experiment, performed with constant magnetic field and pulses of radio frequency. The radio frequency is generated by a controlled pulse through a transmitter coil from a computer. (12) Free induction decays (FID) show measured resonances in the NMR analysis. A Fourier Transformation (FT) of the FID data results in a spectrum with intensities over different frequencies. The resolution varies depending on the tuning, matching and shimming of the instrument, together with matrix effects. (13)

The isotope  $^{35}\text{Cl}$  usually displays broad signals in NMR due to interactions with other spin-active nuclei (both inter- and intramolecular) especially with increasing molecular size. To integrate an NMR spectrum, it should possess a good signal-to-noise (S/N) ratio. Also, during integration the baseline of the spectrum should be flat. This could be a problem when integrating a weak signal nearby a large solvent peak, or when using a broad spectral window, where a rolling baseline can be an issue. (15)

In NMR, when searching for signals over a broad spectral range, this can lead to misinterpretation of the spectrum. Near-lying signals outside of the spectral window can be folded or aliased into the spectral window, but at an incorrect frequency. Often this is detected by phase changes for the apparent signal relative to the true signal within the correct spectral window. In this case, when the spectral peak lacks correct definition, the signal must be aliased back into correct part of the spectrum by correct centering of the spectral window. (15)

### 2.3.2. Instrumental parameters

During NMR analysis there are several factors that affect the results. Firstly, the solvent is chosen according to analyte and matrix, since the analyte must be soluble and not give rise to any disruptive signal in the spectrum. The solvent should be deuterated, with hydrogen atoms substituted by deuterium atoms since it would decrease the interference of analyte peaks (for proton spectra) and permits a locking of the magnetic field onto the deuterium signal. Incomplete deuteration of the solvent will result in overly large solvent peaks, which may mask the desired signals and reduce dynamic range. Two common solvents are deuterium oxide ( $\text{D}_2\text{O}$ ) and deuterated dimethyl sulfoxide ( $\text{d}_6\text{-DMSO}$ ). (15)

For an integration to be reliable, one important factor is the relaxation time. The time between scans, that is the delay before the next radio frequency pulse is initiated, is important for the excited magnetic moment to relax back to its ground state. If the next pulse would start before total relaxation, this would lead to saturation of the signal and inaccurate integration results.

(14) The parameters governing the radio frequency pulse are the power level ( $B_1$ , measured in Watt) and pulse width ( $t_p$ , measured in microseconds). Together, they affect the result of the pulse centred at operating frequency  $\omega_1$ , where a short hard pulse will excite all the nuclei in a broad spectral range, meanwhile a long weak pulse will selectively excite only a small portion of the spectral window centred around  $\omega_1$ . The power level must be adjusted to the spectral window so that all frequencies within the window are excited and that all nuclei that precess become phase coherent. The central frequency therefore is preferably adjusted according to the analyte of interest. (16)

In NMR, the magnetic moments of spin active nuclei are assumed to adopt random orientations in a spherical shape defined by z-, x- and y-axes. When the sample is introduced to the probe in the centre of the instrument, the magnetic field aligns the bulk magnetization of the spin-active nuclei in the direction of the z-axis (see Figure 1). The magnetic moment has orientations with and against the field in z-axis, creating two population states. Absorption of a radio frequency pulse with the correct frequency results in the excitation from the lower state, with the field, to the higher state, against the field. By applying the right radio-frequency pulse in the coils of the instrument, this causes the vector to align in the x- and y-plane. When the pulse vector goes down exactly to these axes (Figure 1) it is called a  $90^\circ$  pulse, which often maximizes the signal in NMR experiments. After the pulse, the magnetization is not in equilibrium, which leads to the magnetization vector relaxing back to the z-axis. Due to the electric field and the spin-active property of the nuclei the pulse vector is also going to spin around the z-axis until total relaxation when the magnetization is in equilibrium (see Figure 1). The parameters to adjust to achieve a  $90^\circ$  pulse are both the pulse power (in Watt), but also the pulse length (in microseconds), the duration for which the pulse is applied. (13)

In an NMR spectrum, the S/N ratio is affected by the number of scans since the S/N is proportional to the square root of the number of scans. More scans over a smaller spectral range however results in longer analysis time compared to using a broader spectral range. (13)

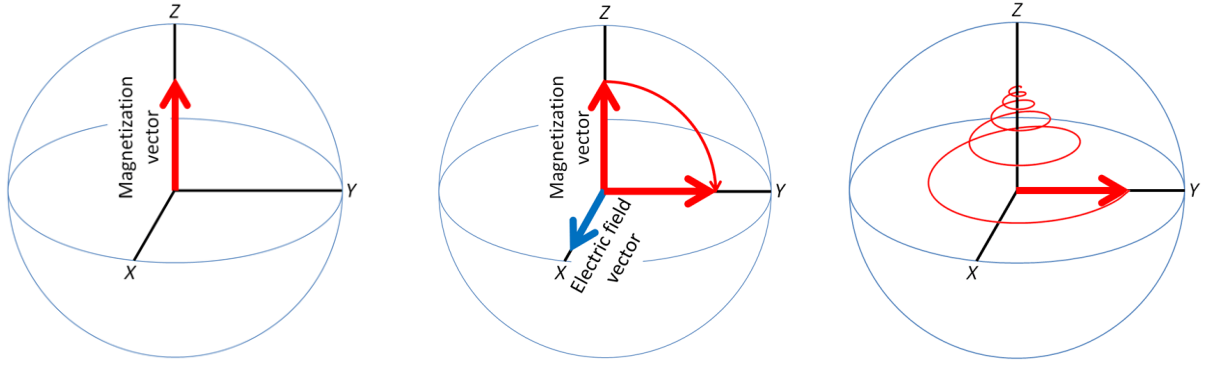


Figure 1 the magnetic field vector of radio frequency pulse in nuclear magnetic resonance (NMR) in equilibrium (left) and the 90° radio frequency pulse from z-axis towards x- and y-axis (middle), and relaxation back to z-axis (right) (13)

## 2.4. Quantification – Pulse-length based concentration determination (PULCON) NMR spectroscopy

The quantification method used in this thesis is PULCON, pulse length-based concentration determination, NMR Spectroscopy. It is especially applicable for samples which are viscous or difficult to weigh. The quantification requires an NMR spectrum from a reference sample with known concentration acting as an external standard, and the sample of interest with unknown concentration. It is very important that the same parameters, such as 90° pulse length, receiver gain and relaxation time are utilized for recording of both spectra. The resulting spectra are integrated and their values inserted into the calculation, shown below.

(17)

$$c_x = f c_R * \frac{I_X A_R T_X \theta_X n_R}{I_R A_X T_R \theta_R n_X}$$

The formula for concentration ( $c$ ) is simplified when having the same parameters (mass number ( $A$ ), sample temperature ( $T$ ), 90° pulse length ( $\theta$ ) and number of scans ( $n$ )) for both analyte ( $X$ ) and reference ( $R$ ) sample. The ratio ( $f$ ) can also be adjusted to 1 to simplify the calculation if the receiver gain and pulse program are the same. (17) The remaining parameters are then the concentration ( $c$ ) and intensity ( $I$ ) for analyte and reference.

## 2.5. Validation and verification

Accuracy is a measure of trueness and precision, indicating the ability of a method to obtain results close to the true value with low deviation. For qualitative reliability the confirmation of the identity of an analyte is required. To ensure selectivity and specificity, it is important that the method is capable of producing correct results even in presence of similar compounds and without interference from the matrix. The precision also includes obtaining the same test results independent of different conditions, such as different analysis days, external standards or operators. (18)

A quantitative and qualitative analysis method, such as NMR, has both a limit of detection (LOD) and quantification (LOQ) for each analyte where it with certainty gives reliable results. A working range, where the linearity has acceptable accuracy, also needs to be established (18)

NFC follows a certain protocol when establishing a validation plan on a new or existing analysis technique. This ensures that the requirements for the method are met and that it is appropriate for the purpose. A validation plan is developed, showing the validation parameters which are deemed relevant to demonstrate that the method is fit-for-purpose. (18) In this thesis, linearity, LOD, LOQ, selectivity, ruggedness and accuracy (including trueness, intermediate precision and repeatability) are included in the validation plan.

## 2.6. Previous studies

### 2.6.1. Analysis of chlorine in drug samples

Analysis of narcotics by  $^{35}\text{Cl}$ -NMR has previously been studied by Guillou and Schönberger (5). The analysis in the report is designed for forensic investigations, especially determination and quantification of narcotics in hydrochloride salt form. They mentioned that  $^{35}\text{Cl}$  has a fast relaxation time (0.04 s in  $\text{D}_2\text{O}$ ) but gives broad signals. The conclusion was that NMR is suitable for this type of analysis, due to fast analysis without additional sample preparation. For quantification, an external calibration was used, resulting in a calibration factor. The quantitative NMR (qNMR) analysis was performed according to International Organization for Standardization (ISO) method 17025(19). The LOD was determined as S/N exceeding 3, and LOQ when exceeding 6. The parameter settings of the analysis is shown in Table 6. (5)

Table 1. Instrumental parameters of NMR used in the study of Guillou and Schönberger. (5)

Parameter	Unit
90° pulse width	17.5 $\mu$ s
Acquisition time	0.39 s
Number of scans	160
Relaxation delay	0.01 s

### 2.6.2. NMR Method development and validation

Monakhova et al (20) published a study on the development and validation of NMR methods. The acquisition parameters identified as being critical for qNMR from the study include 90° pulse length, number of scans, and relaxation time adjusted for the compound and matrix, where the relaxation delay + acquisition time must be at least five times the  $T_1$  relaxation time. The qNMR analysis was made according to ISO 17025, and an analyte peak, not overlapping with other signals, was chosen for quantification. For verification and identification, both single analytes and mixtures were analyzed. (20)

## 3. Method

### 3.1. Materials

The chemicals used in the thesis were as follows: Sodium chloride (NaCl), potassium perchlorate (KClO<sub>4</sub>) and sodium chlorate (NaClO<sub>3</sub>) of >99.5 % purity obtained from Merck (Darmstadt, Germany). NaCl with higher purity (99.999 %) obtained from Sigma-Aldrich (St. Louis, The United States) and cocaine hydrochloride from Apoteksbolaget (Stockholm, Sweden). Dimethyl sulfoxide D6 (CAS 2206-27-1) with 99.8 % purity (stored over drying agent) obtained from VWR Chemicals (Leuven, Belgium). Deuterium oxide, D<sub>2</sub>O, (stored over drying agent) with purity of 99.8 atom% D (CAS 7789-20-0) obtained from Thermo Fischer Scientific (Basel, Switzerland).

The following equipment was used: Analytical balance by Swiss Made (ISO 17025), 5 mL glass tubes with lids, laboratory spatula, Heidolph Reax 2 laboratory tube rotator, 5 mm NMR tubes with lids. Thermo Scientific micropipettes 10-100  $\mu$ L and 100-1000  $\mu$ L with corresponding Finntip filter sterile 1000  $\mu$ L pipette tips (Helsinki, Finland), Duran Wheaton Kimble (Wertheim, Germany) disposable glass Pasteur pipettes 150 mm (ISO 7712), microcentrifuge tubes and an Elmi Sky-Line vortex mixer. The NMR instrument used in the project was a Bruker NMR Ascend 400 MHz, 9.4 Tesla, equipped with a Sample Jet sample carousel and BBO broadband probehead.

### 3.2. Sample preparation

The samples were prepared by gravimetrically weighing the salts into 5 mL tubes on a 5 decimal place laboratory balance. The solvent was then added to the tube with a micropipette and the tube was rotated in a laboratory tube rotator for about 2 minutes, until complete dissolution was achieved. About 700  $\mu$ L of the solution was then transferred into an NMR tube with a glass Pasteur pipette. After labelling the tube it was ready to be introduced to the instrument.

Volume of solvent and which samples were prepared are explained under sample preparation for each experiment.

### 3.3. Procedure

The purpose of these experiments was to find the most appropriate parameter settings suitable for all the analytes of interest in this thesis, listed in Table 2. The instrument parameters of choice were operating frequency (O1 spectral width (SW), choice of solvent, 90° pulse length ( $P_1$ ), relaxation time ( $T_1$ ), number of scans (NS) and relaxation delay ( $D_1$ ). For additional validation of the method, the intermediate precision, trueness, linearity, LOD, LOQ and ruggedness should also be determined.

*Table 2 Analytes of interest related to explosive precursors and narcotic salts to be analyzed in  $^{35}\text{Cl}$  NMR and their chemical formula.*

Compound.	Chemical formula
Potassium chlorate	$\text{KClO}_3$
Potassium perchlorate	$\text{KClO}_4$
Sodium chlorate	$\text{NaClO}_3$
Sodium perchlorate	$\text{NaClO}_4$
Cocaine hydrochloride	$\text{C}_{17}\text{H}_{22}\text{ClNO}_4$

#### 3.3.1. Calibration of working window and choice of solvent

The first part of the method that was investigated was the establishment of suitable working spectral windows, where the signals from different Cl environments listed in Table 2 were located. The parameters to define are the central operating frequency (O1) and the spectral width (SW). The chemical shifts between different compounds and their relative responses were investigated to indicate the suitability of external standards for quantification.

The samples to be analyzed were NaCl and KClO<sub>4</sub> in both D<sub>2</sub>O and d<sub>6</sub>-DMSO and two mixed solutions of NaCl + KClO<sub>4</sub> and NaCl + NaClO<sub>3</sub>, respectively both in D<sub>2</sub>O as solvent. Also, a sample of cocaine · HCl in d<sub>6</sub>-DMSO was included in the study.

#### 3.3.1.1. Data evaluation

Based on tabulated spectral information, the signal of <sup>35</sup>Cl should appear at 39.204 Tesla when recorded on a 400 MHz NMR instrument. Analysis was then enabled by proceeding using this as the central frequency, with standard settings for the acquisition parameters retrieved from the Bruker software. When interpreting the results of this spectrum, the working window and spectral width should be adjusted to enable an optimal integration of the peak(s) of interest.

The most suitable solvent, either D<sub>2</sub>O or d<sub>6</sub>-DMSO, for each compound should be selected based on several factors. Firstly, the sample of interest should be soluble in the solvent to enable the NMR analysis and secondly depending on if a specific solvent is used in other routine NMR analyzes upon the analyte. The spectral peak should be distinguishable and integrable, with as high S/N ratio as possible.

*Table 3 Standard acquisition parameter settings for <sup>35</sup>Cl analysis obtained from computer software of the NMR instrument.*

Parameter	Unit
TD - Time domain	32768
P <sub>1</sub> – Pulse length	21 μs
D <sub>1</sub> – delay between scans	0.1 s
SW - Spectral width	1000 ppm
NS – number of scans	128 scans

#### 3.3.1.2. Sample preparation

Solutions of NaCl (Merck), NaClO<sub>3</sub> and KClO<sub>4</sub> (0.1 mol/L) were prepared in both D<sub>2</sub>O and d<sub>6</sub>-DMSO as solvents, and two mixed 0.1 mol/L solutions of NaCl + KClO<sub>4</sub> and NaCl + NaClO<sub>3</sub>, respectively in D<sub>2</sub>O as solvent. A solution of ~7 mg cocaine hydrochloride in 700 μL d<sub>6</sub>-DMSO was also prepared.

### 3.3.2. Determination of 90° pulse – $P_1$ experiment

To determine the optimal 90° pulse length which places the bulk magnetization into the x- and y-plane (see Figure 1) and thereby obtain maximal signal strength a series of different values of  $P_1$  with constant pulse power was investigated. According to recommended settings of the computer software, the starting value of the pulse length should be set at 5  $\mu$ s with an increase of 5  $\mu$ s for each analysis (ie. 5, 10, 15, ... $\mu$ s). The compilation of the measurements at each pulse length result in a near sinusoidal pattern of intensity vs time (see Figure 7). The first maximum corresponds to the 90° pulse length, the first zero point to the 180° pulse length, the largest negative peak to the 270° pulse length, and finally the second zero point to the pulse length giving a 360° rotation of the bulk magnetization. Whilst the Bruker software can determine the pulse length giving the highest peak automatically a more accurate measurement of the 90° pulse length can be found by establishing the value of  $P_1$  for a 360° pulse.  $P_1(360^\circ)$  then would result in 0 signal (since the magnetization is flipped all the way back to its starting position), which is easier to interpret than judging the point of the highest intensity. The estimated 90° pulse length from this experiment was therefore multiplied by 4 and  $P_1$  then adjusted around that value until the pulse length resulting in 0 signal for the analyte was located. Dividing this 360° pulse length value by 4 gave a more exact 90° pulse length.

The same procedure for triplicates of 0.1 mol/L NaCl and KClO<sub>4</sub> in D<sub>2</sub>O and d<sub>6</sub>-DMSO, respectively, was performed to obtain an average value of  $P_1$  in each solvent.

#### 3.3.2.1. *Sample preparation*

Three solutions of 0.1 mol/L NaCl (Merck) and three solutions of 0.1 mol/L KClO<sub>4</sub> were prepared, in both D<sub>2</sub>O and d<sub>6</sub>-DMSO as solvent.

### 3.3.3. Linearity and limit of detection and quantification - Number of scans

To investigate the linearity as well as limit of detection (LOD) and limit of quantification (LOQ), a sequential series of concentrations in mol/L (1, 0.1, 0.01, 0.001, 0.0001) of NaCl and KClO<sub>4</sub> in D<sub>2</sub>O and d<sub>6</sub>-DMSO were analyzed. Each series of experiments was performed with 512, 1024, 2048, and 4096 scans. The S/N ratio was automatically calculated using



Brukers *sinocal* function. These values were noted and further used to make a calibration curve, where the LOD ( $S/N = 3$ ) and LOQ ( $S/N = 6$ ) could be determined.

#### 3.3.3.1. Sample preparation

Solutions of 1 mol/L NaCl (Sigma-Aldrich) and  $KClO_4$  in 2 mL of either  $D_2O$  or  $d_6$ -DMSO were prepared. These stock solutions were then diluted using the following procedure: From the 1 mol/L solution, 100  $\mu$ L was transferred into a 1.5 mL microcentrifuge tube, together with 900  $\mu$ L solvent. After mixing in a vortex mixer for 5-10 seconds, 100  $\mu$ L of the solution was transferred to a new microcentrifuge tube with another 900  $\mu$ L solvent. The 1:10 dilution series was then continued until having a series of 1 mol/L, 0.1 mol/L, 0.01 mol/L, 0.001 mol/L, and 0.0001 mol/L was obtained. Same procedure was utilized for NaCl and  $KClO_4$  in both solvents.

For NaCl in  $d_6$ -DMSO, and  $KClO_4$  in  $D_2O$  and  $d_6$ -DMSO, the salts did not dissolve completely at the 1 mol/L level. The starting stock was therefore 0.1 mol/L for these series.

#### 3.3.4. Relaxation time - $T_1$ determination

For the reason of simplicity, it would be desirable if the same relaxation time ( $T_1$ ), i.e. the delay between scans to ensure total relaxation, could be used for the analysis of all compounds of interest. Thus, the relaxation time would need to be adjusted to that for the compound with longest longitudinal relaxation time ( $T_1$ ), to ensure total relaxation of all nuclei. Two experiments of NaCl in  $D_2O$  were first made with different series of delay times, shown in Table 4. Firstly, a series of experiments with a wider range (Experiment 1) was performed to better define the most appropriate range for the second experiment (Experiment 2) to obtain a more exact  $T_1$  value. The  $T_1$  for NaCl in  $d_6$ -DMSO and  $KClO_4$  in  $D_2O$  were also measured to indicate which solution gave the longest  $T_1$ . Parameter settings in Experiment 3 with 10 delay times were used for these analyzes. For  $KClO_4$  in  $d_6$ -DMSO, the expected  $T_1$  was significantly longer than for the other combinations according to previous studies and test analyzes performed, and the delay times shown under Experiment 4 were used.

The final delay time needed for quantitative measurements is calculated using the following formula:  $5 * T_1$

Table 4 Delay times used in the pulse program for the  $T_1$  relaxation time determinations.

Measuring point	Experiment 1 (s)	Experiment 2 (s)	Experiment 3 (s)	Experiment 4 (s)
1	0.0010	0.00010	0.00010	0.00010
2	0.050	0.00050	0.00050	0.00050
3	0.10	0.0010	0.0010	0.0050
4	0.25	0.0050	0.0050	0.010
5	0.50	0.010	0.010	0.020
6	0.80	0.10	0.050	0.050
7	-	-	0.10	0.10
8	-	-	0.25	0.25
9	-	-	0.50	0.80
10	-	-	1.0	1.0

#### 3.3.4.1. Sample preparation

Solutions of NaCl (Sigma-Aldrich) and  $\text{KClO}_4$  of 0.1 mol/L prepared in both  $\text{D}_2\text{O}$  and  $\text{d}_6$ -DMSO as solvents.

#### 3.3.5. Repeatability

To measure the intermediate precision and repeatability of the method, a series of analyses (see Table 5) were run during one day to indicate any variations due to same-day instrumental fluctuations and at the same time investigate the effect of variation introduced when using different external standards. Pulse length-based concentration determination (PULCON) quantification was used to quantify the content. Analysis series (see Table 5) were run both for NaCl in  $\text{D}_2\text{O}$ , NaCl in  $\text{d}_6$ -DMSO and  $\text{KClO}_4$  in  $\text{D}_2\text{O}$ . Standard deviation (STDEV) and relative standard deviation (RSD) for each day and reference were calculated in Microsoft Excel (22).

Table 5 Analysis series sequence order for repeatability test of each solution (NaCl in  $\text{D}_2\text{O}$ , NaCl in  $\text{d}_6$ -DMSO and  $\text{KClO}_4$  in  $\text{D}_2\text{O}$ ) and the number of measurements of each sample in the series.

Sample	# Measurements
Reference sample 1	1
Reference sample 2	1
Reference sample 3	1
0.1 mol/L test solution	10

#### 3.3.5.1. Sample preparation

The three reference solutions of NaCl (Sigma-Aldrich) and KClO<sub>4</sub> in each solvent were prepared as ~0.1 mol/L solutions with accurately weighed masses. Test solutions of 0.1 mol/L NaCl and KClO<sub>4</sub> respectively, with known masses and exact concentrations were also prepared.

#### 3.3.6. Intermediate precision and ruggedness

To measure the intermediate precision and ruggedness, the same samples were analyzed over eight sequential days to indicate any day-to-day variations within the results. The purpose was to identify the precision, but also to indicate for how long time the external standard is acceptable to use for analysis – if it is required to run the external standard within the same sequence as the sample or if this is not a factor affecting the result. Analysis series (see Table 6) were run both for NaCl in D<sub>2</sub>O, NaCl in d<sub>6</sub>-DMSO and KClO<sub>4</sub> in D<sub>2</sub>O. PULCON quantification was used to calculate theoretical mass of each analyte with each of the three references as external standards. STDEV and RSD over all days for each reference solution as external standard were calculated in Microsoft Excel (22).

The intermediate precision was defined by studying the RSD of the 10 measurements between days, to indicate the spread of measurements and the stability of the result over time. The ruggedness was defined by studying the RSD of the average measured masses over the eight days and between the three reference samples, to indicate how robust the method is for slight errors introduced during the preparation of external standards.

*Table 6 Analysis series order for intermediate precision test of each solution (NaCl in D<sub>2</sub>O, NaCl in d<sub>6</sub>-DMSO and KClO<sub>4</sub> in D<sub>2</sub>O) and the number of measurements of each sample in the series.*

Sample	# Measurements
Reference sample 1	1
Reference sample 2	1
Reference sample 3	1
0.1 mol/L test solution	10

#### 3.3.6.1. Sample preparation

The three reference solutions of each compound (NaCl and KClO<sub>4</sub>) in each solvent were prepared as ~0.1 mol/L solutions, with accurately weighed masses. Test solutions of 0.1 mol/L NaCl and KClO<sub>4</sub> respectively, with known masses and exact concentrations were also used in these experiments.

### 3.3.7. Trueness

To measure the trueness of the method, test samples of  $\text{KClO}_4$ ,  $\text{NaCl}$  and cocaine  $\cdot$   $\text{HCl}$  with different masses (approx. 1 mg, 4 mg, 8 mg, 12 mg, 20 mg) were analyzed. With three external references of similar compounds ( $\text{KClO}_4$  for  $\text{KClO}_4$ ,  $\text{NaCl}$  for  $\text{NaCl}$  and cocaine  $\cdot$   $\text{HCl}$  for cocaine  $\text{HCl}$ ) used as external standards, their in-weighed masses could be compared with the determined masses from the  $^{35}\text{Cl}$  qNMR experiments. The analysis series is shown in Table 7. PULCON quantification was used for calculation in Excel (22), where the average measured mass of analyte in each solution was subtracted from the actual weighed masses and calculated as percentage from the actual in-weighed masses, to indicate the deviation from the true value.

*Table 7 Analysis series sequence for each  $\text{NaCl}$  in  $\text{D}_2\text{O}$ ,  $\text{KClO}_4$  in  $\text{D}_2\text{O}$  and cocaine  $\text{HCl}$  in  $d_6$ -DMSO and number of measurements for each sample for trueness test. Exact weighed masses of test samples shown in Appendix.*

Sample	# Measurements
External standard 1	3
External standard 2	3
External standard 3	3
1 mg Test sample	3
4 mg Test sample	3
8 mg Test sample	3
12 mg Test sample	3
20 mg Test sample	3

#### 3.3.7.1. Sample preparation

The three reference solutions of each compound ( $\text{NaCl}$  and  $\text{KClO}_4$ ) in each solvent were prepared as  $\sim 0.1$  mol/L solutions, with accurately weighed masses. Test solutions of  $\text{NaCl}$ , cocaine  $\cdot$   $\text{HCl}$  and  $\text{KClO}_4$  respectively were prepared with approximate masses of 1 mg, 4 mg, 8 mg, 12 mg and 20 mg in 700  $\mu\text{L}$  solvent.

The 20 mg  $\text{KClO}_4$  sample did not dissolve completely in  $\text{D}_2\text{O}$ , and some particles were left in the 5 mL tube after transferring the solution into the NMR tube. This should be taken into consideration when interpreting the quantitative results of this sample.

## 4. Results and discussion

### 4.1. Choice of solvent and working window

The first step of the process was to determine the optimum working window in which the  $^{35}\text{Cl}$  signals appear. Theoretically, the  $^{35}\text{Cl}$  nuclei should resonate around 39.204 Tesla for a 400 MHz NMR instrument. In the first tests of NaCl and  $\text{KClO}_4$ , the operating frequency ( $\delta$ ) was set to -39.99 ppm with a spectral width of 1000 ppm. NaCl gave a distinguishable peak at ~0 ppm. A 0.1 mol/L solution of NaCl in  $\text{D}_2\text{O}$  is the accepted chemical shift reference standard in  $^{35}\text{Cl}$  NMR. When decreasing the spectral width to 500 ppm the S/N increased (see Figure 2), and this spectral width was therefore chosen since an integrable peak was obtained, with -39.99 ppm as central frequency. For  $\text{KClO}_4$ , no peak was shown in the spectral window when having  $\text{O}_1 = -39.99$  ppm and spectral width 1000 ppm. By increasing the spectral width to 2000 ppm  $\text{KClO}_4$  gave a peak at ~1000 ppm. The central frequency ( $\delta$ ) was therefore adjusted to 1000 ppm, with a spectral width of 1000 ppm, see Figure 3. These settings resulted in a distinguishable peak of  $\text{KClO}_4$ . For the test solution of cocaine hydrochloride, the peak also appeared around 0 ppm with a spectral width of 1000 ppm, see Figure 6.

Both  $\text{D}_2\text{O}$  and  $\text{d}_6\text{-DMSO}$  were used as solvent for the compounds considering that the investigated salts are soluble in both. After baseline correction of the spectrum, all compounds NaCl and  $\text{KClO}_4$  received high S/N ratios for  $\text{D}_2\text{O}$ .  $\text{d}_6\text{-DMSO}$  resulted in lower S/N ratios. Even after increasing number of scans for both samples, the ratio increased but still was lower than for  $\text{D}_2\text{O}$ .

When interpreting a mixed solution, containing both NaCl and  $\text{NaClO}_3$  in  $\text{D}_2\text{O}$ , only one signal appeared in the spectra (see Figure 4), whereas two peaks were expected to be shown.  $\text{NaClO}_3$  has lower symmetry than both NaCl and  $\text{KClO}_4$ . The non-spherical electron-density surrounding chlorate results in strong interactions of its electric dipole with its surroundings, leading to a signal often too broad to detect. This is the reason why covalently bound Cl nuclei are also almost impossible to detect except in small spherical molecules like  $\text{CCl}_4$ . The single signal at 0 ppm for the  $\text{NaClO}_3$  solutions is suspected to arise from traces of NaCl in the stock material. In in-house methods the DA section often use  $\text{d}_6\text{-DMSO}$  as solvent for NMR analysis. Therefore, this solvent was assumed preferable in the aspect that it is commonly used as solvent for the analysis of narcotics in salt form. In the CT-section, the preferable solvent is  $\text{D}_2\text{O}$ , since focus there is upon inorganic salts within this application.

The mixed solution of NaCl and  $\text{KClO}_4$  resulted in spectral peaks of distinct difference in intensities, see Figure 5. The exact reason for this previously seen phenomenon (16) needs to be investigated. Shorter pulse lengths can give more even excitation over a broad spectral range. This would need to be tested in future studies and will also require an optimization of the number of scans to achieve an equivalent S/N. To be able to perform quantitative measurements on both compounds, it was concluded to use external standards which matched the analytes. That is to say that a chloride external standard must be used for a chloride determination and a perchlorate external standard for a perchlorate quantitation.

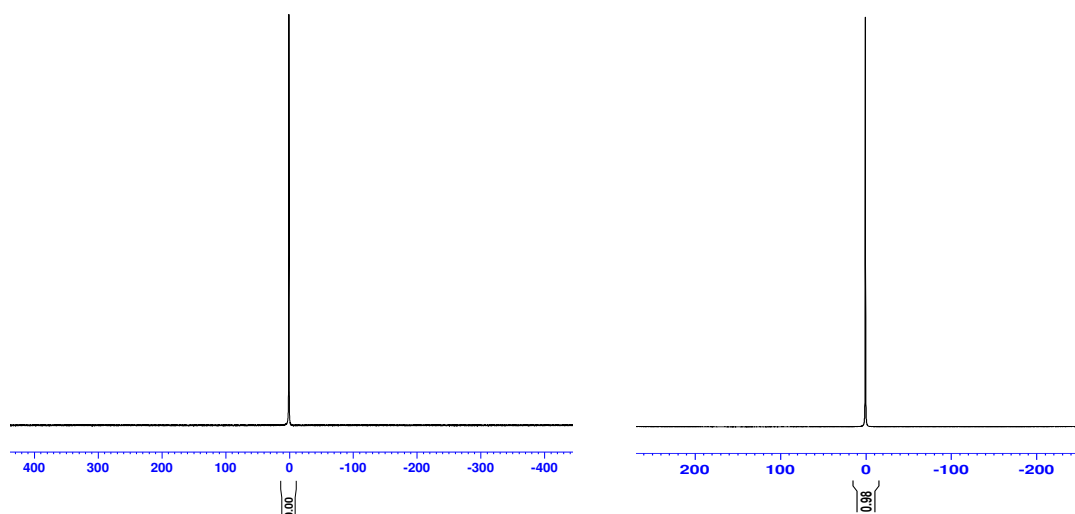


Figure 2 Spectra of NaCl in  $\text{D}_2\text{O}$  with sweep width 1000 ppm (left) and 500 ppm (right), remaining parameters according to standard settings.

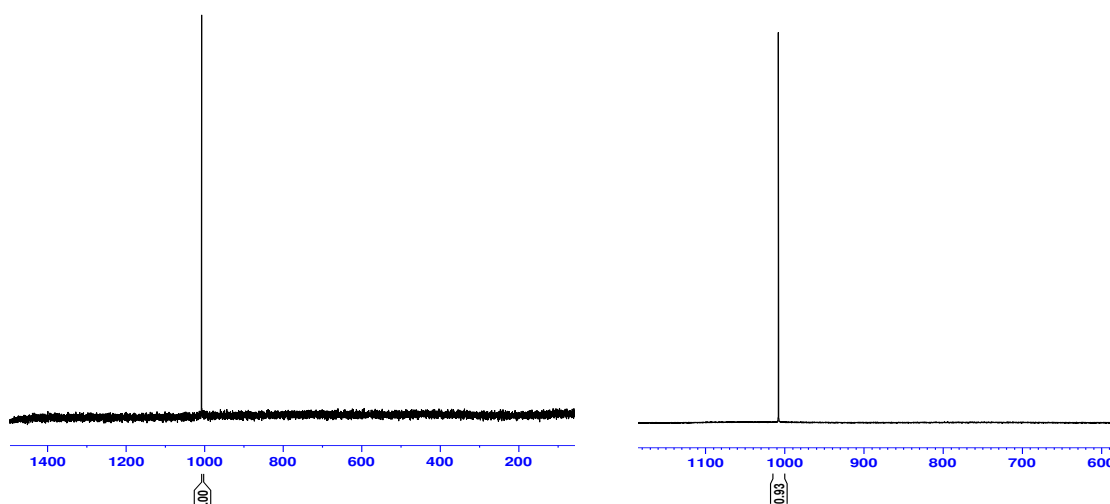


Figure 3 Spectra of  $\text{KClO}_4$  in  $\text{D}_2\text{O}$  with sweep width 2000 ppm (left) and 1000 ppm (right), and standard settings for remaining acquisition parameters.

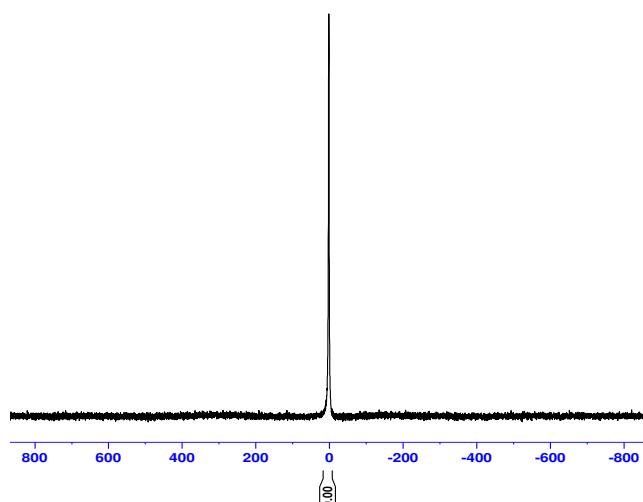


Figure 4 Spectra of the 0.1 mol/L mixed solution of NaCl and KClO<sub>4</sub> in D<sub>2</sub>O, with spectral width 1000 ppm and standard settings for remaining acquisition parameters.

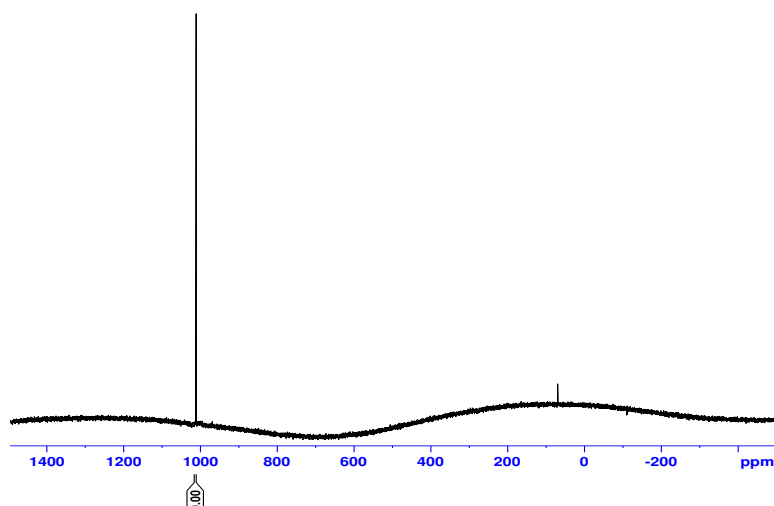


Figure 5 NMR spectra of 0.1 mol/L mixed solution of NaCl and KClO<sub>4</sub>, with spectral width 1000 ppm and standard settings for remaining acquisition parameters.

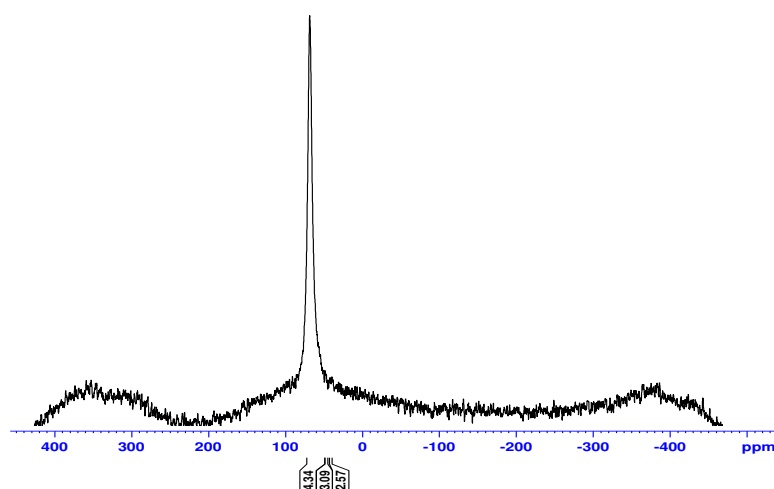


Figure 6 NMR spectra of 0.1 mol/L solution of cocaine · HCl in d<sub>6</sub>-DMSO, with spectral width 1000 ppm and number of scans 8192, with standard settings for remaining acquisition parameters.

## 4.2. Determination of 90° pulse length - P<sub>1</sub> optimization

Figure 7 shows the peak intensity of a series of experiments where the pulse length was increased in 5 μs increments for a 0.1 mol/L NaCl in D<sub>2</sub>O solution, resulting in a sinusoidal pattern. The computer software interpreted the highest measured peak (closest to 90° pulse) at 20 μs, which indicated that the 360° pulse would be around 80 μs (20 μs \* 4). Analyzes was made stepwise from 78.0 μs up to 85.0 μs to identify the pulse length which received 0 signal for the analyte. For the third replicate of NaCl in D<sub>2</sub>O, the exact 360° pulse receiving 0 signal was at 83.0 μs, see Figure 8. For the second and third replicate there was no need to test all the delay values. Since the P<sub>1</sub> of the first solution was identified this could indicate in which range to look further in the other solutions. The average value for the 360° pulse of the triplicates was then divided by 4 to yield the 90° pulse length.

The same procedure was made for NaCl in d<sub>6</sub>-DMSO, and for KClO<sub>4</sub> in D<sub>2</sub>O and d<sub>6</sub>-DMSO. Results of the final P<sub>1</sub> 90° pulse length are shown in Table 8. These values were further used in analyzes to obtain more accurate spectra.

When comparing with the previous study of Guillou and Schönberger (5) there was no significant difference between the measured P<sub>1</sub>. In their study it was measured to 17.5 μs for NaCl in D<sub>2</sub>O, and in this study it was measured to 21.0 μs. A probable reason for this difference is the field strength of the instruments, where Guillou and Schönberger used a 500 MHz instrument and in this study a 400 MHz instrument was used. The field strength changes the energy levels of the transitions, thereby altering the pulse length and other parameters. Therefore, the difference between the studies does not reflect any uncertainty of the P<sub>1</sub> results of this method. It was also concluded by Guillou and Schönberger that different solutions displayed only very slight variations in P<sub>1</sub> of the 90° pulse, making pulse optimization for every sample unnecessary. Hence, the variations between each sample and solvent have explainable reasons, especially since power settings were not given in that study.



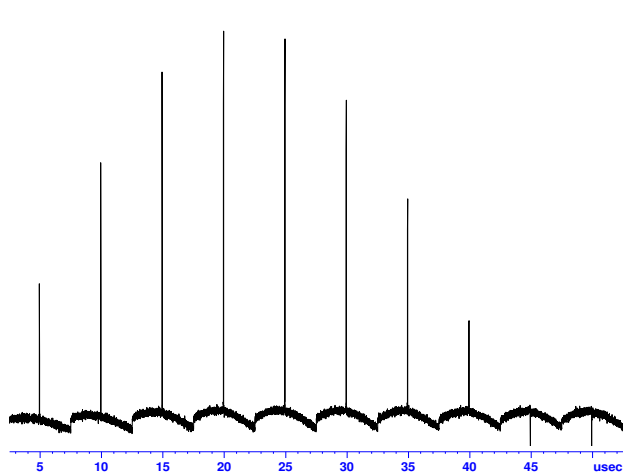


Figure 7 Composite diagram of  $P_1$   $90^\circ$  pulse length determination of 0.1 mol/L NaCl in  $D_2O$ , over pulse lengths in  $\mu s$  vs intensities.

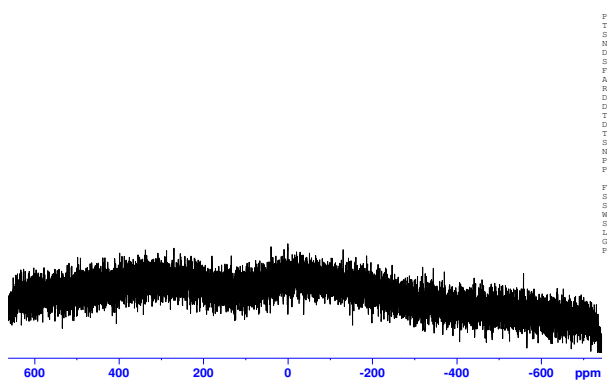


Figure 8 Spectrum of replicate 3 of NaCl in  $D_2O$  with  $P_1$  83.0  $\mu s$  yielding zero analyte signal, ie the exact  $360^\circ$  pulse length.

Table 8 Measured  $P_1$  for  $360^\circ$  pulse lengths in  $\mu s$  receiving 0 signal for NaCl and  $KClO_4$  in  $D_2O$  and  $d_6$ -DMSO, and average value of replicates divided by 4 giving the exact  $90^\circ$  pulse length.

NaCl					
Solvent	$P_1$ replicate 1 ( $360^\circ$ pulse)	$P_1$ replicate 2 ( $360^\circ$ pulse)	$P_1$ replicate 3 ( $360^\circ$ pulse)	Average $P_1$ ( $360^\circ$ pulse)	$P_1$ $90^\circ$ pulse (Avg $P_1$ ( $360^\circ$ )/4)
$D_2O$	84.800	84.700	83.000	84.167	21.042
$d_6$ -DMSO	79.800	79.500	79.700	79.667	19.917
$KClO_4$					
Solvent	$P_1$ replicate 1	$P_1$ replicate 2	$P_1$ replicate 3	Average $P_1$	Exact $90^\circ$ pulse (Average $P_1$ /4)
$D_2O$	87.100	86.900	87.300	87.100	21.775
$d_6$ -DMSO	86.500	86.800	86.300	86.533	21.633

### 4.3. Relaxation time - $T_1$ determination

Experiment 1 of NaCl in  $D_2O$  using a wide range of delay times (see Table 4) resulted in a plot from which the final longitudinal relaxation time  $T_1$  (see Figure 9) could be determined. A script within the Bruker software was used to calculate and define the actual  $T_1$  value and the result was 30.457 ms, where  $5 * T_1$  resulted in 152.3 ms. This value was further used when adjusting the settings of different  $T_1$  values of the narrower measurement range in Experiment 2. The second experiment (Figure 9) resulted in a  $T_1$  of 30.55 ms, and therefore the final  $T_1$  was calculated to 152.8 ms ( $5 * 30.55$  ms).

For the remaining samples, only one  $T_1$  experiment was made for each sample. When studying the final recycle delay values ( $T_1 * 5$ ) in Table 9 one can see that  $KClO_4$  requires significantly longer recycle delays than NaCl, in both solvents, especially  $KClO_4$  in  $d_6$ -DMSO which requires a recycle delay of 2660 ms. It was therefore concluded to assume  $D_2O$  is more suitable for  $KClO_4$  analysis in the future, to shorten analysis times. The final recycle delays used in further analysis were the measured  $T_1 * 5$  for each compound, shown in Table 9.

The time delay between scans (recycle delay) used must be five times the actual  $T_1$  to ensure total relaxation. Since relaxation occurs both during the acquisition time of the experiment and the time delay between experiments ( $D_1$ ) it is the total of these two which must be greater than  $5 * T_1$ . The relaxation delay  $D_1$ , the delay between scans, must therefore be proportioned so that this is achieved. The  $D_1$  of all chloride analytes was therefore set to 0.01 seconds, since the acquisition time was longer than the calculated  $5 * T_1$ . However, for perchlorate analytes, a  $D_1$  of 1.0 seconds was necessary, since the acquisition time was only 0.42 seconds.

The previous study of Guillou and Schönberger (5) which studied NaCl in  $D_2O$  measured the relaxation time as 0.04 s, which is similar to the results in this study (0.031 s). That the result of this sample is similar to the previous study indicates reliability. They also used the same  $D_1$  (0.01 s) as was chosen in this study. The previous study of Woods (21) also indicated similarities, since they measured the  $T_1$  of  $KClO_4$  in  $D_2O$  to be 168 ms, compared to this study's 208.5 ms. One can assume the results of the  $T_1$  experiment in this study to be reliable also for the remaining samples.

Table 9 Measured longitudinal relaxation time  $T_1$  between NMR scans and  $T_1 * 5$  for 0.1 mol/L NaCl and  $\text{KClO}_4$  in each solvent.

Sample	$T_1$ (ms)	$T_1 * 5$ (ms)
NaCl in $\text{D}_2\text{O}$	30.55	152.8
NaCl in $\text{d}_6$ -DMSO	6.309	31.55
$\text{KClO}_4$ in $\text{D}_2\text{O}$	208.5	1043
$\text{KClO}_4$ in $\text{d}_6$ -DMSO	532.0	2660

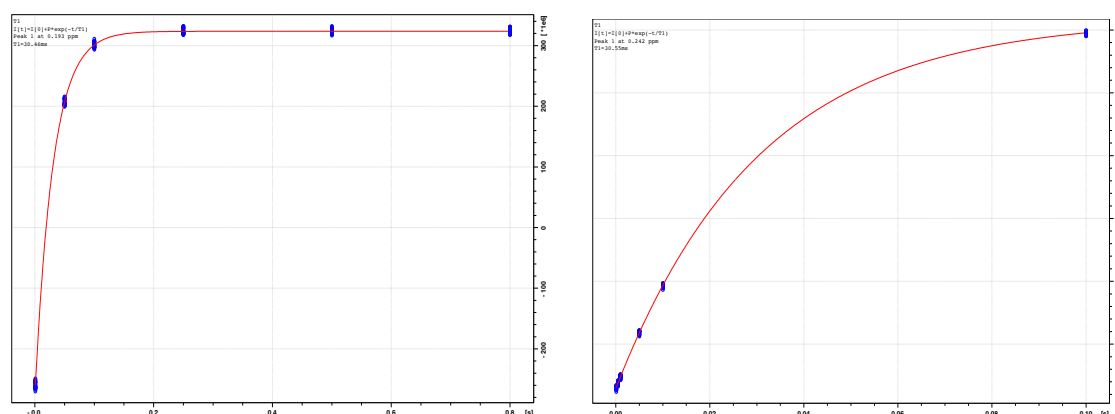


Figure 9 Plots used for the determination of relaxation time  $T_1$  experiment 1 (left) and experiment 2 (right) of 0.1 mol/L NaCl in  $\text{D}_2\text{O}$

## 4.4. Validation

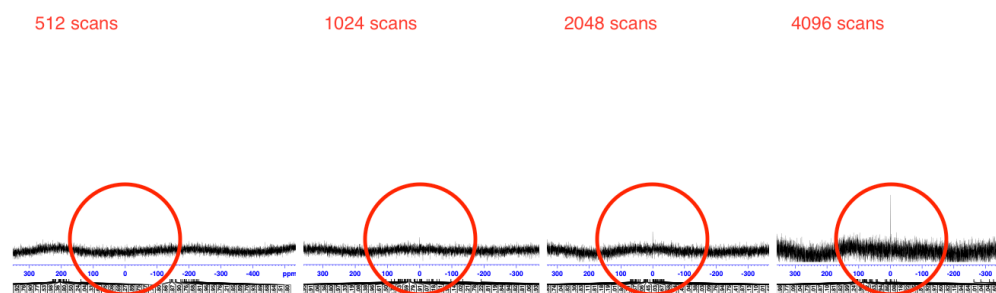
### 4.4.1. Linearity and limit of detection/quantification - Number of scans

There is a distinct trend of increasing S/N ratio with increased number of scans (see Figure 10), but it also results in longer analysis times. None of the scan numbers tested for the 0.0001 mol/L solution resulted in a distinguishable peak in the spectrum. By making a calibration curve for respective dilution series with different number of scans, the limit of detection (LOD) and quantification (LOQ) of the method could be estimated at each setting of the number of scans using the values  $S/N = 3$  for LOD and  $S/N = 6$  for LOQ. Results are shown in Table 10.

To decide which number of scans to use in further analysis the concentrations of the samples of interest was considered. The LOQ of a method when analyzing explosive precursors are often not a factor to consider since the concentrations are most likely to be of the order of g/L in bulk materials. There is no need to increase the number of scans unnecessarily above this limit since the analysis time preferably should be as short as possible. An LOQ of at least  $\sim 0.150$  g/L is way below the levels expected for such applications. The decision as to which number of scans to use for each sample is shown as marked in green in Table 10.

1024 scans were chosen for NaCl in D<sub>2</sub>O, KClO<sub>4</sub> in D<sub>2</sub>O and KClO<sub>4</sub> in d<sub>6</sub>-DMSO. For NaCl in d<sub>6</sub>-DMSO none of the number of scans resulted in a LOQ below 0.150 g/L. Therefore 4096 scans were used to lower the LOQ. For further analysis it is important to keep in mind that the LOQ of this analysis is remarkably higher than the other samples, 0.651 g/L) although considerably below the normal working range of 4 mg/mL – 15 mg/mL for NMR samples.

In the previous study of Guillou and Schönberger (5) the number of scans for NaCl in D<sub>2</sub>O was 160, when performed on a higher field instrument. The increase in number of scans compared to previous studies is not something that affects the reliability, especially when instrument time is not a determining factor at NFC.



*Figure 10 NMR spectra of 0.001 mol/L NaCl in D<sub>2</sub>O with 512, 1024, 2048, and 4096 scans, with spectral width 1000 ppm. Encirclement defining the window of the expected peak position.*

Table 10 Signal-to-noise (S/N) ratios calculated using Brukers sinocal function for different molarities of NaCl and KClO<sub>4</sub> in D<sub>2</sub>O respectively d<sub>6</sub>-DMSO. (1) means no distinguishable peak shown in spectra. Also calculated limit of detection (LOD) and limit of quantification (LOQ), where green-marking showing which number of scans will be used for further analysis

<b>NaCl in D<sub>2</sub>O</b>				
<b>Molar conc. (mol/L)</b>	<b>512 scans</b>	<b>1024 scans</b>	<b>2048 scans</b>	<b>4096 scans</b>
1	1830	2681	3734	5611
0.1	196	252	366	714
0.01	20	26	38	51
0.001	(1)	2.6	3.3	5.8
0.0001	(1)	(1)	(1)	(1)
LOD (S/N 3)	1.7 mmol/L 99.3 mg/L	1.4 mmol/L 81.8 mg/L	0.97 mmol/L 56.7 mg/L	1.8 mmol/L 80.5 mg/L
LOQ (S/N 6)	3.2 mmol/L 187 mg/L	2.60 mmol/L 152 mg/L	1.80 mmol/L 105 mg/L	1.79 mmol/L 105 mg/L
<b>NaCl in d<sub>6</sub>-DMSO</b>				
<b>Molar conc. (mol/L)</b>		<b>1024 scans</b>	<b>2048 scans</b>	<b>4096 scans</b>
0.1		17.1	23.7	34.1
0.01		4.7	5.9	9.2
0.001		(1)	(1)	(1)
0,0001		(1)	(1)	(1)
LOD (S/N 3)		6.9 mmol/L 405 mg/L	3.7 mmol/L 215 mg/L	1.8 mmol/L 104 mg/L
LOQ (S/N 6)		27 mmol/L 1551 mg/L	18 mmol/L 1027 mg/L	11 mmol/L 651 mg/L
<b>KClO<sub>4</sub> in D<sub>2</sub>O</b>				
<b>Molar conc. (mol/L)</b>		<b>1024 scans</b>	<b>2048 scans</b>	<b>4096 scans</b>
0.1		118	143	195
0.01		50	65	92
0.001		6.0	8.7	12
0.0001		(1)	(1)	(1)
LOD (S/N 3)		0.41 mmol/L 56.8 mg/L	0.31 mmol/L 43.0 mg/L	0.22 mmol/L 30.5 mg/L
LOQ (S/N 6)		1.02 mmol/L 141 mg/L	0.78 mmol/L 108 mg/L	0.55 mmol/L 76.2 mg/L
<b>KClO<sub>4</sub> in d<sub>6</sub>-DMSO</b>				
<b>Molar conc. (mol/L)</b>		<b>1024 scans</b>	<b>2048 scans</b>	<b>4096 scans</b>
0.1		207	332	483
0.01		36	54	75
0.001		7.2	9.3	11
0.0001		(1)	(1)	(1)
LOD (S/N 3)		0.26 mmol/L 35.6 mg/L	0.15 mmol/L 20.7 mg/L	0.15 mmol/L 20.2 mg/L
LOQ (S/N 6)		1.13 mmol/L 156 mg/L	0.73 mmol/L 101 mg/L	0.55 mmol/L 76.7 mg/L

#### 4.4.2. Repeatability

The repeatability was measured for eight sequential days for NaCl in D<sub>2</sub>O, and four sequential days for NaCl in d<sub>6</sub>-DMSO and KClO<sub>4</sub> in D<sub>2</sub>O. By PULCON quantification, the content of the 0.1 mol/L test solution was quantified from external standards of known concentrations.

Results of the average estimated mass for each day and the external standard used are shown in Figure 11 for NaCl in D<sub>2</sub>O. Since the same volume of solvent was used to prepare each external standard (700 µL) the PULCON quantification can be used to calculate the mass of analyte used to make up each test solution.

For NaCl in D<sub>2</sub>O the RSD of the 10 measurements carried out each day for 8 days ranged from 0.40 % to 1.98 % (see Table 11). For NaCl in d<sub>6</sub>-DMSO the RSD of each day varied from 0.58 % - 1.80 %, similar to D<sub>2</sub>O as solvent. KClO<sub>4</sub> in D<sub>2</sub>O also resulted in similar RSD each of the four measuring days, 1.06 % - 1.85 %. The repeatability measurements consistently showed RSD values below 2%, which is relatively standard for routine qNMR measurements at NFC.

*Table 11 RSD (in %) for each of the eight or four sequential days of repeatability measurements of NaCl in D<sub>2</sub>O and d<sub>6</sub>-DMSO and KClO<sub>4</sub> in D<sub>2</sub>O.*

Sample	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
NaCl (D <sub>2</sub> O)	1.71	1.98	0.46	0.49	0.62	0.40	0.52	0.48
NaCl (d <sub>6</sub> -DMSO)	1.80	1.60	0.58	1.12	-	-	-	-
KClO <sub>4</sub> (D <sub>2</sub> O)	1.43	1.85	1.06	1.43	-	-	-	-

#### 4.4.3. Intermediate precision and ruggedness

The ruggedness and intermediate precision of the method were measured over eight sequential days for NaCl in D<sub>2</sub>O, and for four sequential days for NaCl in d<sub>6</sub>-DMSO and KClO<sub>4</sub> in D<sub>2</sub>O. By quantifying the content of each compound with three reference samples with different concentrations it is possible to indicate any differences over time. No sign of evaporation or degradation was seen over the course of any of the measurements.

When studying Figure 11 one can see slight differences over time for NaCl in D<sub>2</sub>O. There is some variation of the obtained masses both between the three references and between the different days. All three reference samples gave similar variation over the course of the study.

When looking at the intermediate precision as RSD for each reference it is from 0.86 % to 1.0 % (see Table 12), which can be assumed as robust. The ruggedness displayed by  $\text{KClO}_4$  in  $\text{D}_2\text{O}$  is of similar amplitude, 1.26 % to 3.35 %. When looking at Figure 12 one can indicate a very slight trend of increasing average mass dependent on the day of measurement. Due to lack of time these measurements were only made during four sequential days. Additional tests of the ruggedness over eight days are required for this sample also in order to indicate if this increasing trend was a coincidence or if it is something to consider in future analyzes. The masses calculated using external standards Ref 2 and 3 show very good precision, suggesting that a weighing or pipetting error may be present in Ref 1.

The ruggedness results for Reference 1 and 2 of  $\text{NaCl}$  in  $\text{d}_6$ -DMSO were less than ideal and were not of the same amplitude, with RSD of 12.6 % and 15.7 %, which is remarkably higher than Reference 3 (1.82 %), see Table 12. When looking at the trend of each reference sample (see Figure 13) one can see that Reference 1 and 2 have a decreasing trend. When studying the measured intensities of both reference and sample measurements one could indicate that the source of error originated from the reference samples. The measured intensities of the samples were consistent over the four days, but for Reference 1 and 2 there were an increasing trend of intensity each day. This results in remarkable decrease in mass each day for the test sample. Viewing the spectra for Reference samples 1 and 2 one can immediately see that something was wrong with the sample shimming in these spectra. A suggestion is to measure during eight sequential days instead of four, analyze the results on the same day in order to discover shimming problems and correct them and to prepare new external standards more accurately by weighing the solvent instead of pipetting.

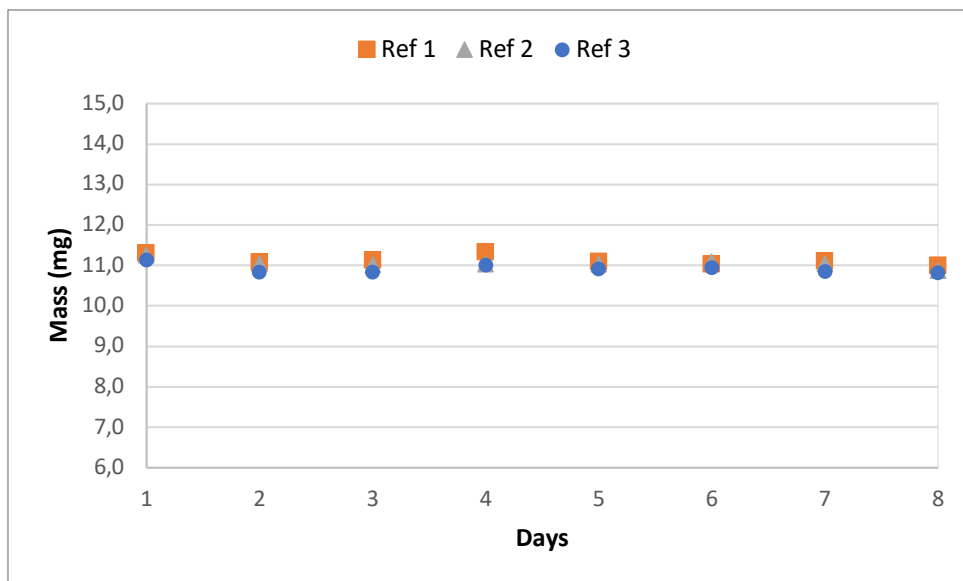


Figure 11 Average measured masses based on Reference samples 1-3 as external standards in quantification over eight days for NaCl in  $D_2O$ .

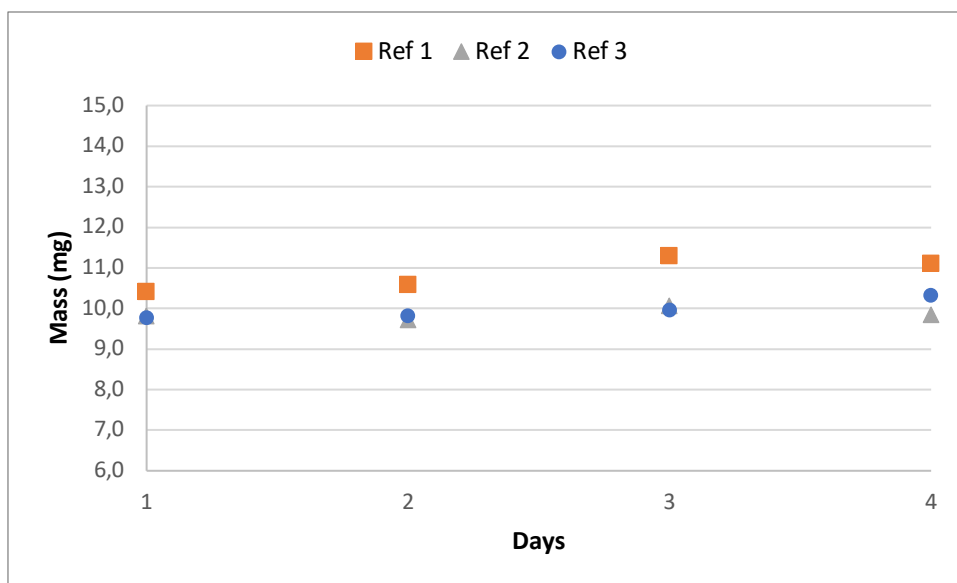


Figure 12 Average measured masses based on Reference samples 1-3 as external standards in quantification over four days for  $KClO_4$  in  $D_2O$ .



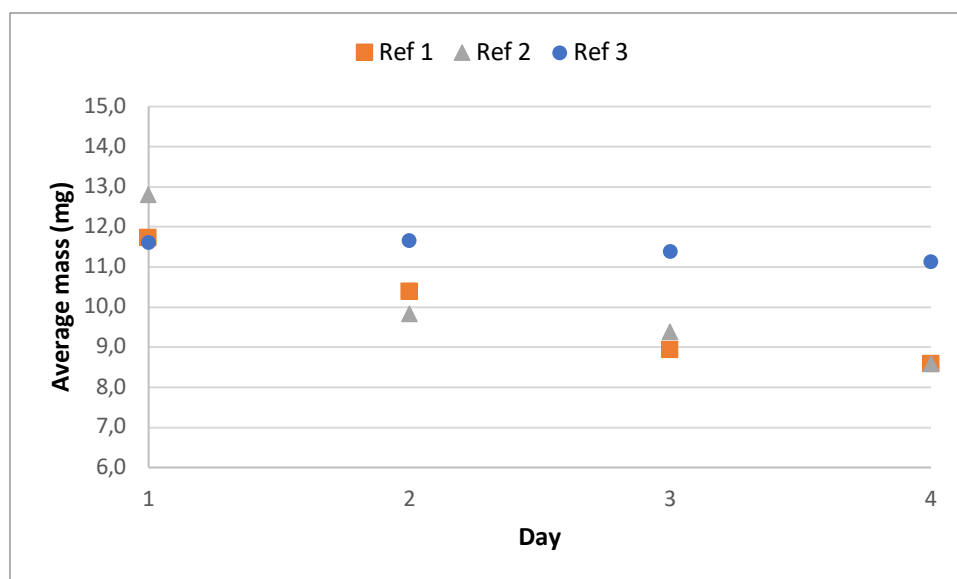


Figure 13 Average measured masses based on Reference samples 1-3 as external standards in quantification over four days for NaCl in  $d_6$ -DMSO.

Table 12 Average mass, STDEV and RSD for each reference sample of NaCl in  $D_2O$ , NaCl in  $d_6$ -DMSO, over eight sequential days for NaCl ( $D_2O$ ) and four sequential days for NaCl ( $d_6$ -DMSO) and  $KClO_4$  ( $D_2O$ ).

Sample	Actual weighed mass (mg)	Average mass over 8 (4) days (mg)	MASS STDEV (mg)	MASS RSD (%)
<b>NaCl (<math>D_2O</math>)</b>	<b>11.1</b>			
Ref 1		11.1	0.11	1.02
Ref 2		11.1	0.10	0.86
Ref 3		10.9	0.10	0.92
<b>NaCl (<math>d_6</math>-DMSO)</b>	<b>10.1</b>			
Ref 1		9.91	1.25	12.6
Ref 2		10.2	1.60	15.7
Ref 3		11.5	0.21	1.82
<b><math>KClO_4</math> (<math>D_2O</math>)</b>	<b>10.0</b>			
Ref 1		10.9	0,63	3.35
Ref 2		9.87	0.12	1.26
Ref 3		9.98	0.21	2.16

#### 4.4.4. Trueness

The trueness of the method was measured for NaCl in D<sub>2</sub>O, KClO<sub>4</sub> in D<sub>2</sub>O and cocaine · HCl in d<sub>6</sub>-DMSO, to see how precise the quantification was over a range of concentrations. The trueness was partly determined by linearity of actual weighed mass vs experimentally determined mass. The range of where the experimental masses give a linear response compared to the in-weighed masses is the range of masses indicating accurate results. The slope should be as close to 1 as possible together with a zero intercept corresponding to 0 mg in the sample should receive 0 signal, and the  $R^2$  as close to 1 as possible, in the range from 0.99-1.01. The recovery was also calculated as a trueness measurement. It was calculated by the formula  $\frac{m_{determined}}{m_{actual}} * 100$  where  $m_{determined}$  is the average determined masses and  $m_{actual}$  is the actual weighed masses.

When studying KClO<sub>4</sub> in D<sub>2</sub>O (Figure 14) one can see a linearity up to at least 8 mg. At masses >8 mg the slope of the curve is decreasing. When looking at recovery of each mass there is also a distinct decrease for masses 12 mg and 20 mg, 61 % – 98 % in average (see Table 13). A probable reason for this result is that the analyte did not dissolve completely, which was noted during sample preparations. When only studying masses of 1-8 mg (see Figure 14) a better linearity is displayed for all three reference samples, with optimal  $R^2$  of 0.9999 and accurate interception with slopes of 1.03 and 1.14. The recovery of this range of masses is ~109 % for all reference samples (see Table 13), which is not perfect but still close enough to assume it as acceptable for quantification. Hence, to obtain most accurate quantification of KClO<sub>4</sub> the masses in 700 µL D<sub>2</sub>O should be between 5-10 mg. If there is reason to weigh masses >12 mg an increased solvent volume is required in order to dissolve the sample completely. The trueness of masses <1 mg has not been studied in this study and should be investigated in further studies to indicate the exact minimum mass receiving good trueness.

Both NaCl in D<sub>2</sub>O and cocaine · HCl in d<sub>6</sub>-DMSO displayed linearity for the whole range, 1-20 mg. For NaCl, lines of best fit for all three references possessed  $R^2$  value of 0.9999 which is optimal. The slope of Reference 2 and Reference 3 indicated more true results, 1.05 and 1.08, than Reference 1 for which the slope was 1.22 (see Figure 15). Also when studying the recovery of each reference, Reference 1 is an exception with an increased percentage of ~120 % compared to Reference 2 and 3 which were of ~104 %. There is a probability that the

source of error is originating from weighing or pipetting. Small deviations from the actual weighed mass can have remarkable impact on the final calculations. But since the two other references indicated good trueness for masses 1-20 mg one can assume the quantification to be reliable. The mass range for NaCl in 700  $\mu$ L D<sub>2</sub>O was decided to be 5 mg - 15 mg, to ensure reliable quantification. Masses >20 mg have not been studied in this investigation.

As mentioned, cocaine · HCl displayed linearity over the studied concentrations range, see Figure 16. The  $R^2$  of the two references was of 0.9967, which was acceptable, even though one could indicate some decrease of the slope for 20 mg in mass. When excluding the 20 mg measuring points and only studying the range 1 mg - 12 mg, the  $R^2$  is closer to 1 (0.9992). When looking at the slope it was also an improvement of ranges from 0.88-0.94 to 0.94-1.01 when excluding the 20 mg measuring points. The mass range was therefore chosen to be 5 mg - 15 mg for cocaine · HCl in 700  $\mu$ L d<sub>6</sub>-DMSO.

*Table 13 Recovery measurement of KClO<sub>4</sub> in D<sub>2</sub>O, NaCl in D<sub>2</sub>O, and cocaine · HCl in d<sub>6</sub>-DMSO, calculated as the difference between actual weighed mass and theoretical calculated mass, for each reference sample and the average purity of each sample.*

Sample (Approximate mass)	Recovery (%) Reference 1	Recovery (%) Reference 2	Recovery (%) Reference 3	Recovery (%) Average
<b>KClO<sub>4</sub> in D<sub>2</sub>O</b>				
1 mg	110.3	101.0	111.5	107.6
4 mg	113.5	103.9	114.7	110.7
8 mg	112.5	103.0	113.6	109.7
12 mg	101.3	92.76	102.4	98.82
20 mg	63.15	57.80	63.79	61.58
<b>NaCl in D<sub>2</sub>O</b>				
1 mg	117.7	104.3	101.9	108.0
4 mg	122.0	108.1	105.6	111.9
8 mg	119.4	105.8	103.4	109.6
12 mg	120.4	106.7	104.2	110.5
20 mg	121.5	107.7	105.2	111.5
<b>Cocaine · HCl in d<sub>6</sub>-DMSO</b>				
1 mg	118.3	110.0	-	114.2
4 mg	105.8	98.44	-	102.1
8 mg	100.0	93.05	-	96.54
12 mg	102.8	95.59	-	99.17
20 mg	94.68	88.07	-	91.37

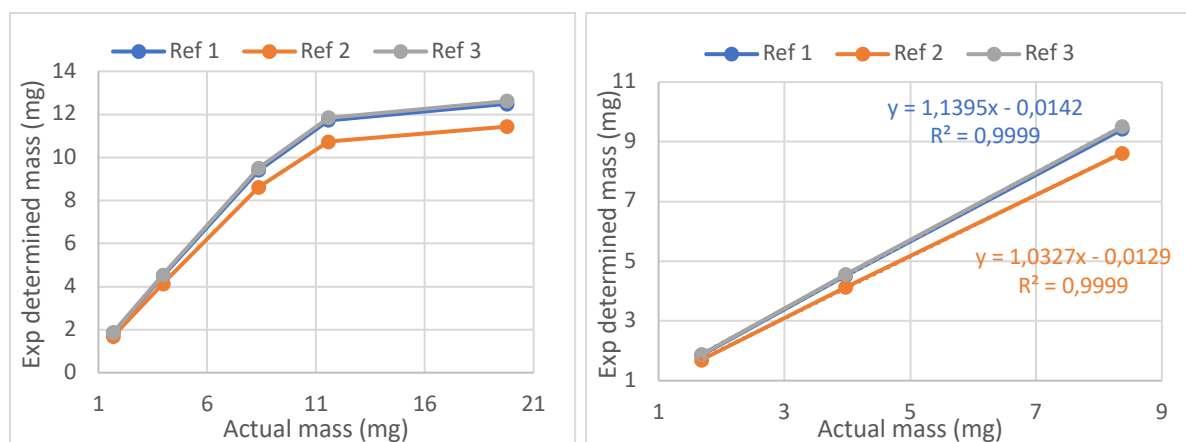


Figure 14 Graph of actual weighed mass vs theoretical calculated masses, 1-20 mg range (left) and 1-8 mg range (right) of each Reference 1-3 of  $\text{KClO}_4$  in  $\text{D}_2\text{O}$ .

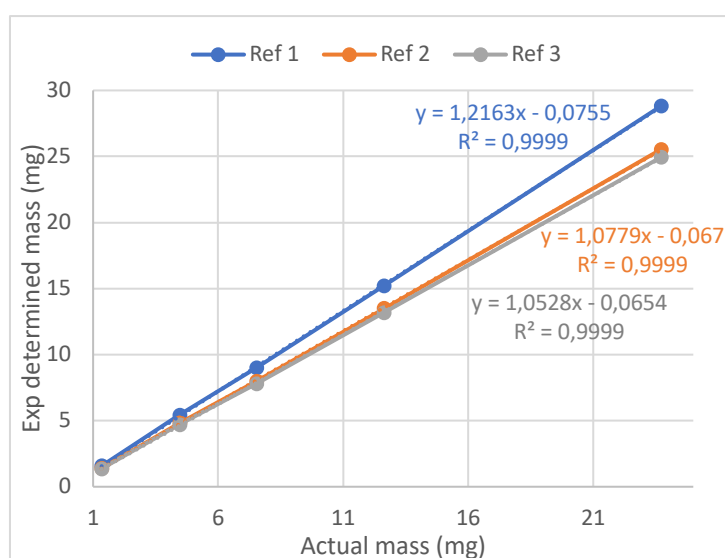


Figure 15 Graph of actual weighed mass vs theoretical calculated masses, 1-20 mg range of each Reference 1-3 of  $\text{NaCl}$  in  $\text{D}_2\text{O}$ .

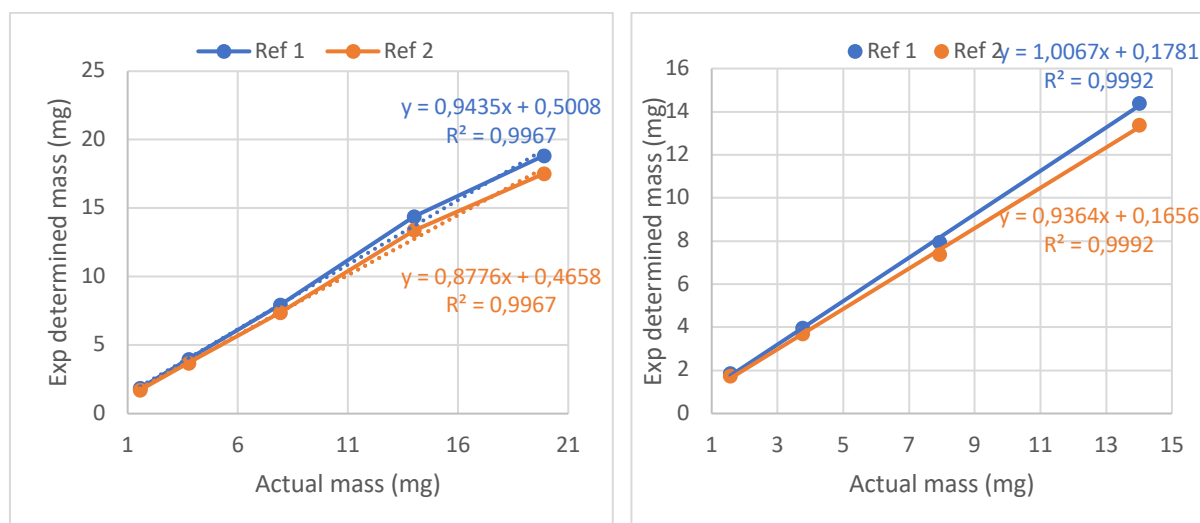


Figure 16 Graph of actual weighed mass vs theoretical calculated masses, 1-20 mg range of each Reference 1-3 of cocaine HCl in  $d_6\text{-DMSO}$ .

## 4.5. Summary

### 4.5.1. Final parameters for analysis

The final measured acquisition parameters arrived at in this study are shown in Table 14. These parameters can be further used for future analysis since they result in the most accurate spectra and enable reliable quantification. The parameters were ensured with validation analyzes which indicated a robustness, repeatability, and a limit of detection considerably lower than samples of interest. The mass range in 700  $\mu$ L solvent also resulted in good trueness. Though, since the intermediate precision over several sequential days showed a very slight increasing trend for  $\text{KClO}_4$  in  $\text{D}_2\text{O}$  and decreasing trend for  $\text{NaCl}$  in  $\text{d}_6$ -DMSO, this must be further investigated. Sources of errors in the results can originate from precipitation of reference samples, as well as accurate weighing and pipetting takes time to master and could have significant consequences on the results.

*Table 14 Measured values of instrumental parameters for NaCl and  $\text{KClO}_4$  in respective solvents, including chemical shift ( $\delta$ ) central frequency ( $\delta$ ), spectral width (SW), relaxation delay ( $D_1$ ), Number of scans (NS), Pulse length ( $P_1$ ) and minimum delay time for relaxation time ( $5 * T_1$ ). Also mass range in 700  $\mu$ L solvent.*

Compound	Mass range (mg)	Solvent	$\delta$ (ppm)	SW (ppm)	$D_1$ (s)	NS	$P_1$ ( $\mu$ s)	Recycle delay (ms)
NaCl	5-15	$\text{D}_2\text{O}$	0	500	0.01	1024	21.042	152.76
NaCl / Cocaine · HCl	5-10	$\text{d}_6$ -DMSO	0	500	0.01	4096	19.917	31.545
$\text{KClO}_4$	5-8	$\text{D}_2\text{O}$	1000	500	0.01	1024	21.775	1042.5
$\text{KClO}_4$	-	$\text{d}_6$ -DMSO	1000	500	0.01	1024	21.633	2659.9

### 4.5.2. Future analyzes

In this project, the suitability of the quantitative  $^{35}\text{Cl}$ -NMR method applicable for explosive precursors and narcotic hydrochloric salts was studied for the first time at NFC. The results achieved within the study are encouraging. The most important acquisition parameters, including spectral window, pulse length, relaxation delay and number of scans have been determined, however there are several additional tests required before being implemented at NFC.

To continue the project of developing a dependable  $^{35}\text{Cl}$ -NMR quantification method, some additional parameter and validation studies are required. To complement the measurement of ruggedness in this study a suggestion is to let other operators perform the analysis.

Reproducibility studies on another NMR instrument are not possible since there is only one instrument at NFC. Since the use of an external standard is extremely sensitive to concentration errors, weighing of the solvent instead of pipetting may lead to more accurate results.

In the beginning of this work, there was also an ambition to analyze additional analytes with other spin-active nuclei, such as  $^{79}\text{Br}$  and  $^{15}\text{N}$ . Unfortunately time was not available. By developing NMR quantification methods and establishing suitable acquisition parameter for these nuclei a broader range of samples related to explosives and narcotics could be analyzed.

#### 4.5.3. Conclusion

The conclusion that can be drawn from the results in this thesis is that additional studies are required to ensure an accurate and reliable method, before implementation at NFC. However, the analyzes performed so far have indicated that it with great certainty can function as a quantitative method for explosive precursors and narcotic hydrochloric salts in the future. In particular, the method places great importance upon the accuracy of the preparation of the external standard. In this light, the ruggedness can be studied additionally by another more-experienced operator. Since no general method was found for all analytes, the spectral width could be reduced to the areas of interest in an effort to simplify integration through baseline improvement and increased resolution.

Compared with previous studies, the measured relaxation times were similar to those measured in this thesis. Though, the number of scans and the  $90^\circ$  pulse length deviated from the previous study differences in, due to the field strength of the instruments used in each study since it affects the parameter settings of each analysis.

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## 5. Appendix

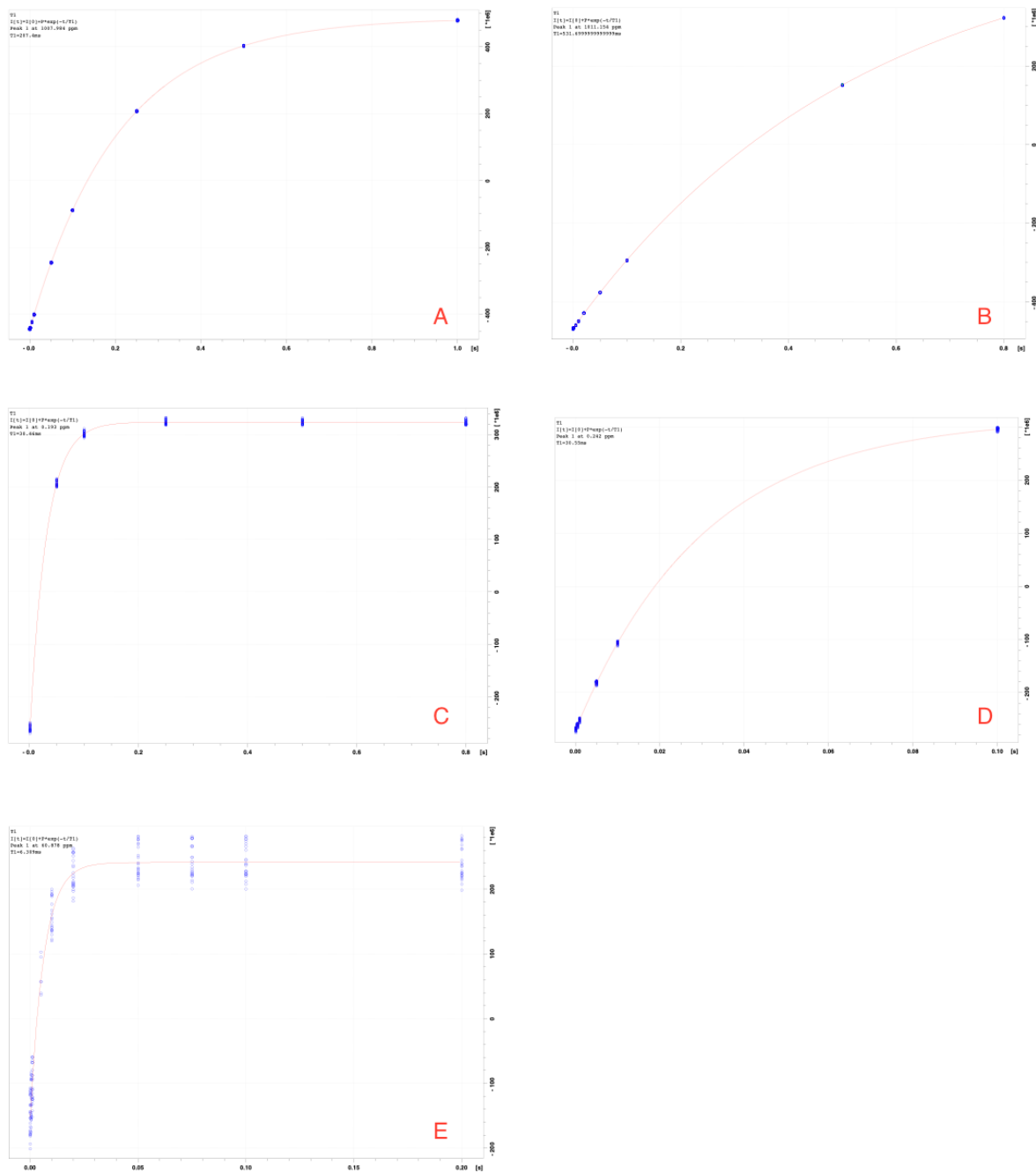


Figure A1 T1 experiment for 0.1 mol/L solutions of  $\text{KClO}_4$  in  $\text{D}_2\text{O}$  (A),  $\text{KClO}_4$  in  $d_6$ -DMSO (B), experiment 1 NaCl in  $\text{D}_2\text{O}$  (C) experiment 2 for NaCl in  $\text{D}_2\text{O}$  (D) and NaCl in  $d_6$ -DMSO (E).

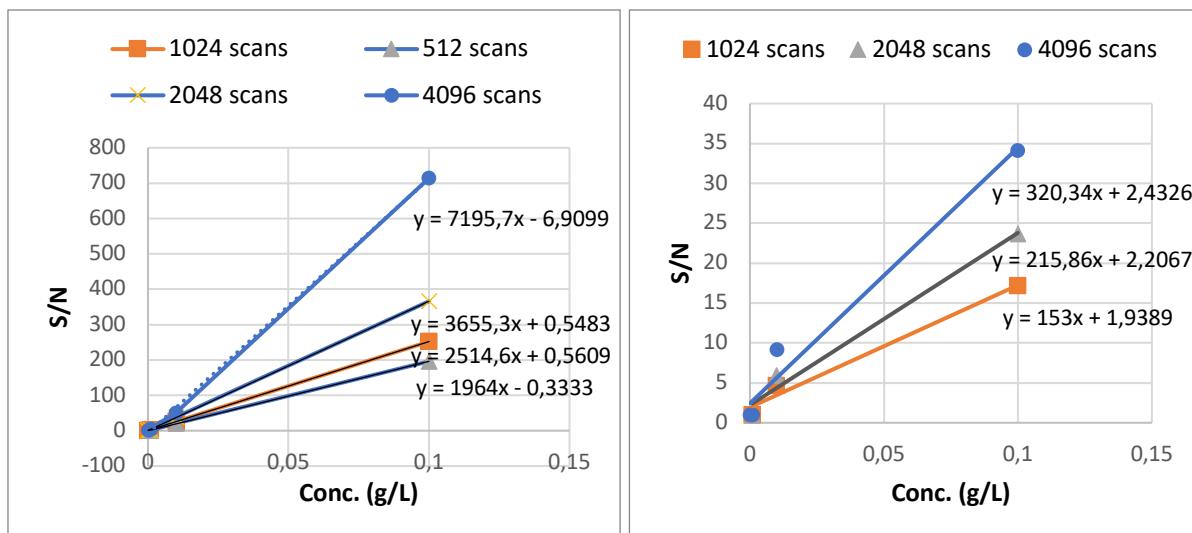


Figure A2 Measured S/N vs concentration of NaCl in  $D_2O$  (left) and  $d_6$ -DMSO (right) and the measured equation of the calibration curves of each number of scans.

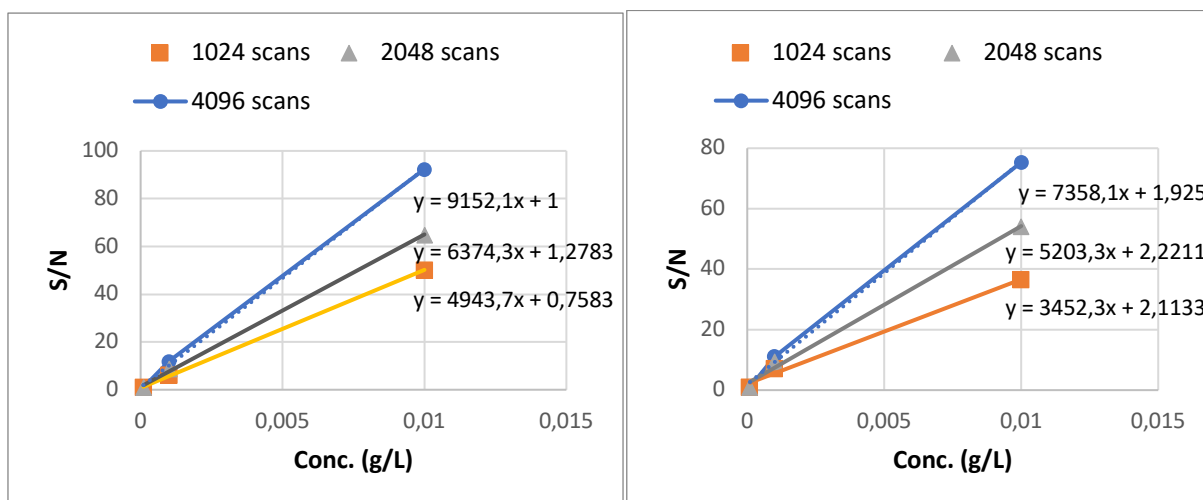


Figure A3 Measured S/N vs concentration of  $KClO_4$  in  $D_2O$  (left) and  $d_6$ -DMSO (right) and the measured equation of the calibration curves of each number of scans.

*Table A1 The calculated average mass (in mg), standard deviation (STDEV) and relative standard deviation (RSD %) of each reference sample 1-3 for NaCl in D<sub>2</sub>O.*

Day	1	2	3	4	5	6	7	8
<b>Ref 1</b>								
Avg mass (mg)	11.3	11.1	11.1	11.3	11.1	11.0	11.1	11.0
Mass STDEV (mg)	0.19	0.22	0.05	0.06	0.07	0.04	0.06	0.05
Mass RSD (%)	1.71	1.98	0.46	0.49	0.62	0.40	0.52	0.48
<b>Ref 2</b>								
Avg. mass (mg)	11.3	11.1	11.0	11.0	11.0	11.1	11.1	10.9
Mass STDEV (mg)	0.19	0.22	0.05	0.05	0.07	0.04	0.06	0.05
Mass RSD (%)	1.71	1.98	0.46	0.49	0.62	0.40	0.52	0.48
<b>Ref 3</b>								
Avg. mass (mg)	11.1	10.8	10.9	11.0	10.9	11.0	10.9	10.8
Mass STDEV (mg)	0.20	0.23	0.05	0.06	0.07	0.05	0.06	0.05
Mass RSD (%)	1.80	2.09	0.48	0.52	0.66	0.43	0.55	0.50

*Table A 2 The calculated average mass (in mg), standard deviation (STDEV) and relative standard deviation (RSD %) of each reference sample 1-3 for KClO<sub>4</sub> in D<sub>2</sub>O.*

Day	1	2	3	4
<b>Ref 1</b>				
Average mass (mg)	10.4	10.6	11.3	11.1
MASS STDEV (mg)	0.15	0.20	0.12	0.16
MASS RSD (%)	1.43	1.85	1.07	1.43
<b>Ref 2</b>				
Average mass (mg)	9.82	9.73	10.1	9.85
MASS STDEV (mg)	0.14	0.18	0.11	0.14
MASS RSD (%)	1.43	1.85	1.07	1.43
<b>Ref 3</b>				
Average mass (mg)	9.78	9.83	9.97	10.3
MASS STDEV (mg)	0.14	0.18	0.11	0.15
MASS RSD (%)	1.43	1.85	1.07	1.43

Table A 3 Table A2 The calculated average mass (in mg), standard deviation (STDEV) and relative standard deviation (RSD %) of each reference sample 1-3 for NaCl in  $d_6$ -DMSO.

Day	1	2	3	4
<b>Ref 1</b>				
Average mass (mg)	11.7	10.9	8.95	8.09
MASS STDEV (mg)	0.21	0.17	0.05	0.09
MASS RSD (%)	1.80	1.60	0.58	1.12
<b>Ref 2</b>				
Average mass (mg)	12.8	9.83	9.39	8.58
MASS STDEV (mg)	0.23	0.17	0.05	0.10
MASS RSD (%)	1.80	1.78	0.58	1.12
<b>Ref 2</b>				
Average mass (mg)	11.6	10.6	8.86	8.29
MASS STDEV (mg)	0.21	0.17	0.05	0.09
MASS RSD (%)	1.80	1.60	0.58	1.12

Table A 4 Actual weighed masses of respective compound (NaCl,  $KClO_4$ , cocaine HCl) and theoretical calculated masses according to each reference sample for trueness test.

$m_{\text{actual}}(\text{mg})$	$m_{\text{theoretical}}(\text{mg})$ Ref 1	$m_{\text{theoretical}}(\text{mg})$ Ref 2	$m_{\text{theoretical}}(\text{mg})$ Ref 3
<b><math>KClO_4 \cdot D_2O</math></b>			
1.68	1.85	1.70	1.87
3.97	4.51	4.13	4.55
8.37	9.42	8.62	9.51
11.6	11.7	10.7	11.8
19.8	12.5	11.4	12.6
<b>NaCl <math>D_2O</math></b>			
1.34	1.58	1.40	1.37
4.46	5.44	4.82	4.71
7.55	9.02	7.99	7.81
12.6	15.2	13.5	13.2
23.7	28.8	25.5	24.9
<b>Cocaine <math>\cdot</math> HCl <math>d_6</math>-DMSO</b>			
1.57	1.86	1.73	-
3.76	3.98	3.70	-
7.94	7.94	7.39	-
14.0	14.4	13.4	-
19.9	18.8	17.5	-