A comparative study of the NMR spectra of parallel potencies of Sulphur with reference to similarities of concentration and dynamisation.

By

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Durban Institute of Technology.

I, Johannes Francois Malan do hereby declare that this dissertation is representative of my own work.

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Dedication

This work is dedicated to the pursuit of a greater understanding of Homoeopathy.

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Abstract

The purpose of this study was to analyse and compare the NMR spectra of homoeopathic Sulphur (the most well-known and often tested homoeopathic remedy) in two commonly used potencies, namely the centesimal (CH) and decimal (DH) potencies. Both potencies were prepared according to the Hahnemannian method. In order to assess the differences and similarities between these two potency scales, remedies with the same levels of deconcentration, and remedies with the same numbers of succussions were tested.

The Control substance used was Water-Ethanol 87% without lactose or Sulphur. The Control substance was prepared in the same way as the Sulphur i.e. potentised as the Sulphur.

Chemical shift and relative integration values of the H_2O, OH and CH_2 peaks were recorded, calculated and compared.

The investigation was designed as a scientific experiment. Firstly, the Sulphur remedies were compared to the Controls. Secondly, Sulphur remedies were compared to Sulphur remedies, and Controls to Controls. The following criteria were used:

- Equal deconcentrations of the centesimal scale were compared to their equivalent decimal scale.
- Equal numbers of succussions of the centesimal scale were compared to their equivalent decimal scale.

The following potencies were assessed for both Sulphur and Control (Water-Ethanol):

- 6CH, 12CH, 24CH and 48CH.

- 12DH, 24DH, and 48DH.

It was hypothesized that significant differences exist between remedies of the CH scale compared to the DH scale, even though they may have similar deconcentrations and/or similar numbers of succussions.

Preparation and NMR Analysis

- Sample volumes of 20ml were prepared in Durban and transported to Pietermaritzburg for NMR spectroscopy analysis.
- Fourteen samples were analysed using a Varian 500 MHz INOVA spectrometer.
- Acquisition time was set at 1.9 seconds and 16 transients were analysed per run.
- The pulse angle was set at 20.4 degrees and a relatively constant temperature of 24° C was maintained during analysis.
- 0.6ml of each sample was extracted using a new micropipette.
- Acetone was used as an external lock and as an internal reference substance.
- The data was recorded in the form of NMR spectra, listing chemical shift values and indicating integration values.

Statistical Analyses and Results

The chemical shift and relative integration values of the H_2O, OH and CH_2 peaks were calculated from the above and used as input to the Mann-Whitney U-test, and the independent sample unpaired t-test statistical analysis programs:

- The level of significance for all tests was set at $\alpha = 0.05$ ($p \leq 0.05$).
- **Sulphur vs. Controls**. No statistically significant differences were found in the comparison of the homoeopathic Sulphur to its potentised Water-Ethanol

Control, for either the chemical shift or relative integration values ($p > 0.05$). Therefore the null hypothesis is accepted.

- **Chemical Shift: Sulphur vs. Sulphur; Control vs. Control**. No significant differences were found in the chemical shift values when comparing equivalent deconcentration potencies, or equivalent number of succussions remedies, for either Sulphur or Control. Therefore the null hypothesis is accepted.
- **Relative Integration: Sulphur vs. Sulphur; Control vs. Control**. Statistically significant differences were found in the relative integration values for equal deconcentrations and equal number of succussions comparisons for both Sulphur and Control. Therefore the null hypothesis is rejected.

Final conclusions drawn from the T-Test:

- **Sulphur vs. Sulphur**. The significant differences for the Sulphur homoeopathic remedy comparisons were only found in the H_2O peaks.
    - Equal levels of deconcentration: $p = 0.050$
    - Equal number of succussions: $p = 0.022$
- **Control vs. Control**. The significant differences for the potentised Water-Ethanol Control comparisons were only found in the OH peaks.
    - Equivalent levels of deconcentration: $p = 0.001$
    - Equivalent number of succussions: $p = 0.003$

The results of this study are similar to previous results obtained by international studies of the NMR spectra of homoeopathic substances. As in this study, significant differences were noted in the relative integration values of the OH peaks.

Abstract

The verification of a significant model of homoeopathic remedies remains beyond the scope of this investigation. However, the results of this study have confirmed that significant differences exist between centesimal and decimal Hahnemannian potencies, even though they have similar deconcentrations and similar number of succussions. It has also confirmed that NMR spectroscopy remains a valuable tool in the investigation of the properties of homoeopathic remedies.

The process of conducting this study highlighted the large number of external factors that could influence the results (both unspecified and unquantified) of the NMR spectroscopy analysis of homoeopathic substances.

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Table of Abbreviations

CH	-	Centesimal Hahnemannienne. Used interchangeably with C
CH_2	-	Methylene group
CH_3	-	Methyl group
DH	-	Decimal after Hahnemann. Used interchangeably with D
K	-	Korsakovian
LM	-	Quinquagenimillesimal potency
OH	-	Hydroxyl group
µl	-	Microlitre

Definition of Terms

Centesimal: The concentration scale originally introduced by Hahnemann, and the most frequently used homeopathic potency scale in current homeopathic practice. Dilution steps are 1:100. It may be indicated as CH (centesimal Hahnemannian) or C, or it is assumed as the potency scale when no scale is indicated.

Chemical shift: The difference in absorption frequency of a proton, dependent upon the group to which the hydrogen atom is bonded. This is measured in parts per million (ppm) and is directly proportional to the field strength or to the oscillator frequency of the NMR spectrometer. It is measured as a shift from a reference standard.

Clathrate: A compound formed when the small molecules of one substance fill in the holes in the structural lattice of another. Therefore, Clathrates are intermediate between mixtures and true compounds.

Decimal: The concentration scale primarily used in Germanic countries. The dilution steps are 1:10. It may be indicated as DH (decimal Hahnemannian) or D. Homoeopaths often use it interchangeably with the centesimal scale based on equal deconcentrations.

Deconcentration: The level of concentration reached by serial dilution of the original substance in a diluent. The level depends on the potency scale that is employed e.g. the centesimal scale diluted a substance one hundredfold at each successive step.

Hydration: In chemistry, the combination of water and another substance to produce a single product. It is the opposite of dehydration. In homoeopathy water is utilised to some degree at almost all levels of remedy preparation.

LM: Quinquagenimillesimal – a homeopathic potency scale, introduced by Hahnemann, in which the rate of deconcentration at each potency stage is 1:50,000. Deconcentration is achieved in two stages; 1:100 and then 1:500.

NMR Spectroscopy: A method of chemical analysis whereby the protons of a test substance are exposed to a magnetic field and an interacting radio frequency in order to achieve resonance. The energy absorption and emission results in a spectrum of the resonant protons.

Potentisation: The process of preparing a homeopathic remedy by repeated dilution and succussion or trituration. It is believed to involve the transfer of information from the original substance onto a carrier. The scope and strength of the effect of the substance is believed to increase through this process.

Posology: The methodology of dosage in general, by which the size, strength and frequency of repetition of a dose is prescribed.

Relative integration: The process of finding the area under the respective peaks on a graph. Relative integration is calculated by dividing the integration values of each peak by the sum of all integration values of the run. The value is proportional to the number of protons generating each of the peaks.

Succussion: The action of vigorously shaking a liquid dilution of a homeopathic medication in its container, whereby each stroke ends in a jolt. This is usually done by holding the container in one hand, and pounding it on the palm of the other hand (or on a solid surface).

Chapter 1 – Introduction

According to Lessell (1994), the main cause of the difficulty of potency prescription has been a failure to supply a reasonable and cohesive theory of the process of potentisation and methodology of dosage. The phenomenon of homoeopathic preparation and prescription is, from a scientific point of view, in relative disarray. Not only do we not know the scientific nature of what we prescribe, but we also do not know the magnitude or strength of what we prescribe.

There are several potentising techniques currently employed. The Hahnemannian potentising techniques are widely available and therefore used. Their easy availability results from the fact that the Hahnemannian methods provide for the best and most clearly defined standardisation in manufacture, and thus between the various laboratories preparing the remedies for commercial distribution. The Hahnemannian potencies have been selected for this study.

The three Hahnemannian potencies primarily used in Homeopathic practice are:

- Centesimal (C or CH) potencies
- Decimal (D or DH) potencies
- LM potencies

According to Gaier (1991), the centesimal potencies are the most commonly used in the francophone and English-speaking countries, whereas the decimal potencies are more often used in the German-speaking countries. Furthermore, Gaier states that although, in theory the LM potencies are available in all countries, in practice they are infrequently used.

Perhaps the infrequent use of the LM potencies mentioned by Gaier (1991) is due to the fact that their method of preparation only become known in the posthumous publication of the 6th edition of Hahnemann's Organon. Another contributing factor may be that their method of preparation is more labour-intensive, thus more costly. However, the frequency of use of the LM potencies varies by location as is illustrated by the almost 70% of all remedies prepared for the Glasgow Homeopathic Hospital (Scotland) being LM potencies in the month of July 2002.

According to Gaier (1991), research has been conducted on the effect of certain CH and DH homoeopathic potencies. An equivalency was found between the effect of 7CH and 14DH (i.e. equal deconcentrations), but this does not necessarily imply a structural difference, or similarity between these potency levels. However, this study is not to test the effect of the various potency types, but to test for any measurable structural differences that might exist in the remedy substance itself.

From the aforementioned one can see why the CH and DH potencies are, at times used interchangeably. However, we need to ascertain whether there is a scientifically measurable difference between CH and DH potencies with respect to both the level of deconcentration and the number of succussions.

For this study NMR Spectroscopy has been selected. NMR Spectroscopy has emerged as a valuable tool in the assessment of homoeopathic remedies as illustrated in studies by Smith and Boericke (1966, 1968), Weingartner (1990), Demangeat *et al* (1992), Ross (1997), Sukul *et al* (2000) and Davies (2001). The structural implications of the observed differences in the above studies remain open to speculation.

1.1 The Aim of the Study

The purpose of this study is to evaluate centesimal (CH) and decimal (DH) Hahnemannian potency equivalency in homoeopathic pharmacy.

In order to evaluate the above, the following was carried out.

i) Comparison of centesimal (CH) and decimal (DH) Hahnemannian potencies with:

- Equivalent levels of deconcentration
- Equivalent number of succussions

ii) Two potentised substances were used:

- Remedy: Sulphur
- Control: Water-Ethanol

iii) Nuclear Magnetic Resonance (NMR) Spectroscopy was used to measure the substances

1.2 The Statement of Objectives

1.2.1 The First Objective

Sulphur CH. To compare and evaluate the NMR spectra of Sulphur 6CH, 12CH, 24CH and 48CH potencies with respect to the chemical shift and relative integration values of the H_2O, OH and CH_2 signals. The Sulphur substances have been potentised using Hahnemannian methods. The objective is to determine their respective physical structures, and any differences that may exist (see **4.4**).

1.2.2 The Second Objective

Sulphur DH. To compare and evaluate the NMR spectra of Sulphur 12DH, 24DH and 48DH potencies with respect to the chemical shift and relative integration values of the H_2O, OH and CH_2 signals. The Sulphur substances have been potentised using Hahnemannian methods. The objective is to determine their respective physical structures, and any differences that may exist (see **4.4**).

1.2.3 The Third Objective

Control CH. To compare and evaluate the NMR spectra of the Control (Water-Ethanol) 6CH, 12CH, 24CH and 48CH potencies with respect to the chemical shift and relative integration values of the H_2O, OH and CH_2 signals. The Controls have been potentised using Hahnemannian methods. The objective is to determine their respective physical structures, and any differences that may exist (see **4.4**).

1.2.4 The Fourth Objective

Control DH. To compare and evaluate the NMR spectra of the Control (Water-Ethanol) 12DH, 24DH and 48DH potencies with respect to the chemical shift and relative integration values of the H_2O, OH and CH_2 signals. The Controls have been potentised using Hahnemannian methods. The objective is to determine their respective physical structures, and any differences that may exist (see **4.4**).

1.2.5 The Fifth Objective

Equal deconcentrations. To compare and evaluate parallel potencies in terms of substance versus substance and control versus control, so that any differences, or similarities between potencies with similar theoretical deconcentrations can, in terms of NMR spectra and statistical analysis be elucidated (see **4.2**, **4.3**).

1.2.6 The Sixth Objective

Equal number of succussions. To compare and evaluate parallel potencies in terms of substance versus substance and control versus control, so that any differences, or similarities between potencies with similar number of succussions can, in terms of NMR spectra and statistical analysis be elucidated (see **4.2**, **4.3**).

1.3 The Hypotheses

1.3.1 The First Hypothesis

Equal deconcentrations. It is hypothesized that significant differences exist between the chemical shift and relative integration values of H_2O, OH and CH_2 signals of group 6CH, 12CH, 24CH and group 12DH, 24DH, 48DH potency levels, and that these indicate inherent differences between remedies with the same deconcentrations, but different methods of preparation. Substances have been potentised using Hahnemannian methods.

1.3.2 The Second Hypothesis

Equal number of succussions. It is hypothesized that significant differences exist between the chemical shift and relative integration values of H_2O, OH and CH_2 signals of group 12CH, 24CH, 48CH and group 12DH, 24DH, 48DH potency levels, and that these indicate inherent differences between remedies with similar number of succussions, but different methods of preparation. Substances have been potentised using Hahnemannian methods.

1.3.3 The Third Hypothesis

It is hypothesized that the amount of solute transferred during the steps of potentisation, as well as the number of succussions, plays an important role in the development of distinct physico-chemical properties of potentised homoeopathic substances. Therefore, it is hypothesized that statistically significant differences exist between parallel potencies of substance versus control, substance versus substance and control versus control, with regard to the chemical shift and relative integration values of the H_2O, OH and CH_2 signals of the respective potency levels.

Chapter 2 – Review of the Related Literature

2.1 The Introduction

It is clear, and has been well established through many years of clinical experience and clinical trials, that homoeopathy does have a measurable effect on patients. The question of what happens to the substance before prescription remains to be answered.

Many aspects of the homoeopathic process remain to be clarified and structured, not least of these being the process of the preparation of the remedies. What really happens when solutions are serially potentised, and what is carried forward into the ultra-molecular stages?

This homoeopathic process, according to Gaier (1991), if performed according to the prescribed mathematico-mechanical attenuation procedures for potentisation, increases both the physical solubility and the physiological assimilability of the drug, while also changing its therapeutic activity in its use as a homoeopathic remedy.

Ross (1997) and Power (1999) demonstrated that there are differences between LM potencies and their controls, and that these differences may increase as the potencies increased. Davies (2001) demonstrated that there are statistically significant differences between Korsakovian and Hahnemannian parallel potencies and that these are more than likely structural differences as indicated by NMR spectroscopy. Within the context of this study we aim to clarify, at least with regard to the commonly used CH and DH homoeopathic potencies, whether a difference exists between parallel potencies as measured by NMR Spectroscopy.

2.2 Homoeopathic Posology

Posology is the methodology of dosage. Unfortunately the whole matter of homoeopathic posology is in a state of chaos.

As mentioned earlier, Lessell (1994) comments that the root of the posology dilemma has been a failure to establish a reasonable and cohesive theory of the process of potentisation. Homoeopathic posology appears to fail due to its inability to define not only the exact nature of the remedies, but also their concrete magnitude.

Whether a homoeopathic remedy is regarded as being low, medium or high potency is subjective and depends to a large degree on the homoeopath, the homoeopathic schools, or even the country in which homoeopathy is practised. For example, some define a low potency as being the tincture up to 3CH or 6DH; a medium potency as above that up to 12CH or 24DH; a high potency as above that up to 30CH or 60DH; as a very high potency all potencies beyond 30CH or 60DH. Others disagree, claiming that below a 12CH is low, and 12CH to 30CH is medium, and 200CH and above is high. Another example is France. Here it is illegal to potentize remedies over 30CH (Gaier, 1991), and yet one of their most popular remedies, which is not prescribed based on the Law of Similars, and is commercially available without prescription, is a 200K. One could argue, as some do, that 200K is equal to an 8CH, but this point is made only on the level of deconcentration, and not the vast difference in the amount of succussions.

The amount per dose of one pill, four pills or an entire vial, and the frequency with which the dose is repeated, also varies greatly. For example, some may give four pills every hour; others will give a single dose (regarded as a more classical prescription), until the effects of that dose has warn off. This may be a short period of time or may extend to several years before repetition.

2.3 Decimal and Centesimal Scales of Potency

What are the differences and similarities between the DH and CH potencies?

Similarities:

- Both are produced through a process of serial dilution
- Both are produced through a process of succussion after serial dilution
- Separate vials of diluent are used at each successive step
- A similar number of succussions is applied to the diluted substance, although this may vary from 2 to100
- Deconcentration increases exponentially, but at a sustained rate

Differences:

- The amount of substance transferred between dilutions differs by a factor of 10
- Deconcentration is 10 times greater per step in the CH as opposed to the DH potency
- The step by which nothing of the original diluted substance remains is reached much sooner in the CH as opposed to the DH potency. Theoretically, this is done in half the amount of steps

The following tables serve to illustrate the similarities between CH and DH potencies used in this study with regard to equal deconcentrations and equal number of succussions.

Table 2.1 *Similarities in CH and DH potencies. Equal deconcentration.*

Concentration	CH	DH
$1{:}10^{12}$	6C	12D
$1{:}10^{24}$	12C	24D
$1{:}10^{48}$	24C	48D
$1{:}10^{96}$	48C	96D*

- **CH Potencies**: dilution of 1 part Substance to 99 parts Water-Ethanol (or Lactose where applicable) per step of potentisation (see **Appendix A**)
- **DH Potencies**: dilution of 1 part Substance to 9 parts Water-Ethanol (or Lactose where applicable) per step of potentisation

Table 2.2 *Similarities in CH and DH potencies. Equal number of succussions*

Numbers of Succussions				'Final' Level	
B/F from Previous Level	Intermediate Levels	'Final' Level	Total	CH	DH
0	2 = 20	100	**120**	6C	6D*
120	5 = 50	100	**270**	12C	12D
270	11 = 110	100	**480**	24C	24D
480	23 = 230	100	**810**	48C	48D

- Potencies are succussed 10 x at intermediate levels of potentisation during preparation
- Potencies are succussed 100 x at each 'final' level

* Potencies not used in this study (6D, 96D)

2.4 Models of Homoeopathic Remedies

Various authors have attempted to provide science and homoeopathy with an effective theoretical model to explain the unconventional effects and claims of homoeopathy. Modern technological development in the form of X-ray spectroscopy, Raman laser spectroscopy, UV spectroscopy, and most often NMR spectroscopy, has given science the ability to investigate, formulate and test many new hypotheses of what homoeopathic remedies are and how they work.

Barnard (1965) investigated homoeopathic remedies in his viscosity studies. He developed the idea that the i) solvent carries the information content of the original substance, and that ii) the solvent acts as the remedy rather than the original substance. He believed that water polymers were formed by succussion. These structures were determined by the original structures of the dissolved substance, and that these caused spatial changes in the solvent. He believed that these polymers were induced to grow and split by the energy provided by succussion.

Smith and Boericke (1968) conducted NMR spectroscopy analysis of homoeopathic Sulphur. They concluded that the act of succussion increased the area of the NMR hydroxyl (OH) spectrum in water-ethanol (13%:87%) when compared to the identical unsuccussed dilutions containing the same solute. They also speculate that it is the formation of water polymers that may be responsible for their observed results.

The properties and information content of a microscopic system, such as remedy information, cannot be understood by starting from the properties and information content of its molecules. Resch and Gutmann (1991) believe the molecular concept must be

extended to include the continuous relationship between the molecules based on the concept of super-molecular system organisation. They believe the conditions of potentisation lead to considerable improvement not only of the system organisation, but also of the precision of the remedy information, with increased differentiation at high levels of deconcentration. They postulate that further improvement is achieved as alcohol is added to the aqueous solution used for potentisation.

Anagnostatos *et al* (1991) propose a clathrate model. This model proposes that the organised hydrogen bonded molecules of the solvent form shells around the original substance. During the process of succussion or trituration, some of these clathrates are broken, leaving empty clathrates (structure specific shells) that in turn produced their own surrounding clathrates from the solvent.

Antonchenko and Ilyin (1992) propose that water structures in hydration shells are dissipative structures, and that movement of protons along spiral water molecule chains within these structures provide stabilisation. Homoeopathic potentisation produces cavitation in a liquid, which when cavitation collapses, releases protons necessary to stabilise the dissipative structures. This process of proton 'hopping' in a water-diluted medium was put forward by Smith and Best (1989).

The preceding theories are largely dependent on the formation of complex structures within water supported by hydrogen bonds. An objection to these theories is that the increase in cluster size of these polymers may change both boiling and freezing points of the diluent, and that the expected lifetime of a hydrogen bond in water is in the order of sub-nanoseconds. If the hydrogen bonds are disintegrating and re-forming at new sites to form new clusters, this may explain the general structure stability to some degree.

Lessell (1994) proposes a model that incorporates the previously described geometrical models, a dynamic field model, and a sub-molecular model. The dynamic field model proposes the following:

- The solid produces an individually characteristic vibrating electromagnetic field, with a unique frequency related to its components and their relative molecular presence
- The various components of the diluent become attuned to this frequency and collectively resonate in accordance with it
- The vibrations of the characteristic electromagnetic field and the resonantly vibrating components of the diluent maintain each other mutually, so that the vibrations persist even after the removal of the intended solute by serial dilution

The sub-molecular theory hinges on the NMR spectroscopy observations that the OH groups (the water molecule itself is essentially a bifurcate OH group) have shown significant changes in their relative integration values. The wave-particle duality, as described by quantum physics, is also of importance. In the Bohr model of the atom, electrons move in definite orbits around the nucleus, with only certain orbits 'allowable'. It is proposed that electrons are promoted by the application of appropriate energy, and that they therefore jump from lower to their next higher orbital. When the electron returns to its lower orbital, a quantum of electromagnetic radiation is released, which bears a frequency characteristic of the orbital with resultant specific electromagnetic radiation. These resonantly promoted electrons of the OH groups possess energies characteristic of those of the highest orbitals of the solute.

Another important point concerns the energetic stability of the promoted electrons. Promoted electrons, when demoted, return to their unpotentised ground state, thus prohibiting the formation of aberrant compounds. The inactivation of homoeopathic remedies by strong ultraviolet radiation, and strong magnetic fields, verifies this orbital instability, as the UV frequencies coincide with valence electron frequencies. The promoted electrons exhibit a degree of meta-stability rather than gross instability. This would not be possible if the energy of succussion or trituration could not be stored for electronic excitation and promotion to occur.

It is proposed that some of the energy during collision is utilised to produce certain virtual particles, called orbitons. Although this may smack of science fiction, it is a well-established concept in quantum physics. The orbitons become attached to the lone pair electrons. When a sufficient number of identical frequency have accumulated on a particular electron, the electron jumps to the appropriate target orbital, thus contributing to the change of the diluent molecule. However, this orbital energy theory does not appear to explain the matter of potency or strength of a remedy. Neither does it appear to offer an explanation of any differences between centesimal, decimal and LM scales of potency.

Lessell proposes that the concept of potency has two facets, i) the major one concerning therapeutic range, and ii) the lesser concerning the force or strength of action. We cannot base the differences in action of a potency purely upon either the number or the concentration of the imprinted diluent molecules. We must take into account the oscillatory motions of the diluent molecules associated with an electromagnetic field, with similar vibrational character, and that the field becomes an energising entity and not a mere passive product of molecular oscillation. When we succuss the diluent molecules, in accordance with the principle of super-radiance, they are caused to oscillate in harmony

with a particular frequency, supported by the energetic field of their own making. When we succuss again, the oscillatory frequency of both diluent and the field increases.

In combination, the field and the molecules constitute a type of frequency capacitor. The total energy of the system is determined by the sum of the energy of the field itself (which is related to its frequency) and that of the oscillatory motions of the molecules. Unlike the field, the molecular oscillatory energy is not only determined by frequency, but also by amplitude. The greater the amplitude of oscillation at a given frequency, the greater the kinetic energy will be. Thus, as a result of simple dilution, kinetic energy is shed from the oscillating molecules by a reduction in amplitude. Once a diluent molecule exhibits maximum amplitude for a given frequency, having gained this by agitation, it can no longer be accelerated to a higher frequency, unless its amplitude is reduced by dilution. In this way, the diluent molecules control the development of the frequency of the field itself, for they must maintain a state of harmony. This explains the necessity of serial dilution to produce higher and higher field frequencies.

Based on the above-mentioned model the following physical differences between the three major potency scales (CH, DH, LM) can be considered:

- Dilution itself reduces the concentration of promoted diluent molecules. This reduction is theoretically proportional to the degree of dilution.
- Dilution, within limits, causes little or no change in dynamic field frequency, and thus potency.
- Dilution, within limits, favours the increase in frequency and thus potency, if followed by significant agitation.
- Succussion increases the number of promoted molecules of diluent.

- Succussion increases field frequency and thus potency. However, blocking of this process will occur when the molecules have reached maximum oscillatory amplitude.

By using this combined model we can also explain; the neutralisation of homoeopathic remedies by UV and electromagnetic radiation; how serial dilution and agitation affects potency; why there is a difference between the various potency scales.

2.5 Nuclear Magnetic Resonance Spectroscopy

It is well known that electrons have spin, and that protons and neutrons similarly have spin. Therefore, depending on the arrangement of protons and neutrons, a nucleus could have spin. A nucleus with spin will act like a bar magnet, similar to the electron although many times smaller in magnitude. Examples of nuclei with spin are hydrogen protons and carbon-13. Although nuclear magnetism is much smaller than that of electrons, with the correct equipment it is easily seen, and it forms the basis of Nuclear Magnetic Resonance (NMR) spectroscopy, now one of the most important methods for determining molecular structure.

The essential features of NMR can be seen if you consider the proton. Like the electron, the proton has two spin states. In the absence of a magnetic field, these spin states have the same energy, but in the field of a strong magnet, they have different energies. If the proton magnet is aligned with the external magnetic field, with its south pole facing the north pole of the external magnetic field, it will have lower energy. If the proton magnet is at 180 degrees to the external magnetic field, with its south pole facing the south pole of

the external magnetic field, it will have high energy. Now if the proton in the lowest spin state is irradiated with electromagnetic waves of the proper frequency, the proton will change to the higher spin state. The frequency absorbed by the proton depends on the

magnitude of the magnetic field. Since electrons have their own magnetic fields and the electrons surround each proton, the magnetic environment of a proton depends on what other atom (which will have its own electrons with their own magnetic fields) it is bonded to.

2.5.1 Precession: Rotating Particle in a Magnetic Field

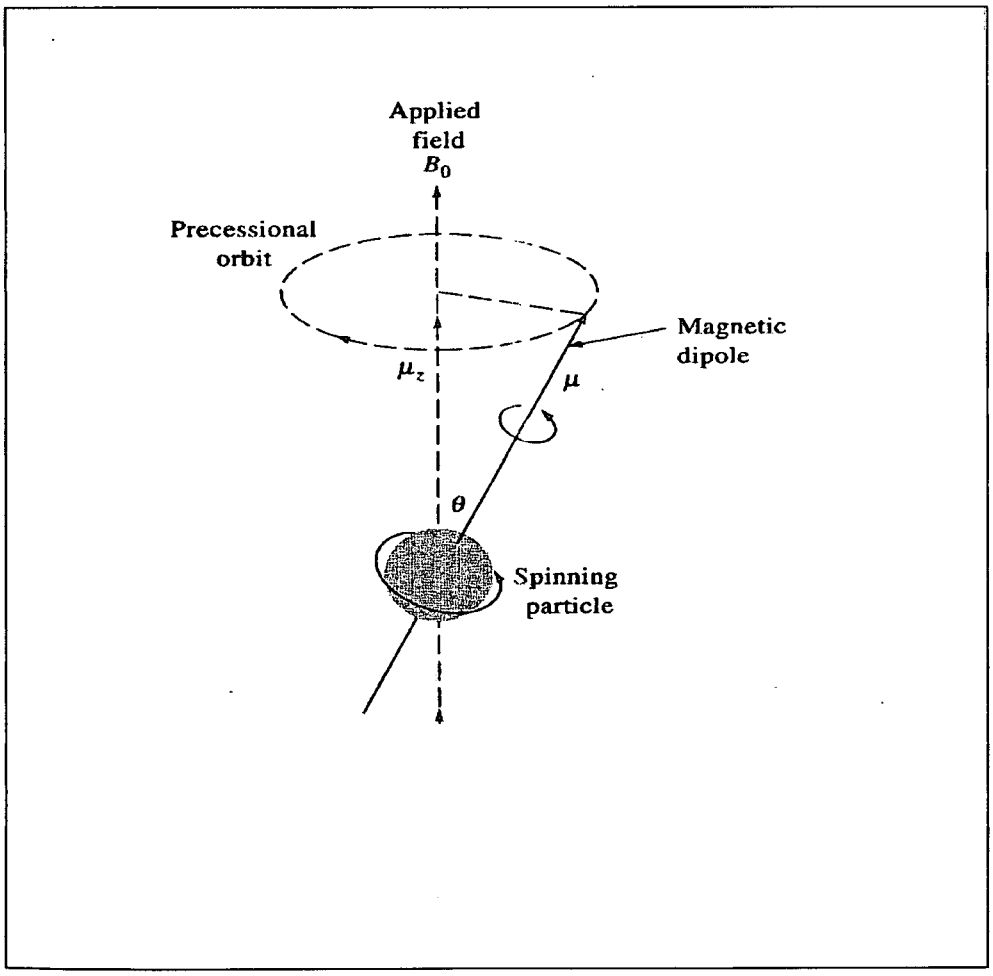


Figure 2.1 *NMR Spectroscopy. Precession of a rotating particle in a magnetic field*

The most important consequence of the extraordinary sharpness of nuclear magnetic resonance lines in liquids is the possibility of measuring the chemical shifts i.e. the

separation between NMR lines from nuclear spins of the same species, but in different molecular environments.

The physical origin of chemical shifts is as follows. An external magnetic field polarizes the closed electron shells of the atoms, and produces a small magnetic field proportional to the external magnetic field. This shifts the NMR line with respect to its position for the bare nucleus, i.e. one that is devoid of electrons. The bare nucleus itself is never observed. The atomic diamagnetic shifts that correspond to atoms located in different molecular sites are slightly different, and it is their differences that produce the chemical shifts. As an example, the proton NMR spectrum of ethanol, with the formula CH_3-CH_2-OH, exhibits three peaks, with relative weights or intensities of 3:2:1. In more complicated molecules such spectra contain much chemical information and can help in the determination of unknown molecular structures.

2.5.2 Fourier NMR Spectrometer

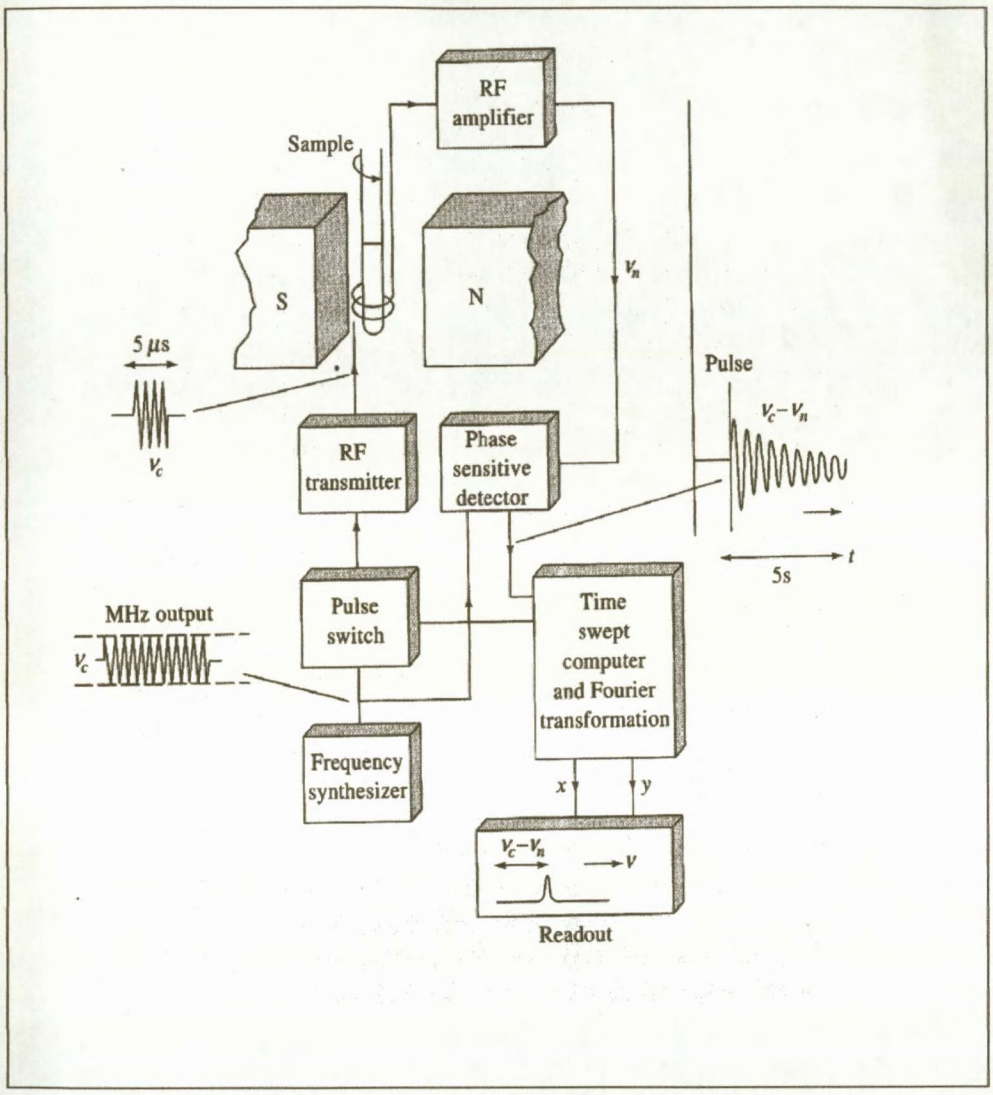


Figure 2.2 *NMR Spectroscopy. Block Diagram of a Fourier NMR Spectrometer*

High-resolution nuclear magnetic resonance has become one of the most prized tools in the fields of organic chemistry and biochemistry. On the experimental side, the requirements to be met by the equipment are severe. In order to match natural line widths of a fraction of a cycle, the applied magnetic fields must have a relative stability and homogeneity throughout the sample better than one part in 100 million (Skoog *et al* 1998:445-476).

2.5.3 Example of NMR Spectrum for Sulphur 6CH

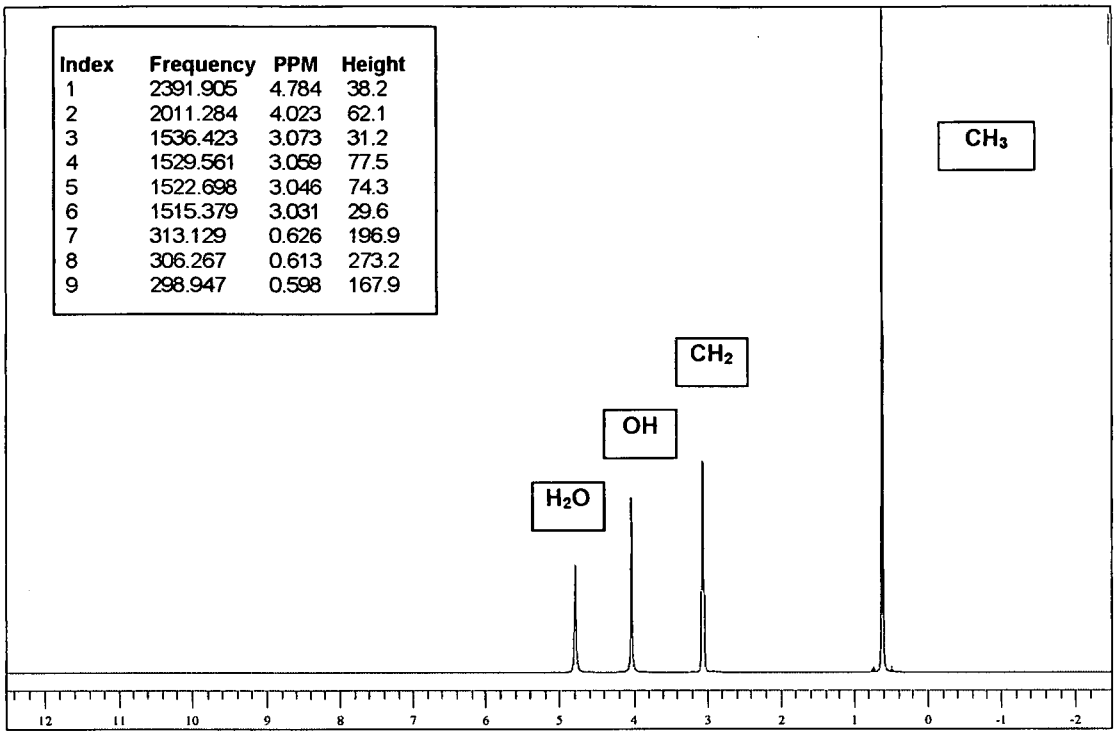


Figure 2.3 *NMR Spectrum of Sulphur 6CH.*

Peaks from left to right:

- 1st H_2O
- 2nd OH
- 3rd CH_2
- 4th CH_3

Table 2.3 *NMR Spectrum. Table of peak indices for Sulphur 6CH. PPM values are used for Chemical Shift calculations.*

Peaks		Index	Frequency	PPM	Height
1st	H_2O	1	2391.905	4.784	38.2
2nd	OH	2	2011.284	4.023	62.1
3rd	CH_2	3	1536.423	3.073	31.2
		4	1529.561	3.059	77.5
		5	1522.698	3.046	74.3
		6	1515.379	3.031	29.6
4th	CH_3	7	313.129	0.626	196.9
		8	306.267	0.613	273.2
		9	298.947	0.598	167.9

Each peak is made up of one or more peak indices.

PPM values = chemical shift

Calc.: Chemical Shift per peak:
(Sum of peak's PPM indices)
÷
(no. of indices for the peak)

Relative Integration is calculated from the integration values that are indicated below each peak on the laboratory printed NMR spectra (see **3.4** for calculation; **Appendix B**).

2.6 NMR Research in Homoeopathy

A great demand has developed over the last century for objective instrumental analysis of homoeopathic remedies. NMR spectroscopy analysis has made a major contribution to this end over the last thirty-five years. Smith and Boericke (1966) analysed various potencies of homoeopathic Sulphur. They concluded that significant differences were indicated in the integration values of the OH groups when compared to the other peaks of a water ethanol solution. It was surmised that these indicated differences in the solvent structure of the homoeopathic remedy when compared to unsuccussed controls.

Sacks (1983) used NMR spectroscopy to analyse various homoeopathic remedies and also to compare 16 different potencies of Sulphur. Distinct differences were noticed between succussed and diluted homoeopathic remedies and their controls. It is interesting to note that both decimal and centesimal potencies of Sulphur were analysed, and yet the correlations of the concentration and numbers of succussions were not given specific attention. Once again differences in the OH groups were most notable.

Weingartner (1990) demonstrated differences in the intensity of the H_2O and OH signals of Sulphur 23D whereas the signals of the CH_2 and CH_3 peaks did not vary significantly. He suggested that the lowering of the NMR spectrum peaks indicated accelerated proton exchange. His probability value of > 99.9% indicated a possible flaw in his research methodology.

Another area of NMR spectroscopy analysis relates to proton relaxation time. Demangeat *et al* (1992) analysed the proton relaxation time T_1 and the ratio T_1/T_2 in saline solutions of a silica/lactose vortexed remedy, versus pure saline solvent and found

statistically significant values for $p < 0.034$ (T_1) and $p < 0.018$ (T_1/T_2). They suggested that the results could be due to the interaction of solutes with water molecules through hydrogen bonding, and electrostatic forces linked to their dipolar moment.

Ross (1997) analysed the NMR spectra of LM potencies of Sulphur compared to a lactose-based control. Statistically significant differences were found between parallel potencies and between potencies within the Sulphur and lactose based control groups. The most significant differences were noticed between Sulphur LM10 and the control, and it was therefore suggested that higher LM potencies be investigated as they may elucidate the differences.

This was taken up by Power (1999) who analysed LM6, LM14 and LM22 of Tin and Lead, using both potentised and unpotentised controls. Both chemical shift and relative integration values showed significant differences between substance and control and substance versus substance. No differences were found within the control groups or between control groups.

An NMR analysis of the comparison between NaCl Hahnemannian and Korsakovian potencies (9C, 30C and 200C) and their respective controls, was done by Davies (2001). He found statistically significant differences with relation to the chemical shift values between these two potency types, but not significant results when comparing relative integration values.

By using three different NMR spectrometers (80 MHz, 200 MHz and 500 MHz respectively), Cason (2002) analysed the differences between the spectra derived from the three spectrometer frequencies for Sulphur and a water-ethanol control. Comparison of

the spectra at parallel frequency strengths revealed no significant differences between the Sulphur and control. She postulated that differences in chemical shift values are unlikely to be a reflection of physico-chemical differences present prior to measurement, but are rather a result of an aspect of the analytical procedure. A variation in chemical shift values is likely to be the result of a change in temperature during the NMR spectroscopy analysis. The most significant difference between the NMR spectrometers, besides their frequencies, is the fluctuation in temperature during measurements. Modern instruments with superior technology will ensure more accurate chemical shift and relative integration values, and more stable temperature control. Therefore, the more modern instruments are recommended to analyse homoeopathic remedies.

Aabel *et al* (2001) conducted NMR research on the proton relaxation times T_1 of Sulphur 4D to 30D at frequencies of 300 and 500 MHz and also of Betula alba 30C at 20 MHz and only found significant differences in Sulphur 4D, seemingly as a result of the high concentration of dissolve material. This was in an attempt to reproduce results that had previously been claimed. A criticism of this experiment is that the substances were potentised in plastic containers, therefore not following the normal procedures of homoeopathic pharmacy.

Milgrom *et al* (2001) attempted to reproduce the results of Conte *et al* (1996) whereby the low resolution NMR T_2 spin-spin relaxation times of homoeopathic Nitric acid were analysed using similar experimental protocols and instrumentation. They failed to reproduce the results, and ascribed the previous results to the use of the low quality soda glass NMR tubes.

Demangeat *et al* (2001) caution that NMR is a complex technique, fraught with experimental pitfalls. Furthermore, that the homoeopathic medical community has believed that it was slowly accumulating physical results that verify the existence and character of homoeopathic remedies. However, this cannot be done until a basic methodology for NMR spectroscopy analysis of homoeopathic remedies is established.

2.7 Variables in the Process of Homoeopathic Manufacture

The process of standardization within homoeopathic pharmacy has a long way to go. Several components of the process of the manufacturing and dispensing of homoeopathic remedies need to be standardised. This does not and cannot exclude all variables. To begin with, it is important that the raw materials used are standardised as far as possible. This is more easily achieved with minerals than with plant or animal substances, as the quality of living material is greatly affected by the environment and conditions in which it grew and developed.

Even with standardisation it is extremely difficult to control and eliminate all forms of contamination. As we now know, environments previously thought to be sterile are not. Certain microbes can survive and even grow in the most extreme conditions. Postgate (1994) describes two such cases, where human breath in the one instance, and the evaporation of alcohol in another, served as 'bacterial food'. Postgate describes an incident where bacteria grew on a tap where alcohol vapour had been diffusing towards the tap, dissolving in each drop of water. This enabled the bacteria to multiply. Another source of volatile bacterial food is human sweat. The stale smell of sweat is compounded in part by ethyl and butyl alcohols, acetone and chemicals called isoprene and toluene; the first three can be 'eaten' by many kinds of bacteria, as well as some micro-fungi. All three dissolve

readily in water. Exhaled breath can contain several of these substances, too. Art galleries and museums, as well as sites of prehistoric paintings, often suffer the bad effects of the exhaled breaths and perspiration of their visitors.

Further variables are differences in atmospheric conditions. To regulate atmospheric conditions such as atmospheric gas composition, room temperature and atmospheric pressures, as well as exposure to certain levels of electromagnetic radiation, can be very expensive. Unfortunately, such expense is outside the budgets of ordinary homoeopathic remedy manufacturers.

2.7.1 Variables in Trituration

The process of trituration is essential to homoeopathic pharmacy, as many solid or insoluble substances have to be triturated to a certain deconcentration level before they can be dissolved. Thereafter, further potentisation by serial dilution and succussion is needed. According to Dellmour (1994), after 1835, Hahnemann produced all his remedies, whether from soluble or insoluble base substances, by the process of trituration of the base substance in dry lactose powder, with each potency level being grinded for one hour.

Trituration is mostly done by hand. This introduces the following variables:

- Variation in the pressure exerted by the mortar on the lactose powder between various people, and changes of the pressure exerted by each person as they tire.
- Variation in the speed of grinding between people, and by each person during the course of each trituration.
- Variation in the symmetry of grinding motions by various people, and by each person during the process.

- Differences between people in the method used to scrape down the lactose powder, and variations in their speed and intensity.
- When grinding by hand, the person breathes on the substance being triturated. The volume and composition of exhaled breath will differ from person to person.
- Lactose powder readily absorbs moisture and other substances. This may contaminate the lactose powder.

2.7.2 Variables in Succussion

Succussion of liquid substances is probably the most commonly used mechanism in homoeopathic pharmacy. Even where potency scales differ, succussion usually takes place, although there may be disagreement as to how many times something is succussed. Therefore succussion can vary from 2 blows to 100, or many more.

Even when following the standard guidelines for succussion, the following variables are present:

- Variation in the rate of leaching of sodium ions into the preparation, depending on the type and shape of soda glass bottle and the force of succussion. Lessell (1994) mentions that most potencies are prepared in soda glass, although he does later mention that this may be an advantage due to the protective layer formed by the leaching sodium.
- The force of succussion may vary between people or as each person tires.
- The speed of succussion may vary between people or as each person tires or becomes hurried or lazy.
- The method of holding the bottle, and therefore the temperature conducted from the hand to the bottle, and the effect of the succussion blow because of the way the bottle is held, is likely to vary from person to person.

- The movement of the hand, its course during acceleration, and impact and rebound will vary from person to person.
- The time lapse between succussions may vary.
- The consistency of the material onto which the succussive blow strikes is not prescribed adequately and may also create variances.

2.8 Variables in the Process of NMR Sampling

NMR Spectroscopy is a sensitive analysis of the chemical and structural properties of a substance. It is also sensitive to the effects of contaminants and other external environmental influences. Some of these environmental influences include:

- Atmospheric pressure changes
- Atmospheric temperature changes
- Electromagnetic radiation
- Composition of the atmospheric gasses

Contaminants can be introduced during the process of taking multiple samples of a single substance, as a result of:

- Exposure to the atmosphere when opening and closing the bottle repeatedly
- The process of cleaning the NMR tubes between samples
- Conditions of storage of substances between sampling

As the period of time increases over which multiple samples are taken, the likelihood of contamination also increases significantly. These influences probably occur randomly, and therefore cannot be seen as a linear trend when taking samples in a rotating, overlapping manner.

2.9 Summary

The problem of manufacturing homoeopathic remedies, and then testing them through NMR spectroscopy, is that there are many variables involved in both processes. If all the variables during the process of manufacture can be kept constant across the samples, we can at least analyse similar differences in each sample. The significant problem arises that the time lapse over which multiple samples are taken during NMR spectroscopy may exaggerate the effect of variables that are not identified and more importantly not quantified.

For practical reasons, and in normal homoeopathic practice, the usual guidelines from the various homoeopathic pharmacopoeias can be followed. However, when we wish to carry out a valid scientific evaluation of the effects of the process of homoeopathic manufacture, it becomes apparent that very strict guidelines will have to be followed so that reasonable conclusions can be drawn.

Chapter 3 – Materials and Methods

3.1 Production of Sample Potencies

14 sample substances were produced and tested, 7 Sulphur remedies and 7 Water-Ethanol Controls. (CH: 6, 12, 24, 48 and DH: 12, 24, 48 potencies). The detailed materials and methodology is given in **Appendix A**.

Trituration and Dilution

- For dilution the ethanol and distilled water were of standard purity.
- For trituration the lactose powder and Flowers of Sulphur were of the standardised purity as supplied and used by the Homoeopathic pharamceutic laboratory of D.I.T.
- All equipment was autoclaved and sterilised before use.
- All potencies were prepared in a Labaire laminar flow unit at a constant pressure.

Numbers of Succussions

The procedure adopted for this study were those used in previous MNR studies of homoeopathic Sulphur (Cason, 2002), and are as follows:

- 10 succussion were applied to each intermediate potency level
- 100 succussions were applied to each of the 6, 12, 24, and 48 potency levels

Although the 6DH Sulphur and Water-Ethanol substances were not included in this study, they were succussed 100 times rather than 10 times as would normally be the case for intermediate potencies. This was necessary for the comparisons using equal numbers of succussions.

3.1.1 Sulphur Remedies

Sulphur homoeopathic potencies were triturated and diluted according to the German Homoeopathic Pharmacopoeia (1985: 22-24) with modifications as explained below.

Method 6: Triturations.

This method was followed. However, substances were triturated up to and including the 3rd potency rather than the prescribed 4th (4CH or DH), as the potencies required for this study had to include the 6th (6CH or DH) potency. Therefore, for this study, before 87% alcohol dilution, the lactose triturate had to be dissolved in distilled water (4CH or DH level), and then in 30% alcohol (5CH or DH level). 87% alcohol dilution was made for the 6th potency.

Method 8a: Liquid preparations made from triturations.

This method was followed. However, Method 8a prescribes the use of 43% alcohol. This was replaced by 87% alcohol and 13% water, as this was the standard used in the previous NMR experiments.

3.1.2 Water-Ethanol Controls

The Control (87% Water-Ethanol) was produced by the same method of serial dilution and succussion as the Sulphur remedies, without adding the triturated lactose and Flowers of Sulphur. As this study compares equal deconcentrations and equal numbers of succussions for both Sulphur remedies and the Water-Ethanol Controls, the following was done:

- 1 drop of 87% Water-Ethanol was diluted with distilled water and 30% alcohol to equal the triturate dilutions for both CH and DH potencies.
- Up to and including the 3rd potencies, no succussions were applied in order to equal the number of succussions applied to the Sulphur remedies.

3.2 Preparation of Sample Potencies for Analysis

The sample potencies required for this study were produced as detailed in **Appendix A**:

- Volumes of 20 ml of 87% alcohol were succussed 100 times in a 50 ml amber glass screw-top bottle and labelled as the sample potency. Although the 5ml bottles are more commonly used for remedy preparation, 50ml bottles were used as a larger volume is required for NMR analysis (Davies, 2001).
- The seven Hahnemannian potencies (6CH, 12DH, 12CH and 24DH, 24CH and 48DH, 48CH) and their 7 related Water-Ethanol Controls were labelled as such, wrapped in soft tissue paper, placed in a secure cardboard box and transported to the Chemistry Department of the University of Natal Pietermaritzburg by car. There they were handed to Mr. Grimmer for NMR spectroscopy analysis.

3.3 NMR Measurement of the Samples

One sample was drawn from each remedy and control substance submitted. Mr. C. Grimmer, the laboratory technician, drew the samples, and ran the NMR spectra.

Multiple samples of each substance were not drawn as this introduces unspecified and unquantified variables. Even if samples are drawn in an overlapping cyclical order, the external variables are not constant and linear over time, and therefore cannot be seen as a linear correlation pattern.

The most accurate NMR spectra are obtained from one sample with sufficient transients per run (in this instance 16). Therefore each spectrum is the result of 16 measurements (Grimmer, 2002).

The instrument used was a Varian 500 MHz INOVA Spectrometer:

- It operated at a frequency of 499.9832268 MHz.
- The magnet was shimmed before each run to ensure a homogenous field around each sample before it was tested.
- The acquisition time was 1.9 seconds times 16 transients per run.
- The pulse angle was set at 20.4 degrees.
- The temperature was maintained at 24 degrees ± 0.5 degrees C.

The sample volumes of 600 µl were drawn by micropipette and injected into a coaxial tube. Acetone was used as both the external lock and reference substance, as it provides a very reliable chemical shift value outside of the range of the other peaks (Grimmer, 2002).

Data was recorded in the form of NMR spectra indicating the chemical shifts and integration values.

3.4 Statistical Analysis

i) Chemical shift and relative integration values of H_2O, OH, CH_2 and CH_3, peaks of the NMR spectra were recorded and calculated to six decimal places.

- The chemical shift values of peaks with multiple splitting were averaged to give a single value for each peak.
- Relative integration values were calculated by dividing the integration values of each peak by the sum of all integration values for the run.
- Only H_2O, OH and CH_2 values were used for the subsequent statistical analysis.

ii) For analysis of both the Sulphur remedies and the Controls

- Two potentising methods were used, viz. centesimal (C) and decimal (D).

- The Sulphur groups were compared to the Control groups
- Comparative deconcentration groups were compared.
- Comparative number of succussion groups were compared.

iii) Data was transferred to a Microsoft Excel© 2000 and analysed with SPSS© Base 10.0. This data underwent statistical evaluation according to the Mann-Whitney U- test and the Independent Sample Unpaired T-Test.

3.4.1 The Mann-Whitney U-Test

The Mann-Whitney U-Test evaluates samples to see if two independent random samples come from two populations with the same median. It was therefore used to compare methods in order to find out where actual differences lie.

The hypotheses therefore are stated as:

- H_0 : The two independent population groups have identical medians.
- H_1 : The two independent population groups have different medians.

The Mann-Whitney U-Test is a nonparametric alternative to the Independent-samples T-test. Rather than being based on parameters of a normal distribution like mean and variance, the Mann-Whitney statistics are based on ranks.

The equation for the Mann-Whitney U is:

$$U = N_1N_2 + [\, N_1(N_1+1)]/2 - T_1$$

Where N_1 and N_2 are the sample sizes of the two groups, and T_1 is the sum of the ranks of one of the samples. The level of significance is set at 0.05 ($p \leq 0.05$).

3.4.2 The Two-sample Unpaired T-Test

The two-sample unpaired t-test is used to compare two unpaired or independent samples X and Y, where both X and Y are random samples drawn from respective parent populations having normal distributions and respective means μ_1 and μ_2 and variances σ_1^2 and σ_2^2. The two samples are independent of each other and have a common unknown variance σ^2.

If these assumptions have been satisfied, the equality of the two population means are tested as follows:

H_0: $\mu_1 = \mu_2$ (null hypothesis)

H_1: $\mu_1 \neq \mu_2$ (alternative hypothesis)

α = level of significance of test = 0.05

- The H_0 is rejected if the absolute value of the calculated t-statistic (t_{cal}) is greater than the tabulated t-value (t_{tab}).

- If the absolute value of t_{cal} is less than or equal to t_{tab} the H_0 is accepted (i.e. $\mu_1 = \mu_2$).

The calculated and tabulated t-values are given as follows:

$$t_{cal} = \frac{\bar{X} - \bar{Y}}{S_p \left(1/n_1 + 1/n_2 \right)^{1/2}}$$

$$\text{where } S_p^2 = \frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2}$$

= the pooled error variance;

t_{tab} = $t(df)_{\alpha/2}$ where

df = $n_1 + n_2 - 2$ = degrees of freedom of the t-statistic

α = level of significance of the test

The values of t_{tab} are read from the t-distribution table.

The unpaired t-tests were run at the 95% ($\alpha = 0.05$) level of significance. Confidence intervals for $\mu_1 - \mu_2$ were calculated according to the formula:

$$\mu_1 - \mu_2 \in [(\bar{X} - \bar{Y}) - L , (\bar{X} - \bar{Y}) + L]$$

where L is the margin of error as given below:

$$L = t_{tab}.S_p.(1/n_1 + 1/n_2)^{1/2}$$

If the 95% confidence interval for $\mu_1 - \mu_2$ contained 0, and / or the p-value < α [0.05] the H_0 was accepted (i.e. $\mu_1 = \mu_2$). Otherwise H_0 was rejected at the same level.

(Ross 1997: 27-28)

Chapter Four – Results

Chapter Contents

a) Discussion

Three different groups of comparison were done i.e. i) Substance (Sulphur) versus Control (Water-Ethanol), ii) equal levels of deconcentration for CH and DH potencies and iii) equal numbers of succussions for CH and DH potencies. Therefore the levels of comparison that had to be done were threefold.

As a result, Chapter 4 is quite lengthy. Included in this chapter are separate tables (therefore pages) for each level of comparison per type of analysis done:

- Mann-Whitney U-Test: 10 levels of comparison
- T-Test: 10 levels of comparison
- NMR Spectroscopy: 6 levels of comparison

The same data from the NMR spectra (see **Appendix B**) was input for the Mann-Whitney and T-Test analyses, and was used to generate the bar charts for the NMR analysis. The raw data is given in **Appendix D**.

Statistical Analyses

The results of the T-tests support those of the Mann-Whitney U-tests, except for the CH_2 peaks that were found significant by the Mann-Whitney U-Test.

As regards the acceptance or rejection of the null hypothesis the results of the tests are as shown below. (See **Appendix C** for tables of all p-values).

NULL HYPOTHESIS ACCEPTED

p-values for peaks: greater than 0.05

Comparison Group	Comparison Type	M-W Refs.	T-T Refs.
Sulphur vs. Controls	Chemical Shift	4.2.1.1	4.3.1.1
	Relative Integration	4.2.1.2	4.3.1.2
	Chemical Shift		
Sulphur C vs. Sulphur D	Equal deconcentrations	4.2.2.1	4.3.2.1
	Equal succussions	4.2.2.2	4.3.2.2
Control C vs. Control D	Equal deconcentrations	4.2.2.3	4.3.2.3
	Equal succussions	4.2.2.4	4.3.2.4

(M-W = Mann-Whitney U-Test; T-T = T-Test)

NULL HYPOTHESIS REJECTED

p-values for various peaks: equal to or less than 0.05

Comparison Group	Comparison Type	M-W	T-T
	Relative Integration		
Sulphur C vs. Sulphur D	Equal deconcentrations	4.2.3.1	4.3.3.1
	Equal succussions	4.2.3.2	4.3.3.2
Control C vs. Control D	Equal deconcentrations	4.2.3.3	4.3.3.3
	Equal succussions	4.2.3.4	4.3.3.4

(M-W = Mann-Whitney U-Test; T-T = T-Test)

Levels of comments and discussions are structured as follows:

- At each level of comparison (i.e. per table), the significance of the p-values is noted.
- Summary comments are given at the intermediary levels:

  4.2.1 (Mann-Whitney); **4.3.1** (T-Test)

  4.2.2 (Mann-Whitney); **4.3.2** (T-Test)

  4.2.3 (Mann-Whitney); **4.3.3** (T-Test)

- An overall p-value significance per group of comparisons is given as a discussion:

  4.2 (Mann-Whitney); **4.3** (T-Test)

NMR Laboratory Data

A detailed scientific interpretation of NMR output falls outside of this study. However, by examining the graphs some interesting patterns for CH and DH potencies are apparent. (See **4.4**)

b) Analyses and Test Criteria

i) 2 Substances:

- Sulphur Remedies: Sulphur, Lactose, Water-Ethanol 13% : 87%
- Controls: Water-Ethanol 13% : 87%

Potencies prepared for each:

- 4 × C potencies 6, 12, 24, 48
- 3 × D potencies - 12, 24, 48

- Total of 14 samples: 7 Sulphur + 7 Water-Ethanol samples

ii) Analysis of 14 samples by:

- Chemical Shift
- Relative Integration

For the 3 Peaks:

- H_2O, OH and CH_2

iii) Methods used for the analysis:

- Mann-Whitney U-Test: Statistical Analyses
  - (× 10)
- T-Test: Statistical Analyses
  - Group Statistics and Equality of Means (× 10)
- NMR Spectroscopy: NMR Spectra
  - Spectrum per substance (× 14) (see **Appendix B**)
  - Bar Chart per peak (× 6)

iv) Substance groups used for statistical analyses:

- Sulphur vs. Control
- Equal deconcentrations: Sulphur vs. Sulphur; Control vs. Control
- Equal succussions: Sulphur vs. Sulphur; Control vs. Control

iv) NMR Spectra analyses:

- Sulphur and Controls (separately by peak and potency scale)

4.1 Criteria Governing the Admissibility of Data

a) Sample equivalencies for deconcentration and number of succussions

Sulphur and Controls: Equivalency between CH potencies and DH potencies: (see **2.3**)

Equal deconcentrations	6C=12D; 12C=24D; 24C=48D, 48C no equivalent
Equal Succussions	6C no equivalent; 12C=12D; 24C=24D; 48C=48D

b) Preparation of the Sulphur and Controls

See **2.7, 3.1, Appendix A.**

c) NMR Spectroscopy Analysis

See **2.8, 3.3**

d) Analysis of Data

See **3.4**.

The sample size was too small for a meaningful Intra-Group analysis (see **Chapter 5**).

e) Discussion

The efficacy of Homoeopathic remedies is said to be the result of several physical processes that are applied to the selected substances during their preparation. Within this context it is not possible to exclude all unspecified and unquantified variables, or external factors, that may affect the nature of the Homoeopathic remedy during the process of manufacture, transport, testing and storage.

By preparing and testing multiple samples of the same substance (which by necessity is done over a period of time) one exposes the substances to additional variables, which could have an effect on the results (see **2.7**, **2.8**).

The NMR Spectra were generated from sixteen transients (passes) of each sample, rather than four or eight as in the past analyses. Thus each sample was analysed 16 times. Therefore, the results are already the mean of sixteen transients (see **Appendix B**).

4.2 Statistical Results: Mann-Whitney U-Test

Mann-Whitney U-Test: Main Analyses
4.2.1 Sulphur vs. Controls
4.2.2 Chemical Shift: Sulphur and Controls
4.2.3 Relative Integration: Sulphur and Controls

Discussion on Mann-Whitney U-Test: Statistical Differences

1) Sulphur vs. Control: Chemical Shift and Relative Integration

- There are no significant differences in the comparison of Sulphur vs. Control.

2) Chemical Shift: Sulphur and Controls.

Equal deconcentrations and equal number of succussions:

- There are no significant differences between Sulphur CH versus Sulphur DH potencies.
- There are no significant differences between Control CH versus Control DH potencies.

3) Relative Integration: Sulphur and Controls.

Equal deconcentrations and equal number of succussions:

- The Controls show potentised qualities of CH and DH potencies similar to Sulphur CH and DH.
- The Sulphur groups CH and DH show significant differences in the H_20 peaks.
    - Sulphur: equal deconcentrations: H_20 peaks: p=0.050
    - Sulphur: equal number of succussions: H_20 peaks: p=0.050
- The Control groups CH and DH shows significant differences in the OH and CH_2 peaks.
    - Control: equal deconcentrations: OH peaks: p=0.050; CH_2 peaks: p=0.050
    - Control: equal number of succussions: OH peaks: p=0.050; CH_2 peaks: p=0.050

4.2.1 Sulphur vs. Controls

a) List of Analyses: Mann-Whitney U-Test. Sulphur vs. Control

Remedies:	Sulphur	6C, 12C, 24C, 48C, 12D, 24D, 48D
Controls:	Water-Ethanol	6C, 12C, 24C, 48C, 12D, 24D, 48D

4.2.1.1 Chemical Shift: Sulphur vs. Control
Table 4.1

4.2.1.2 Relative Integration: Sulphur vs. Control
Table 4.2

b) Comments: Mann-Whitney U-Test. Sulphur vs. Control

- Shows no p-values equal to or less than 0.05.
- This indicates that there are no significant differences in the Sulphur vs. Control potencies for either **chemical shift**, or **relative integration** values.

4.2.1.1 Chemical Shift: Sulphur vs. Control

Table 4.1 *Mann-Whitney U-Test. Chemical Shift: Sulphur vs. Control*

Chemical Shift: Ranks				
	Group	N	Mean Rank	Sum of Ranks
H_20	1.00	7	6.43	45.00
	2.00	7	8.57	60.00
	Total	14		
0H	1.00	7	6.57	46.00
	2.00	7	8.43	59.00
	Total	14		
CH_2	1.00	7	5.43	38.00
	2.00	7	9.57	67.00
	Total	14		

Group 1.00
Sulphur: 6C, 12C, 24C, 48C, 12D, 24D, 48D

Group 2.00
Control: 6C, 12C, 24C, 48C, 12D, 24D, 48D

Chemical Shift: Test Statistics (b)			
	H_20	0H	CH_2
Mann-Whitney U	17.000	18.000	10.000
Wilcoxon W	45.000	46.000	38.000
Z	-.966	-.842	-1.938
Asymp. Sig. (2-tailed)	.334	.400	.053
Exact Sig. [2*(1-tailed Sig.)]	.383(a)	.456(a)	.073(a)
a Not corrected for ties			
b Grouping Variable: VAR00001			

Asymp. Sig. (2-tailed): p-values

- Chemical Shift: No p-values equal to or less than 0.05 for H_20, 0H, CH_2
- ∴ Null hypothesis accepted

4.2.1.2 Relative Integration: Sulphur vs. Control

Table 4.2 *Mann-Whitney U-Test. Relative Integration: Sulphur vs. Control*

Relative Integration: Ranks				
	Group	N	Mean Rank	Sum of Ranks
H_20	1.00	7	6.71	47.00
	2.00	7	8.29	58.00
	Total	14		
0H	1.00	7	8.29	58.00
	2.00	7	6.71	47.00
	Total	14		
CH_2	1.00	7	6.71	47.00
	2.00	7	8.29	58.00
	Total	14		

Group 1.00
Sulphur: 6C, 12C, 24C, 48C, 12D, 24D, 48D

Group 2.00
Control: 6C, 12C, 24C, 48C, 12D, 24D, 48D

Relative Integration: Test Statistics (b)			
	H_20	0H	CH_2
Mann-Whitney U	19.000	19.000	19.000
Wilcoxon W	47.000	47.000	47.000
Z	-.703	-.703	-.703
Asymp. Sig. (2-tailed)	.482	.482	.482
Exact Sig. [2*(1-tailed Sig.)]	.535(a)	.535(a)	.535(a)
a Not corrected for ties			
b Grouping Variable: VAR00001			

Asymp. Sig. (2-tailed): p-values

- Relative Integration: No p-values equal to or less than 0.05 for H_20, 0H, CH_2
- ∴ Null hypothesis accepted

4.2.2 Chemical Shift: Sulphur and Controls

a) List of Analyses: Mann-Whitney U-Test. Chemical Shift: Sulphur and Controls

4.2.2.1 Sulphur (C) vs. Sulphur (D): Equal Deconcentrations
Table 4.3
Remedies: Sulphur (C) 6, 12, 24
Remedies: Sulphur (D) 12, 24, 48

4.2.2.3 Sulphur (C) vs. Sulphur (D): Equal Succussions
Table 4.4
Remedies: Sulphur (C) 12, 24, 48
Remedies: Sulphur (D) 12, 24, 48

4.2.2.3 Control (C) vs. Control (D): Equal Deconcentrations
Table 4.5
Controls: Control (C) 6, 12, 24
Controls: Control (D) 12, 24, 48

4.2.2.4 Control (C) vs. Control (D): Equal Succussions
Table 4.6
Controls: Control (C) 12, 24, 48
Controls: Control (D) 12, 24, 48

b) Comments: The Mann-Whitney U-Test. Chemical Shift. Sulphur and Controls

Equal deconcentrations and equal numbers of succussions:

- Shows no significant difference in **chemical shifts** when comparing Sulphur CH and Sulphur DH potencies.
- Shows no significant difference in **chemical shifts** when comparing Controls of CH and Controls of DH potencies.

4.2.2.1 Sulphur (C) vs. Sulphur (D): Equal Deconcentrations

Table 4.3 *Mann-Whitney U-Test. Chemical Shift. Equal deconcentrations: Sulphur C: 6, 12, 24 vs. Sulphur D: 12, 24, 48*

Chemical Shift: Ranks				
	Group	N	Mean Rank	Sum of Ranks
H_20	1.00	3	2.50	7.50
	2.00	3	4.50	13.50
	Total	6		
0H	1.00	3	2.83	8.50
	2.00	3	4.17	12.50
	Total	6		
CH_2	1.00	3	2.67	8.00
	2.00	3	4.33	13.00
	Total	6		

Group 1.00
Sulphur: 6C, 12C, 24C

Group 2.00
Sulphur: 12D, 24D, 48D

Chemical Shift: Test Statistics (b)			
	H_20	0H	CH_2
Mann-Whitney U	1.500	2.500	2.000
Wilcoxon W	7.500	8.500	8.000
Z	-1.328	-.886	-1.091
Asymp. Sig. (2-tailed)	.184	.376	.275
Exact Sig. [2*(1-tailed Sig.)]	.200(a)	.400(a)	.400(a)
a Not corrected for ties			
b Grouping Variable: VAR00001			

Asymp. Sig. (2-tailed): p-values

- Chemical Shift: No p-values equal to or less than 0.05 for H_20, 0H, CH_2
- ∴ Null hypothesis accepted

4.2.2.2 Sulphur (C) vs. Sulphur (D): Equal Succussions

Table 4.4 *Mann-Whitney U-Test. Chemical Shift. Equal succussions: Sulphur C: 12, 24, 48 vs. Sulphur D: 12, 24, 48*

Chemical Shift: Ranks				
	Group	N	Mean Rank	Sum of Ranks
H_20	1.00	3	3.50	10.50
	2.00	3	3.50	10.50
	Total	6		
0H	1.00	3	3.67	11.00
	2.00	3	3.33	10.00
	Total	6		
CH_2	1.00	3	3.17	9.50
	2.00	3	3.83	11.50
	Total	6		

Group 1.00
Sulphur: 12C, 24C, 48C

Group 2.00
Sulphur: 12D, 24D, 48D

Chemical Shift: Test Statistics (b)			
	H_20	0H	CH_2
Mann-Whitney U	4.500	4.000	3.500
Wilcoxon W	10.500	10.000	9.500
Z	.000	-.218	-.443
Asymp. Sig. (2-tailed)	1.000	.827	.658
Exact Sig. [2*(1-tailed Sig.)]	1.000(a)	1.000(a)	.700(a)
a Not corrected for ties			
b Grouping Variable: VAR00001			

Asymp. Sig. (2-tailed): p-values

- Chemical shift: No p-values equal to or less than 0.05 for H_20, 0H, CH_2
- ∴ Null hypothesis accepted

4.2.2.3 Control (C) vs. Control (D): Equal Deconcentrations

Table 4.5 *Mann-Whitney U-Test. Chemical Shift. Equal deconcentrations: Control C: 6, 12, 24 vs. Control D: 12, 24, 48*

Chemical Shift: Ranks				
	Group	N	Mean Rank	Sum of Ranks
H_20	1.00	3	2.17	6.50
	2.00	3	4.83	14.50
	Total	6		
0H	1.00	3	4.67	14.00
	2.00	3	2.33	7.00
	Total	6		
CH_2	1.00	3	2.50	7.50
	2.00	3	4.50	13.50
	Total	6		

Group 1.00
Water-Ethanol: 6C, 12C, 24C

Group 2.00
Water-Ethanol: 12D, 24D, 48D

Chemical Shift: Test Statistics (b)			
	H_20	0H	CH_2
Mann-Whitney U	.500	1.000	1.500
Wilcoxon W	6.500	7.000	7.500
Z	-1.826	-1.623	-1.581
Asymp. Sig. (2-tailed)	.068	.105	.114
Exact Sig. [2*(1-tailed Sig.)]	.100(a)	.200(a)	.200(a)
a Not corrected for ties			
b Grouping Variable: VAR00001			

Asymp. Sig. (2-tailed): p-values

- Chemical Shift: No p-values equal to or less than 0.05 for H_20, 0H, CH_2
- ∴ Null hypothesis accepted

4.2.2.4 Control (C) vs. Control (D): Equal Succussions

Table 4.6 *Mann-Whitney U-Test. Chemical Shift. Equal succussions: Control C: 12, 24, 48 vs. Control D: 12, 24, 48*

Chemical Shift: Ranks				
	Group	N	Mean Rank	Sum of Ranks
H_20	1.00	3	2.50	7.50
	2.00	3	4.50	13.50
	Total	6		
0H	1.00	3	4.83	14.50
	2.00	3	2.17	6.50
	Total	6		
CH_2	1.00	3	2.67	8.00
	2.00	3	4.33	13.00
	Total	6		

Group 1.00
Water-Ethanol: 12C, 24C, 48C

Group 2.00
Water-Ethanol: 12D, 24D, 48D

Chemical Shift: Test Statistics (b)			
	H_20	0H	CH_2
Mann-Whitney U	1.500	.500	2.000
Wilcoxon W	7.500	6.500	8.000
Z	-1.348	-1.771	-1.179
Asymp. Sig. (2-tailed)	.178	.077	.239
Exact Sig. [2*(1-tailed Sig.)]	.200(a)	.100(a)	.400(a)
a Not corrected for ties			
b Grouping Variable: VAR00001			

Asymp. Sig. (2-tailed): p-values

- Chemical Shift: No p-values equal to or less than 0.05 for H_20, 0H, CH_2
- ∴ Null hypothesis accepted

4.2.3 Relative Integration: Sulphur and Controls

a) List of Analyses: Mann-Whitney U-Test. Relative Integration. Sulphur and Controls

4.2.3.1 Sulphur (C) vs. Sulphur (D): Equal Deconcentrations
Table 4.7
Remedies: Sulphur (C) 6, 12, 24
Remedies: Sulphur (D) 12, 24, 48

4.2.3.2 Sulphur (C) vs. Sulphur (D): Equal Succussions
Table 4.8
Remedies: Sulphur (C) 12, 24, 48
Remedies: Sulphur (D) 12, 24, 48

4.2.3.3 Control (C) vs. Control (D): Equal Deconcentrations
Table 4.9
Controls: Control (C) 6, 12, 24
Controls: Control (D) 12, 24, 48

4.2.3.4 Control (C) vs. Control (D): Equal Succussions
Table 4.10
Controls: Control (C) 12, 24, 48
Controls: Control (D) 12, 24, 48

b) Comments: Mann-Whitney U-Test. Relative Integration. Sulphur and Controls

Equal deconcentrations and equal number of succussions:

- Shows significant differences in **relative integration** of the H_20 peaks when comparing Sulphur CH and Sulphur DH potencies. The significant differences in the H_20 peaks are:
  - Sulphur: equal deconcentrations: H_20 peaks: p=0.050
  - Sulphur: equal number of succussions: H_20 peaks: p=0.050
- Shows significant differences in **relative integration** of the OH and CH_2 peaks when comparing Controls of CH and Controls of DH potencies. The significant differences in the OH and CH_2 peaks are:
  - Control: equal deconcentrations: OH peaks: p=0.050; CH_2 peaks: p=0.050
  - Control: equal number of succussions: OH peaks: p=0.050; CH_2 peaks: p=0.050

4.2.3.1 Sulphur (C) vs. Sulphur (D): Equal Deconcentrations

Table 4.7 *Mann-Whitney U-Test. Relative Integration. Equal deconcentrations: Sulphur C: 6, 12, 24 vs. Sulphur D: 12, 24, 48*

Relative Integration: Ranks				
	Group	N	Mean Rank	Sum of Ranks
H_20	1.00	3	5.00	15.00
	2.00	3	2.00	6.00
	Total	6		
0H	1.00	3	4.00	12.00
	2.00	3	3.00	9.00
	Total	6		
CH_2	1.00	3	3.67	11.00
	2.00	3	3.33	10.00
	Total	6		

Group 1.00
Sulphur: 6C, 12C, 24C

Group 2.00
Sulphur: 12D, 24D, 48D

Relative Integration: Test Statistics (b)			
	H_20	0H	CH_2
Mann-Whitney U	.000	3.000	4.000
Wilcoxon W	6.000	9.000	10.000
Z	-1.964	-.655	-.218
Asymp. Sig. (2-tailed)	.050	513	827
Exact Sig. [2*(1-tailed Sig.)]	.100(a)	.700(a)	1.000(a)
a Not corrected for ties			
b Grouping Variable: VAR00001			

Asymp. Sig. (2-tailed): p-values

- Relative Integration: p-value equal to 0.05 for H_20
- ∴ Null hypothesis rejected

4.2.3.2 Sulphur (C) vs. Sulphur (D): Equal Succussions

Table 4.8 *Mann-Whitney U-Test. Relative Integration. Equal succussions: Sulphur C: 12, 24, 48 vs. Sulphur D: 12, 24, 48*

Relative Integration: Ranks				
	Group	N	Mean Rank	Sum of Ranks
H_20	1.00	3	5.00	15.00
	2.00	3	2.00	6.00
	Total	6		
0H	1.00	3	3.00	9.00
	2.00	3	4.00	12.00
	Total	6		
CH_2	1.00	3	4.33	13.00
	2.00	3	2.67	8.00
	Total	6		

Group 1.00
Sulphur: 12C, 24C, 48C

Group 2.00
Sulphur: 12D, 24D, 48D

Relative Integration: Test Statistics (b)			
	H_20	0H	CH_2
Mann-Whitney U	.000	3.000	2.000
Wilcoxon W	6.000	9.000	8.000
Z	-1.964	-.655	-1.091
Asymp. Sig. (2-tailed)	.050	513	275
Exact Sig. [2*(1-tailed Sig.)]	.100(a)	.700(a)	.400(a)
a Not corrected for ties			
b Grouping Variable: VAR00001			

Asymp. Sig. (2-tailed): p-values

- Relative Integration: p-value equal to 0.05 for H_20
- ∴ Null hypothesis rejected

4.2.3.3 Control (C) vs. Control (D): Equal Deconcentrations

Table 4.9 *Mann-Whitney U-Test. Relative Integration. Equal deconcentrations: Control C: 6, 12, 24 vs. Control D: 12, 24, 48*

Relative Integration: Ranks				
	Group	N	Mean Rank	Sum of Ranks
H_20	1.00	3	4.00	12.00
	2.00	3	3.00	9.00
	Total	6		
0H	1.00	3	5.00	15.00
	2.00	3	2.00	6.00
	Total	6		
CH_2	1.00	3	2.00	6.00
	2.00	3	5.00	15.00
	Total	6		

Group 1.00
Water-Ethanol: 6C, 12C, 24C

Group 2.00
Water-Ethanol: 12D, 24D, 48D

Relative Integration: Test Statistics (b)			
	H_20	0H	CH_2
Mann-Whitney U	3.000	.000	.000
Wilcoxon W	9.000	6.000	6.000
Z	-.655	-1.964	-1.964
Asymp. Sig. (2-tailed)	.513	**.050**	**.050**
Exact Sig. [2*(1-tailed Sig.)]	.700(a)	.100(a)	.100(a)
a Not corrected for ties			
b Grouping Variable: VAR00001			

Asymp. Sig. (2-tailed): p-values

- Relative Integration: p-values equal to 0.05 for 0H and CH_2
- ∴ Null hypothesis rejected

4.2.3.4 Control (C) vs. Control (D): Equal Succussions

Table 4.10 *Mann-Whitney U-Test. Relative Integration. Equal succussions: Control C: 12, 24, 48 vs. Control D: 12, 24, 48*

Relative Integration: Ranks				
	Group	N	Mean Rank	Sum of Ranks
H_20	1.00	3	3.67	11.00
	2.00	3	3.33	10.00
	Total	6		
0H	1.00	3	5.00	15.00
	2.00	3	2.00	6.00
	Total	6		
CH_2	1.00	3	2.00	6.00
	2.00	3	5.00	15.00
	Total	6		

Group 1.00
Water-Ethanol: 12C, 24C, 48C

Group 2.00
Water-Ethanol: 12D, 24D, 48D

Relative Integration: Test Statistics (b)			
	H_20	0H	CH_2
Mann-Whitney U	4.000	.000	.000
Wilcoxon W	10.000	6.000	6.000
Z	-.218	-1.964	-1.964
Asymp. Sig. (2-tailed)	.827	.050	.050
Exact Sig. [2*(1-tailed Sig.)]	1.000(a)	.100(a)	.100(a)
a Not corrected for ties			
b Grouping Variable: VAR00001			

Asymp. Sig. (2-tailed): p-values

- Relative Integration: p-values equal to 0.05 for 0H and CH_2
- ∴ Null hypothesis rejected

4.3 Statistical Results: T-Test

T-Test: Main Analyses
4.3.1 Sulphur vs. Controls
4.3.2 Chemical Shift: Sulphur and Controls
4.3.3 Relative Integration: Sulphur and Controls

Discussion on T-Tests: Statistical Differences

Except for the CH_2 peaks, the T-Test results confirm those of the Mann Whitney U-Test.

1) Sulphur vs. Control: Chemical Shift and Relative Integration

- There are no significant differences in the comparison of Sulphur vs. Control.

2) Chemical Shift: Sulphur and Controls.

Equal deconcentrations and equal number of succussions:

- There are no significant differences between Sulphur CH and Sulphur DH potencies.
- There are no significant differences between Control CH and Control DH potencies.

3) Relative Integration: Sulphur and Controls.

Equal deconcentrations and equal number of succussions:

- The Controls show potentised qualities of CH and DH potencies similar to Sulphur CH and DH.
- The Sulphur groups CH and DH show significant differences in the H_20 peaks.
    - Sulphur: equal deconcentrations: H_20 peaks: p=0.050
    - Sulphur: equal number of succussions: H_20 peaks: p=0.022
- The Control groups CH and DH shows significant differences in the OH peaks.
    - Control: equal deconcentrations: OH peaks: p=0.001
    - Control: equal number of succussions: OH peaks: p=0.003

4.3.1 Sulphur vs. Controls

a) List of Analyses: T-Test. Sulphur vs. Control

Remedies:	Sulphur	6C, 12C, 24C, 48C, 12D, 24D, 48D
Controls:	Water-Ethanol	6C, 12C, 24C, 48C, 12D, 24D, 48D

4.3.1.1 Chemical Shift: Sulphur vs. Controls
Table 4.11

4.3.1.2 Relative Integration: Sulphur vs. Controls
Table 4.12

b) Comments: T-Test. Sulphur vs. Controls

- Shows no p-values equal to or less than 0.05.
- This indicates that there is no significant difference in the Sulphur vs. Controls for either **chemical shift**, or **relative integration** values.
- It is interesting to note however, that the comparison of the **chemical shifts** of the CH_2 peaks:
    - Shows a p-value of close to 0.05
    - Shows a t-value of 2.147. This is close to the required 2.179, which would indicate a level of significant difference.

4.3.1.1 Chemical Shift: Sulphur vs. Control

Table 4.11 *T-Test. Chemical Shift: Sulphur vs. Control*

Chemical Shift: Group Statistics					
	VAR00001	N	Mean	Std. Deviation	Std. Error Mean
H_20	1.00	7	4.79328571	5.6778601E-03	2.1460294E-03
	2.00	7	4.79642857	1.2724180E-03	4.8092881E-04
0H	1.00	7	4.02642857	3.7352886E-03	1.4118064E-03
	2.00	7	4.02700000	1.2909944E-03	4.8795004E-04
CH_2	1.00	7	3.06028571	4.4239607E-03	1.6721000E-03
	2.00	7	3.06403571	1.3340772E-03	5.0423378E-04

Group 1.00
Sulphur: 6C, 12C, 24C, 48C, 12D, 24D, 48D

Group 2.00
Control: 6C, 12C, 24C, 48C, 12D, 24D, 48D

Chemical Shift: T-Test for Equality of Means								
		t	df	Sig. (2-tailed) p	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
H_20	Equal variances assumed	-1.429	12	.179	-3.14285714E-03	2.1992578E-03	-7.93462817E-03	1.6489139E-03
	Equal variances not assumed	-1.429	6.601	.199	-3.14285714E-03	2.1992578E-03	-8.40765706E-03	2.1219428E-03
0H	Equal variances assumed	-.383	12	.709	-5.71428571E-04	1.4937512E-03	-3.82603276E-03	2.6831756E-03
	Equal variances not assumed	-.383	7.413	.713	-5.71428571E-04	1.4937512E-03	-4.06406020E-03	2.9212031E-03
CH_2	Equal variances assumed	-2.147	12	.053	-3.75000000E-03	1.7464736E-03	-7.55523911E-03	5.5239107E-05
	Equal variances not assumed	-2.147	7.082	.068	-3.75000000E-03	1.7464736E-03	-7.87004639E-03	3.7004639E-04

Asymp. Sig. (2-tailed): p-values

- Chemical Shift: No p-values equal to or less than 0.05 for H_20, 0H, CH_2
- ∴ Null hypothesis accepted

4.3.1.2 Relative Integration: Sulphur vs. Control

Table 4.12 *T-Test. Relative Integration: Sulphur vs. Control*

Relative Integration: Group Statistics					
	VAR00001	N	Mean	Std. Deviation	Std. Error Mean
H_20	1.00	7	.14854386	1.0148434E-03	3.8357475E-04
	2.00	7	.14912443	1.1739570E-03	4.4371403E-04
0H	1.00	7	.15033814	5.6549556E-03	2.1373723E-03
	2.00	7	.14555614	4.7760877E-03	1.8051915E-03
CH_2	1.00	7	.27393371	2.7834747E-03	1.0520545E-03
	2.00	7	.27489129	3.1886133E-03	1.2051825E-03

Group 1.00
Sulphur: 6C, 12C, 24C, 48C, 12D, 24D, 48D

Group 2.00
Control: 6C, 12C, 24C, 48C, 12D, 24D, 48D

Relative Integration: T-Test for Equality of Means								
		t	df	Sig. (2-tailed) p	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
H_20	Equal variances assumed	-.990	12	.342	-5.80571429E-04	5.8652513E-04	-1.85849991E-03	6.9735705E-04
	Equal variances not assumed	-.990	11.754	.342	-5.80571429E-04	5.8652513E-04	-1.86147076E-03	7.0032790E-04
0H	Equal variances assumed	1.709	12	.113	4.7820000E-03	2.7976913E-03	-1.31364570E-03	1.0877646E-02
	Equal variances not assumed	1.709	11.673	.114	4.7820000E-03	2.7976913E-03	-1.33262717E-03	1.0896627E-02
CH_2	Equal variances assumed	-.599	12	.561	-9.57571429E-04	1.5997761E-03	-4.44318420E-03	2.5280413E-03
	Equal variances not assumed	-.599	11.785	.561	-9.57571429E-04	1.5997761E-03	-4.45024864E-03	2.5351058E-03

Asymp. Sig. (2-tailed): p-values

- Relative Integration: No p-values equal to or less than 0.05 for H_20, 0H, CH_2
- ∴ Null hypothesis accepted

4.3.2 Chemical Shift: Sulphur and Controls

a) List of Analyses: T-Test. Chemical Shift. Sulphur and Control

4.3.2.1 Sulphur (C) vs. Sulphur (D): Equal Deconcentrations
Table 4.13
Remedies: Sulphur (C) 6, 12, 24
Remedies: Sulphur (D) 12, 24, 48

4.3.2.2 Sulphur (C) vs. Sulphur (D): Equal Succussions
Table 4.14
Remedies: Sulphur (C) 12, 24, 48
Remedies: Sulphur (D) 12, 24, 48

4.3.2.3 Control (C) vs. Control (D): Equal Deconcentrations
Table 4.15
Controls: Control (C) 6, 12, 24
Controls: Control (D) 12, 24, 48

4.3.2.4 Control (C) vs. Control (D): Equal Succussions
Table 4.16
Controls: Control (C) 12, 24, 48
Controls: Control (D) 12, 24, 48

b) Comments: T-Test. Chemical Shift. Sulphur and Controls

Equal deconcentrations and equal number of succussions:

- Shows no significant difference in **chemical shifts** when comparing Sulphur CH and Sulphur DH potencies.
- Shows no significant difference in **chemical shifts** when comparing Controls of CH and Controls of DH potencies.

4.3.2.1 Sulphur (C) vs. Sulphur (D): Equal Deconcentrations

Table 4.13 *T-Test. Chemical Shift. Equal deconcentrations: Sulphur C: 6, 12, 24 vs. Sulphur D: 12, 24, 48*

Chemical Shift: Group Statistics					
	Substance	N	Mean	Std. Deviation	Std. Error Mean
H_20	CH	3	4.78933333	5.0332230E-03	2.9059326E-03
	DH	3	4.79500000	4.5825757E-03	2.6457513E-03
0H	CH	3	4.02400000	1.0000000E-03	5.7735027E-04
	DH	3	4.02700000	4.0000000E-03	2.3094011E-03
CH_2	CH	3	3.05733333	4.6120314E-03	2.6627576E-03
	DH	3	3.06233333	3.7941841E-03	2.1905732E-03

CH
Sulphur: 6C, 12C, 24C

DH
Sulphur: 12D, 24D, 48D

Chemical Shift: T-Test for Equality of Means								
		t	df	Sig. (2-tailed) p	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
H_20	Equal variances assumed	-1.442	4	.223	-5.66666667E-03	3.9299420E-03	-1.65779350E-02	5.2446017E-03
	Equal variances not assumed	-1.442	3.965	.223	-5.66666667E-03	3.9299420E-03	-1.66156781E-02	5.2823447E-03
0H	Equal variances assumed	-1.260	4	.276	-3.00000000E-03	2.3804761E-03	-9.60926133E-03	3.6092613E-03
	Equal variances not assumed	-1.260	2.249	.323	-3.00000000E-03	2.3804761E-03	-1.22287646E-02	6.2287646E-03
CH_2	Equal variances assumed	-1.450	4	.221	-5.00000000E-03	3.4480268E-03	-1.45732572E-02	4.5732572E-03
	Equal variances not assumed	-1.450	3.857	.223	-5.00000000E-03	3.4480268E-03	-1.47151819E-02	4.7151819E-03

Asymp. Sig. (2-tailed): p-values

- Chemical Shift: No p-values equal to or less than 0.05 for H_20, HO, CH_2
- ∴ Null hypothesis accepted

4.3.2.2 Sulphur (C) vs. Sulphur (D): Equal Succussions

Table 4.14 *T-Test. Chemical Shift. Equal succussions: Sulphur C: 12, 24, 48 vs. Sulphur D: 12, 24, 48*

Chemical Shift: Group Statistics					
	Substance	N	Mean	Std. Deviation	Std. Error Mean
H_20	CH	3	4.79466667	5.0332230E-03	2.9059326E-03
	DH	3	4.79500000	4.5825757E-03	2.6457513E-03
0H	CH	3	4.02700000	4.3588989E-03	2.5166115E-03
	DH	3	4.02700000	4.0000000E-03	2.3094011E-03
CH_2	CH	3	3.06091667	2.2684429E-03	1.3096861E-03
	DH	3	3.06233333	3.7941841E-03	2.1905732E-03

CH
Sulphur: 12C, 24C, 48C

DH
Sulphur: 12D, 24D, 48D

Chemical Shift: T-Test for Equality of Means								
		t	df	Sig. (2-tailed) p	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
H_20	Equal variances assumed	-.085	4	.936	-3.33333333E-04	3.9299420E-03	-1.12446017E-02	1.0577935E-02
	Equal variances not assumed	-.085	3.965	.937	-3.33333333E-04	3.9299420E-03	-1.12823447E-02	1.0615678E-02
0H	Equal variances assumed	.000	4	1.000	.00000000	3.4156503E-03	-9.48336543E-03	9.4833654E-03
	Equal variances not assumed	.000	3.971	1.000	.00000000	3.4156503E-03	-9.51090666E-03	9.5109067E-03
CH_2	Equal variances assumed	-.555	4	.608	-1.41666667E-03	2.5522321E-03	-8.50279909E-03	5.6694658E-03
	Equal variances not assumed	-.555	3.268	.615	-1.41666667E-03	2.5522321E-03	-9.17510280E-03	6.3417695E-03

Asymp. Sig. (2-tailed): p-values

- Chemical Shift: No p-values equal to or less than 0.05 for H_20, 0H, CH_2
- ∴ Null hypothesis accepted

4.3.2.3 Control (C) vs. Control (D): Equal Deconcentrations

Table 4.15 *T-Test. Chemical Shift. Equal deconcentrations: Control C: 6, 12, 24 vs. Control D: 12, 24, 48*

Chemical Shift: Group Statistics					
	Substance	N	Mean	Std. Deviation	Std. Error Mean
H_20	CH	3	4.79650000	2.3804761E-03	1.1902381E-03
	DH	3	4.79733333	1.1547005E-03	6.6666667E-04
0H	CH	3	4.02850000	2.3804761E-03	1.1902381E-03
	DH	3	4.02600000	1.0000000E-03	5.7735027E-04
CH_2	CH	3	3.06300000	.00000000	.00000000
	DH	3	3.06483333	1.5877132E-03	9.1666667E-04

CH
Water-Ethanol: 6C, 12C, 24C

DH
Water-Ethanol: 12D, 24D, 48D

Chemical Shift: T-Test for Equality of Means								
		t	df	Sig. (2-tailed) p	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
H_20	Equal variances assumed	-.550	5	.606	-8.33333333E-04	1.5147424E-03	-4.72710255E-03	3.0604359E-03
	Equal variances not assumed	-.611	4.512	.571	-8.33333333E-04	1.3642255E-03	-4.45751813E-03	2.7908515E-03
0H	Equal variances assumed	1.679	5	.154	2.5000000E-03	1.4888474E-03	-1.32720416E-03	6.3272042E-03
	Equal variances not assumed	1.890	4.227	.128	2.5000000E-03	1.3228757E-03	-1.09644655E-03	6.0964466E-03
CH_2	Equal variances assumed	-2.390	5	.062	-1.83333333E-03	7.6693836E-04	-3.80481114E-03	1.3814448E-04
	Equal variances not assumed	-2.000	2.000	.184	-1.83333333E-03	9.1666667E-04	-5.77743167E-03	2.1107650E-03

Asymp. Sig. (2-tailed): p-values

- Chemical Shift: No p-values equal to or less than 0.05 for H_20, 0H, CH_2
- ∴ Null hypothesis accepted

4.3.2.4 Control (C) vs. Control (D): Equal Succussions

Table 4.16 *T-Test. Chemical Shift. Equal succussions: Control C: 12, 24, 48 vs. Control D: 12, 24, 48*

Chemical Shift: Group Statistics					
	Substance	N	Mean	Std. Deviation	Std. Error Mean
H_20	CH	3	4.79600000	1.0000000E-03	5.7735027E-04
	DH	3	4.79733333	1.1547005E-03	6.6666667E-04
0H	CH	3	4.02800000	1.0000000E-03	5.7735027E-04
	DH	3	4.02600000	1.0000000E-03	5.7735027E-04
CH_2	CH	3	3.06358333	1.0103630E-03	5.8333333E-04
	DH	3	3.06483333	1.5877132E-03	9.1666667E-04

CH
Water-Ethanol: 12C, 24C, 48C

DH
Water-Ethanol: 12D, 24D, 48D

Chemical Shift: T-Test for Equality of Means								
		t	df	Sig. (2-tailed) p	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
H_20	Equal variances assumed	-1.512	4	.205	-1.33333333E-03	8.8191710E-04	-3.78192776E-03	1.1152611E-03
	Equal variances not assumed	-1.512	3.920	.207	-1.33333333E-03	8.8191710E-04	-3.80176192E-03	1.1350953E-03
0H	Equal variances assumed	2.449	4	.070	2.0000000E-03	8.1649658E-04	-2.66957936E-04	4.2669579E-03
	Equal variances not assumed	2.449	4.000	.070	2.0000000E-03	8.1649658E-04	-2.66957936E-04	4.2669579E-03
CH_2	Equal variances assumed	-1.150	4	.314	-1.25000000E-03	1.0865337E-03	-4.26670127E-03	1.7667013E-03
	Equal variances not assumed	-1.150	3.392	.325	-1.25000000E-03	1.0865337E-03	-4.49257751E-03	1.9925775E-03

Asymp. Sig. (2-tailed): p-values

- Chemical Shift: No p-values equal to or less than 0.05 for H_20, 0H, CH_2
- ∴ Null hypothesis accepted

4.3.3 Relative Integration: Sulphur and Controls

a) List of Analyses: T-Test. Relative Integration. Sulphur and Control

4.3.3.1 Sulphur (C) vs. Sulphur (D): Equal Deconcentrations
Table 4.17
Remedies: Sulphur (C) 6, 12, 24
Remedies: Sulphur (D) 12, 24, 48

4.3.3.2 Sulphur (C) vs. Sulphur (D): Equal Succussions
Table 4.18
Remedies: Sulphur (C) 12, 24, 48
Remedies: Sulphur (D) 12, 24, 48

4.3.3.3 Control (C) vs. Control (D): Equal Deconcentrations
Table 4.19
Controls: Control (C) 6, 12, 24
Controls: Control (D) 12, 24, 48

4.3.3.4 Control (C) vs. Control (D): Equal Succussions
Table 4.20
Controls: Control (C) 12, 24, 48
Controls: Control (D) 12, 24, 48

b) Comments: T-Test. Relative Integration. Sulphur and Controls

Equal deconcentrations and equal number of succussions:

- Shows significant differences in **relative integration** of the H_20 peaks when comparing Sulphur CH and Sulphur DH potencies. The significant differences in the H_20 peaks are:
  - Sulphur: equal deconcentrations: H_20 peaks: p=0.050
  - Sulphur: equal number of succussions: H_20 peaks: p=0.022
- Shows significant differences in **relative integration** of the OH peaks when comparing Controls of CH and Controls of DH potencies. The significant differences in the OH peaks are:
  - Control: equal deconcentrations: OH peaks: p=0.001
  - Control: equal number of succussions: OH peaks: p=0.003

4.3.3.1 Sulphur (C) vs. Sulphur (D): Equal Deconcentrations

Table 4.17 *T-Test. Relative Integration. Equal deconcentrations: Sulphur C: 6, 12, 24 vs. Sulphur D: 12, 24, 48*

Relative Integration: Group Statistics					
	Substance	N	Mean	Std. Deviation	Std. Error Mean
H_20	CH	3	.14927633	7.5677496E-04	4.3692422E-04
	DH	3	.14765967	6.6027595E-04	3.8121050E-04
0H	CH	3	.15352600	8.2647498E-03	4.7716555E-03
	DH	3	.14821833	7.1566356E-04	4.1318855E-04
CH_2	CH	3	.27329967	1.9282978E-03	1.1133032E-03
	DH	3	.27269733	9.4107191E-04	5.4332812E-04

CH
Sulphur: 6C, 12C, 24C

DH
Sulphur: 12D, 24D, 48D

Relative Integration: T-Test for Equality of Means								
		t	df	Sig. (2-tailed) p	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
H_20	Equal variances assumed	2.788	4	.049	1.6166667E-03	5.7984845E-04	6.7492825E-06	3.2265841E-03
	Equal variances not assumed	2.788	3.928	.050	1.6166667E-03	5.7984845E-04	-4.98715172E-06	3.2383205E-03
0H	Equal variances assumed	1.108	4	.330	5.3076667E-03	4.7895116E-03	-7.99014930E-03	1.8605483E-02
	Equal variances not assumed	1.108	2.030	.382	5.3076667E-03	4.7895116E-03	-1.50109110E-02	2.5626244E-02
CH_2	Equal variances assumed	.486	4	.652	6.0233333E-04	1.2388097E-03	-2.83715389E-03	4.0418206E-03
	Equal variances not assumed	.486	2.902	.661	6.0233333E-04	1.2388097E-03	-3.41687074E-03	4.6215374E-03

Asymp. Sig. (2-tailed): p-values

- Relative Integration: p-values equal to or less than 0.05 for H_20
- ∴ Null hypothesis rejected

4.3.3.2 Sulphur (C) vs. Sulphur (D): Equal Succussions

Table 4.18 *T-Test. Relative Integration. Equal succussions: Sulphur C: 12, 24, 48 vs. Sulphur D: 12, 24, 48*

Relative Integration: Group Statistics					
	Substance	N	Mean	Std. Deviation	Std. Error Mean
H_20	CH	3	.14945333	4.8730928E-04	2.8134814E-04
	DH	3	.14765967	6.6027595E-04	3.8121050E-04
0H	CH	3	.14826767	1.7998534E-03	1.0391459E-03
	DH	3	.14821833	7.1566356E-04	4.1318855E-04
CH_2	CH	3	.27591867	3.4107111E-03	1.9691750E-03
	DH	3	.27269733	9.4107191E-04	5.4332812E-04

CH
Sulphur: 12C, 24C, 48C

DH
Sulphur: 12D, 24D, 48D

Relative Integration: T-Test for Equality of Means								
		t	df	Sig. (2-tailed) p	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
H_20	Equal variances assumed	3.786	4	.019	1.7936667E-03	4.7379133E-04	4.7821105E-04	3.1091223E-03
	Equal variances not assumed	3.786	3.680	.022	1.7936667E-03	4.7379133E-04	4.3189095E-04	3.1554424E-03
0H	Equal variances assumed	.044	4	.967	4.9333333E-05	1.1182794E-03	-3.05550812E-03	3.1541748E-03
	Equal variances not assumed	.044	2.617	.968	4.9333333E-05	1.1182794E-03	-3.82312600E-03	3.9217927E-03
CH_2	Equal variances assumed	1.577	4	.190	3.2213333E-03	2.0427569E-03	-2.45026893E-03	8.8929356E-03
	Equal variances not assumed	1.577	2.303	.239	3.2213333E-03	2.0427569E-03	-4.54833154E-03	1.0990998E-02

Asymp. Sig. (2-tailed): p-values

- Relative Integration: p-values less than 0.05 for H_20
- ∴ Null hypothesis rejected

4.3.3.3 Control (C) vs. Control (D): Equal Deconcentrations

Table 4.19 *T-Test. Relative Integration. Equal deconcentrations: Control C: 6, 12, 24 vs. Control D: 12, 24, 48*

Relative Integration: Group Statistics					
	Substance	N	Mean	Std. Deviation	Std. Error Mean
H_20	CH	3	.14976467	1.6901362E-03	9.7580058E-04
	DH	3	.14861367	4.4311661E-04	2.5583350E-D04
0H	CH	3	.14941533	8.6248324E-04	4.9795493E-04
	DH	3	.14053133	1.1670760E-03	6.7381163E-04
CH_2	CH	3	.27334867	1.9027460E-03	1.0985509E-03
	DH	3	.27744067	3.0002889E-03	1.7322176E-03

CH
Water-Ethanol: 6C, 12C, 24C

DH
Water-Ethanol: 12D, 24D, 48D

Relative Integration: T-Test for Equality of Means								
		t	df	Sig. (2-tailed) p	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
H_20	Equal variances assumed	1.141	4	.318	1.1510000E-03	1.0087802E-03	-1.64982294E-03	3.9518229E-03
	Equal variances not assumed	1.141	2.274	.360	1.1510000E-03	1.0087802E-03	-2.72531231E-03	5.0273123E-03
0H	Equal variances assumed	10.603	4	.000	8.8840000E-03	8.3784320E-04	6.5577744E-03	1.1210226E-02
	Equal variances not assumed	10.603	3.683	.001	8.8840000E-03	8.3784320E-04	6.4765487E-03	1.1291451E-02
CH_2	Equal variances assumed	-1.995	4	.117	-4.09200000E-03	2.0511928E-03	-9.78702420E-03	1.6030242E-03
	Equal variances not assumed	-1.995	3.385	.130	-4.09200000E-03	2.0511928E-03	-1.02194411E-02	2.0354411E-03

Asymp. Sig. (2-tailed): p-values

- Relative Integration: p-values less than 0.05 for 0H
- ∴ Null hypothesis rejected

4.3.3.4 Control (C) vs. Control (D): Equal Succussions

Table 4.20 *T-Test. Relative Integration. Equal succussions: Control C: 12, 24, 48 vs. Control D: 12, 24, 48*

Relative Integration: Group Statistics					
	Substance	N	Mean	Std. Deviation	Std. Error Mean
H_20	CH	3	.14900033	1.3076928E-03	7.5499676E-04
	DH	3	.14861367	4.4311661E-04	2.5583350E-04
0H	CH	3	.14900167	4.0842666E-04	2.3580524E-04
	DH	3	.14053133	1.1670760E-03	6.7381163E-04
CH_2	CH	3	.27297733	2.1069486E-03	1.2164473E-03
	DH	3	.27744067	3.0002889E-03	1.7322176E-03

CH
Water-Ethanol: 12C, 24C, 48C

DH
Water-Ethanol: 12D, 24D, 48D

Relative Integration: T-Test for Equality of Means								
		t	df	Sig. (2-tailed) p	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
H_20	Equal variances assumed	.485	4	.653	3.8666667E-04	7.9716428E-04	-1.82661620E-03	2.5999495E-03
	Equal variances not assumed	.485	2.453	.668	3.8666667E-04	7.9716428E-04	-2.50209749E-03	3.2754308E-03
0H	Equal variances assumed	11.865	4	.000	8.4703333E-03	7.1388110E-04	6.4882817E-03	1.0452385E-02
	Equal variances not assumed	11.865	2.483	.003	8.4703333E-03	7.1388110E-04	5.9056434E-03	1.1035023E-02
CH_2	Equal variances assumed	-2.109	4	.103	-4.46333333E-03	2.1166771E-03	-1.03401711E-02	1.4135044E-03
	Equal variances not assumed	-2.109	3.587	.111	-4.46333333E-03	2.1166771E-03	-1.06171075E-02	1.6904409E-03

Asymp. Sig. (2-tailed): p-values

- Relative Integration: p-values less than 0.05 for 0H
- ∴ Null hypothesis rejected

4.4 Laboratory Results: NMR Spectroscopy Analyses

NMR Spectroscopy: Main Analyses
4.4.1 Chemical Shift
4.4.2 Relative Integration

Discussion on NMR Spectroscopy Results: Peak Values H_2O, OH, CH_2

The analysis and comparison of the raw data does not indicate significant trends within the scope of this study, except for the Sulphur C and Control C potencies as far as **chemical shift** is concerned (which is not statistically significant). However, these results show interesting patterns for the potencies.

1) Chemical Shift

i) Sulphur C:

- For H_2O, OH and CH_2 peaks, the Sulphur C potencies show a linear increase in potency from the lowest to the highest chemical shift values i.e. 6C, 12C, 24C, 48C.

ii) Sulphur D:

- For H_2O, OH and CH_2 peaks, the Sulphur D potencies show an irregular but consistent pattern of potency i.e. lowest 24D, middle 48D, and highest 12D chemical shift values.

iii) Control C:

- For H_2O, OH and CH_2 peaks, the Control C potencies show a linear increase in potency from the lowest to the highest chemical shift values i.e. 6C, 12C, 24C, 48C. The potency/chemical shift pattern is the same as for Sulphur C.

iv) Control D:

- For H_2O, and CH_2 peaks, the Control D potencies show an irregular but consistent pattern of potency i.e. lowest 48D, middle 12D, and highest 24D chemical shift values.
- For the OH peaks, the Control D potencies show a linear decrease in potency from the lowest to the highest chemical shift values i.e. 48D, 24D, 12D.

2) Relative Integration

i) Sulphur C:

- For H_2O and CH_2 peaks, values of the Sulphur C show irregular and inconsistent potency/relative integration patterns.
- For OH peaks, the values of Sulphur C show a linear decrease in potency from the lowest to the highest relative integration values i.e. 48C, 24C, 12C, 6C.

ii) Sulphur D:

- For H_2O peaks, values of the Sulphur D show a linear decrease in potency from the lowest to the highest relative integration values i.e. 48D, 24D, 12D.
- For OH peaks, Sulphur D showed irregular and inconsistent potency/relative integration patterns.
- For the CH_2 peaks, values of Sulphur D show a linear increase in potency from the lowest to the highest relative integration values i.e. 12D, 24D, 48D.

iii) Control C:

- For H_2O, OH and CH_2 peaks, values of the Control C potencies showed irregular and inconsistent potency/relative integration patterns.

iv) Control D:

- For H_2O, OH and CH_2 peaks, values of the Control D potencies showed irregular and inconsistent potency/relative integration patterns.

4.4.1 Chemical Shift: Sulphur and Controls

a) List of Analyses: NMR Spectroscopy. Chemical Shift. Sulphur and Controls

Remedies:	Sulphur	6C, 12C, 24C, 48C, D12, D24, D48
Controls:	Water-Ethanol	6C, 12C, 24C, 48C, D12, D24, D48

4.4.1.1 Chemical Shift: Sulphur and Control H_2O Peaks
Chart 4.1

4.4.1.2: Chemical Shift: Sulphur and Control OH Peaks
Chart 4.2

4.4.1.3 Chemical Shift: Sulphur and Control CH_2 Peaks
Chart 4.3

b) Chemical Shift: Peak H_2O OH, CH_2 Values

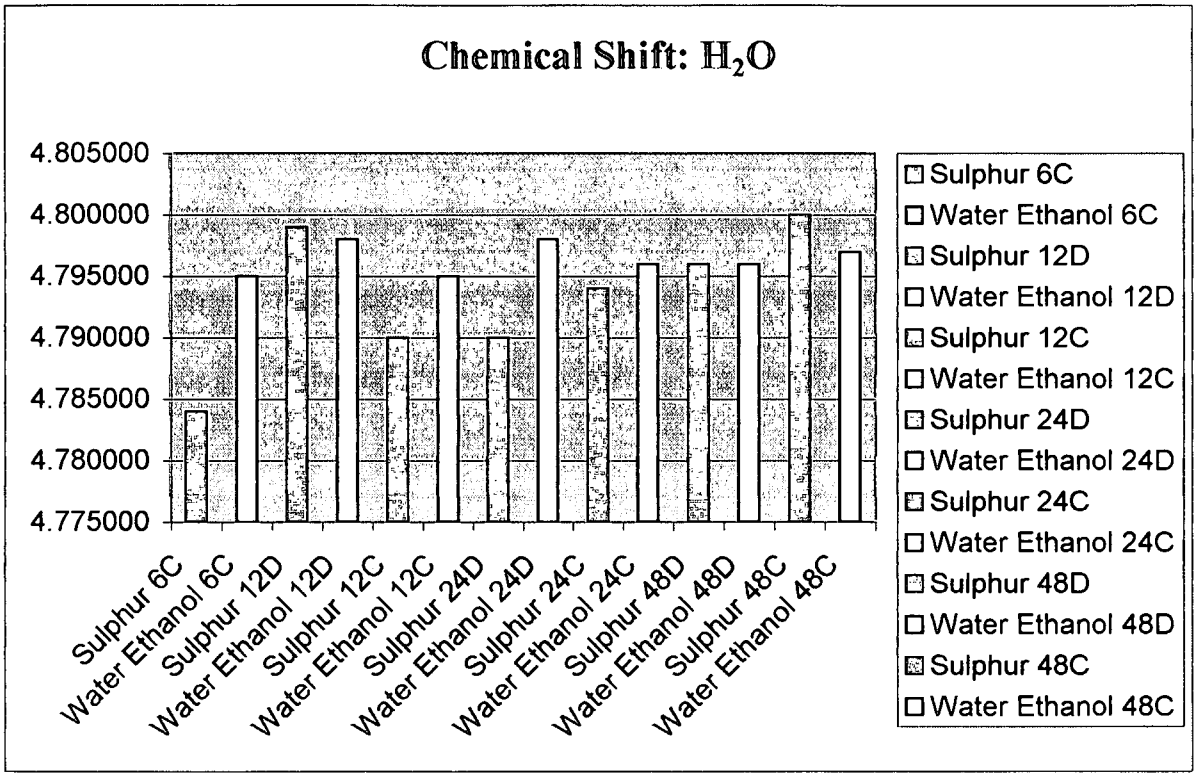
Table 4.21 *NMR Spectroscopy. Chemical Shift. Peak values: Sulphur and Controls*

Chemical Shift: Potency – Lowest to Highest

Substance	H_2O	OH	CH_2
Sulphur 6C	4.784000	4.023000	3.052250
Water Ethanol 6C	4.795000	4.027000	3.063000
Sulphur 12D	4.799000	4.031000	3.065750
Water Ethanol 12D	4.798000	4.027000	3.065750
Sulphur 12C	4.790000	4.024000	3.058500
Water Ethanol 12C	4.795000	4.027000	3.063000
Sulphur 24D	4.790000	4.023000	3.058250
Water Ethanol 24D	4.798000	4.026000	3.065750
Sulphur 24C	4.794000	4.025000	3.061250
Water Ethanol 24C	4.796000	4.028000	3.063000
Sulphur 48D	4.796000	4.027000	3.063000
Water Ethanol 48D	4.796000	4.025000	3.063000
Sulphur 48C	4.800000	4.032000	3.063000
Water Ethanol 48C	4.797000	4.029000	3.064750

4.4.1.1 Chemical Shift: Sulphur and Control H_2O Peaks

Chart 4.1 *NMR Spectroscopy. Chemical Shift: H_2O Peaks. Sulphur & Controls*



Chemical Shift: H_2O Peaks – Lowest to Highest

Substance	H_2O
Sulphur 6C	4.784000
Sulphur 12C	4.790000
Sulphur 24D	4.790000
Sulphur 24C	4.794000
Water Ethanol 6C	4.795000
Water Ethanol 12C	4.795000
Sulphur 48D	4.796000
Water Ethanol 24C	4.796000
Water Ethanol 48D	4.796000
Water Ethanol 48C	4.797000
Water Ethanol 12D	4.798000
Water Ethanol 24D	4.798000
Sulphur 12D	4.799000
Sulphur 48C	4.800000

Sulphur: 6C, 12C, 24D, 24C, 48D, 12D, 48C
- CH: 6, 12, 24, 48
- DH: 24, 48, 12

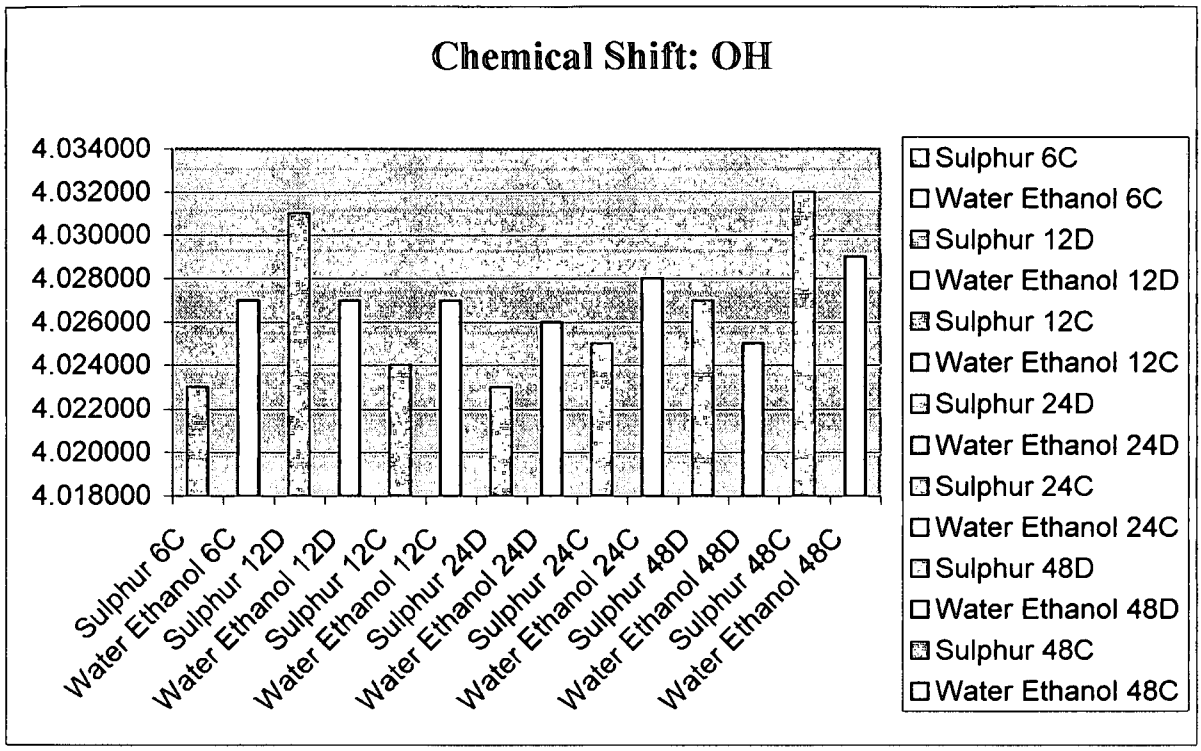
Water-Ethanol: 6C, 12C, 24C, 48D, 48C, 12D, 24D
- CH: 6, 12, 24, 48
- DH: 48, 12, 24

Values are very stable. Values differ by very small margins, and often coincide.

H_2O Peaks are often used as a reference peak, due to its stability (value between 4.7 and 4.8)

4.4.1.2 Chemical Shift: Sulphur and Control OH Peaks

Chart 4.2 *NMR Spectroscopy. Chemical Shift: OH Peaks. Sulphur & Controls*



Chemical Shift: OH Peaks – Lowest to Highest

Substance	OH
Sulphur 6C	4.023000
Sulphur 24D	4.023000
Sulphur 12C	4.024000
Sulphur 24C	4.025000
Water Ethanol 48D	4.025000
Water Ethanol 24D	4.026000
Sulphur 48D	4.027000
Water Ethanol 6C	4.027000
Water Ethanol 12C	4.027000
Water Ethanol 12D	4.027000
Water Ethanol 24C	4.028000
Water Ethanol 48C	4.029000
Sulphur 12D	4.031000
Sulphur 48C	4.032000

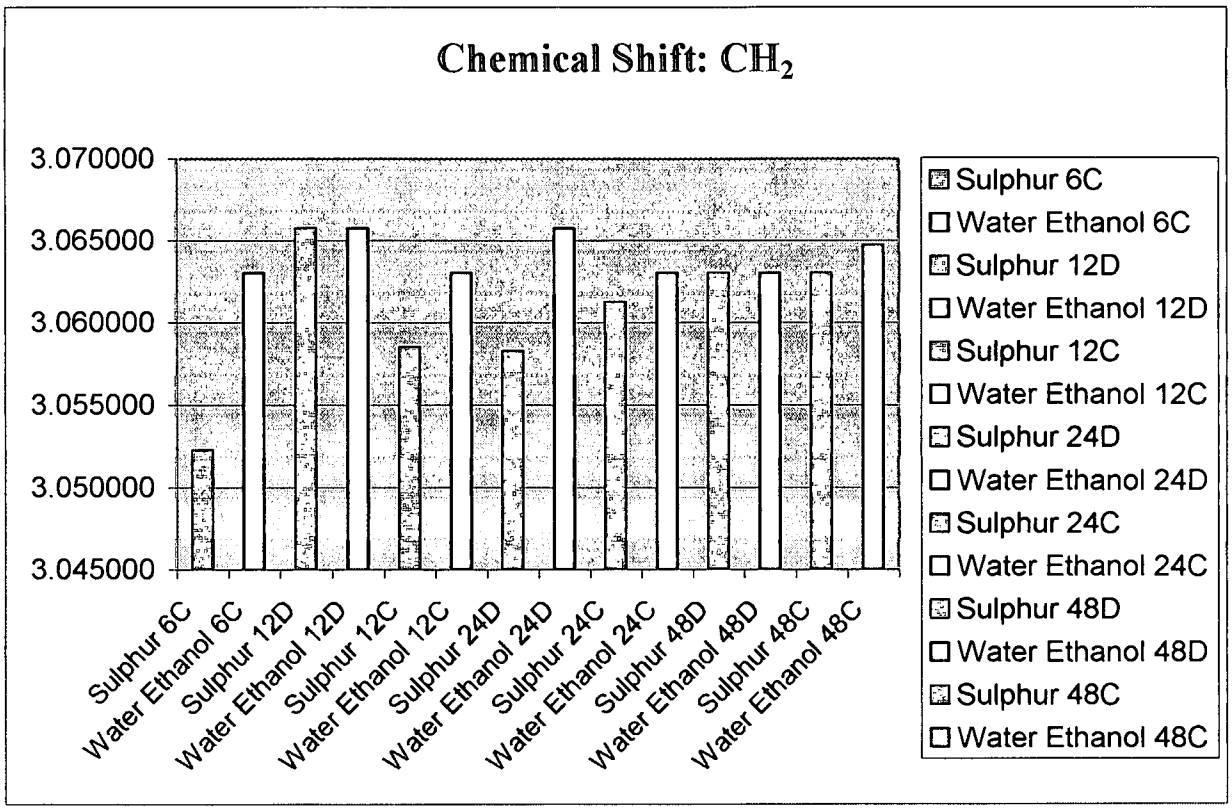
Sulphur: 6C, 24D, 12C, 24C, 48D, 12D, 48C
- CH: 6, 12, 24, 48
- DH: 24, 48, 12

Water-Ethanol: 48D, 24D, 6C, 12C, 12D, 24C, 48C
- CH: 6, 12, 24, 48
- DH: 48, 24, 12

Values are stable, and often coincide.

4.4.1.3 Chemical Shift: Sulphur and Control CH_2 Peaks

Chart 4.3 *NMR Spectroscopy. Chemical Shift: CH_2 Peaks. Sulphur & Controls*



Chemical Shift: CH_2 Peaks – Lowest to Highest

Substance	CH_2
Sulphur 6C	3.052250
Sulphur 24D	3.058250
Sulphur 12C	3.058500
Sulphur 24C	3.061250
Sulphur 48C	3.063000
Sulphur 48D	3.063000
Water Ethanol 6C	3.063000
Water Ethanol 12C	3.063000
Water Ethanol 24C	3.063000
Water Ethanol 48D	3.063000
Water Ethanol 48C	3.064750
Sulphur 12D	3.065750
Water Ethanol 12D	3.065750
Water Ethanol 24D	3.065750

Sulphur: 6C, 24D, 12C, 24C, 48C, 48D, 12D
- CH: 6, 12, 24, 48
- DH: 24, 48, 12

Water-Ethanol: 6C, 12C, 24C, 48D, 48C, 12D, 24D
- CH: 6, 12, 24, 48
- DH: 48, 12, 24

Values are stable, and often coincide.

4.4.2 Relative Integration

a) List of Analyses: NMR Spectroscopy. Relative Shift. Sulphur and Controls

Remedies:	Sulphur	6C, 12C, 24C, 48C, D12, D24, D48
Controls:	Water-Ethanol	6C, 12C, 24C, 48C, D12, D24, D48

4.4.2.1 Relative Integration: Sulphur and Control H_2O Peaks
Chart 4.4

4.4.2.2: Relative Integration: Sulphur and Control OH Peaks
Chart 4.5

4.4.2.3 Relative Integration: Sulphur and Control CH_2 Peaks
Chart 4.6

b) Relative Integration: Peak H_2O OH, CH_2 Values

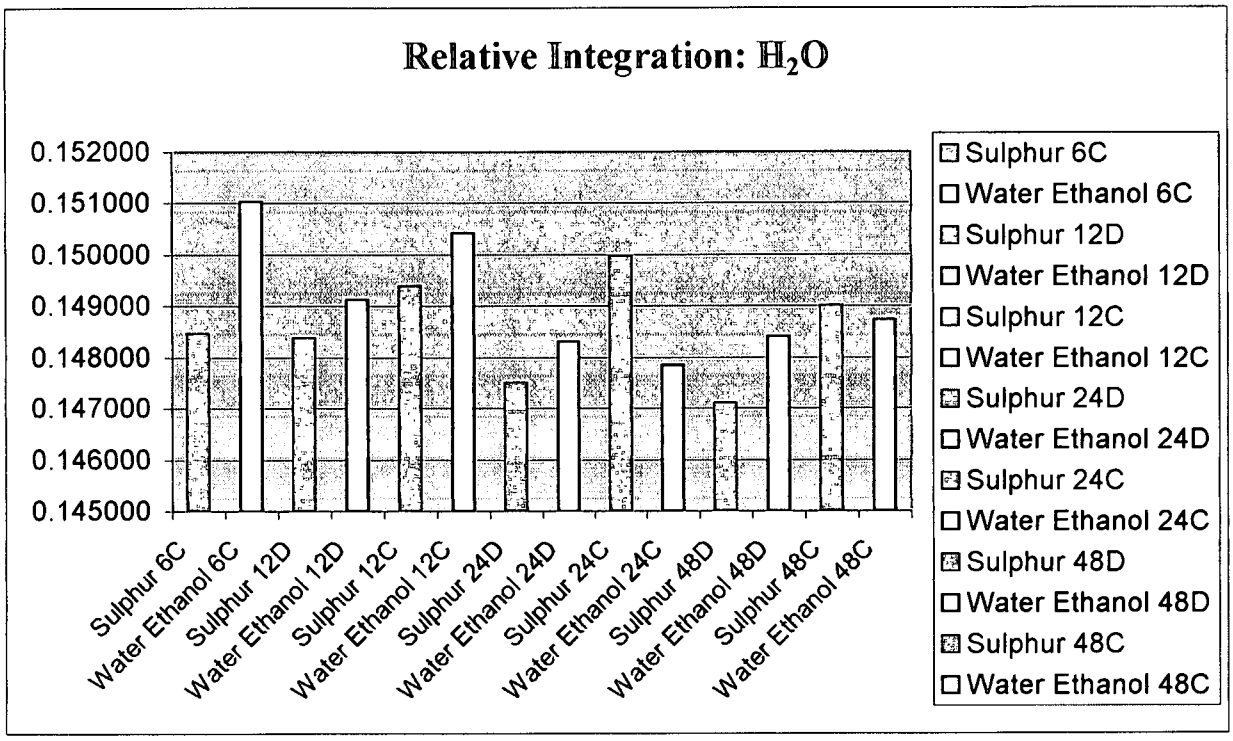
Table 4.22 *NMR Spectroscopy. Relative Integration. Peak values: Sulphur & Controls*

Relative Integration: Potency – Lowest to Highest

Substance	H_2O	OH	CH_2
Sulphur 6C	0.148468	0.162909	0.271688
Water Ethanol 6C	0.151029	0.150294	0.272985
Sulphur 12D	0.148387	0.148925	0.271613
Water Ethanol 12D	0.149122	0.140394	0.280894
Sulphur 12C	0.149393	0.150343	0.275436
Water Ethanol 12C	0.150420	0.149382	0.275407
Sulphur 24D	0.147494	0.147494	0.273178
Water Ethanol 24D	0.148309	0.139439	0.275474
Sulphur 24C	0.149968	0.147326	0.272775
Water Ethanol 24C	0.147845	0.148570	0.271654
Sulphur 48D	0.147098	0.148236	0.273301
Water Ethanol 48D	0.148410	0.141761	0.275954
Sulphur 48C	0.148999	0.147134	0.279545
Water Ethanol 48C	0.148736	0.149053	0.271871

4.4.2.1 Relative Integration: Sulphur and Control H_2O Peaks

Chart 4.4 *NMR Spectroscopy. Relative Integration: H_2O Peaks. Sulphur & Controls*



Relative Integration: H_2O Peaks – Lowest to Highest

Substance	H_2O
Sulphur 48D	0.147098
Sulphur 24D	0.147494
Water Ethanol 24C	0.147845
Water Ethanol 24D	0.148309
Sulphur 12D	0.148387
Water Ethanol 48D	0.148410
Sulphur 6C	0.148468
Water Ethanol 48C	0.148736
Sulphur 48C	0.148999
Water Ethanol 12D	0.149122
Sulphur 12C	0.149393
Sulphur 24C	0.149968
Water Ethanol 12C	0.150420
Water Ethanol 6C	0.151029

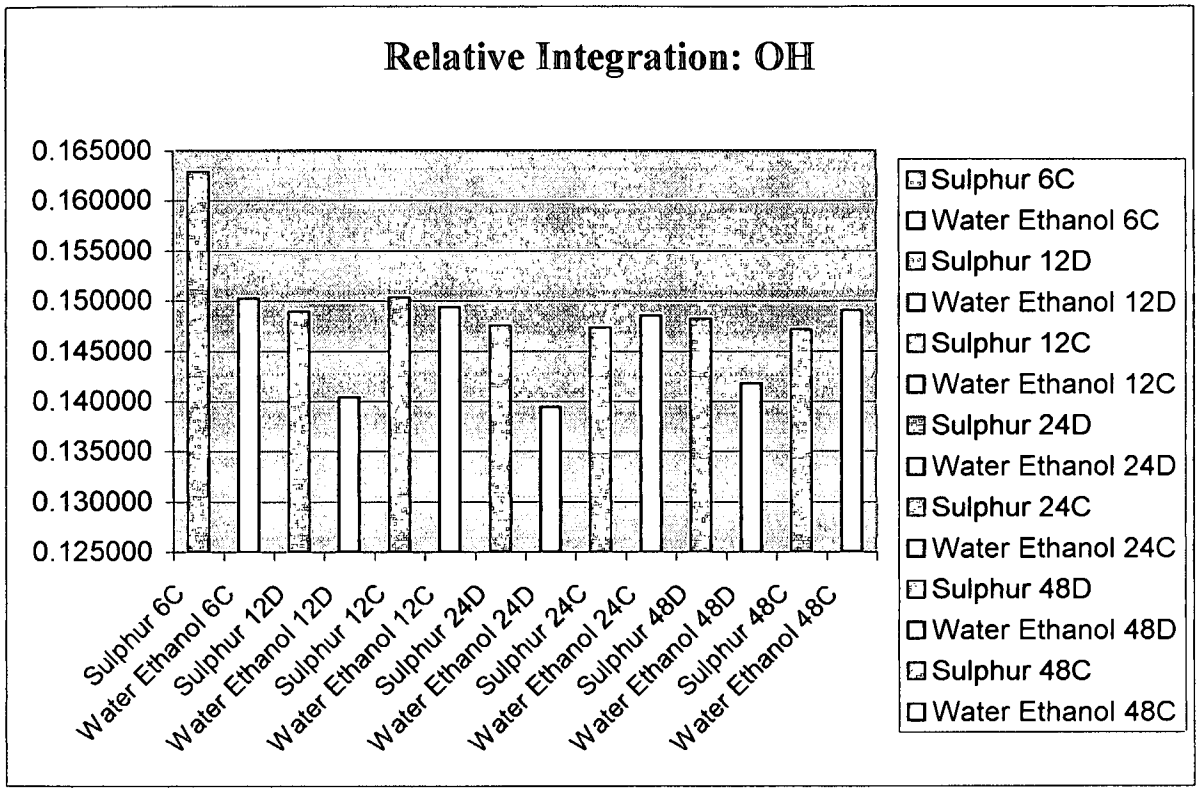
Sulphur: 48D, 24D, 12D, 6C, 48C, 12C, 24C
- CH: 6, 48, 12, 24
- DH: 48, 24, 12

Water-Ethanol: 24C, 24D, 48D, 48C, 12D, 12C, 6C
- CH: 24, 48, 12, 6
- DH: 24, 48, 12

All values differ.
There are no matches within a small but significant range.

4.4.2.2 Relative Integration: Sulphur and Control OH Peaks

Chart 4.5 *NMR Spectroscopy. Relative Integration: OH Peaks. Sulphur & Controls*



Relative Integration: OH Peaks – Lowest to Highest

Substance	OH
Water Ethanol 24D	0.139439
Water Ethanol 12D	0.140394
Water Ethanol 48D	0.141761
Sulphur 48C	0.147134
Sulphur 24C	0.147326
Sulphur 24D	0.147494
Sulphur 48D	0.148236
Water Ethanol 24C	0.148570
Sulphur 12D	0.148925
Water Ethanol 48C	0.149053
Water Ethanol 12C	0.149382
Water Ethanol 6C	0.150294
Sulphur 12C	0.150343
Sulphur 6C	0.162909

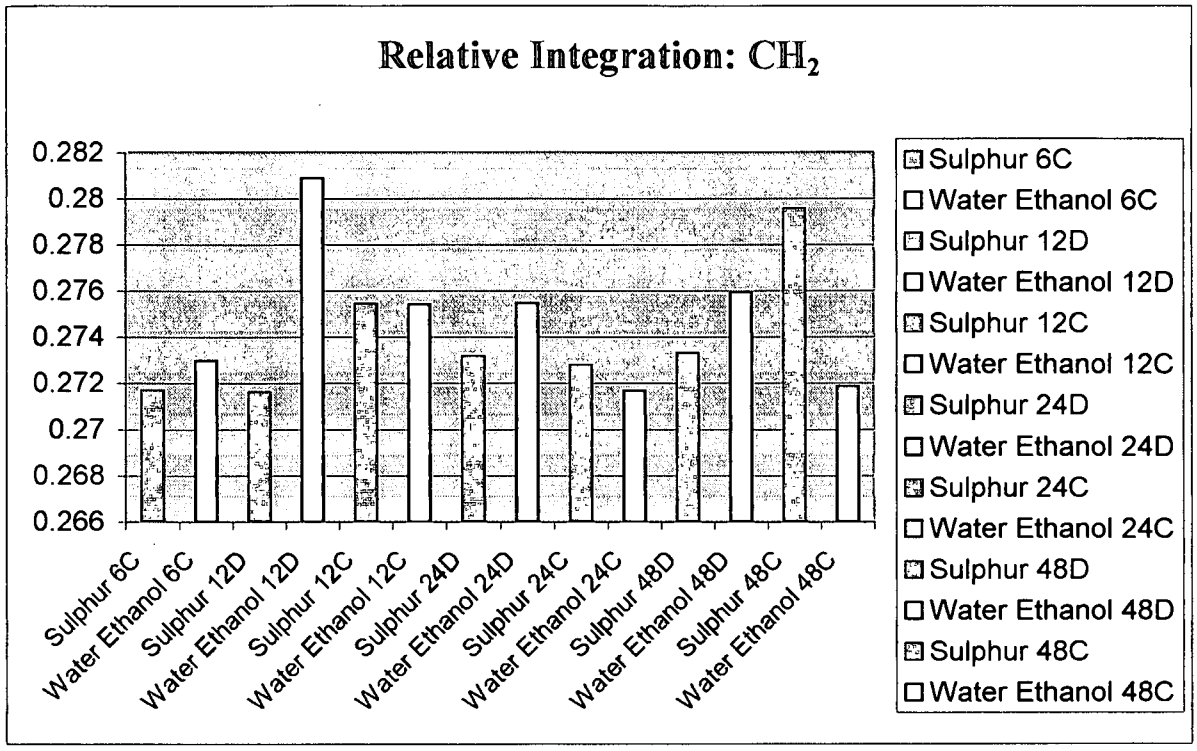
Sulphur: 48C, 24C, 24D, 48D, 12D, 12C, 6C
- CH: 48, 24, 12, 6
- DH: 24, 48, 12

Water-Ethanol: 24D, 12D, 48D, 24C, 48C, 12C, 6C
- CH: 24, 48, 12, 6
- DH: 24, 12, 48

All values differ.
There are no matches within a relatively large range.

4.4.2.3 Relative Integration: Sulphur and Control CH_2 Peaks

Chart 4.6 *NMR Spectroscopy. Relative Integration: CH_2 Peaks. Sulphur & Controls*



Relative Integration: CH_2 Peaks – Lowest to Highest

Substance	CH_2
Sulphur 12D	0.271613
Water Ethanol 24C	0.271654
Sulphur 6C	0.271688
Water Ethanol 48C	0.271871
Sulphur 24C	0.272775
Water Ethanol 6C	0.272985
Sulphur 24D	0.273178
Sulphur 48D	0.273301
Water Ethanol 12C	0.275407
Sulphur 12C	0.275436
Water Ethanol 24D	0.275474
Water Ethanol 48D	0.275954
Sulphur 48C	0.279545
Water Ethanol 12D	0.280894

Sulphur: 12D, 6C, 24C, 24D, 48D, 12C, 48C
- CH: 6, 24, 12, 48
- DH: 12, 24, 48

Water-Ethanol: 24C, 48C, 6C, 12C, 24D, 48D, 12D
- CH: 24, 48, 6, 12
- DH: 24, 48, 12

All values differ.
Differences are small.

Chapter 5 – Discussion

The results of this investigation suggest that significant differences exist between centesimal (CH) and decimal (DH) Hahnemannian potencies for:

- Equal levels of deconcentration
- Equal number of succussions

i) No statistically significant differences were found between homoeopathic Sulphur and the potentised Water-Ethanol Control.

ii) For Sulphur and Control with equal deconcentration and equal number of succussions:

- Significant differences were found to exist for relative integration values.
- Chemical shift values did not show significant differences.

This would suggest that the amount of transferred substance at each step of deconcentration, and the number of succussions at each step, play an important role in the development of distinct physico-chemical properties of homoeopathic remedies.

iii) Is also interesting to note:

- Only the H_2O peaks showed significant differences in the comparisons of homoeopathic Sulphur potencies, but that these differences were observed consistently for both deconcentration and number of succussions comparisons.
- For the Control comparisons, only the OH peaks showed significant differences for their integration values when comparing parallel deconcentrations and number of succussions. Note: CH_2 peaks showed significant differences in the Mann-Whitney U-Test but not in the T-Test.

iv) That the H_2O and OH peaks showed significant differences in their relative integration values is consistent with previous international research.

v) The significant relative integration values suggest that there are differences in the relative proton densities at the H_2O and OH peaks of the different Hahnemannian potencies. These findings suggest that it is indeed the OH groups (H_2O is regarded as a bifurcate OH) that play a significant part in the development of the properties of a homoeopathic remedy.

vi) It is of interest that significant differences were not noticed for any peaks in the chemical shift values, at any of the levels of comparison. This is in line with general NMR spectroscopy analysis where certain peaks such as H_2O have very stable chemical shift values, and are used as reference substances.

Chemical shift values are primarily used for the identification of the type of substance analysed. In this analysis, it is clear that all the substances tested are Water-Ethanol as far as the chemical shift values and the spin-spin splitting of the peaks are concerned. In this instance, chemical shift values only are likely to differ as a result of significant temperature changes during measurement. This will be exaggerated over the long time period of multiple sampling (see **2.6** and **2.8**).

vi) However, the relative integration values that do show a significant difference, indicate that there is a structural difference in the basic Water-Ethanol. Relative integration is an indication of the amount or density of hydrogen protons participating in the formation of a peak.

vii) As far as relative integration is concerned:

Sulphur remedies:

- H_2O peaks in equal deconcentration comparison: p= 0.05.
- H_2O peaks in equal number of succussions comparison: p= 0.022.

Control substances :

- OH peaks in equal deconcentration comparison: p= 0.001.
- OH peaks in equal number of succussions comparison: p= 0.003.

Finding a statistically significant difference between wide varieties of components may not be of real relevance, especially if one is not careful of what one is comparing. Statistical analysis of objectives that fall outside of the scope of a particular experiment may lead to greater confusion, rather than clarification and the establishment of scientific principles.

Consultations with SPSS©, and Statistica©, and the statistical expert of a large international research company (Millward Brown Impact (Pty) Ltd.), confirmed that sample sizes for this type of statistical analyses used for homoeopathic NMR research are far, far too small. In their opinion, whether one, ten or twenty samples of each substance was analysed, it would make no difference. Thus, the results of these statistical analyses are likely to be wide open to misinterpretation, incorrect allocation of groups and variables, and inaccuracies. We must therefore be very careful not to try to fit a valid scientific inquiry into the wrong statistical model and vice versa.

In Summary:

The structural implications of the findings of this study and how that relates to the information imprinting of a homoeopathic substance on a carrier, still remains open to speculation.

If we are to formulate a congruent theory that is able to explain the wide ranging phenomena associated with a homoeopathic remedy, the model may require a combination of geometric, dynamic field and sub-molecular models as proposed by Lessell (1994). This is necessary, as we have to explain not only that information is imprinted onto a carrier, but also how the scope and intensity of this information may change with an increase in homoeopathic potency. Furthermore, we have to explain how homoeopathic remedies can be affected by external influences, such as UV light.

What we can conclude, within the context of this study, is that there is a significant difference between the CH and DH homoeopathic potencies, although they have the same level of deconcentration and/or the same number of succussions.

Trituration is considered an important phase in potentisation. The Control excluded the trituration process (see **3.1** and **Appendix A**). However, the Control substances showed qualities of potentisation. It was shown that deconcentration and succussion can give rise to potentisation without trituration. Therefore it is necessary to quantify the potentisation contributions made by trituration versus deconcentration versus succussion.

Further studies, based on the findings of this study, should compare:

- LM potencies to centesimal potencies of equal deconcentration
- LM potencies to decimal potencies of equal deconcentration

This will complete the comparisons of the three Hahnemannian potency scales.

It is also suggested that the aspect of succussion be further investigated by comparing succussed Water-Ethanol to unsuccussed Water-Ethanol, without adding a homoeopathic base substance such as Sulphur, and without adding lactose. Various amounts of succussions may be compared, e.g. 1, 2, 10,100 either to each other or to substances without succussions.

For homoeopathy to establish itself as a science it would have to be grounded in conventional notions of science. As Ernst (1996) says, scientific proof really means the replication and convergence of evidence, both empirical and theoretical. Since complementary and alternative medicine is often based on non-conformist medical ideologies, the demand for evidence may be higher and more methodologically rigorous than is usually expected.

According to Bellavite and Signorini (1995), if research studies are conducted with correct methods and yield results, which are reproducible in different laboratories, then they cannot be refuted on the basis of arguments such as the placebo effect, and thus simply dismissed. Part of the reason for homeopathy's unacceptability is, presumably, because there is no 'plausible mechanism of action' as Ernst (1996) put it.

NMR spectroscopy, and perhaps other types of spectroscopy may begin to build the required scientific basis for this 'plausible mechanism of action'.

Chapter 6 – Conclusions and Recommendations

6.1 Conclusions

The results of this study showed that:

- Of the substances used in this study, there are certain recognisable differences between different potency types within the general context of homoeopathic pharmacy, and that these differences can be shown by NMR spectroscopy.
- These differences show distinct physico-chemical structures for different homoeopathic remedies.

However, we cannot as yet claim that these differences are responsible for the homoeopathic results observed in clinical practice. What we can claim is that the CH and DH homoeopathic potencies tested all differ, and that they are not interchangeable.

As regards substance (Sulphur remedies) and Control (potentised Water-Ethanol) the Mann-Whitney U-test indicated:

- Between Sulphur and Control there are no significant differences as far as chemical shift and relative integration values are concerned.
- Between Sulphur and Sulphur, and Control and Control, both equal deconcentration and equal number of succussions comparisons yielded significant results only as far as their relative integration values were concerned.
- In this respect, Sulphur remedies with equal deconcentration and Sulphur remedies with equal number of succussions showed p-values = 0.05 for the H_2O peaks. On the other hand the Water-Ethanol Controls showed significant p-values (p= 0.05) for OH peaks, and p-values (p= 0.05) CH_2 peaks.

The T-test further clarified these results by indicating:

- That the CH_2 peaks for relative integration of the Water-Ethanol Controls were not significantly different
- Significant differences viz. H_20 peaks for Sulphur homoeopathic remedies, with similar deconcentration ($p=0.05$), and similar number of succussions ($p= 0.022$) was confirmed.
- Significant differences viz. OH peaks of the Water-Ethanol Controls, with similar deconcentration ($p=0.001$), and similar number of succussions ($p= 0.003$) was confirmed.

Therefore, this study confirms that both succussion and dilution play important roles in the development of distinctive CH and DH structural potency qualities. Standards for deconcentration procedures are well recorded and applied. But there are no laid down standards for the number of succussions to be applied at the various stages of the manufacture of homeopathic remedies. Regarding the number of succussions, what may be applied by several manufactures and so deemed acceptable by them, does not necessarily make their procedure the standard.

This study adds to the scientific data available for the assessment of homoeopathy. It thus contributes further to the standardisation of homoeopathic practice and manufacture. In particular, any interchangeability between CH and DH potencies, based solely upon the fact of equivalent succussions and/or deconcentrations, cannot be claimed. However, this study does not address the validity or invalidity of interchangeability between CH and DH potencies in their clinical effect in prescribed treatments. Rigorous and exhaustive clinical trials are required.

6.2 Recommendations

NMR spectroscopy is a complex and sensitive means of chemical analysis. The analysis of homoeopathic remedies by means of strict scientific methods (including NMR spectroscopy) is riddled with many unspecified and unquantified variables, due to processes involved in the preparation and multiple sampling of the substances submitted for analysis (see **2.7** and **2.8**). Notwithstanding the aforementioned limitations, NMR spectroscopy remains a valuable tool for analysing the subtle differences in homoeopathic remedies.

We can only formulate a scientific basis once we have established certain fundamental principles. The following components, among others, need to be addressed in order to assist in achieving this:

1. Limitation of the number of aspects tested

In order to draw significant basic conclusions, it is suggested that single variables be isolated and tested. The effect of the combination of variables, to which a homoeopathic remedy is exposed during manufacture, is unknown. As an example, the difference between one and two succussions of the same substance, prepared in exactly the same way, at the same time, under the same conditions, be tested without significant time-lapse between tests.

2. Analyse fewer substances with more samples

Samples need to be tested over the shortest possible period of time, as significant external influences may affect a substance between sampling. Limit the i) taking of multiple samples to a single potency and its Control thus the time taken between sampling will be decreased

ii) the number of different sample groups. By doing so, the number of variables will be reduced. This will enable one to do a better statistical analysis with fewer variables, thus giving less ambiguous results.

3. **Isolate and analyse single steps in the manufacturing process**

An example is the use of a machine for trituration. A machine is suggested, as the process of trituration by hand has too many variables. As an example, a substance could be divided, one being triturated for 30 minutes, the other for an hour. The difference between these substances could then be analysed by Raman laser spectroscopy, which is used to analyse solid substances.

Further examples are:

- Compare the differences between substances that are succussed on mediums with different consistencies, e.g. soft sponge versus a hard plastic surface.
- Test the difference in the NMR spectroscopy of a succussed and unsuccussed Water-Ethanol substance, without adding any active base substance.

4. **Compare the difference between hand and machine succussed remedies.**

Most homoeopaths believe that remedies succussed by hand are homoeopathically more effective than those done by machine. This claim has not, as yet, been scientifically measured. It is obvious that the use of a standardised machine will give more consistent results. One could then compare the difference for a person doing the same amount of succussions as a particular machine.

5. Compare the difference between two different people succussing a substance

It may be interesting to assess the difference between remedies made by different people. E.g. compare one person who is more athletically fit and physically vigorous, who has a stronger, faster and perhaps different pattern of succussions, to a second person, who is less vigorous in succussion.

6. Compare one LM potency to one CH potency with the same deconcentration level

A comparison between CH and LM potencies of the same deconcentration level will finally clarify whether significant differences exist between the traditional Hahnemannian homoeopathic potencies, and what their possible natures may be. The same comparison between a DH and LM potency with equal deconcentration levels should also be done.

7. Assess the influence of time on NMR results

Take samples of a homoeopathic remedy over a period of time with intervals of perhaps 1 week between samples. Assess the difference between samples in sequence to compare the effect of influences over time and the possible changes in the remedy. Energy applied to the remedy before sampling, and stored in the form of potential energy, should surely decrease over time.

8. Compare trituration to succussion

Triturate a soluble substance and compare this to a dissolved and succussed equal potency of the same substance. This may highlight the differences between succussion and trituration, and it may clarify the role played by lactose in the preparation of homoeopathic remedies.

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Appendix A – Preparation of Sample Potencies

Appendix Content:

Prepared substances: **Sulphur**:

- 3CH* Triturated base substance
- 3DH* Triturated base substance
- 6CH, 12CH, 24CH, 48CH
- 6DH*, 12DH, 24DH, 48DH

* Potencies prepared but not used for this study.

Prepared substances: **Water-Ethanol Controls**:

- 3CH* Dilution by distilled water and 30% alcohol only
- 3DH* Dilution by distilled water and 30% alcohol only
- 6CH, 12CH, 24CH, 48CH
- 6DH*, 12DH, 24DH, 48DH

* Potencies prepared but not used for this study.

1) Preparation of Sulphur Remedies

The Sulphur remedies as far as trituration and dilution were essentially prepared in accordance with the German Homoeopathic Pharmacopoeia (1985: 22-24). However, certain procedures were modified to meet the test criteria of this study (see **3.1**).

a) Sulphur 3CH

Purpose: To produce, by hand, a 3CH trituration from Flowers of Sulphur

Apparatus: Unglazed porcelain pestle and mortar
Porcelain spatula
Steel spatula
Mass balance (accurate and calibrated)
Cigarette lighter

Consumables: 96% alcohol (for flaming)
Clean, empty vials
Filter paper
Labels

Ingredients: Pure lactose powder
Flowers of Sulphur

Method: All apparatus and utensils must be clean and odourless

1. Clean the mortar and pestle with water, and flame with 96% alcohol.
2. Flame the spatula.
3. Allow mortar and pestle to cool sufficiently before use.
4. Place a piece of filter paper on the scale and tare it.

5. Mass 0.1g of Flowers of Sulphur onto filter paper.
6. Place a new piece of filter paper on the scale and tare it.
7. Mass 3.3g of pure lactose powder onto filter paper.
8. Repeat step 7 twice more. (Total lactose powder mass: 3 x 3,3g = 9.9g, therefore drug substance to vehicle ratio = 0,1g : 9,9g = 1 : 100).
9. Place 3.3g of lactose into mortar and triturate for a short period.
10. Add the 0.1g crude Sulphur into the mortar.
11. Triturate for 6 minutes and scrape down for 4 minutes with a porcelain spatula. Then triturate for 6 minutes and scrape down for 4 minutes.
    (Trituration time: 2 x 10min = 20min)
12. Add the second portion of 3.3g of lactose powder. Continue as in step 11 above.
13. Finally add the third portion of 3.3g of lactose. Proceed as in step 11 above. (Total trituration time: 20min x 3 = 60min [minimum])
14. Place triturate in a vial and label as Sulphur 1CH.
15. Repeat steps 1-13 when preparing Sulphur 2CH and 3CH, replacing crude Sulphur with Sulphur 1CH and 2CH respectively at each dilution level.

b) Sulphur 3DH

Purpose: To produce, by hand, a 3DH trituration from Flowers of Sulphur

Apparatus: Unglazed porcelain pestle and mortar
Porcelain spatula
Steel spatula
Mass balance (accurate and calibrated)
Cigarette lighter

Consumables: 96% alcohol (for flaming)

Clean, empty vials

Filter paper

Labels

Ingredients: Pure lactose powder

Flowers of Sulphur

Method: All apparatus and utensils must be clean and odourless.

1. Clean the mortar and pestle with water, and flame with 96% alcohol.
2. Flame the spatula.
3. Allow mortar and pestle to cool sufficiently before use.
4. Place a piece of filter paper on the scale and tare it.
5. Mass 1g of Flowers of Sulphur onto filter paper.
6. Place a new piece of filter paper on the scale and tare it.
7. Mass 3g of pure lactose powder onto filter paper.
8. Repeat step 7 twice more. (Total lactose powder mass: 3 x 3g = 9g, therefore drug substance to vehicle ratio = 1g : 9g = 1 : 10).
9. Place 9g of lactose into mortar and triturate for a short period.
10. Add the 1g crude Sulphur into the mortar.
11. Triturate for 6 minutes and scrape down for 4 minutes with a porcelain spatula. Then triturate for 6 minutes and scrape down for 4 minutes. Repeat this step 5 times more. (Total Trituration time: 6 x 10min = 60min)
12. Place triturate in a vial and label as Sulphur 1DH.
13. Repeat steps 1-12 when preparing Sulphur 2DH and 3DH, replacing crude Sulphur with Sulphur 1DH and 2DH respectively at each dilution level.

c) Sulphur 6CH, 12CH, 24CH, 48CH

Purpose: To produce liquid dilutions of Sulphur 6CH, 12CH, 24CH, 48CH from the 3CH trituration.

Apparatus: Mass balance (accurate and calibrated)

5ml clear glass pipettes

Consumables: 50ml amber glass reagent bottles

25ml amber glass dropper bottles

5ml clear glass screw top bottles

Rubber dropper bulbs

Filter paper

Labels

Ingredients: 30% alcohol

87% alcohol

Distilled water

Sulphur 3CH triturate

Method: All apparatus and utensils must be clean and odourless.

1. Place a piece of filter paper on the scale and tare it.
2. Mass 0.1g of Sulphur 3CH on the filter paper. Place it in a 25 ml amber bottle.
3. Add 9.9ml of distilled water and succuss 10 times without stopping. Label as Sulphur 4CH.
4. Place 99 parts 30% alcohol in a 5ml clear glass screw top bottle. Add 1 part Sulphur 4CH. Succuss 10 times without stopping. Label as Sulphur 5CH.

5. Place 99 parts 87% alcohol in a 50ml amber glass bottle and add 1 part Sulphur 5CH. Succuss 100 times without stopping. Label as Sulphur 6CH.
6. Place 99 parts 87% alcohol in a 5ml clear glass screw top bottle. Add 1 part Sulphur 6CH. Succuss 10 times without stopping. Label as Sulphur 7CH.
7. To prepare Sulphur 8CH – 11CH repeat step 6 adding 1 part of the previous potency to 99 parts 87% alcohol at each dilution level.
8. To prepare Sulphur 12CH place 99 parts 87% alcohol in a 50ml amber glass reagent bottle. Add 1 part Sulphur 11CH. Succuss 100 times without stopping. Label as Sulphur 12CH.
9. To prepare Sulphur 13CH – 23CH repeat step 6 adding 1 part of the previous potency to 99 parts 87% alcohol at each dilution level.
10. To prepare Sulphur 24CH place 99 parts 87% alcohol in a 50ml amber glass reagent bottle. Add 1 part Sulphur 23CH. Succuss 100 times without stopping. Label as Sulphur 24CH.
11. To prepare Sulphur 25CH – 47CH repeat step (6) adding 1 part of the previous potency to 99 parts 87% alcohol at each dilution level.
12. To prepare Sulphur 48CH place 99 parts 87% alcohol in a 50ml amber glass reagent bottle. Add 1 part Sulphur 47CH. Succuss 100 times without stopping. Label as Sulphur 48CH.

d) Sulphur 6DH, 12DH, 24DH, 48DH

Purpose: To produce liquid dilutions of Sulphur 6DH, 12DH, 24DH, 48DH from the 3DH trituration

Apparatus: Mass balance (accurate and calibrated)

5ml clear glass pipettes

Rubber dropper bulbs

Consumables: 50ml amber glass reagent bottles

25ml amber glass dropper bottles

5ml clear glass screw top bottles

Filter paper

Labels

Ingredients: 30% alcohol

87% alcohol

Distilled water

Sulphur 3DH triturate

Method: All apparatus and utensils must be clean and odourless.

1. Place a piece of filter paper on the scale and tare it.
2. Mass 1g of Sulphur 3DH on the filter paper and place it in a 25 ml amber bottle.
3. Add 9ml of distilled water and succuss 10 times without stopping. Label as Sulphur 4DH.
4. Place 9 parts 30% alcohol in a 5ml clear glass screw top bottle. Add 1 part Sulphur 4DH. Succuss 10 times without stopping. Label as Sulphur 5DH.
5. Place 9 parts 87% alcohol in a 50ml amber glass bottle. Add 1 part Sulphur 5DH. Succuss 100 times without stopping. Label as Sulphur 6DH.
6. Place 9 parts 87% alcohol in a 5ml clear glass screw top bottle. Add 1 part Sulphur 6DH. Succuss 10 times without stopping. Label as Sulphur 7DH.
7. To prepare Sulphur 8DH – 11DH repeat step 6 adding 1 part of the previous potency to 9 parts 87% alcohol at each dilution level.

8. To prepare Sulphur 12DH place 9 parts 87% alcohol in a 50ml amber glass reagent bottle. Add 1 part Sulphur 11DH. Succuss 100 times without stopping. Label as Sulphur 12DH.
9. To prepare Sulphur 13DH – 23DH repeat step 6 adding 1 part of the previous potency to 9 parts 87% alcohol at each dilution level.
10. To prepare Sulphur 24DH place 9 parts 87% alcohol in a 50ml amber glass reagent bottle. Add 1 part Sulphur 23DH. Succuss 100 times without stopping. Label as Sulphur 24DH.
11. To prepare Sulphur 25DH – 47DH repeat step 6 adding 1 part of the previous potency to 9 parts 87% alcohol at each dilution level.
12. To prepare Sulphur 48DH place 9 parts 87% alcohol in a 50ml amber glass reagent bottle. Add 1 part Sulphur 47DH. Succuss 100 times without stopping. Label as Sulphur 48DH.

2) Preparation of Water-Ethanol Controls

The Water-Ethanol Controls were prepared by the same method of serial dilution and succussion as the Sulphur remedies, without the triturated lactose and Flowers of Sulphur. As equal dilutions and equal numbers of succussions comparisons are used in this study, the identical steps had to be carried out for the Water-Ethanol Controls without a triturated substance. Therefore, a 3CH with the dilution of 30% alcohol and distilled water was prepared from 1 drop of 87% alcohol.

a) Water-Ethanol 3CH, 6CH, 12CH, 24CH, 48CH

Purpose: To produce liquid dilutions of Water-Ethanol Control 3CH, 6CH, 12CH, 24CH, 48CH.

Apparatus: 5ml clear glass pipettes
Rubber dropper bulbs

Consumables: 50ml amber glass reagent bottles
5ml clear glass screw top bottles
Labels

Ingredients: 30% alcohol
87% alcohol
Distilled water

Method: All apparatus and utensils must be clean and odourless.

1. Pipette 99 parts distilled water into a 5ml clear glass screw top bottle. Add 1 part 87% alcohol, and cap. Label as Water-Ethanol 1CH.

2. Pipette 99 parts 30% alcohol into a 5ml clear glass screw top bottle. Add 1 part Water-Ethanol 1CH, and cap. Label as Water-Ethanol 2CH.
3. Pipette 99 parts 87% alcohol into a 5ml clear glass screw top bottle. Add 1 part Water-Ethanol 2CH, and cap. Label as Water-Ethanol 3CH.
4. Pipette 99 parts 87% alcohol into a 5ml clear glass screw top bottle. Add 1 part Water-Ethanol 3CH, and cap. Succuss 10 times without stopping. Label as Water-Ethanol 4CH.
5. Pipette 99 parts 87% alcohol into a 5ml clear glass screw top bottle. Add 1 part Water-Ethanol 4CH, and cap. Succuss 10 times without stopping. Label as Water-Ethanol 5CH.
6. Place 99 parts 87% alcohol in a 50ml amber glass bottle. Add 1 part Water-Ethanol 5CH. Succuss 100 times without stopping. Label as Water-Ethanol 6CH.
7. Place 99 parts 87% alcohol in a 5ml clear glass screw top bottle. Add 1 part Water-Ethanol 6CH. Succuss 10 times without stopping. Label as Water-Ethanol 7CH.
8. To prepare Water-Ethanol 8CH – 11CH repeat step 7 adding 1 part of the previous potency to 99 parts 87% alcohol at each dilution level.
9. To prepare Water-Ethanol 12CH place 99 parts 87% alcohol in a 50ml amber glass reagent bottle. Add 1 part Water-Ethanol 11CH. Succuss 100 times without stopping. Label as Water-Ethanol 12CH.
10. To prepare Water-Ethanol 13CH – 23CH repeat step 7 adding 1 part of the previous potency to 99 parts 87% alcohol at each dilution level.
11. To prepare Water-Ethanol 24CH place 99 parts 87% alcohol in a 50ml amber glass reagent bottle. Add 1 part Water-Ethanol 23CH. Succuss 100 times without stopping. Label as Water-Ethanol 24CH.

12. To prepare Water-Ethanol 25CH – 47CH repeat step 7 adding 1 part of the previous potency to 99 parts 87% alcohol at each dilution level.
13. To prepare Water-Ethanol 48CH place 99 parts 87% alcohol in a 50ml amber glass reagent bottle. Add 1 part Water-Ethanol 47CH. Succuss 100 times without stopping. Label as Water-Ethanol 48CH.

b) Water-Ethanol 3DH, 6DH, 12DH, 24DH, 48DH

Purpose: To produce liquid dilutions of Water-Ethanol 3DH, 6DH, 12DH, 24DH, 48DH.

Apparatus: 5ml clear glass pipettes
Rubber dropper bulbs

Consumables: 50ml amber glass reagent bottles
5ml clear glass screw top bottles
Labels

Ingredients: 30% alcohol
87% alcohol
Distilled water

Method: All apparatus and utensils must be clean and odourless.

1. Pipette 9 parts distilled water into a 5ml clear glass screw top bottle. Add 1 part 87% alcohol, and cap. Label as Water-Ethanol 1DH.
2. Pipette 9 parts 30% alcohol into a 5ml clear glass screw top bottle. Add 1 part Water-Ethanol 1DH, and cap. Label as Water-Ethanol 2DH.

3. Pipette 9 parts 87% alcohol into a 5ml clear glass screw top bottle. Add 1 part Water-Ethanol 2DH, and cap. Label as Water-Ethanol 3DH.
4. Pipette 9 parts 87% alcohol into a 5ml clear glass screw top bottle. Add 1 part Water-Ethanol 3DH, and cap. Succuss 10 times without stopping. Label as Water-Ethanol 4DH.
5. Pipette 9 parts 87% alcohol into a 5ml clear glass screw top bottle. Add 1 part Water-Ethanol 4DH, and cap. Succuss 10 times without stopping. Label as Water-Ethanol 5DH.
6. Place 9 parts 87% alcohol in a 50ml amber glass bottle. Add 1 part Water-Ethanol 5DH. Succuss 100 times without stopping. Label as Water-Ethanol 6DH.
7. Place 9 parts 87% alcohol in a 5ml clear glass screw top bottle. Add 1 part Water-Ethanol 6DH. Succuss 10 times without stopping. Label as Water-Ethanol 7DH.
8. To prepare Water-Ethanol 8DH – 11DH repeat step 7 adding 1 part of the previous potency to 9 parts 87% alcohol at each dilution level.
9. To prepare Water-Ethanol 12DH place 9 parts 87% alcohol in a 50ml amber glass reagent bottle. Add 1 part Water-Ethanol 11DH. Succuss 100 times without stopping. Label as Water-Ethanol 12DH.
10. To prepare Water-Ethanol 13DH – 23DH repeat step 7 adding 1 part of the previous potency to 9 parts 87% alcohol at each dilution level.
11. To prepare Water-Ethanol 24DH place 9 parts 87% alcohol in a 50ml amber glass reagent bottle. Add 1 part Water-Ethanol 23DH. Succuss 100 times without stopping. Label as Water-Ethanol 24DH.
12. To prepare Water-Ethanol 25DH – 47DH repeat step 7 adding 1 part of the previous potency to 9 parts 87% alcohol at each dilution level.

13. To prepare Water-Ethanol 48DH place 9 parts 87% alcohol in a 50ml amber glass reagent bottle. Add 1 part Water-Ethanol 47DH. Succuss 100 times without stopping. Label as Water-Ethanol 48DH.

Sulphur 6C
Acetone-d6 insert

Pulse Sequence: s2pul

APPENDIX B : NMR SPECTRA

Index	Frequency	PPM	Height
1	2391.905	4.784	38.2
2	2011.284	4.023	62.1
3	1536.423	3.073	31.2
4	1529.561	3.059	77.5
5	1522.698	3.046	74.3
6	1515.379	3.031	29.6
7	313.129	0.626	196.9
8	306.267	0.613	273.2
9	298.947	0.598	167.9

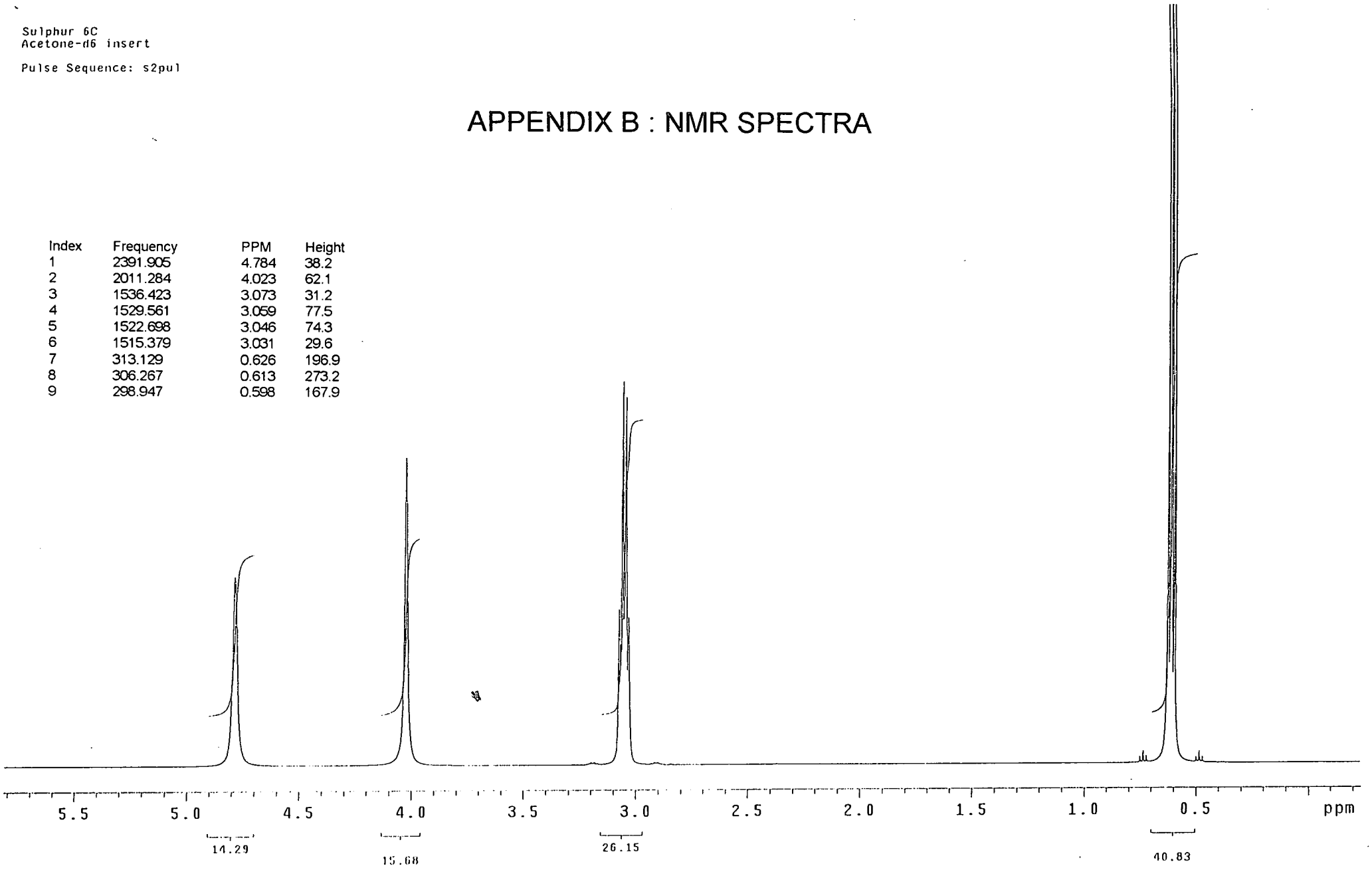

Figure 1: NMR spectrum of Sulphur 6CH

Water-ethanol 6C
Acetone-d6 insert

Pulse Sequence: s2pul

Index	Frequency	PPM	Height
1	2397.395	4.795	34.5
2	2013.571	4.027	55.5
3	1540.998	3.082	19.8
4	1535.050	3.070	46.7
5	1528.188	3.056	44.9
6	1521.784	3.044	18.5
7	318.619	0.637	157.4
8	311.757	0.624	222.4
9	304.437	0.609	136.1

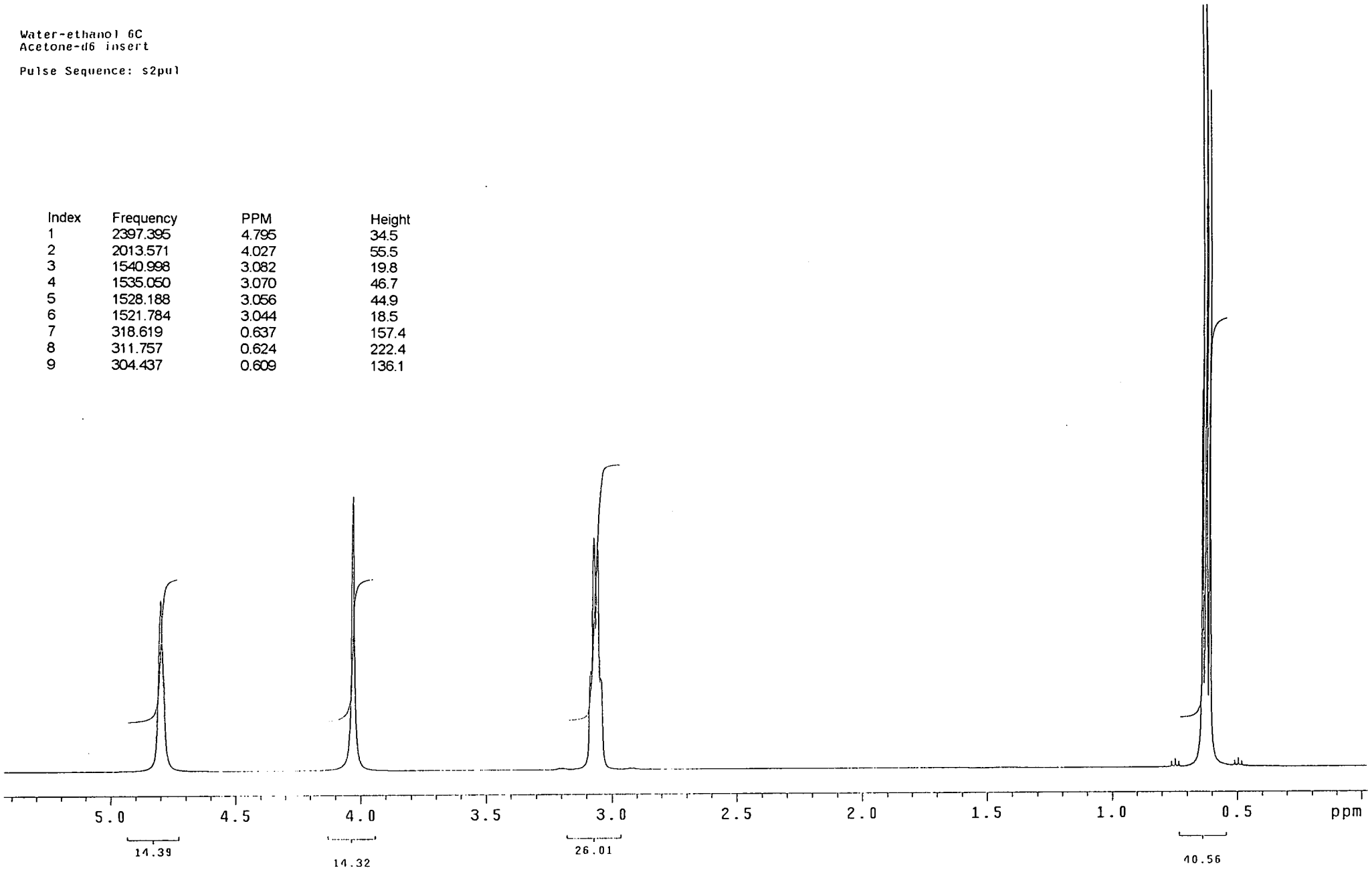

Figure 2 : NMR spectrum of Water-ethanol 6CH

Sulphur 12C
Acetone-d6 insert

Pulse Sequence: s2pul

Index	Frequency	PPM	Height
1	2395.107	4.790	37.2
2	2011.742	4.024	53.7
3	1539.625	3.079	26.6
4	1532.763	3.066	66.1
5	1525.901	3.052	64.0
6	1518.581	3.037	25.9
7	316.789	0.634	142.6
8	309.469	0.619	227.4
9	302.150	0.604	26.3

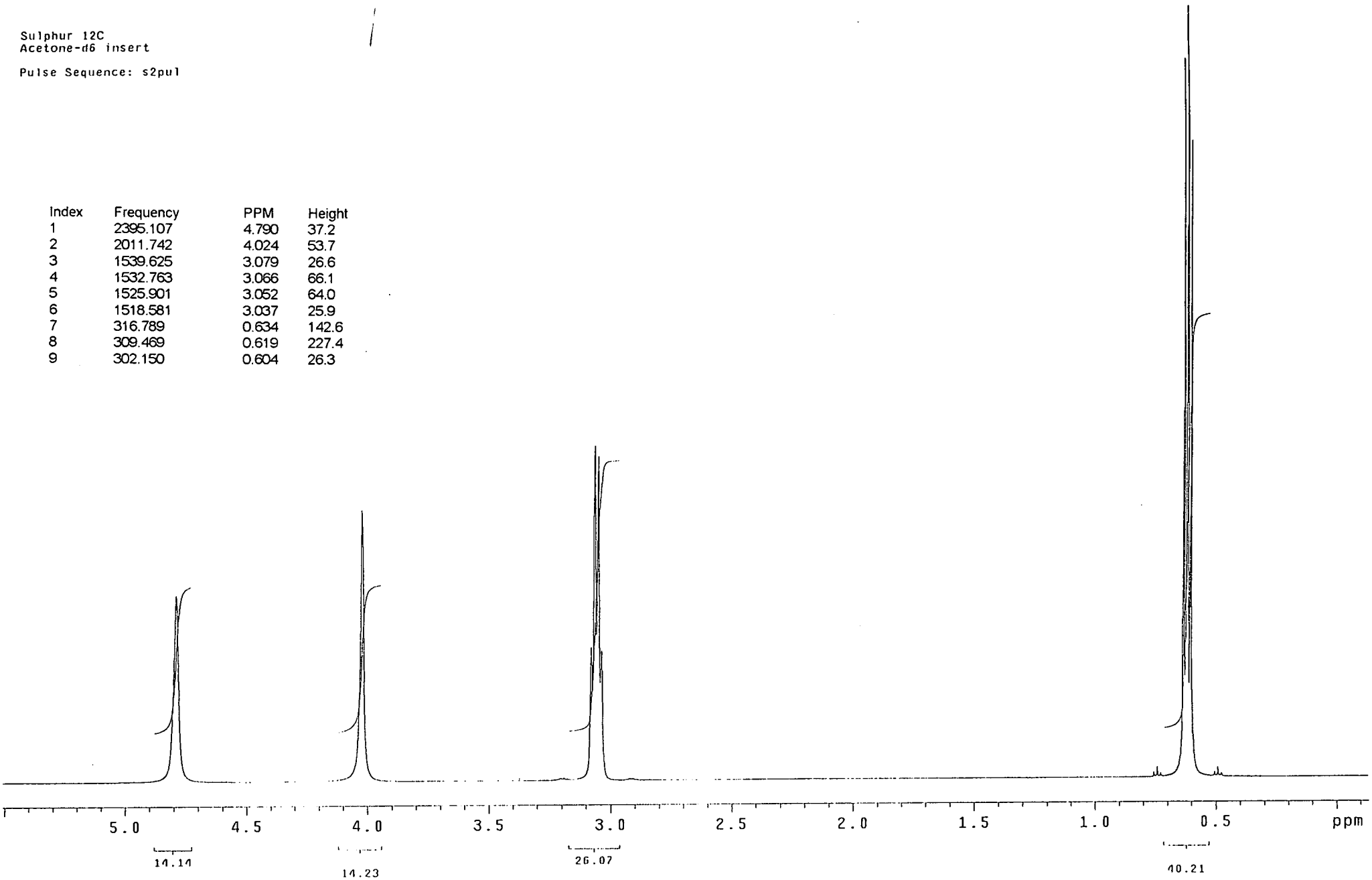

Figure 3: NMR spectrum of Sulphur 12CH

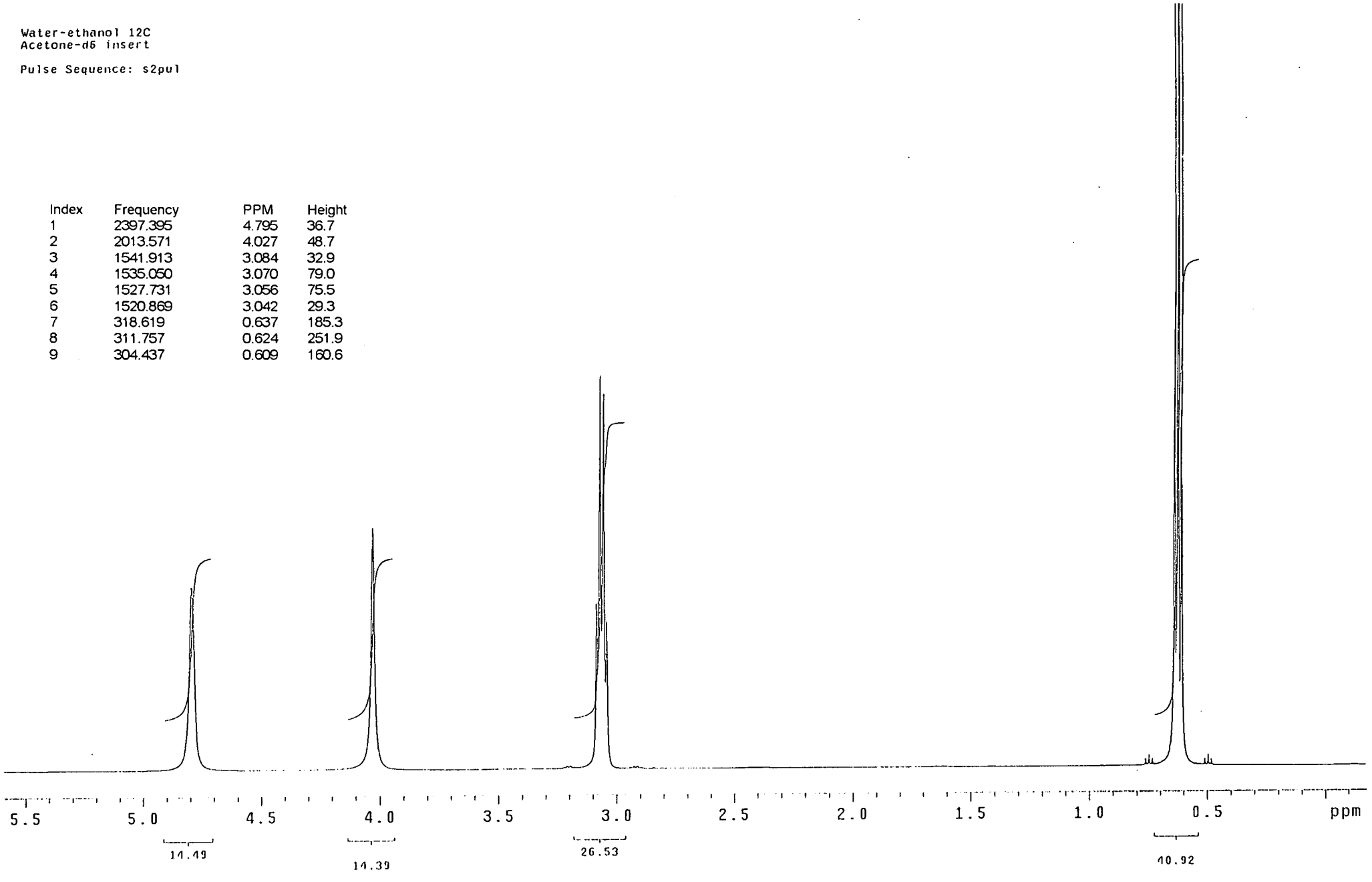

Figure 4: NMR spectrum of Water-ethanol 12CH

Sulphur 24C
Acetone-d6 insert

Pulse Sequence: s2pul

Index	Frequency	PPM	Height
1	2396.937	4.794	47.6
2	2012.656	4.025	70.3
3	1540.998	3.082	34.2
4	1534.135	3.068	82.8
5	1527.273	3.055	78.1
6	1519.954	3.040	30.7
7	318.161	0.636	234.0
8	310.842	0.622	314.4
9	303.980	0.608	194.4

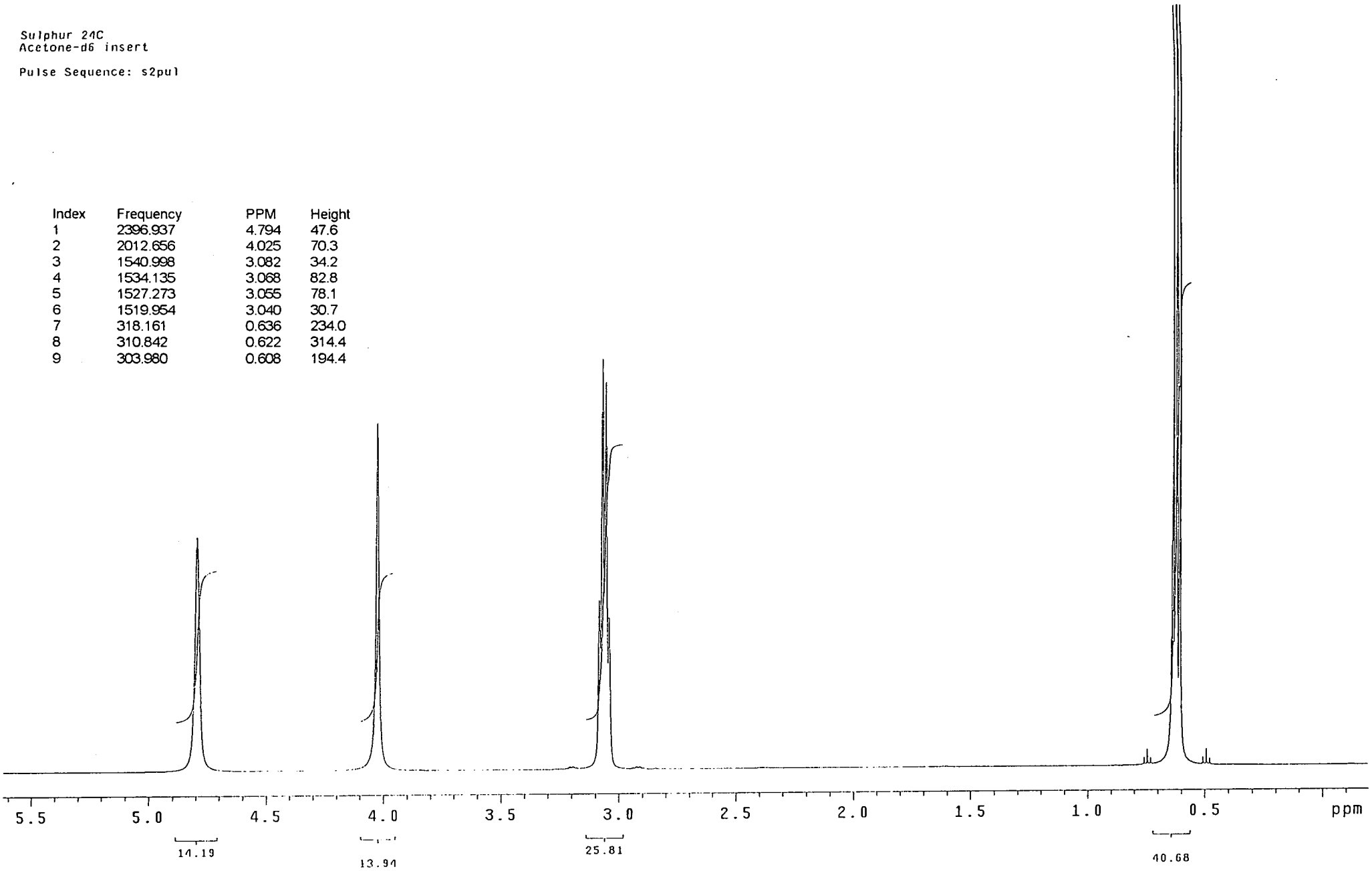

Figure 5: NMR spectrum of Sulphur 24CH

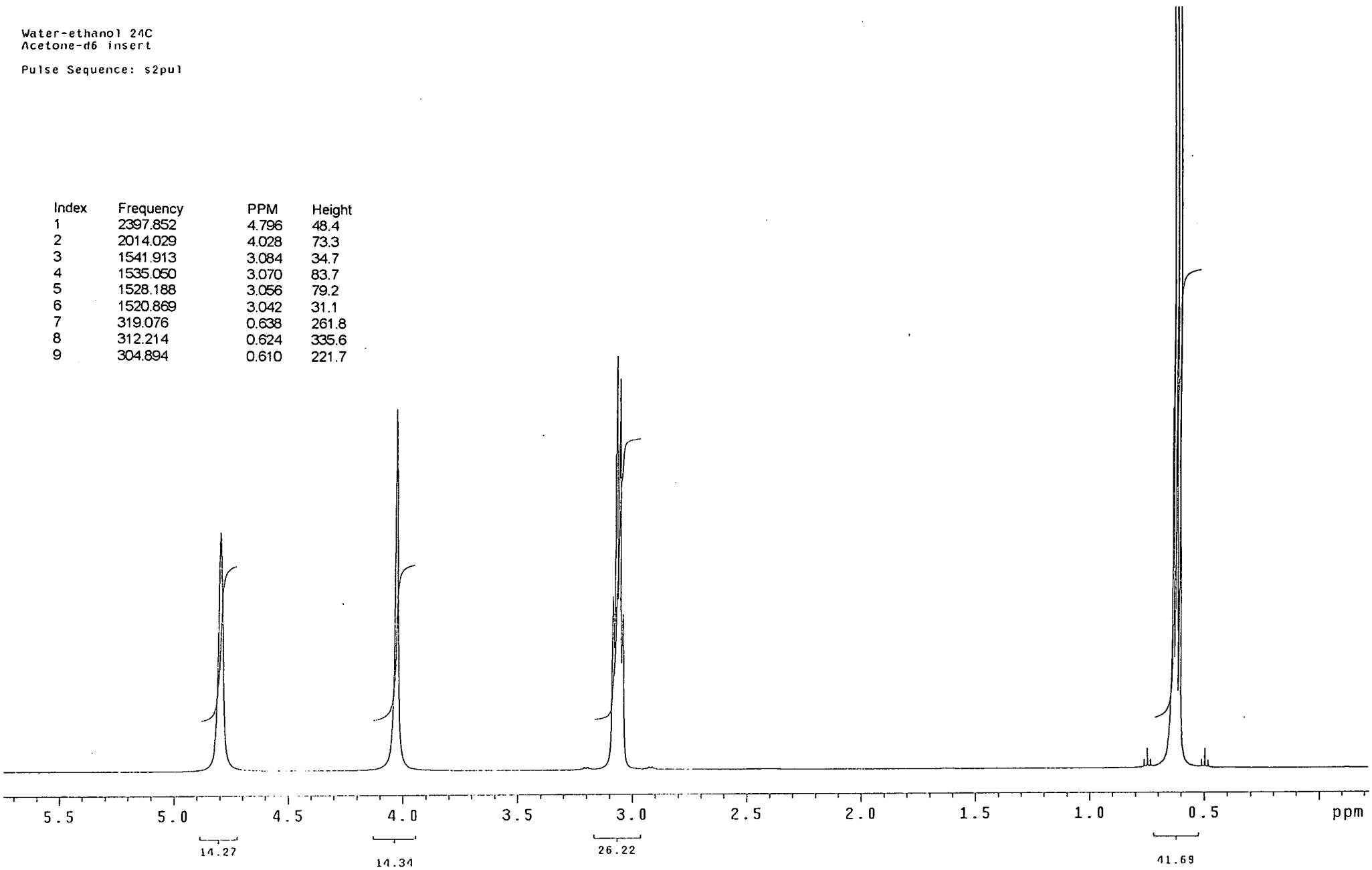

Figure 6: NMR spectrum of Water-ethanol 24CH

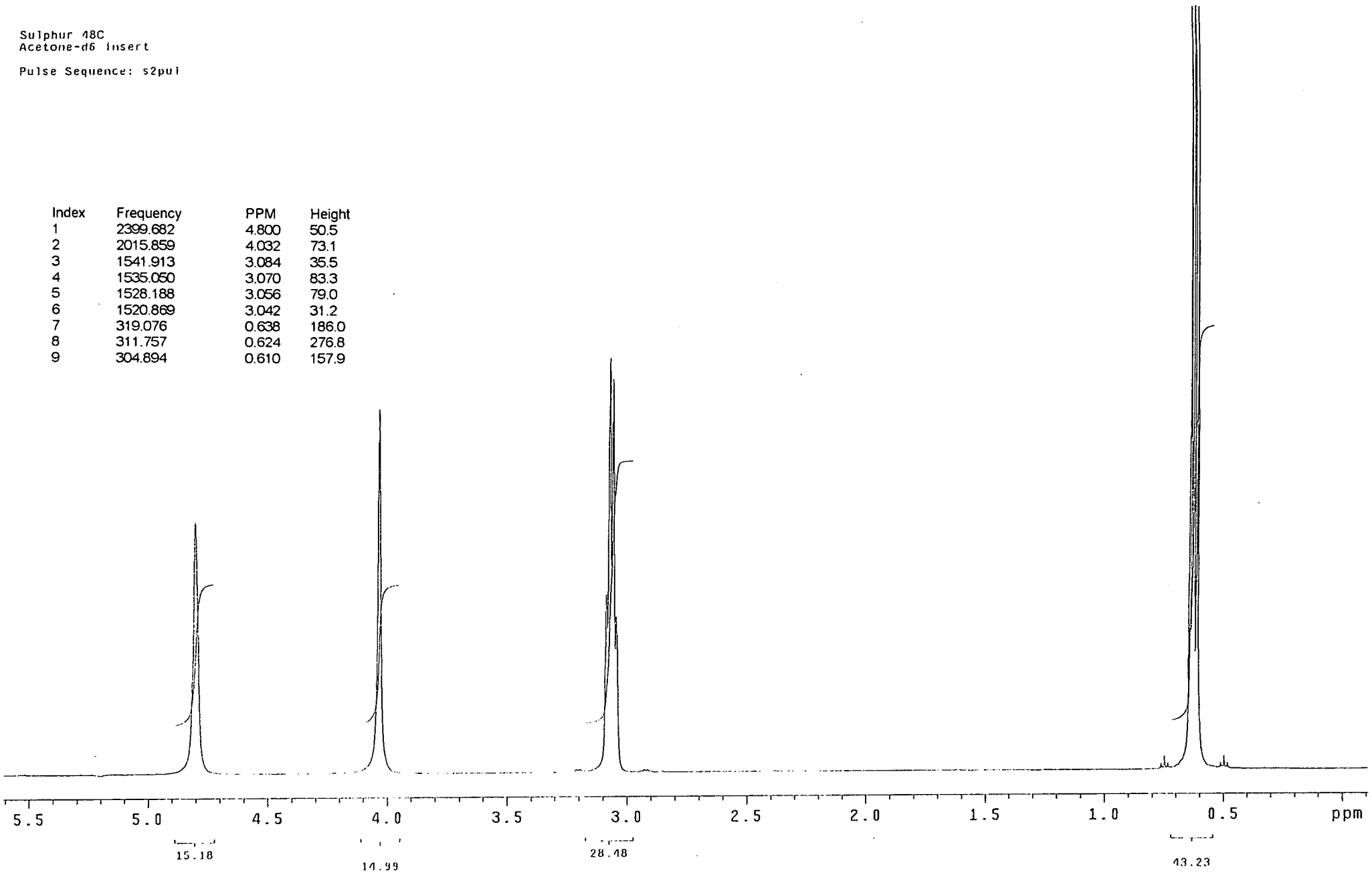

Figure 7: NMR spectrum of Sulphur 48CH

Water-ethanol 48C
Acetone-d6 insert

Pulse Sequence: s2pul

Index	Frequency	PPM	Height
1	2398.310	4.797	47.3
2	2014.486	4.029	72.8
3	1542.827	3.086	32.8
4	1535.965	3.072	77.4
5	1528.646	3.057	73.8
6	1521.784	3.044	29.5
7	319.534	0.639	231.6
8	312.672	0.625	337.8
9	305.352	0.611	198.9

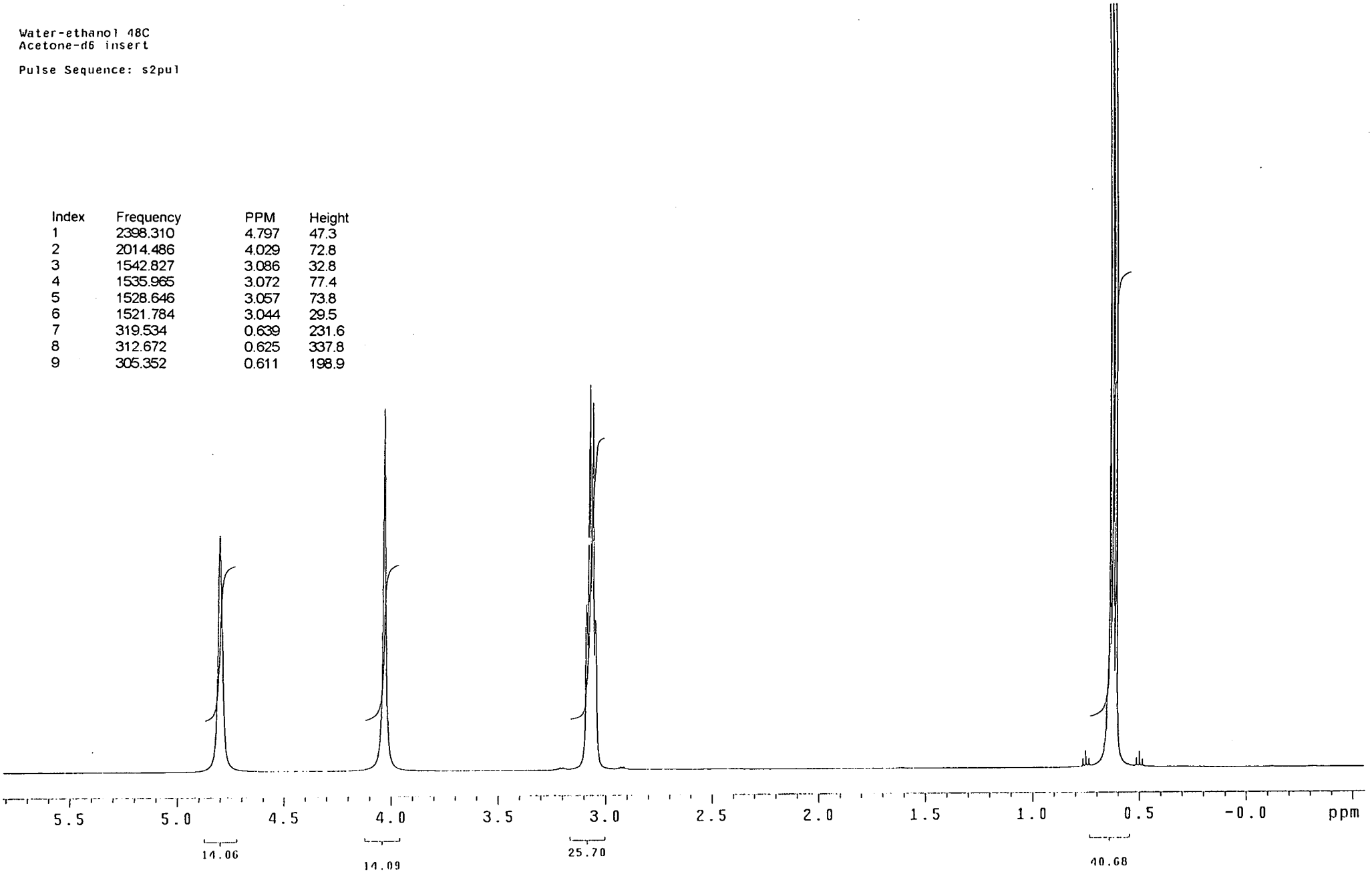

Figure 8: NMR spectrum of Water-ethanol 48CH

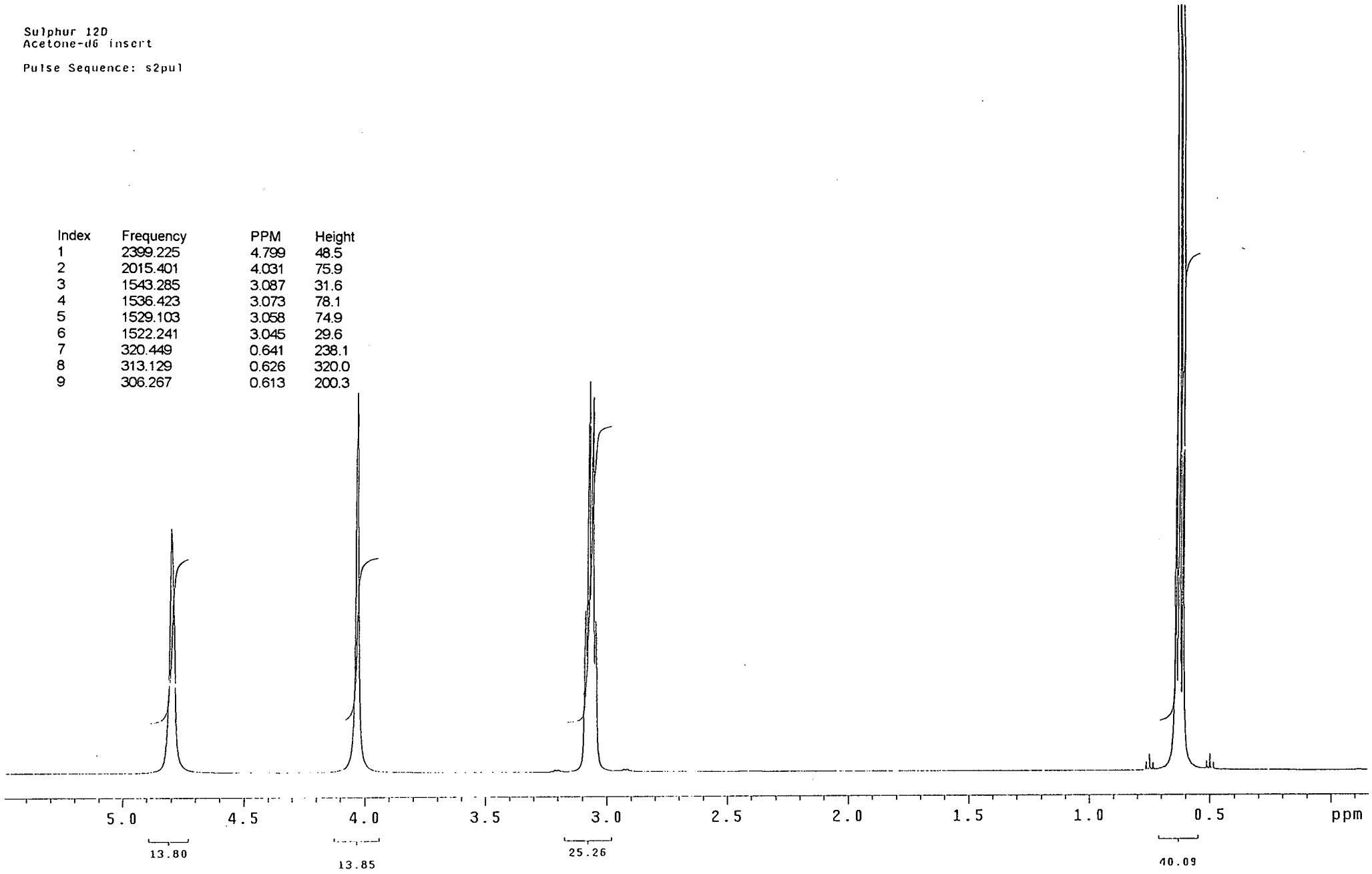

Figure 9: NMR spectrum of Sulphur 12DH

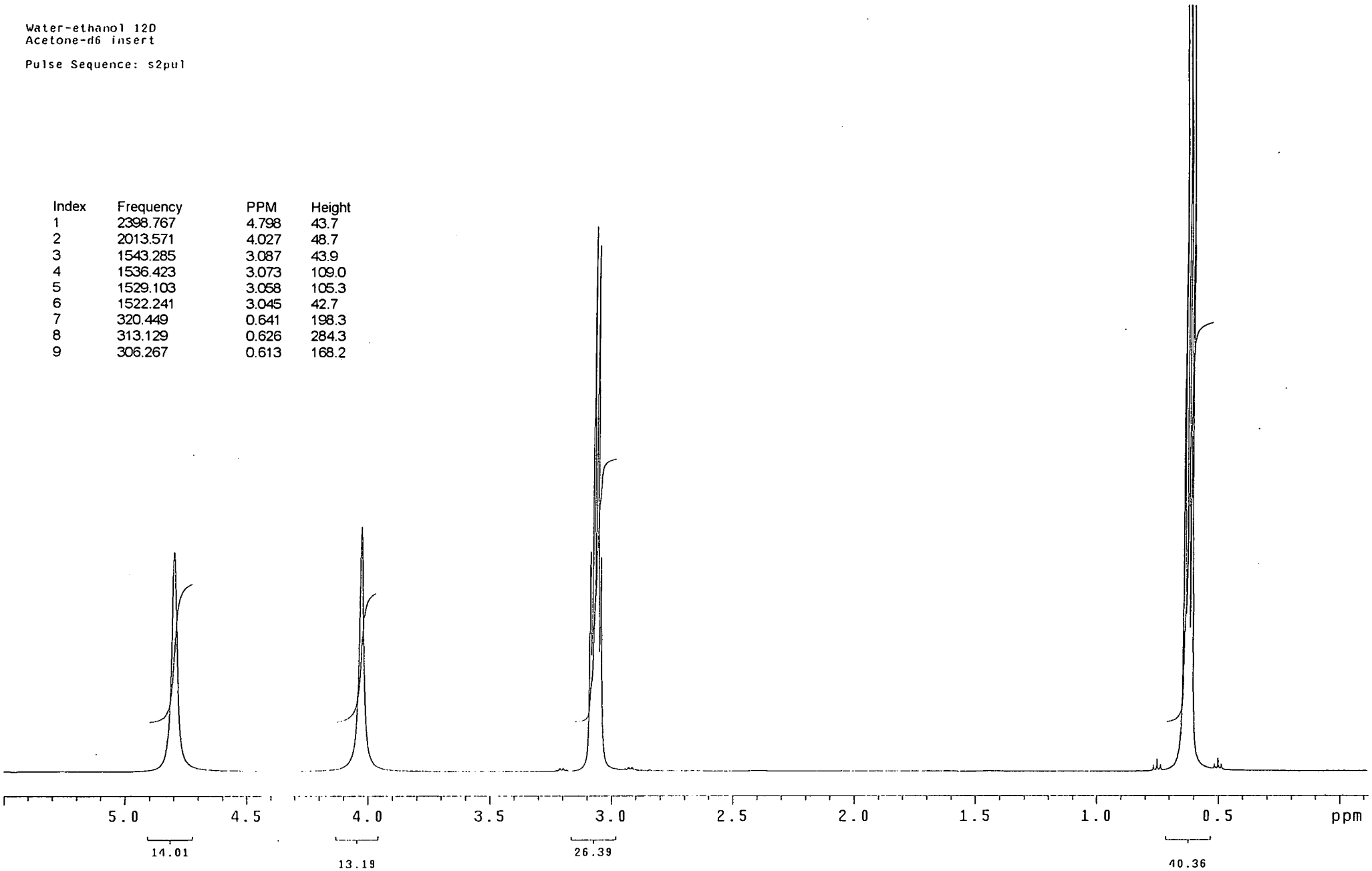

Figure 10: NMR spectrum of Water-ethanol 12DH x

Sulphur 24D
Acetone-d6 insert

Pulse Sequence: s2pul

Index	Frequency	PPM	Height
1	2395.107	4.790	48.0
2	2011.284	4.023	72.7
3	1539.625	3.079	36.5
4	1532.763	3.066	85.2
5	1525.443	3.051	80.0
6	1518.581	3.037	30.9
7	316.789	0.634	231.6
8	309.469	0.619	325.3
9	302.150	0.604	198.1

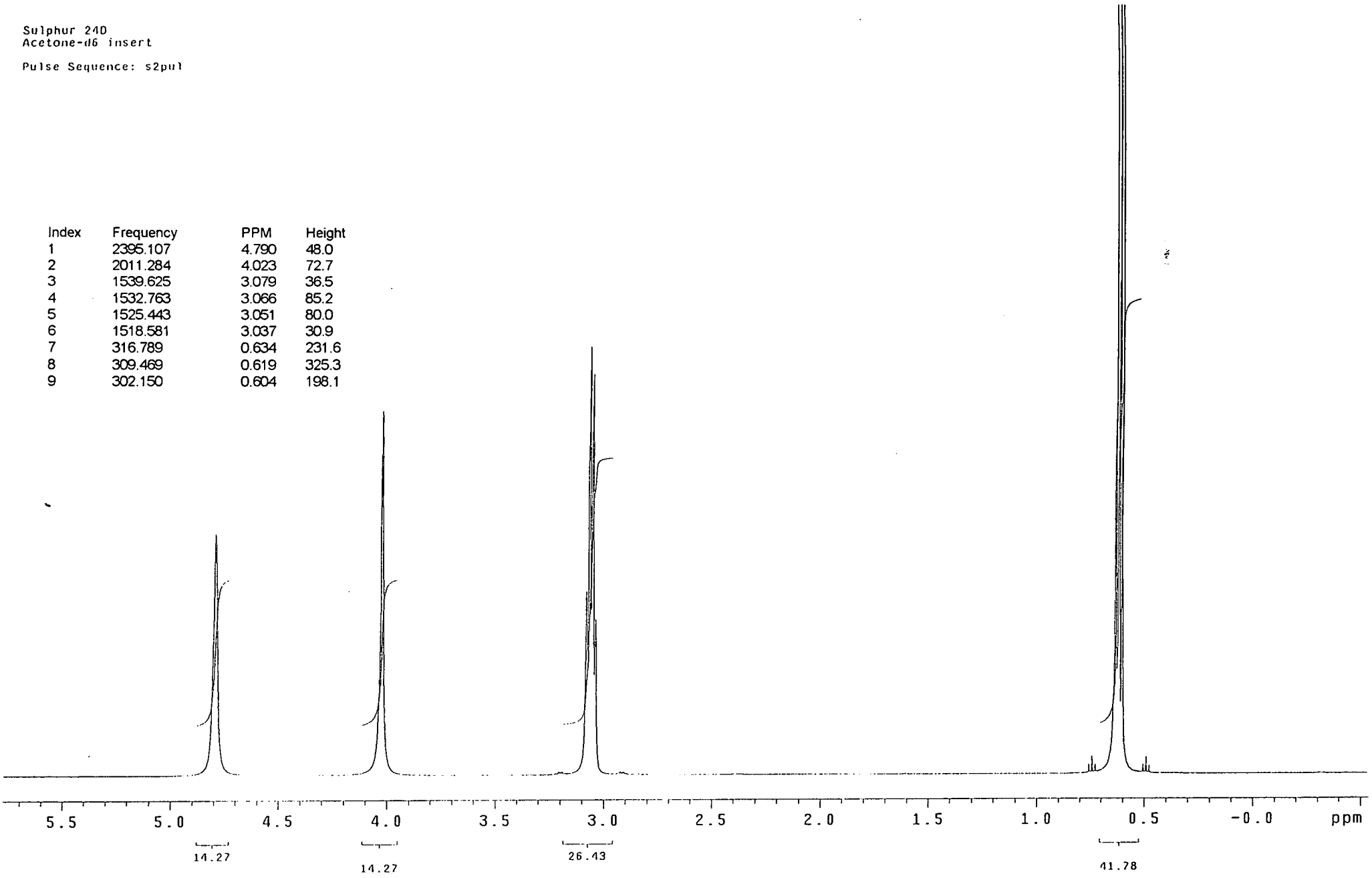

Figure 11: NMR spectrum of Sulphur 24DH

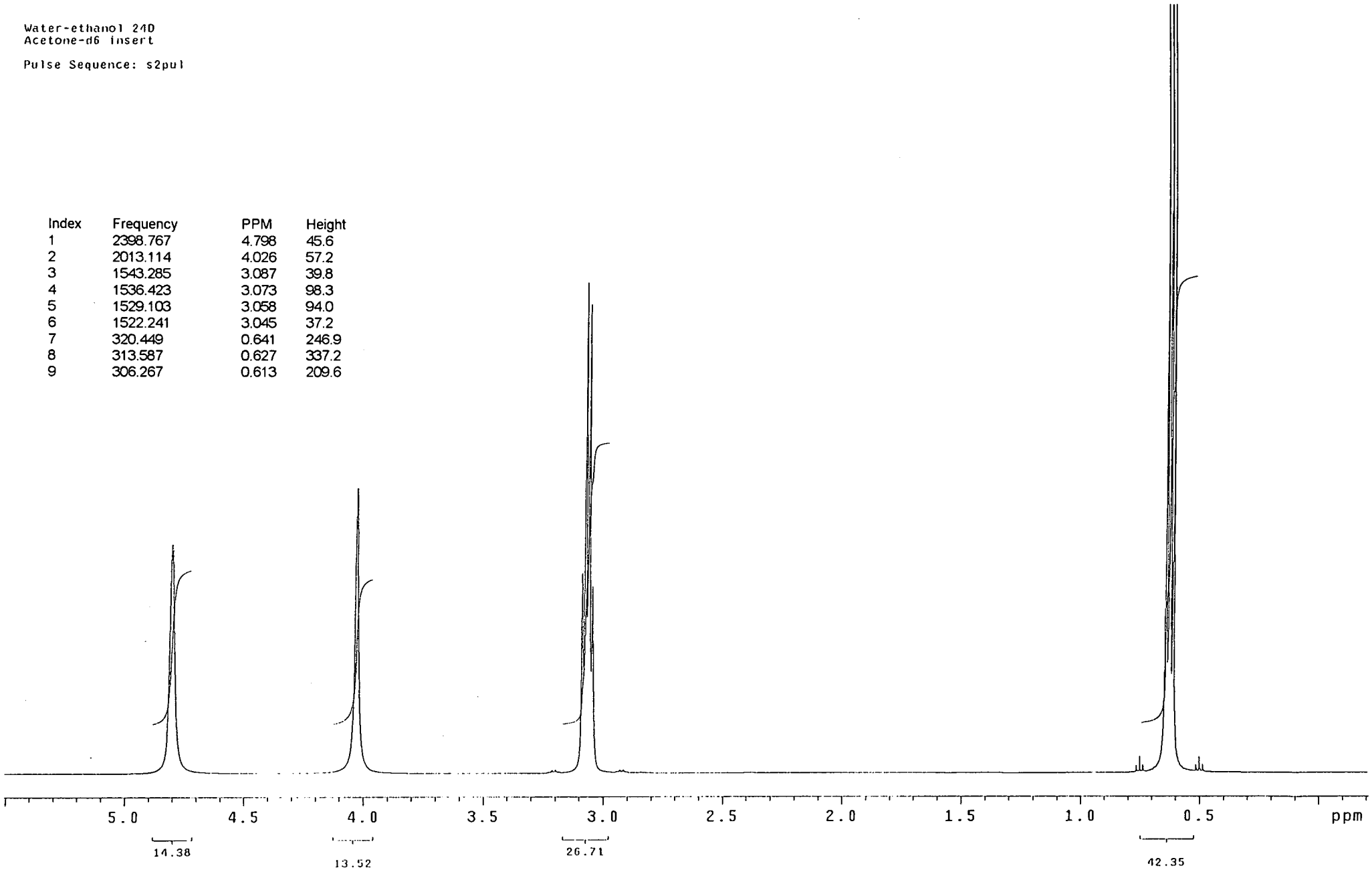

Figure 12: NMR spectrum of Water-ethanol 24DH

Sulphur 48D
Acetone-d6 insert

Pulse Sequence: s2pul

Index	Frequency	PPM	Height
1	2397.852	4.796	47.2
2	2013.571	4.027	67.7
3	1541.913	3.084	37.1
4	1535.050	3.070	89.3
5	1528.188	3.056	84.3
6	1520.869	3.042	32.8
7	318.619	0.637	217.8
8	311.757	0.624	328.0
9	304.437	0.609	185.6

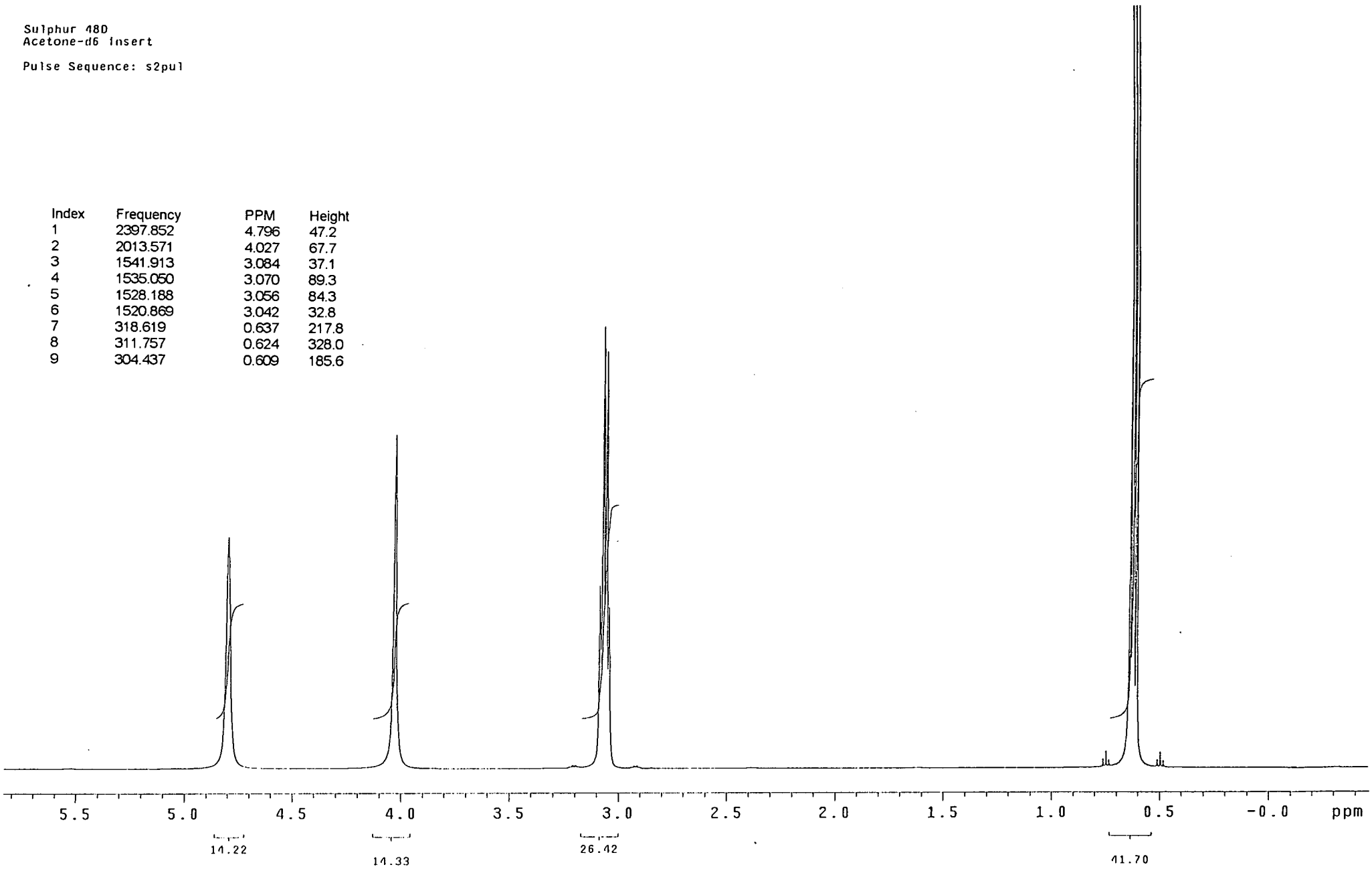

Figure 13: NMR spectrum of Sulphur 48DH

Water-ethanol 48D
Acetone-d6 insert

Pulse Sequence: s2pul

Index	Frequency	PPM	Height
1	2397.852	4.796	45.0
2	2012.199	4.025	56.2
3	1541.913	3.084	41.7
4	1535.050	3.070	97.1
5	1527.731	3.056	92.2
6	1520.869	3.042	35.2
7	318.619	0.637	234.2
8	311.757	0.624	336.8
9	304.437	0.609	203.2

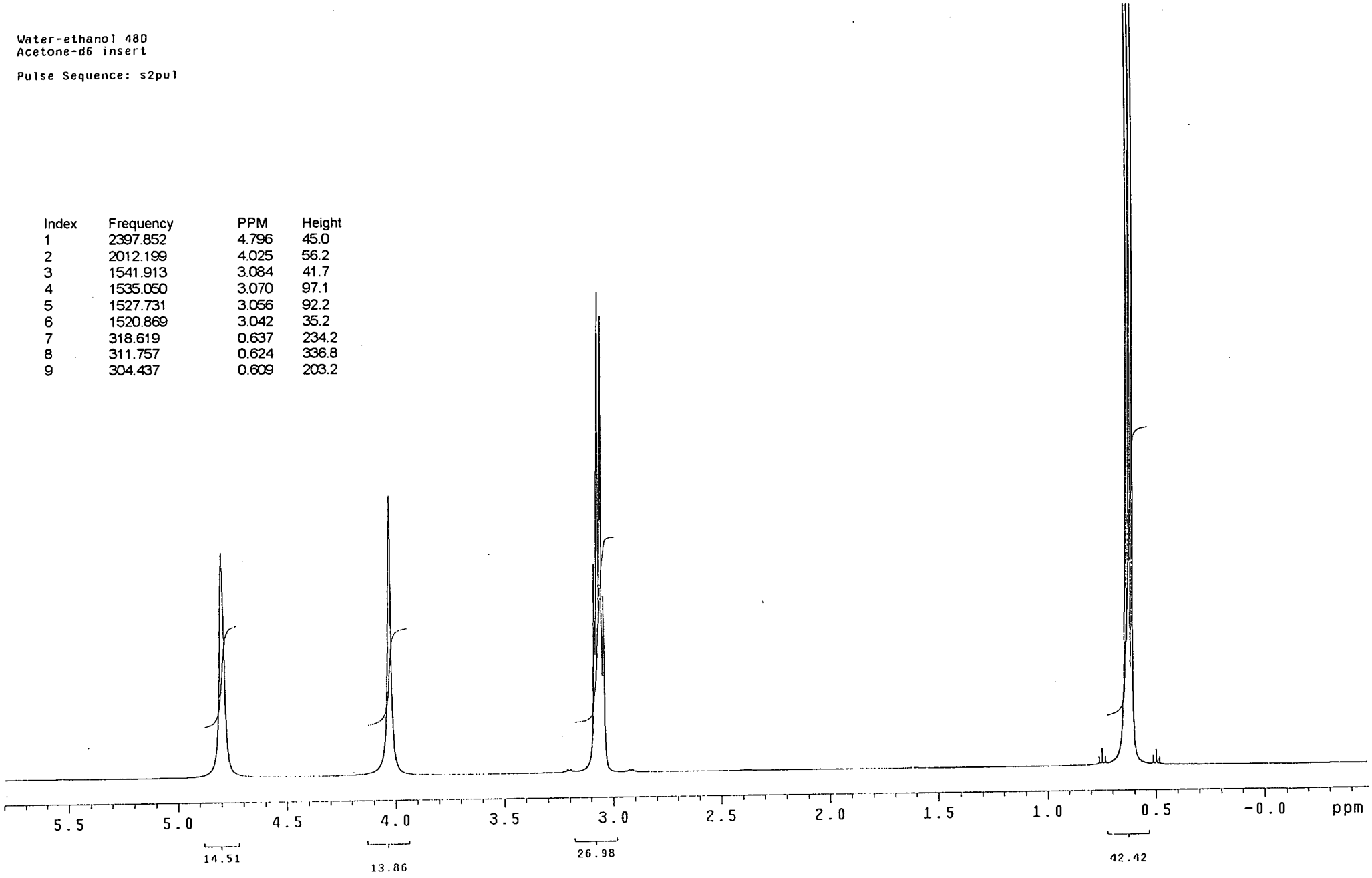

Figure 14: NMR spectrum of Water-ethanol 48DH

Appendix C – Tables of p-values

Appendix Contents

1) Mann-Whitney U-Test: p-values (see 4.2)

Table C.1 *Mann Whitney U-Test. NMR Spectroscopy p-values for H_2O, OH, CH_2 peaks.*

Mann-Whitney U-test p-values for Peaks				
	Comparison	**H_2O**	**OH**	**CH_2**
Substance vs. Control	**Chemical shift**	0.334	0.400	0.053
	Relative integration	0.482	0.482	0.482
Chemical Shift	**Sulphur C / Sulphur D Equal concentration**	0.184	0.376	0.275
	Sulphur C / Sulphur D Equal no. of succussions	1.000	0.827	0.658
	Control C / Control D Equal concentration	0.068	0.105	0.114
	Control C / Control D Equal no. of Succussions	0.178	0.077	0.239
Relative Integration	**Sulphur C / Sulphur D Equal concentration**	**0.050**	0.513	0.87
	Sulphur C / Sulphur D Equal no. of Succussions	**0.050**	0.513	0.275
	Control C / Control D Equal concentration	0.513	**0.050**	**0.050**
	Control C / Control D Equal no. of Succussions	0.827	**0.050**	**0.050**

- The significant p-values are equal to or less than 0.05. Therefore, the null hypothesis is rejected.
- Non-significant p-values are greater than 0.05. Therefore the null hypothesis is accepted.

2) T-Test: p-values (see 4.3)

Table C.2 *T-Test. NMR Spectroscopy p-values for H_2O, OH, CH_2 peaks.*

T-Test p-values for Peaks				
	Comparison	**H_2O**	**OH**	**CH_2**
Substance vs. Control	**Chemical shift**	0.342	0.114	0.561
	Relative integration	0.199	0.713	0.068
Chemical Shift	**Sulphur C / Sulphur D Equal concentration**	0.223	0.323	0.223
	Sulphur C / Sulphur D Equal no. of succussions	0.937	1.000	0.615
	Control C / Control D Equal concentration	0.571	0.128	0.184
	Control C / Control D Equal no. of Succussions	0.207	0.070	0.325
Relative Integration	**Sulphur C / Sulphur D Equal concentration**	**0.050**	0.382	0.661
	Sulphur C / Sulphur D Equal no. of Succussions	**0.022**	0.968	0.239
	Control C / Control D Equal concentration	0.360	**0.001**	0.130
	Control C / Control D Equal no. of Succussions	0.668	**0.003**	0.111

- The significant p-values are equal to or less than 0.05. Therefore, the null hypothesis is rejected.
- Non-significant p-values are greater than 0.05. Therefore the null hypothesis is accepted.

Appendix D – NMR Spectroscopy: Values for Statistics

Appendix Content

The following is the raw data that was used as input to the Mann-Whitney U-Tests and the T-Tests. This data was derived from the NMR Spectra (see **Appendix B**). The input values required calculations:

- Calculation of Chemical Shift values: see **2.5.3**
- Calculation of Relative Integration values: see **3.4**

1) Sulphur vs. Control

See **4.2.1** (Mann-Whitney); **4.3.1** (T-Test)

a) Chemical Shift

Table D.1 *NMR Spectroscopy peak values. Chemical Shift: Sulphur vs. Control*

Chemical Shift	Substance	H_2O	OH	CH_2
Group 1.00 Sulphur	Sulphur 6C	4.784000	4.023000	3.052250
	Sulphur 12C	4.790000	4.024000	3.058500
	Sulphur 12D	4.799000	4.031000	3.065750
	Sulphur 24C	4.794000	4.025000	3.061250
	Sulphur 24D	4.790000	4.023000	3.058250
	Sulphur 48C	4.800000	4.032000	3.063000
	Sulphur 48D	4.796000	4.027000	3.063000
Group 2.00 Control	Water Ethanol 6C	4.795000	4.027000	3.063000
	Water Ethanol 12C	4.795000	4.027000	3.063000
	Water Ethanol 12D	4.798000	4.027000	3.065750
	Water Ethanol 24C	4.796000	4.028000	3.063000
	Water Ethanol 24D	4.798000	4.026000	3.065750
	Water Ethanol 48C	4.797000	4.029000	3.064750
	Water Ethanol 48D	4.796000	4.025000	3.063000

b) Relative Integration

Table D.2 *NMR Spectroscopy peak values. Relative Integration: Sulphur vs. Control*

Relative Integration	Substance	H_2O	OH	CH_2
Group 1.00 Sulphur	Sulphur 6C	0.148468	0.162909	0.271688
	Sulphur 12C	0.149393	0.150343	0.275436
	Sulphur 12D	0.148387	0.148925	0.271613
	Sulphur 24C	0.149968	0.147326	0.272775
	Sulphur 24D	0.147494	0.147494	0.273178
	Sulphur 48C	0.148999	0.147134	0.279545
	Sulphur 48D	0.147098	0.148236	0.273301
Group 2.00 Control	Water Ethanol 6C	0.151029	0.150294	0.272985
	Water Ethanol 12D	0.149122	0.140394	0.280894
	Water Ethanol 24C	0.147845	0.148570	0.271654
	Water Ethanol 24D	0.148309	0.139439	0.275474
	Water Ethanol 48C	0.148736	0.149053	0.271871
	Water Ethanol 48D	0.148410	0.141761	0.275954

2) Chemical Shift: Sulphur and Controls

See **4.2.2** (Mann-Whitney); **4.3.2** (T-Test)

a) Sulphur vs. Sulphur

Table D.3 *NMR Spectroscopy peak values. Chemical Shift: Sulphur (C) vs. Sulphur (D). Equal Deconcentrations*

Chemical Shift	Substance	H_2O	OH	CH_2
Group 1.00 Sulphur CH	Sulphur 6C	4.784000	4.023000	3.052250
	Sulphur 12C	4.790000	4.024000	3.058500
	Sulphur 24C	4.794000	4.025000	3.061250
Group 2.00 Sulphur DH	Sulphur 12D	4.799000	4.031000	3.065750
	Sulphur 24D	4.790000	4.023000	3.058250
	Sulphur 48D	4.796000	4.027000	3.063000

Table D.4 *NMR Spectroscopy peak values. Chemical Shift: Sulphur (C) vs. Sulphur (D). Equal Succussions*

Chemical Shift	Substance	H_2O	OH	CH_2
Group 1.00 Sulphur CH	Sulphur 12C	4.790000	4.024000	3.058500
	Sulphur 24C	4.794000	4.025000	3.061250
	Sulphur 48C	4.800000	4.032000	3.063000
Group 2.00 Sulphur DH	Sulphur 12D	4.799000	4.031000	3.065750
	Sulphur 24D	4.790000	4.023000	3.058250
	Sulphur 48D	4.796000	4.027000	3.063000

b) Control vs. Control

Table D.5 *NMR Spectroscopy peak values. Chemical Shift: Control (C) vs. Control (D). Equal Deconcentrations*

Chemical Shift	Substance	H_2O	OH	CH_2
Group 1.00 Control CH	Water Ethanol 6C	4.795000	4.027000	3.063000
	Water Ethanol 12C	4.795000	4.027000	3.063000
	Water Ethanol 24C	4.796000	4.028000	3.063000
Group 2.00 Control DH	Water Ethanol 12D	4.798000	4.027000	3.065750
	Water Ethanol 24D	4.798000	4.026000	3.065750
	Water Ethanol 48D	4.796000	4.025000	3.063000

Table D.6 *NMR Spectroscopy peak values. Chemical Shift: Control (C) vs. Control (D). Equal Succussions*

Chemical Shift	Substance	H_2O	OH	CH_2
Group 1.00 Control CH	Water Ethanol 12C	4.795000	4.027000	3.063000
	Water Ethanol 24C	4.796000	4.028000	3.063000
	Water Ethanol 48C	4.797000	4.029000	3.064750
Group 2.00 Control DH	Water Ethanol 12D	4.798000	4.027000	3.065750
	Water Ethanol 24D	4.798000	4.026000	3.065750
	Water Ethanol 48D	4.796000	4.025000	3.063000

3) Relative Integration: Sulphur and Controls

See **4.2.3** (Mann-Whitney); **4.3.3** (T-Test)

a) Sulphur vs. Sulphur

Table D.7 *NMR Spectroscopy peak values. Relative Integration: Sulphur (C) vs. Sulphur (D). Equal Deconcentrations*

Relative Integration	Substance	H_2O	OH	CH_2
Group 1.00 Sulphur CH	Sulphur 6C	0.148468	0.162909	0.271688
	Sulphur 12C	0.149393	0.150343	0.275436
	Sulphur 24C	0.149968	0.147326	0.272775
Group 2.00 Sulphur DH	Sulphur 12D	0.148387	0.148925	0.271613
	Sulphur 24D	0.147494	0.147494	0.273178
	Sulphur 48D	0.147098	0.148236	0.273301

Table D.8 *NMR Spectroscopy peak values. Relative Integration: Sulphur (C) vs. Sulphur (D). Equal Succussions*

Relative Integration	Substance	H_2O	OH	CH_2
Group 1.00 Sulphur CH	Sulphur 12C	0.149393	0.150343	0.275436
	Sulphur 24C	0.149968	0.147326	0.272775
	Sulphur 48C	0.148999	0.147134	0.279545
Group 2.00 Sulphur DH	Sulphur 12D	0.148387	0.148925	0.271613
	Sulphur 24D	0.147494	0.147494	0.273178
	Sulphur 48D	0.147098	0.148236	0.273301

b) Control vs. Control

Table D.9 *NMR Spectroscopy peak values. Relative Integration: Control (C) vs. Control (D). Equal Deconcentrations*

Relative Integration	Substance	H_2O	OH	CH_2
Group 1.00 Control CH	Water Ethanol 6C	0.151029	0.150294	0.272985
	Water Ethanol 12C	0.150420	0.149382	0.275407
	Water Ethanol 24C	0.147845	0.148570	0.271654
Group 2.00 Control DH	Water Ethanol 12D	0.149122	0.140394	0.280894
	Water Ethanol 24D	0.148309	0.139439	0.275474
	Water Ethanol 48D	0.148410	0.141761	0.275954

Table D.10 *NMR Spectroscopy peak values. Relative Integration: Control (C) vs. Control (D). Equal Succussions*

Relative Integration	Substance	H_2O	OH	CH_2
Group 1.00 Control CH	Water Ethanol 12C	0.150420	0.149382	0.275407
	Water Ethanol 24C	0.147845	0.148570	0.271654
	Water Ethanol 48C	0.148736	0.149053	0.271871
Group 2.00 Control DH	Water Ethanol 12D	0.149122	0.140394	0.280894
	Water Ethanol 24D	0.148309	0.139439	0.275474
	Water Ethanol 48D	0.148410	0.141761	0.275954