A Comparative Study of the NMR Spectra of Sulphur 12CH prepared using Hahnemannian method and Sonication

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I Scott Marsh-Brown do declare that this dissertation is representative of my own work, both in conception and execution.

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Dedication

I’d like to dedicate this dissertation to my mother whose support has been constant and invaluable, as well as to my two supervisors. Without your effort and support this accomplishment would not have been possible.

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ABSTRACT

Aim

The aim of this study was to compare the nuclear magnetic resonance spectra of Sulphur 12c samples produced by the traditional Hahnemannian method with Sulphur 12c samples produced using sonication as an alternative method of agitation. Sonication, while not widely employed as an agitating technique in the homoeopathic potentisation process, is a highly effective agitation process which produces effects on liquids that closely resemble the effect of traditional Hahnemannian hand succussion (Bhattacharyya *et al.* 2008). Thus, this study sought to reveal whether or not homoeopathic remedies produced by sonication bore a close enough physicochemical resemblance to traditional hand succussed remedies to be considered as a viable equivalent.

Methodology

Five sample groups were manufactured for analysis, all by means of serial dilution at the centesimal ratio (1:100) to the 12c potency, and with agitation between dilution levels where applicable. Three of the sample groups were experimental, namely the Sulphur 12c Hahnemannian, Sulphur 12c sonicated and Sulphur 12c both (succussion and sonication). The Sulphur 12c Hahnemannian samples were produced by hand according to the German Homoeopathic Pharmacopoeia (Benyunes 2005), which includes an agitation phase of 10 hand succussions. Sonicated samples were produced according to the Hahnemannian method as far as possible, however the agitation phase consisted of 30 seconds of sonication in a sonication bath at 40Hz in accordance with related studies (Sukul, Sinhabau, and Sukul 1999: 58-59; Sukul *et al*. 2001a: 187). Sulphur 12c both (succussion and sonication) samples underwent ten hand succussions and 30 seconds of sonication at 40Hz between dilution levels.

Two of the sample groups were controls, namely Sulphur 12c unagitated and Lactose 12c unagitated, neither of which underwent agitation between dilution phases but were otherwise produced according to the German Homoeopathic Pharmacopoeia specification (Benyunes 2005). All samples were raised to the 12c potency level in 87% alcohol from a 3CH triturate. The Lactose 12c unagitated control was derived from a 3CH triturate of lactose, while the other samples were all derived from a 3CH triturate of Sulphur.

The sample groups were sent for nuclear magnetic resonance (NMR) spectroscopy at the Department of Chemistry at Stellenbosch University. The NMR device used was the Varian ^{Unity}*Inova* 600 NMR Spectrometer ®, with a Deuterated DMSO insert added as an instrument frequency lock. Samples were drawn and analysed by Dr D.J. Brand. One sample was drawn from each sample group.

The chemical shift and relative integration values for the OH, H_2O, CH_2, and CH_3 peaks of the NMR spectra were captured and tabulated using Microsoft Excel© 2013. The statistical analysis was performed with the aid of SPSS Version 22. The chemical shift and relative integration values for the H_2O, OH, CH_2 and CH_3 peaks were used in the statistical analysis. The Kruskal-Wallis method was performed for the five sample groups to ascertain whether or not a statistically significant difference existed between the five sample groups. Comparisons between individual paired groups were conducted by means of the non-parametric Mann-Whitney test. The significance interval was set at $\alpha = 0.05$.

Results

The chemical shift values of the CH_2 peaks of the samples showed a clear similarity between the samples produced by Hahnemannian hand succussion, sonication and both (succussion and sonication) as well as a clear difference between these three samples and the two controls. The relative integration values, however, showed no clear trends in support of or detracting from the hypotheses.

Conclusion

In terms of the CH_2 peak chemical shift values it can be concluded that distinct similarities exist between 12c potency level of Sulphur produced by Hahnemannian hand succussion and sonication, and that the two methods of agitation produce similar structural properties in samples of the 12c potency level. Furthermore in terms of the chemical shift values, succussion and sonication develop remedies that are distinct from unagitated remedies of equivalent potency level. Thus, these findings support the use of sonication as a potentially viable alternative to hand succussion as a method of agitation in the potentisation process.

Further studies need to be conducted however, with the inclusion of a greater variety of potency levels in order to possibly reveal more trends in terms of the relative integration values as these values were inconclusive in this study.

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ANOVA	Analysis of Variance
B_o	External magnetic field
CH	Centesimal Hahnemannien
CH_2	Methylene group
CH_3	Methyl group
FID	Free induction decay
FT	Fourier Transform
g	gram
H_2O	Water
Hz	Hertz
J	Coupling constants
ml	Millilitre
NMR	Nuclear Magnetic Resonance
OH	Hydroxyl group
ppm	Parts per million
RF	Radio frequency
T_1, T_2	Relaxation times
TMS	Trimethylsilane
δ	Delta
µl	Microlitre

DEFINITION OF TERMS

Agitation

The application of a disturbing force to a liquid.

Analysis of Variance

A method of statistical analysis in which the differences between group means are analysed.

Avogadro's number

The number of units in a mole of any substance equal to 6.022×10^{23}

Centesimal

The most commonly used dilution scale used in homoeopathic pharmacy. It represents a dilution level of 1:100 so that each succeeding potency thus contains one hundredth part of the preceding potency. The centesimal scale is denoted by CH or C.

Chemical shift

In NMR spectroscopy, it indicates the resonance frequency of nuclei in an external electromagnetic field relative to a reference standard such as Trimethylsilane. It is measured in parts per million (ppm) of the operating frequency of the spectrometer.

Cavitation

The formation of vapour cavities or voids within a liquid subjected to varying pressures. Such cavities implode when the pressure of the liquid increases and release shockwaves into the surrounding liquid.

Integration

The relative intensity of individual NMR peaks. It is indicative of the relative number of hydrogens present at each signal.

Larmor frequency

The characteristic frequency of the precessional motion of a charged nucleus when placed in an external magnetic field during NMR experimentation. The Larmor frequency is specific to each nucleus.

Mean (x)

A measure of central tendency defined as the sum of the values in the sample divided by the sample size.

NMR-spectroscopy

An analytical method that employs an applied magnetic field to measure its effect on the protons present within organic compounds and thereby reveal information about the structure of such compounds. The interaction between the applied magnetic field and the compound is recorded as a series of peaks known as an NMR spectrum.

Physical structure

The three-dimensional arrangement of atoms and molecules in a compound or mixture.

Potency

An altered state of material substance used as a homoeopathic medicine. The potency level is indicative of the deconcentration level of that remedy.

Potentisation

A process used in the preparation of homoeopathic medicines to raise potency levels. The remedy undergoes serial dilution and agitation, typically by succussion and/or trituration, in a specified ratio.

Precession

The gyration of the rotation axis of spinning nuclei exposed to an external magnetic force.

Relative integration values

The integration, or area beneath a particular peak, relative to the total area beneath all the peaks on an NMR graph. It is calculated by dividing the integration values of each peak by the sum of all the integration values of the run.

Remedy

A medicine produced in accordance with the homoeopathic pharmaceutical process and homoeopathic philosophy.

Sonication

An agitation technique that employs the passage of sound waves through a substance.

Sonicator

A device used to perform sonication.

Spectrum (NMR)

A graph depicting, by means of a series of peaks, the resonant frequencies at which protons of different types of hydrogen atoms of a compound absorb electromagnetic radiation. Ethanol for example produces four peaks: H_2O, OH, CH_2, and CH_3.

Standard deviation

A measure of dispersion in statistics in which all of the data points are used to indicate the variation that exists within the data set.

Succussion

Agitation of a liquid homoeopathic remedy that employs firm swift shakes in a vertical plane ending with a jolt. It is often performed by hand by pounding the remedy container repeatedly against an elastic surface.

Trituration

The process of grinding lactose and a medicinal starting substance together by means of a mortar and pestle for a prolonged period of time in order to

reduce the particle size of the substance and render insoluble substances soluble.

T-tests

In statistics, an analytical method that is employed to determine the differences between the means of two groups.

Unagitated

Having not undergone an agitation process such as hand succussion or sonication during the potentisation process of homoeopathic remedy production.

CHAPTER 1: INTRODUCTION

Since the inception of Homoeopathy and Homoeopathic medicines in 1796 there has been much experimentation and development of different methods of remedy manufacture. The aim of such experimentation and innovation was largely to reduce the time required during and cost of manufacture (Winston 1999). Currently many different methods of manufacture are employed, with a variety of potentisation techniques being utilised. The original method, the Hahnemannian method, was developed by the founder of Homoeopathy, Samuel Hahnemann. Many homoeopaths believe that this is the gold standard of homoeopathic remedy production, as at least in part the succussive or shaking action employed by this method of manufacture develops the medicinal properties of the homoeopathic remedy (Kayne 1997).

One particular alternative method of manufacture employs sonication to agitate the homoeopathic solution during manufacture, replacing or in addition to the succussive phase found in the Hahnemannian method of manufacture (Sukul *et al.* 1999; Bhattacharyya *et al.* 2008). Indeed, the nature of the agitation produced by sonication, specifically the production of high and low pressure zones as a result of the passage of sound energy through remedy samples being produced very closely resembles the high and low pressure zones that are theorised to occur within a sample being succussed (Bhattacharyya *et al*. 2008). This striking similarity between the two agitation techniques may in theory result in remedies with very similar physicochemical profiles. Although sonication is currently not widely used, this method of production could have the potential to be an alternative to the traditional Hahnemannian style of remedy agitation during production, and could possibly surpass the traditional Hahnemannian succussive style in terms of easy of use and time consumption.

Little published research exists that supports the use of sonication as an alternative or adjuct to Hahnemannian succussion. Thus, sonication as a method of remedy agitation deserves further investigation in order to establish whether or not it could be a viable equivalent to Hahnemannian succussion. To address this issue of the viability of sonication as an alternative to Hahnemannian succussion, this study will serve to analyse and compare the

physicochemical properties of remedies prepared by the two techniques. If the two styles of remedy agitation produce remedy samples that are alike in terms of physicochemical properties, then this may justify further investigation such as homoeopathic drug proving trials to test whether the two methods of agitation produce remedies that have the same or similar medicinal effects when administered to patients.

To analyse the samples produced by Hahnemannian succussion and sonication, as well as the controls, Nuclear Magnetic Resonance (NMR) spectroscopy was selected as the most appropriate method of spectroscopy for the purpose of this study. NMR spectroscopy is well suited to the analysis of the physicochemical properties of homoeopathic remedies due to the high degree of analytical sensitivity (Young 1975: 8-16).

1.1 THE AIM OF THE STUDY

The aim of this study is to compare the Nuclear Magnetic Resonance (NMR) spectra of Sulphur 12c prepared using serial dilution and succussion (Hahnemannian method) versus serial dilution and sonication. Five samples were produced, namely:

1) Sulphur 12c Hahnemannian method (succussion).
2) Sulphur 12c Sonication.
3) Sulphur 12c Hahnemannian method and sonication (both).
4) Sulphur 12c Unagitated.
5) Lactose 12c Control.

All samples employed serial dilution at the centesimal (1:99) dilution scale.

1.2 THE OBJECTIVES OF THE STUDY

1.2.1 The first objective

To compare the chemical shift and integration values of the CH_2, CH_3, H_2O, and OH signals of NMR spectra of Sulphur 12c prepared by the Hahnemannian method, with those of the other four sample groups.

1.2.2 The second objective

To compare the chemical shift and integration values of the CH_2, CH_3, H_2O, and OH signals of NMR spectra of Sulphur 12c prepared by sonication, with those of those of the other four sample groups.

1.2.3 The third objective

To compare the chemical shift and integration values of the CH_2, CH_3, H_2O, and OH signals of NMR spectra of Sulphur 12c prepared by the Hahnemannian method and sonication (both), with the other four sample groups.

1.2.4 The fourth objective

To compare the chemical shift and integration values of the CH_2, CH_3, H_2O, and OH signals of NMR spectra of Sulphur 12c prepared without agitation with the other four sample groups.

1.2.5 The fifth objective

To compare the chemical shift and integration values of the CH_2, CH_3, H_2O, and OH signals of NMR spectra properties of the Lactose 12c control, with the other four sample groups.

1.3 THE HYPOTHESES

1.3.1 The first hypothesis

It was hypothesised that little or no difference existed between the chemical shift and integration values of CH_2, CH_3, H_2O signals produced by 12c potencies manufactured by the Hahnemannian method, sonication, or a combination of the two.

1.3.2 The second hypothesis

It was hypothesised that a statistically significant difference in terms of chemical shift and relative integration values for the H_2O, OH, CH_2 and CH_3 peak values would exist between the samples produced by the Hahnemannian method, sonication, and both Hahnemannian and sonication and the control samples, namely the Lactose 12c control and the Sulphur 12c unagitated.

CHAPTER TWO: REVIEW OF THE RELATED LITERATURE

2.1 INTRODUCTION

Various methods of homeopathic remedy production have been employed throughout the history of Homoeopathy. Many of these alternative techniques were developed and used without a scientific understanding of how the remedies produced by such means may alter the physicochemical properties of the remedy. Thus, the modern science of homoeopathy has an obligation to scientifically explore the validity of alternative methods of homoeopathic remedy production and find a way of standardising the Homoeopharmaceutics industry (Botha 2005).

The Hahnemannian method is the original method of homeopathic remedy production, and considered by many to be the gold standard. Sonication, the application of sound waves to agitate a solution, is an alternative method of production employed in homoeopathic remedy production (Sukul *et al.* 2001b; Bhattacharyya *et al.* 2008). The effect of sonication on the physicochemical properties of a remedy has not been thoroughly researched. Thus this review will serve to explore the importance of potentisation, the theoretical effect of sonication on remedies, and NMR spectroscopy as a means of analysing high dilution homoeopathic remedy samples.

2.2 HISTORY OF HAHNEMANNIAN POTENTISATION

Samuel Hahnemann (1755-1843), the founder of Homeopathy, spent many decades developing and refining the production method of high dilution homoeopathic medicines. By 1801 Hahnemann had published a pamphlet, *Cure and Prevention of Scarlet Fever,* in which he specified diluting the medicine in three phases of dilution, and stressed the importance of shaking the solution between dilution phases, as well as vigorous stirring of the medicine before giving a dose. His reason for diluting the medicinal substance was to reduce its toxicity; later he discovered and deduced that physically agitating the medicinal liquid between dilution phases increased the interaction

between the medicine and the organism and gave it ‘power’, i.e. increased its therapeutic efficacy (Barthel 1991).

By 1814, the agitating phase had been standardised and three minutes of vigorous shaking between dilution phases was required. In volume 6 of *Materia Medica Pura*, Hahnemann introduced hand succussion as the method of agitation between dilutions (1821). This involved beating the remedy vial against an elastic surface ten times using “the full strength of the arm”. In Hahnemann’s sixth edition of *Materia Medica Pura* (1987) he postulates that the duration of succussion or trituration affects the strength of the medicine, and expressed concern that medicines succussed or triturated for too long would become harmful. By the late 1830’s, Hahnemann was using varying numbers of succussions ranging from ten to 50 or more between dilution phases. The number of succussions were also adjusted according to the substance being potentised. By 1838 Hahnemann had developed the LM potency (1 in 50000 dilution), and settled on 100 succussions per potency level. This method of production was detailed in the sixth edition of the *Organon* (1921).

2.3 MODERN HOMOEOPATHIC REMEDY PRODUCTION

In modern times, the Hahnemannian method involves hand succussion between dilution phases. The number of succussions differs from one pharmacopoeia to another, ranging from ten to 100 succussions.

Dilutions typically occur in three deconcentrations (Gaier 1991):

i) Decimal, in which each potency contains 1/10 of the preceding potency.
ii) Centesimal, in which each potency contains 1/100 of the preceding potency.
iii) Quinquagenimillesimal, in which each potency contains 1/50000 of the preceding potency.

While hand succussion as performed by Hahnemann was perhaps the original form of agitation employed in the potentisation process, many different methods of agitation have since been employed. The Skinner continuous fluxion method has been commonly used to produce very high potencies including 10M (1 in 100 dilution 10000 times) and CM (1 in 100 dilution 100000 times) potencies. This method employs a device in which a single vial is automatically emptied and refilled under a pressurised flow of water (Kayne 2006: 98-99). The water flows over the vial, simultaneously creating turbulence within the vial and diluting the contents. Dr Bernhardt Fincke developed another fluxion technique in which water is pumped through the bottom of a cylinder containing 30c potency of the remedy being potentised (Kaercher 2000). The top of the cylinder is narrowed to increase the pressure of the water entering the cylinder and to create turbulence within the cylinder. The 30c potency is thereby simultaneously diluted and agitated, thus raising its potency level.

Under the school of anthroposophical medicine, homoeopathic dilutions are agitated by means of vortex formation (Erasmus 2004). Wala preparations are agitated by swiftly shaking the container in a horizontal plane back and forth by hand. Weleda preparations are agitated by swirling the container in a figure of eight. Both techniques create vortex formations within the liquid contents, thereby subjecting the contents to shearing forces and cavitation.

Standardised mechanical arm pounding against a hard surface is commonly used by homeopharmaceutical companies (Bell and Koithan 2012). Such semiautomatic potentizers ensure that the number of succussions per dilution remains constant and that each succussion is performed with the same force with each stroke (Bhattacharyya, Mandal and Biswas 2008).

2.4 AGITATION BY SONICATION

Sonication is a means of solution agitation commonly employed in chemistry laboratories, which involves passing sound waves through a substance. Very little literature exists which explores the physicochemical effect that sonication has on Homoeopathic remedies, and whether the agitating effect is capable of producing remedies that are similar or identical to those produced by the

Hahnemannian method. Sonication, when employed in Homoeopathic remedy production, is more commonly used as an adjunct technique to the Hahnemannian method. Recently, a number of studies have been published in which homoeopathic remedies were potentised by means of Hahnemannian succussion and sonication (Sukul *et al.* 1999; Sukul *et al.* 2001a). A study by Chikramane *et al.* (2012) revealed that there were similarities between microbubbles in high dilutions produced by succussion and sonication. Bhattacharyya *et al.* (2008) performed various spectroscopy studies which revealed that little difference existed between the highest potencies of a homoeopathic remedy produced by hand jerk, vortexing and sonication.

Sonication is probably the most effective mechanical technique for the reduction of aggregations in a suspension, and is widely used in the production of nanofluids (fluids containing nanometer-sized particles) and other industry (Ruan and Jacobi 2012). Sonication agitates a liquid by creating compression (high pressure) and decompression (low pressure) zones within the liquid. These waves of high and low pressure zones move through the liquid being sonicated (Suslick 1988b). Cavitation, the formation of tiny cavities within the liquid structure, occurs during a decompression phase and is essentially a tiny vacuum within the solution structure. Such cavities then implode when under the pressure of a compression zone, and as a result emit a significant amount of energy into the surrounding liquid. During implosion, extremely high temperatures and pressures are reached, shock waves are emitted, and a jet of water is ejected from the collapsing cavity (Suslick 1988a). Thus, many types of energies are at play during sonication, all of which contribute to the dissolution of the solute in a solvent.

Auerbach theorised that compression and decompression waves are formed within a liquid during Hahnemannian succussion, and that cavitation similar to that seen in sonicated liquids also occurs in succussed liquids (Endler and Schulte 1996). Thus in theory, sonication and succussion have similar effects on liquids and so sonication may prove to be a viable method of potentisation.

2.5 IMPORTANCE OF POTENTISATION

Homoeopathic potentisation is a term used to describe the process of serial dilution and succussion employed in homoeopathic remedy production; this is believed to increase the therapeutic strength of homoeopathic remedies (Kayne 2006). Succussion is vigorous shaking of the remedy, and apart from simply dissolving the solute in the solvent it is thought to play a vital role in developing the medicinal properties of a remedy. According to Vithoulkas (1980) succussion adds kinetic energy to a solution, which is crucially important for the efficacy of the remedy. Clinical observations have revealed that sufficient agitation is vitally important for the therapeutic efficacy of a remedy; dilution alone is not sufficient (Jones and Jenkins 1983). As posited by Sukul *et al.* (2001), succussed and unsuccussed remedies differ considerably in their physicochemical characteristics as displayed by electronic, infra-red and nuclear magnetic resonance spectra. Thus the agitation or succussion process is certainly necessary for the production of clinically effective remedies.

Bell and Koithan (2012) demonstrated that high dilution remedies produced by very gentle agitation versus dynamisation (powerful succussion) show considerable differences in terms of the NMR relaxation times. Lyell (2004) demonstrated by means of NMR chemical shift and relative integration values that parallel potencies of Natrum Muriaticum prepared with differing numbers of succussions were dissimilar, thus suggesting that the number of succussions also influence the physicochemical properties of the remedy.

The exact mechanism of how potentisation conveys the medicinal properties of high dilution homoeopathic remedies is not well understood. High dilution remedies at the 12CH potency level and higher have theoretically exceeded Avogardo's constant, meaning that statistically there is little possibility of even a single molecule of the original substance being present (Barthel 1991). Thus, the biological or medicinal effect of homoeopathic remedies has been attributed to water's ability to act as a repository and transmitter of biologically significant information (Bellavite 2014). Indeed, there is much evidence ultilising various methods of spectroscopy that demonstrates that the potentisation process results in high dilutions that are distinct from similarly prepared controls.

Ultra-high dilutions of samples prepared with water as the solvent by the 1/100 dilution technique displayed distinctly different heats of mixing compared to the corresponding heats of mixing of the untreated aqueous solvent controls. Heat of mixing is a measure of the amount of heat that is absorbed or released during the mixing of two non-reacting chemical substances. The authors concluded that this indicated that a permanent alteration of the physicochemical properties of the solvent water must have occurred due to the presence of the original solute (Elia *et al.* 2004). A study that employed thermoluminescence revealed that the networks of hydrogen bonds of ultra-high dilutions produced with pure water as the solvent and sodium chloride as the solute displayed thermoluminescence peaks that differed from those of pure water that had undergone the same dilution process (Van Wijk *et al.* 2006). This indicates a structural reorganisation in response to the original solute that then persisted at high dilution. Nuclear magnetic resonance spectra of ultra-high dilutions suggest evidence of supramolecular organisation of water in ultra-diluted samples (Becker-Witt 2003; Demangeat 2009). The above mentioned studies all serve to provide evidence of the structure or physicochemical altering effects that potentisation has on an aqueous high dilution.

A complete understanding of water, its structural properties and behaviour is not yet complete. What is certain, however, is that water is extremely complex as revealed by its interesting behaviour seen during phase changes and during its liquid state. The current understanding of water is interpreted through our understanding of its short-range interactions, namely hydrogen bonds and van der Waals forces. Such interactions link water molecules into an intricate network (Ball 2008). Hydrogen-oxygen bonds are polar covalent bonds that create electropositivity around the hydrogen atoms and electronegativity around the oxygen atom. This results in linking of neighbouring water molecules and the formation of chains and irregular networks. Due to the particular orientation of the hydrogen atoms to the oxygen atom within a water molecule, a dipole moment is formed. In other words, the one end of the water molecule is more electropositive than the other. This dipole moment plays a role in the coherence phenomena which is essentially the restructuring of water molecules in response to the presence of a solute. The structure of the water molecules

adjusts in a manner that is dependent on the properties of the added solute molecule. Enormous structural reorganisation takes place at the interface of the water molecules and the macromolecules or solute. This structural reorganisation extends a considerable distance from the solute (Cheng, Rossky 1999; Drost-Hansen 1982; Kitano, Gemmei-Ide 2010). These structural properties of water have been used by many researchers to explain how aqueous homoeopathic high dilutions are capable of conveying biological effects dispite not containing a single molecule of the original medicinal substance.

Various theories have therefore arisen which have attempted to explain how the solvent, whether lactose powder, aqueous or ethanol solution, may be capable of conveying the therapeutic effect of the original drug substance.

The replicating clathrate model introduced by Anagnostatos (1994) suggests that clathrates may be responsible for the retention of the medicinal properties of high dilution remedies. Clathrates are essentially clusters of molecules in a liquid structure which occur in response to a contaminant or an externally induced fluctuation. The structure of the liquid becomes altered; some bond lengths are shortened and others are lengthened in order to accommodate the contaminant. Clathrates replicate and the cluster lattice or structure persists even after the original contaminant disintegrates, thus allowing for the possibility of information storage within the structure of the solvent without the presence of the original ‘contaminant’ or drug substance, as would be the case in homoeopathic remedies.

Berezin (1994) proposed that the interaction of atomically identical, isotopically different atoms forms the basis of an ordered framework within a liquid. He proposes that the sequence of isotopic diversity (isotopicity) may be the means by which information is stored within the structure of a liquid. Del Giudice and Preparata (1998) developed a dynamic model of information storage in condensed matter, based on coherent regions of electromagnetic fields which exist in such matter. Such regions of electromagnetic coherence are theorised to act as information carriers and may explain how high dilution remedies have therapeutic effect.

Resch and Gutmann (1991) proposed that water as a solvent has information carrying properties by virtue of its highly ordered “super molecular system”. Dissolved contaminants and gases help to shape an ordered lattice structure. Further, ‘solvation spheres’ develop around hydrophilic solutes, and ‘inner surface’ molecules form around hydrophobic solutes. They postulate that with the aid of agitation such as succussion a substance or solute will modify the water structure, thereby imparting information.

The nanoparticle theory suggests that nanoparticles of the original drug substance persist even in high dilution homoeopathic remedies, and are the means by which high dilution homoeopathic remedies have therapeutic effect. Chikramane *et al.* (2010) demonstrated the presence nanoparticles of particular metals including gold, zinc, copper and tin (used as starting substances) in extremely high dilutions. It was also discovered that despite large differences in the degree of dilution from 6c to 200c, no major difference occurred in the nature of the particles of the starting material and their absolute concentrations. The samples were analysed by means of Transmission Electron Microscopy (TEM), electron diffraction and chemical analysis by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES).

Bell and Koithan (2012) proposed that the trituration phase, as well as agitating techniques such as succussion and sonication performed in the potentisation of homoeopathic remedies, develop nanoparticles of the starting substance as well as silica nanoparticles from silica leached from the glassware used to prepare the remedies. Such silica nanoparticles are modified by the starting substance which alters their three dimensional shape, thereby giving unique structural properties. These silica nanoparticles are thought to facilitate ‘nanoseeding’, the transference of the unique structural properties of the silica nanoparticles of preceding potencies to the next potency level, and are thus thought to convey medicinal power in high potencies.

Demangeat (2015) built a convincing case for the nanobubble superstructure theory to explain how high dilution homoeopathic remedies carry medicinal properties. Nanobubbles exist in liquids in varying amounts and have an effect on the structure of the liquid. This has been demonstrated by a number of

spectroscopy techniques including dynamic small-angle laser-light (LLS) spectroscopy, neutron scattering, and atomic force microscopy (ATM) studied by Upadhyay and Nayak (2011 cited in Demangeat 2015).

Certain factors promote the formation of nanobubbles (Demangeat 2015). The presence of a hydrophobic solute, such as a homeopathic starting substance, acts as a nucleation centre around which nanobubbles congregate forming bulk nanobubbles. Demangeat proffered that shells of highly organised water molecules develop around the nucleation centres and prevent the solute from dissolving out. These nucleation centres, along with their highly organised nanobubble and water molecule 'shells', were postulated to be replicated and carried across the dilution spectrum. Thus, the nanobubbles form a 'superstructure' within the solvent. Evidence of such superstructures was demonstrated by means of NMR relaxation times. The superstructures appeared to grow in size as higher dilution stages were reached, and showed greatest development in the 15c to 30c range.

The role of potentisation in the formation of nanobubbles is crucial. The presence of a hydrophobic solute increases the number of nanobubbles, however gas supersaturation of the solvent and cavitation both greatly increases the number of nanobubbles and facilitates development of nanobubble superstructuring. Agitation techniques such as succussion and vortexing saturate the solvent with gases, and cause a degree of cavitation, thereby favouring good nanobubble formation. Sonication is one of the most effective means of inducing cavitation in a solvent. Therefore both means of potentisation should produce highly developed nanobubble superstructures.

Demangeat also demonstrated by means of NMR relaxation studies that high dilution homeopathic liquids differed considerably from controls when an agitation technique such as vigorous shaking was applied.

2.6 NMR SPECTROSCOPY

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most powerful techniques available for studying the structure of molecules. NMR spectroscopy has progressed significantly since it was first used commercially in 1952 to analyse organic compounds such as oil. With the development of ever stronger magnets, NMR spectroscopy has become increasingly sensitive and currently has detection limits approaching nanogram levels (Robinson, *et al.* 2005).

Nuclear Magnetic Resonance enables the study of the shape and structure of molecules, and provides information on the spatial orientation of atoms in a molecule. It can also be used to identify the quantity of a known substance present in a sample, and therefore a method that is used for quantitative and qualitative analyses.

Certain nuclei, called spin-active nuclei, absorb electromagnetic radiation when placed in a strong electromagnetic field. The frequency at which spin-active nuclei resonate can be determined by NMR spectroscopy (Williams 1986). Due to their electrical charge and spin, spin-active nuclei produce their own magnetic fields around themselves. This magnetic field is called a magnetic moment (μ). When placed in a strong external electromagnetic field (Bo), such as an NMR spectroscopy device, the spin-active nuclei orientate themselves in a particular fashion. For instance protons orientate themselves either with, or against, the external electromagnetic field (Bo). Nuclei in a low energy state orientate themselves with the external magnetic field, while those in a high energy state orientate themselves away from the external magnetic field. In the absence of a magnetic field, nuclei orientation is random (Robinson *et al.* 2005). See Figure 2.1 below for a visual depiction of spin-active nuclei orientation in a magnetic field.

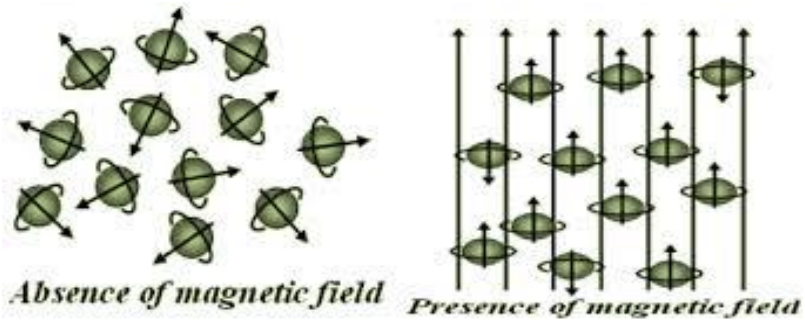

Figure 2.1 - Spin-active nuclei orientation in the presence of an external magnetic field (Jahasultana *et al.* 2008).

When exposed to a particular quantity of radiofrequency, a nucleus can absorb energy and move from a low to a high energy state. Different nuclei require exposure to different amounts of radiofrequence in order to move them to a high energy state. The orientation of the nucleus relative to the external magnetic field will change as a result. Protons in the low energy state will orientate themselves with the external electromagnetic field, while those in a high energy state will oppose the external magnetic field and orientate in the opposite direction. The nucleus will at some point emit the absorbed energy and return to its low energy state and former orientation, but will absorb more energy and regain its higher energy state if radiofrequency is still applied. This continual oscillation between a high and low energy state is called resonance (v). When a nucleus returns to a low energy state from a high energy state, energy is emitted. This emitted energy is detected by the receiver of the NMR device. Refer to figure 2.2 for a visual depiction an NMR spectrometer. Every nucleus, when charged or exposed to radiofrequency, resonates at its own particular frequency. This characteristic frequency is known as Larmor frequency (Kaseman 2010).

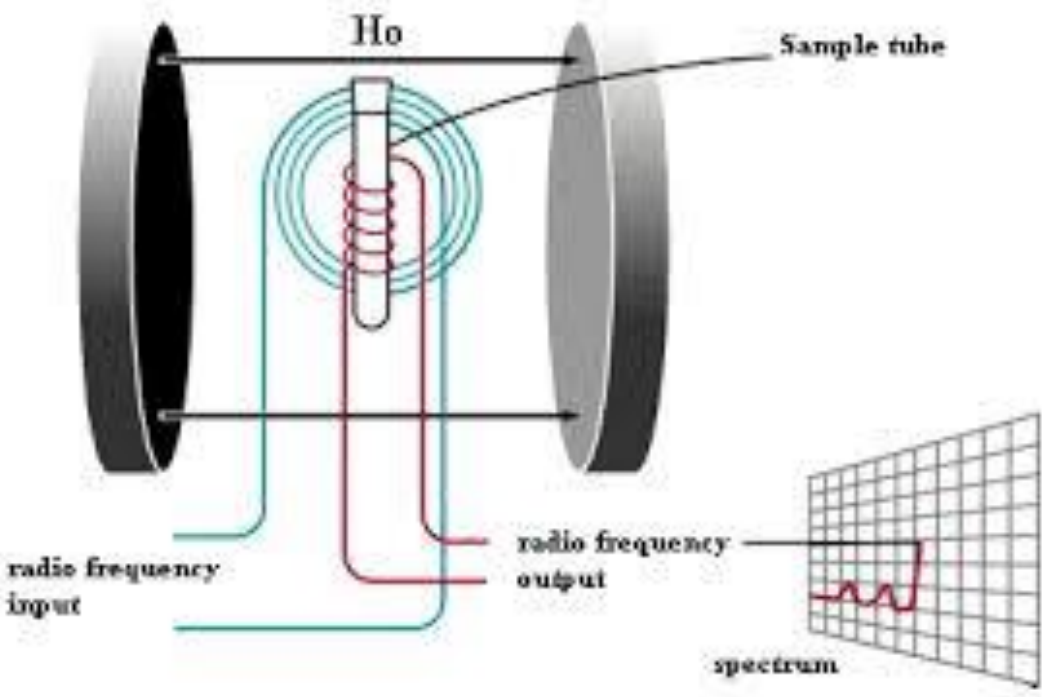

Figure 2.2 - Diagram of NMR spectrometer (Carey 2000).

Four main parameters are considered in NMR spectroscopy, namely, chemical shifts, coupling constants, relaxation times and integrations.

a) Chemical shifts (δ)

Chemical shift (δ), measured in parts per million (ppm), is a measure of how the resonance frequencies of sample nuclei differ from a standard such as tetramethylsilane (TMS). The electrons bound to and orbiting a nucleus produce an electromagnetic field, which effectively shields the nucleus from an external electromagnetic field (Bo). When an atom is isolated, it resonates at its Larmor frequency. In molecules, however, electrons are shared among neighbouring nuclei, and will orbit more closely to some than they will to others due to the differing electronegativity of different nuclei. This results in more of an electromagnetic shielding effect over some areas of the molecule, and less shielding over others. The influence of the external magnetic field on each nucleus bound in a molecule will thus be either greater or lesser, depending on how much that nucleus is shielded by electrons. As a

result, spin-active nuclei will not resonate at their Larmor frequencies when bound to other nuclei.

As the resonance absorptions which produce NMR spectra produced by spin-active nuclei differ when bound in a molecule, compared with when in isolation, a standard is employed (usually TMS). The absolute frequency of resonance of nuclei is particularly difficult to measure, however differences in the frequencies of resonances of different substances can be compared more easily. Therefore a standard such as TMS is set, and resonance frequencies are measured relative to it. Consequently, the position of these resonances in the spectrum are usually measured with respect to their 'shift' from a standard which has been added to a sample.

Chemical shift values therefore provide information about the electromagnetic environment, nature of the bonds with neighbouring nuclei, and degree of shielding that a nucleus possesses.

b) Coupling constants (J)

Peaks on an NMR spectra often appear as a number of peaks very close together. Two peaks that appear next to each other are known as a doublet; three peaks are a triplet; etcetera. The reason for this 'splitting' of peaks is that neighbouring nuclei exert an electromagnetic field that interacts with the external magnetic field (Bo), and can enhance it (if orientated with it), or weaken it (if orientated against it), or not affect it. Thus the electromagnetic field felt by the nucleus being analysed will differ depending on the number of neighbouring nuclei, as well as their orientation relative to the external magnetic field. This differing external electromagnetic field thus produces peaks with slightly different chemical shift values. The distance between these peaks remains constant, however, and is known as a coupling constant (J) which is measured in Hertz (Hz) (Robinson, Frame and Frame 2005). The number of peaks

produced is related to the number of adjacent spin-active nuclei (Williams 1986).

c) Relaxation times (T_1, T_2)

Relaxation describes the loss of energy from a nucleus that is in a high energy or excited state, thereby returning to a low energy or unexcited state. Relaxation time is the time spent in the excited state.

Longitudinal relaxation ($T_{1)}$ describes relaxation in which energy from nuclei moving from an excited to an unexcited state is lost to the entire sample or 'lattice'. This energy is absorbed by the lattice in the form of increased vibrational and rotational energy (Robinson, Frame and Frame 2005).

Transverse relaxation (T_2) describes relaxation in which energy from a nucleus which is moving from an excited state to an unexcited state is transferred to a nearby nucleus which is entering an excited state. There is no net change in energy to the system, however the average excited state lifetime decreases (Robinson *et al*. 2005).

Relaxation times affect the width of absorption lines on NMR spectra. Typically liquids have longer relaxation times and produce narrower absorption lines. The opposite is true of solids.

d) Integrations

Integrations provide information about the relative number of spin-active nuclei present in separate resonance peaks. The area under the NMR signal is proportional to the number of spin-active nuclei causing the signal. Thus if one peak has an area twice that of another peak, it will have twice the number of spin-active nuclei. The absolute number of spin-active nuclei is difficult to ascertain, however, the number of spin-active nuclei relative to other resonance peaks can be fairly accurately compared. Therefore, integrations are relative values (Williams 1986).

During NMR analysis a large amount of radiation is emitted by the device and is received by the receiver as electronic noise. The NMR signals have to be distinguished from this electronic noise by means of electronic filtering in order to gain useful data. This filtration of signals becomes even more important when analysing highly dilute substances such as homoeopathic remedies, due to the fact that there are fewer spin-active nuclei present in the sample and as a result fewer NMR signals being produced.

Fourier Transformation (FT) is therefore a signal enhancement technique which is commonly applied in order to render clear NMR signal spectra. The process of FT involves exposing a sample to a powerful pulse of radiofrequency radiation which contains a broad band of frequencies. This causes all the spin-active nuclei of the sample to resonate at their respective Larmor frequencies. The spectrometer detector senses the change in magnetisation of the sample and the decay of the magnetisation with respect to time. This is called free induction decay (FID). Free induction decays are composed of a complex set of interfering wave forms as well as a lot of noise, therefore multiple pulses and subsequent detections of FIDs are performed and the results added together. In this manner, signal-to-noise enhancements can be achieved.

Frequency induced decays are very complex and essentially uninterpretable forms of data which require mathematical conversion so that they appear as normal NMR spectra. A normal NMR spectrum is a set of resonances related by frequency and is considered a frequency-domain spectrum. Frequency induced decays are time-domain spectrums, however, and therefore require mathematical conversion to frequency-domain spectra. This conversion takes place by means of complicated mathematics performed by computer software, which then produces interpretable NMR spectra.

2.7 NUCLEAR MAGNETIC RESONANCE (NMR) USE IN HOMOEOPATHY

Homoeopathic remedies are widely believed to rely on 'the memory of water' as a means of maintaining their medicinal effect when in ultra-high dilution (12c and higher), as not a single molecule of the starting medicinal substance is theoretically present. Nuclear Magnetic Resonance spectroscopy is particularly applicable in the analysis of high dilution homoeopathic remedies due to its sensitivity to the dynamics and structural organisation of water, and has therefore been used to study water in numerous kinds of solutions and macromolecular systems (Demangeat 2013). Nuclear Magnetic Resonance is also sensitive enough to detect differences in homoeopathic remedies that have exceeded Avogadro's number (6.022×10^{23}), i.e. 12CH and higher, and are thus very useful in the analyses of homoeopathic remedies (Young 1976). Significant differences between the spectra of high dilution homoeopathic remedies and controls have been observed by means of NMR spectroscopy (Smith and Boericke 1968).

The history of NMR spectroscopy use in analysing homoeopathic remedies dates back to about 1966, when Smith and Boericke first used NMR spectroscopy to analyse *Sulphur* D12 in 87% alcohol. The NMR spectra showed distinct differences between succussed and unsuccussed dilutions, and that the hydroxyl spectrum in 87% ethanol increased when succussed was compared with unsuccussed dilutions containing the same solute (Smith and Boericke 1968).

A meta-analysis by Botha (2009) concluded that NMR spectroscopy is an effective means of analysing the physicochemical properties of homoeopathic medicines that utilise lactose and water/ethanol bases. A meta-analysis of NMR studies by Demangeat (2013) revealed that T1 and T1/T2 spectra were increased in centesimal dilutions of various solutes including silica-lactose, histamine, and manganese-lactose which had undergone vigorous agitation. No changes were observed in controls. A continuous decrease in T2 and a rise in T1/T2 in silica-lactose dilutions beyond the Avogadro's number (12c dilution), but within the 12c to 24c range, indicated growing structure of the water solvent despite absence of the initial solute. Demangeat (2013) suggested that

supramolecular structures involving water, nanobubbles and ions may explain how homoeopathic high dilutions show NMR spectra distinct from controls.

A study by Botha (2005) which employed NMR spectroscopy, revealed statistically significant physicochemical differences between homoeopathic remedies of different potencies prepared by trituration. An NMR study of the differences of parallel potencies of homoeopathic remedies produced by the Hahnemannian method and Radionic instrumentation showed that significant differences existed between parallel potencies in terms of chemical shift values for H_2O, CH_2 and CH peaks (Allsopp 2010). An NMR study by Lyell (2004) revealed that potencies produced by the Hahnemannian method with a differing number of succussions showed statistically significant differences in terms of relative integration values. It was therefore hypothesised that the number of succussions employed during the manufacture of homoeopathic remedies by the Hahnemannian method may affect the physicochemical properties of the resultant remedies. A study by Davies (2001) revealed that homoeopathic remedies produced by different methods of dilution, in this case Hahnemannian versus Korsakovian methods, produce physicochemically distinct NMR spectra when analysed by NMR spectroscopy. Hahnemannian and Korsakovian dilutions of parallel potencies differed in terms of chemical shift and relative integration values. The method of dilution was therefore theorised to affect the physicochemical properties of the resultant remedy. An NMR study by Ross (1997) revealed statistically significant differences in terms of chemical shift and relative integration values between different potencies of quinquagenimillesimal potencies of Sulphur.

2.8 SUMMARY

From the review of the relevant literature it has become clear that sonication could theoretically be an effective means of potentising homoeopathic remedies. Its agitating effects on liquid solutions is remarkably similar to that of hand succussion, and so it would appear to be a potentially viable alternative method of potentisation. Little research exists, however, that provides scientific evidence that it produces remedies that are similar or identical to those produced by the original Hahnemannian method.

Nuclear Magnetic Resonance spectroscopy has proven to be very valuable in the analysis of high dilution homoeopathic remedies, as it has been able to indicate clear differences between different homoeopathic remedies, even at dilution levels that have exceeded Avogadro's constant. Its ability and extreme sensitivity in the detection of physical structure properties of high dilution solutions makes NMR spectroscopy the most appropriate method of analysis in this research.

The purpose of this research was to analyse and compare the NMR spectra of Hahnemannian Sulphur, sonicated Sulphur, and controls, in order to draw conclusions regarding the similarity or dissimilarity of their physicochemical properties. This investigation should therefore serve to assist in evaluating the validity of the use of sonication in the production of Homoeopathic remedies, seeing as to date there is little scientific evidence substantiating its use.

CHAPTER 3: MATERIALS AND METHODS

3.1 PREPARATION OF POTENCIES

The five samples for analysis were prepared using the traditional Hahnemannian style, as outlined in the German Homoeopathic Pharmacopoeia (Benyunes 2005), as far as possible. All the samples, apart from the control, were produced from the same 3c Sulphur triturate. For the sake of continuity, the control sample was produced from a 3c triturate of pure lactose powder, as lactose is considered a medicinally inert substance. All the samples were produced to the 12c potency. The 12c potency was chosen as it is theoretically the point at which Avogardo's constant has been exceeded, and not a single molecule of the original starting material is believed to be present.

Manufacturing conditions were carefully controlled in order to avoid the introduction of other variables that may have affected the accuracy of the experiment. Samples were produced in temperature controlled laboratories under laminar flow; aseptic techniques were adhered to strictly. All glassware was autoclaved prior to use, and equipment utilised in the production processes was sterilised appropriately. The Sulphur and control (lactose) triturates were produced from a single batch of lactose powder, and liquid potencies were all produced from the same bottle of distilled water and 87% ethanol. In order to minimise differences in the production conditions, parallel potencies of the five sample groups were produced together before producing the next potency levels. For a detailed account of the manufacturing process, consult Appendix A.

3.1.1 Method 6: Trituration by hand (Benyunes 2005)

One part, by weight, of Sulphur powder was triturated together with 99 parts, by weight, of lactose powder. The lactose powder was accurately massed out in three parts, each weighing 3.30 grams. After flaming a mortar and pestle using 96% alcohol and allowing it to cool, one part vehicle was then triturated together with 0.10 grams of Sulphur powder for six minutes followed by four minutes of scraping down. This was followed by a further six minutes of

trituration and four minutes of scraping. The second part of vehicle was then added, and underwent two stages of six minutes of trituration and four minutes of scraping down, as was done to the first part of vehicle. The third part of vehicle was then added, and underwent trituration and scraping as with the first two parts. This collectively resulted in one hour of trituration and scraping, and produced a Sulphur 1CH triturate.

The 2CH triturate was manufactured by using 0.10 grams of the 1CH Sulphur triturate and three batches of 3.30 grams of lactose vehicle. The batches of lactose were added to the 1CH Sulphur in the same three-step trituration and scraping process as employed in the production of the 1CH. This added hour of trituration produced a 2CH Sulphur triturate.

The 3CH Sulphur triturate was manufactured in the same manner as the 2CH, only 0.10 grams of the 2CH triturate was used as a starting material instead of the 1CH. See Appendix A for the exact process used.

For the sake of continuity, the triturate used to produce the control was produced in exactly the same fashion as utilised to produce the Sulphur triturates, only 0.10 grams of lactose powder was used as the starting material instead of Sulphur powder.

3.1.2 Method 8a: Liquid preparations from triturations (Hahnemannian potency) (Benyunes 2005)

From a 3CH Sulphur triturate, a 4CH liquid potency was manufactured by dissolving one part (0.03 grams) of the 3CH triturate in 99 parts (2.97 ml) distilled water in a 5 ml bottle and succussed ten times. One part (0.03 ml) of the 4CH liquid potency was then dissolved in 99 parts (2.97 ml) of 87% alcohol in a 5ml bottle and succussed ten times to produce the 5CH potency. Potencies 6CH through to 11CH were produced in the same fashion as the 5CH potency. The 12CH potency was manufactured by placing 0.15ml of the 11CH in 14.85ml of 87% ethanol in a 25ml screw top bottle and succussing ten times. This produced a Sulphur 12CH Hahnemannian.

3.1.3 Sonicated potency (Sukul *et al.*, 2001)

In the production of the sonicated potencies, method 8a was replicated exactly, however, the succussion phase was replaced with 30 seconds of sonication at 40Hz in a sonication bath.

3.1.4 Hahnemannian potency with sonication (both) (Sukul *et al.*, 2001)

The production of Hahnemannian with sonication potencies employed method a8, however after the succussive phase the potencies underwent 30 seconds of sonication at 40Hz in a sonication bath. This was in keeping with the most common employment of sonication in homoeopathic remedy production.

3.1.5 Unagitated Sulphur potency (Bhattacharyya, Mandal and Biswas 2008)

The production of the unagitated Sulphur potencies employed method a8, however the succussion phase was omitted.

3.1.6 Lactose control (unagitated) (Bhattacharyya, Mandal and Biswas 2008)

The control was produced from the 3c lactose triturate. Serial dilution was performed in the same manner as method a8, however no succussion was performed so that the role of the agitation process employed in the experimental samples could be better understood.

3.2 PREPARATION OF SAMPLES FOR ANALYSIS

The five samples for analysis were produced in 25ml amber glass screw top bottles (soda glass). Amber glass was selected in order to offer protection from light destruction. Fifteen ml of potency were produced; a quantity appropriately sized for the drawing of samples for analysis, but also small enough to allow for succussion space inside the bottle (at least a third of the bottle needs to be free to allow for succussion) (Barthel 1991). All samples sent for analysis were at the 12c potency level (Bhattacharyya, Mandal and Biswas 2008).

The samples were clearly labelled and carefully wrapped in tissue paper inside a small cardboard box immediately after production. This box was packed into a slightly larger box and tissue was used as a cushion between the two boxes. The samples were protected from disturbing stimuli such as noise, vibration, temperature extremes, light and electromagnetic disturbance, as far as was possible. The samples were then couriered to Stellenbosch University were they underwent NMR analysis by Dr D.J. Brand.

3.3 NMR MEASUREMENT OF SAMPLES

The samples were drawn by the Stellenbosch University NMR unit technician Dr D.J.Brand, who also ran the NMR analyses. The sample volumes were 750µl. One sample was drawn from each of the five sample groups by means of new pipettes and transferred to new NMR tubes. A Deuterated DMSO insert is added as an instrument frequency lock. The samples were analysed by a Varian ^{Unity}*Inova* 600 NMR Spectrometer ®. The following factors were noted during the data acquisition:

Spectrometer frequency: 599.99.

Spectral width: 5999.7.

Acquisition time: 3.0000.

Number of scans: 16.

Pulse width: 6.2750.

Pulse sequence: s2pul.

Relaxation delay: 2.5000.

Probe: ldpfg.

Solvent: dsmo.

Temperature: 25.0°C

3.4 RECORDING OF DATA

The NMR spectra produced by the analysis were delivered by e-mail in the form of FID files. The FID files were opened using MestReNova Version 10.0 (see Appendix B for spectra). The raw data for the chemical shift and relative integration values was recorded in a Microsoft Excel 2013 spreadsheet (see Appendix C). Only the relative integration and chemical shift values of the H_2O, OH, CH_2 and CH_3 peaks were considered during collection of the raw data (Allsopp 2010).

Chemical shift values for CH_2 and CH_3 were comprised of two or more peaks, so the average value was calculated and a single value was used. Relative integration values were calculated by dividing the total area beneath each peak by the sum of all the area values for all the peaks recorded in each run (Field 2009).

3.5 STATISTICAL ANALYSIS

The statistical analysis was performed with the aid of SPSS Version 22. The chemical shift and relative integration values for the H_2O, OH, CH_2 and CH_3 peaks were used in the statistical analysis. The Kruskal-Wallis method was performed for the five sample groups to ascertain whether or not a statistically significant difference existed between the five sample groups. Comparisons between individual paired groups was conducted by means of Mann-Whitney test.

3.5.1 Kruskal-Wallis Test

The Kruskal-Wallis test, being the non-parametric equivalent of the one-way ANOVA test, is applicable where three or more experimental conditions are being compared, and reveals whether or not statistical differences exist between the groups (Field 2009). This non-parametric test was selected due to the fact that the distributions within the five groups were not normal, and the sample sizes were small.

The Kruskal-Wallis test is based on ranked data and is performed as follows (Field 2009):

- Scores from all the sample groups are ordered from lowest to highest, ignoring the groups to which they belong.
- The scores are then assigned a rank. The lowest score gets a rank of 1, the next highest a rank of 2, and so on until all the scores are ranked.
- Once the scores are all ranked, they are regrouped according to the sample groups from which they came.
- The ranks for each group are then summed up and denoted by R_i, in which i denotes the particular group.
- The test statistic, H, can then be calculated using the following formula:

$$H = \frac{12}{n(n+1)} \sum_{i=1}^{k} \frac{R_i^2}{n_i} - 3(N+1)$$

  In which R_i is the sum of the ranks for each group, N is the total sample size and n_i is the sample size of a particular group.
- The significance (α) was set at 0.05.

The hypotheses were thus formulated as H_0, where a statistically insignificant differences existed between the sample groups, i.e. there are no differences between the manufacture methods, or H_1, where a statistically significant difference did exist between the sample groups, i.e. there is a difference between the manufacture methods. Thus, H_0 was accepted if the p value was greater than or equal to 0.05, and H_1 was accepted if the p value was less than or equal to 0.05.

3.5.2 Mann-Whitney Test

The Mann-Whitney test is the non-parametric equivalent of the independent *t*-test, and is applicable when data is not normally distributed, and when sample sizes are small (Field 2009). The Mann-Whitney test is performed as follows:

- The scores from both the sample groups are ordered from lowest to highest, ignoring the groups from which they originate.

- The scores are then assigned a rank. The lowest score gets a rank of 1, the next highest a rank of 2, and so on until all the scores are ranked. If two or more scores are identical, then each of the identical scores is assigned a rank which is a mean of the 'potential ranks' (the rank numbers that would have been assigned had the scores not been identical) of the identical scores.
- All the scores with their assigned rankings are then regrouped into the sample groups they originated from, and the sums of the rankings for each sample group is calculated.
- The test statistics U is then calculated using the following formula:

$$U = n_1 n_2 + \frac{N_1(N_1 + 1)}{2} - R_1$$

  In which n_1 and n_2 are the sample sizes of groups 1 and 2 respectively, and R_i is the sum of the ranks for group one.
- The U value is compared to the critical value (α) which is set at 0.05.

The hypotheses were thus formulated as H_0, where a statistically insignificant difference existed between the sample groups, i.e. there are no differences between the manufacture methods, or H_1, where a statistically significant difference did exist between the sample groups, i.e. there is a difference between the manufacture methods. Thus, H_0 was accepted if the p value was greater than or equal to 0.05, and H_1 was accepted is the p value was less than or equal to 0.05.

CHAPTER 4: THE RESULTS

4.1 THE CRITERIA GOVERNING THE ADMISSIBILITY OF DATA

As discussed in chapter three, the manufacture of the samples was performed with great caution given that homoeopathic remedies are reputed to be very sensitive to various stimuli (Kayne 2006) (refer to sections 3.1 and 3.2 for more detail). The samples sent for NMR analysis were handled, stored and drawn with an equal degree of caution due to the sensitivity of the remedies, but also due to the great sensitivity of NMR analysis.

A single bottle of each sample was prepared due to the standard method of preparation. This eliminated additional variables that may have been introduced by multiple samples. One sample was drawn from each bottle in a linear fashion. A new pipette was used to draw each sample. All the sample bottles were stored under the same conditions at all times.

From the spectra produced by the NMR analyses, raw data was obtained and used to determine the chemical shift (δ) values and to calculate the relative integration values. Comparisons of the chemical shift (δ) and relative integration values for the H_2O, OH, CH_2 and CH_3 peaks were performed between all the experimental and control groups. The comparisons were performed by means of statistical analysis as set out in section 3.5 of chapter three.

A table in Appendix C contains the data that was sent for statistical analysis, and Appendix D contains the SPSS analyses of the data.

4.2 KRUSKAL-WALLIS TEST

4.2.1 Comparison of chemical shift (δ) values

Table 4.1: Kruskal-Wallis Test: Comparison of Chemical Shift (δ) Values.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.000	0.000	0.000	0.000

The Kruskal-Wallis analysis of the chemical shift values of the five sample groups showed that a statistically significant difference existed between all of the sample groups.

4.2.2 Comparison of relative integration values

Table 4.2: Kruskal-Wallis Test: Comparison of Relative Integration Values.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.002	0.017	0.015	0.001

The Kruskal-Wallis analysis of the relative integration values of the five sample groups showed that there were statistically significant differences between all of the sample groups.

4.3 MANN-WHITNEY TEST

4.3.1 Chemical shift (δ) values

a) Sulphur 12c Hahnemannian and Sulphur 12c sonicated.

Table 4.3: Mann-Whitney Test, Chemical shift values for Sulphur 12c Hahnemannian and Sulphur 12c sonicated.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.008	0.007	0.095	0.007

The Mann-Whitney analysis of the Sulphur 12c Hahnemannian and Sulphur 12c sonicated samples revealed a statistically significant difference between the two sample groups in terms of the chemical shift values for the H_2O, OH, and CH_3 peaks. The hypothesis therefore is not supported in terms of the chemical shift values for these three peaks. The chemical shift values for the CH_2 peaks showed no statistically significant differences, however, and can thus be considered similar. The hypotheses are therefore supported in this regard.

b) Sulphur 12c Hahnemannian and Sulphur 12c both (Hahnemannian and sonication).

Table 4.4: Mann-Whitney Test, Chemical shift values for Sulphur 12c Hahnemannian and Sulphur 12c both (Hahnemannian and sonication).

	H_2O	OH	CH_2	CH_3
Significance(α)	0.009	0.009	0.050	0.018

The Mann-Whitney analysis of the chemical shift values for the Sulphur 12c Hahnemannian verses the Sulphur 12c Hahnemannian and sonication revealed statistically significant differences between the H_2O, OH and CH_3 peak values. In this regard, the hypotheses are not supported. The analysis did however reveal a statistically insignificant difference between the CH_2 peak values,

indicating that the samples were similar in this regard. In terms of the CH_2 values, the hypotheses are supported.

c) Sulphur 12c Hahnemannian and Lactose 12c control.

Table 4.5: Mann-Whitney Test, chemical shift values for Sulphur 12c Hahnemannian and Lactose 12c control.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.007	0.009	0.005	0.006

The Mann-Whitney analysis of the Sulphur 12c Hahnemannian and the Lactose 12c control showed that a significant difference existed between the sample groups in terms of their H_2O, OH, CH_2 and CH_3 peak values. The hypotheses are supported in this regard.

d) Sulphur 12c Hahnemannian and Sulphur 12c unagitated.

Table 4.6: Mann-Whitney Test, chemical shift values for Sulphur 12c Hahnemannian and Sulphur 12c unagitated.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.009	0.008	0.005	0.007

The Mann-Whitney analysis of the chemical shift values for the H_2O, OH, CH_2 and CH_3 peaks revealed that statistically significant differences exist between all of the peak values. The hypotheses are therefore supported.

e) Sulphur 12c sonicated and Sulphur 12c both (Hahnemannian and sonication).

Table 4.7: Mann-Whitney Test, Chemical shift values for Sulphur 12c sonicated and Sulphur 12c both (Hahnemannian and Sonication).

	H_2O	OH	CH_2	CH_3
Significance(α)	0.008	0.007	0.112	0.006

The Mann-Whitney analysis of the Sulphur 12c sonicated and Sulphur 12c Hahnemannian and sonication sample groups revealed that a significant difference existed between the sample groups in terms of their chemical shift values for the H_2O, OH and CH_3 peaks. In this regard the hypotheses are not supported. The CH_2 peak values showed no difference, however, and therefore in this regard the two sample groups were similar. The hypotheses in terms of the chemical shift values for the CH_2 peaks are thus supported.

f) Sulphur 12c sonicated and Lactose 12c control.

Table 4.8: Mann-Whitney Test, Chemical shift values for Sulphur 12c Sonicated and Lactose 12c control.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.009	0.007	0.008	0.006

A significant difference existed between the chemical shift values for the H_2O, OH, CH_2 and CH_3 peaks of the Sulphur 12C sonicated and Lactose 12C control sample groups. The hypotheses are thus supported.

g) Sulphur 12c sonicated and Sulphur 12c unagitated.

Table 4.9: Mann-Whitney Test, Chemical shift values for Sulphur 12c sonicated and Sulphur 12c unagitated.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.008	0.006	0.009	0.031

The Mann-Whitney analysis of the Sulphur 12c sonicated and Sulphur 12c unagitated sample groups showed that there were significant differences between the chemical shift values of all the peaks. The hypotheses are therefore supported in this regard.

h) Sulphur 12c both (Hahnemannian and sonication) and Lactose 12c control.

Table 4.10: Mann-Whitney Test, Chemical shift values for Sulphur 12c both (Hahnemannian and sonication) and Lactose 12c control.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.007	0.009	0.007	0.005

The Mann-Whitney analysis of the chemical shift values of the H_2O, OH, CH_2 and CH_3 peaks demonstrated a significant difference between the Sulphur 12c Hahnemannian and sonication and Lactose 12c control sample groups. The hypotheses are therefore supported.

i) Sulphur 12c both (Hahnemannian and sonication) and Sulphur 12c unagitated.

Table 4.11: Mann-Whitney Test, Chemical shift values for Sulphur 12c both (Hahnemannian and sonication) and Sulphur 12c unagitated.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.009	0.008	0.008	0.006

Significant differences existed between all of the chemical shift peak values for the Sulphur 12c Hahnemannian and sonication and the Sulphur 12c unagitated sample groups. The hypotheses are supported in this regard.

j) Lactose 12c control and Sulphur 12c unagitated.

Table 4.12: Mann-Whitney Test, Chemical shift values for Lactose 12c control and Sulphur 12c unagitated.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.009	0.008	0.008	0.006

Significant differences existed between the chemical shift values of all of the peaks of the Lactose 12c control and Sulphur 12c unagitated sample groups.

4.3.2 Relative integration values

a) Sulphur 12c Hahnemannian and Sulphur 12c sonicated.

Table 4.13: Mann-Whitney Test, Relative integration values for Sulphur 12c Hahnemannian and Sulphur 12c sonicated.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.009	0.142	0.175	0.016

The Mann-Whitney analysis of the relative integration values for the H_2O and CH_3 values of the Sulphur 12c Hahnemannian and Sulphur 12c sonicated sample groups shows a statistically significant difference between the groups. The hypotheses in this regard are therefore not supported. The relative integration values for the OH and CH_2 peaks according to the Mann-Whitney analysis are similar, and thus the hypotheses are supported in this regard.

b) Sulphur 12c Hahnemannian and Sulphur 12c both (Hahnemannian and sonication).

Table 4.14: Mann-Whitney Test, Relative integration values for Sulphur 12c Hahnemannian and Sulphur 12c both (Hahnemannian and sonication).

	H_2O	OH	CH_2	CH_3
Significance(α)	0.016	0.675	0.009	0.009

The relative integration values for the H_2O, CH_2 and CH_3 peaks of the Sulphur 12c Hahnemannian and Sulphur 12c Hahnemannian and sonication sample groups revealed statistically significant differences according to the Mann-Whitney analysis. The hypotheses are therefore not supported. The OH peaks for the two sample groups showed no significant difference, however, and so the hypotheses are supported in this regard.

c) Sulphur 12c Hahnemannian and Lactose 12c control.

Table 4.15: Mann-Whitney Test, Relative integration values for Sulphur 12c Hahnemannian and Lactose 12c control.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.009	0.175	0.602	0.009

The relative integration values for the H_2O and CH_3 peaks for the Sulphur 12c Hahnemannian and Lactose 12c control show a statistically significant difference between the two sample groups, which is in support of the hypotheses. The H_2O and CH_3 values showed no significant difference, and thus the hypotheses are not supported in this regard.

d) Sulphur 12c Hahnemannian and Sulphur 12c unagitated.

Table 4.16: Mann-Whitney Test, Relative integration values for Sulphur 12c Hahnemannian and Sulphur 12c unagitated.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.175	0.009	0.009	0.753

The Mann-Whitney analysis of the relative integration values for the OH and CH_2 peaks of the Sulphur 12c Hahnemannian and Sulphur 12c unagitated sample groups revealed a statistically significant difference between the groups. This supports the hypotheses. The H_2O and CH_3 peaks, however, were not significantly different. The hypotheses are therefore not supported in this regard.

e) Sulphur 12c sonicated and Sulphur 12c both (Hahnemannian and sonication).

Table 4.17: Mann-Whitney Test, Relative integration values for Sulphur 12c sonicated and Sulphur 12c both (Hahnemannian and sonication).

	H_2O	OH	CH_2	CH_3
Significance(α)	0.009	0.117	0.116	0.009

The relative integration values for the H_2O and CH_3 peaks for the Sulphur 12c sonicated and the Sulphur 12c Hahnemannian and sonication sample groups reveal a statistically significant difference between the two sample groups. This is not in support of the hypotheses. The OH and CH_2 peaks reveal that the sample groups are similar with regard to these peak values and therefore support the hypotheses.

f) Sulphur 12c sonicated and Lactose 12c control.

Table 4.18: Mann-Whitney Test, Relative integration values for Sulphur 12c sonicated and Lactose 12c control.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.251	0.117	0.465	0.175

In terms of the relative integration values, the significance values of the Mann-Whitney analysis of the H_2O, OH, CH_2 and CH_3 peaks of the Sulphur 12c sonicated and Lactose 12c control show that the two sample groups are similar. This does not support the hypotheses.

g) Sulphur 12c sonicated and Sulphur 12c unagitated.

Table 4.19: Mann-Whitney Test, Relative integration values for Sulphur 12c sonicated and Sulphur 12c unagitated.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.016	0.016	0.059	0.076

The Mann-Whitney analysis of the relative integration values for the H_2O and OH peaks shows a statistically significant difference between the two sample groups. This supports the hypotheses. The relative integration values of the CH_2 and CH_3 peaks show no differences between the two sample groups. The hypotheses are therefore not supported in this regard.

h) Sulphur 12c both (Hahnemannian and sonication) and Lactose 12c control.

Table 4.20: Mann-Whitney Test, Relative integration values for Sulphur 12c both (Hahnemannian and sonication) and Lactose 12c control.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.028	0.251	0.600	0.009

The Mann-Whitney analysis of the H_2O and CH_3 peak values of the Sulphur 12c Hahnemannian and sonication and Lactose 12c control revealed a statistically significant difference in terms of relative integration. This supports the hypotheses. The OH and CH_2 peak values, however, showed no significant difference between the two sample groups. This is not in support of the hypotheses.

i) Sulphur 12c both (Hahnemannian and sonication) and Sulphur 12c unagitated.

Table 4.21: Mann-Whitney Test, Relative integration values for Sulphur 12c both (Hahnemannian and sonication) and Sulphur 12c unagitated.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.917	0.009	0.009	0.009

The relative integration values for the OH, CH_2 and CH_3 peaks of Sulphur 12c Hahnemannian and sonication versus Sulphur 12c unagitated reveals that a significant difference exists between the two sample groups. This is in support of the hypotheses. The H_2O peaks showed no significant differences between the sample groups, therefore this is not in support of the hypotheses.

j) Lactose 12c control and Sulphur 12c unagitated.

Table 4.22: Mann-Whitney Test, Relative integration values of Sulphur 12c unagitated and Lactose 12c control.

	H_2O	OH	CH_2	CH_3
Significance(α)	0.076	0.465	0.009	0.009

The Mann-Whitney analysis of the relative integration values of the H_2O and OH peaks for the Lactose 12c control and Sulphur 12c unagitated sample groups shows no significant difference between the two sample groups. This supports the hypotheses. The CH_2 and CH_3 peak values, however, show a significant difference and are thus not in agreement with the hypotheses.

4.4 GROUPED DATA ANALYSES

In order to more effectively analyse the data, the sample pairs were grouped in various combinations in order to highlight any tendencies amongst the chemical shift and relative integration values. In 4.4.1, the sample pairs were arranged by sample group, thus revealing any differences or similarities that existed between the given sample group and all the other sample groups. In 4.4.2, the presence or absence of the medicinal substance, Sulphur, and the presence or absence of an agitation process being either Hahnemannian succussion or sonication, or both, were the measures that were grouped in order to reveal any tendencies attributable to these two components of the sample remedies. The significance values from the Mann-Whitney analyses performed in sections 4.3.1 and 4.3.2 were used to perform the comparisons. Both the chemical shift and relative integration values were included.

4.4.1 Analyses of sample pairs by sample group

a) Hahnemannian.

Table 4.23: Paired group analysis of Mann-Whitney p-values: Hahnemannian.

	Measure	H_20	OH	CH_2	CH_3
H + S	Chemical Shift	0.008	0.007	0.095	0.007
	Relative integ	0.009	0.142	0.175	0.016
H + Both	Chemical Shift	0.009	0.009	0.050	0.018
	Relative integ	0.016	0.675	0.009	0.009
H + Unag	Chemical Shift	0.009	0.008	0.005	0.007
	Relative integ	0.175	0.009	0.009	0.753
H + SVR	Chemical Shift	0.007	0.009	0.005	0.006
	Relative integ	0.009	0.175	0.602	0.009

Analysis of the significance values of the Hahnemannian paired groups as a whole revealed some trends. The Hahnemannian sample group was dissimilar to all of the other sample groups in terms of chemical shift values for the H_2O, OH and CH_3 peaks, as well as the relative integration values for the CH_3 peaks.

b) Sonication.

Table 4.24: Paired group analysis of Mann-Whitney p-values: Sonication.

	Measure	H_20	OH	CH_2	CH_3
S + H	Chemical Shift	0.008	0.007	0.095	0.007
	Relative integ	0.009	0.142	0.175	0.016
S + Both	Chemical Shift	0.008	0.007	0.112	0.006
	Relative integ	0.009	0.117	0.116	0.009
S + Unag	Chemical Shift	0.008	0.006	0.009	0.031
	Relative integ	0.016	0.016	0.059	0.076
S + SVR	Chemical Shift	0.009	0.007	0.008	0.006
	Relative integ	0.251	0.117	0.465	0.175

Analysis of the significance values of the sonicated paired groups as a whole revealed that the sonicated sample group was dissimilar to all of the other sample groups in terms of chemical shift values for the H_2O, OH and CH_3 peaks. The sonicated sample group was similar to all of the other sample groups in terms of relative integration values of the CH_2 peaks.

c) Hahnemannian and sonication (both).

Table 4.25: Paired group analysis of Mann-Whitney p-values: Hahnemannian and sonication (both).

	Measure	H_20	OH	CH_2	CH_3
Both + H	Chemical Shift	0.009	0.009	0.050	0.018
	Relative integ	0.016	0.675	0.009	0.009
Both + S	Chemical Shift	0.008	0.007	0.112	0.006
	Relative integ	0.009	0.117	0.116	0.009
Both + Unag	Chemical Shift	0.009	0.008	0.008	0.006
	Relative integ	0.917	0.009	0.009	0.009
Both + SVR	Chemical Shift	0.009	0.008	0.008	0.006
	Relative integ	0.028	0.251	0.600	0.009

Analysis of the significance values of the paired groups, all including the Hahnemannian and sonication (both) sample group as a whole, revealed that the Hahnemannian and sonication (both) sample group was dissimilar to all of the other sample groups in terms of the chemical shift values for the H_2O, OH and CH_3 peaks. The Hahnemannian and sonication (both) sample group was also dissimilar to all of the other sample groups in terms of the relative integration values of the CH_3 peaks.

d) Unagitated (Sulphur).

Table 4.26: Paired group analysis of Mann-Whitney p-values: unagitated.

	Measure	H_20	OH	CH_2	CH_3
Unag + H	Chemical Shift	0.009	0.008	0.005	0.007
	Relative integ	0.175	0.009	0.009	0.753
Unag + S	Chemical Shift	0.008	0.006	0.009	0.031
	Relative integ	0.016	0.016	0.059	0.076
Unag + Both	Chemical Shift	0.009	0.008	0.008	0.006
	Relative integ	0.917	0.009	0.009	0.009
Unag + SVR	Chemical Shift	0.007	0.008	0.008	0.006
	Relative integ	0.076	0.465	0.009	0.009

The analysis of the sample pairs, all including the unagitated sample group, revealed that the unagitated sample group was dissimilar to all of the other sample groups in terms of the chemical shift values of all the peaks.

e) Lactose control (SVR).

Table 4.27: Paired group analysis of Mann-Whitney p-values: lactose control (SVR).

	Measure	H_20	OH	CH_2	CH_3
SVR + H	Chemical Shift	0.007	0.009	0.005	0.006
	Relative integ	0.009	0.175	0.602	0.009
SVR + S	Chemical Shift	0.009	0.007	0.008	0.006
	Relative integ	0.251	0.117	0.465	0.175
SVR + Both	Chemical Shift	0.009	0.008	0.008	0.006
	Relative integ	0.028	0.251	0.600	0.009
SVR + Unag	Chemical Shift	0.007	0.008	0.008	0.006
	Relative integ	0.076	0.465	0.009	0.009

The analysis of the lactose control paired groups revealed that the lactose control sample group was completely dissimilar to all the other sample groups in terms of the chemical shift values of all of the peaks. The lactose control was similar to all the other sample groups in terms of the relative integration values of the OH peaks.

4.4.2 Grouped analyses according to presence or absence of medicinal substance and agitation process

a) Medicinal substance with agitation versus medicinal substance with agitation.

Table 4.28: Paired group analysis of Mann-Whitney p-values: Medicinal substance with agitation versus medicinal substance with agitation.

	Measure	H_20	OH	CH_2	CH_3
H + S	Chemical Shift	0.008	0.007	0.095	0.007
	Relative integ	0.009	0.142	0.175	0.016
H + Both	Chemical Shift	0.009	0.009	0.050	0.018
	Relative integ	0.016	0.675	0.009	0.009
S + Both	Chemical Shift	0.008	0.007	0.112	0.006
	Relative integ	0.009	0.117	0.116	0.009

Analysis of the sample groups which all contained the medicinal substance and had undergone an agitation process (Hahnemannian succussion, sonication or both) revealed some distinct trends. All three sample pairs were identical in terms of the significance values for the CH_2 peak chemical shift values. All three sample pairs were also identical in terms of the OH relative integration values. All three sample pairs were completely dissimilar in terms of the chemical shift and relative integration values of the H_2O and CH_3 peaks.

b) Medicinal substance with agitation versus medicinal substance without agitation.

Table 4.29: Paired group analysis of Mann-Whitney p-values: Medicinal substance with agitation versus medicinal substance without agitation.

	Measure	H_20	OH	CH_2	CH_3
H + Unag	Chemical Shift	0.009	0.008	0.005	0.007
	Relative integ	0.175	0.009	0.009	0.753
S + Unag	Chemical Shift	0.008	0.006	0.009	0.031
	Relative integ	0.016	0.016	0.059	0.076
Both + Unag	Chemical Shift	0.009	0.008	0.008	0.006
	Relative integ	0.917	0.009	0.009	0.009

Some trends were observable in the comparison of the sample pairs, where samples which contained a medicinal substance with agitation were compared with samples which contained the medicinal substance but had not undergone agitation. All the paired sample groups were dissimilar across all the peaks in terms of the chemical shift values. All the paired sample groups were dissimilar in terms of the chemical shift and relative integration values of the OH peaks.

c) Medicinal substance with agitation versus inert substance without agitation.

Table 4.30: Paired group analysis of Mann-Whitney p-values: Medicinal substance with agitation versus inert substance without agitation.

	Measure	H_20	OH	CH_2	CH_3
H + SVR	Chemical Shift	0.007	0.009	0.005	0.006
	Relative integ	0.009	0.175	0.602	0.009
S + SVR	Chemical Shift	0.009	0.007	0.008	0.006
	Relative integ	0.251	0.117	0.465	0.175
Both + SVR	Chemical Shift	0.009	0.008	0.008	0.006
	Relative integ	0.028	0.251	0.600	0.009

The comparison of the agitated medicinal substance samples with the unagitated inert substance revealed two distinct trends. The chemical shift values of all the peaks indicated that all the sample pairs were completely dissimilar. The relative integration values of the OH and CH_2 peaks revealed that all the samples were similar in this regard.

4.4.3 Chemical shift values of all the Mann-Whitney sample pairs

Table 4.31: Mann-Whitney analysis of chemical shift values.

	Measure	H_20	OH	CH_2	CH_3
H + S	Chemical Shift	0.008	0.007	0.095	0.007
H + Both	Chemical Shift	0.009	0.009	0.050	0.018
S + Both	Chemical Shift	0.008	0.007	0.112	0.006
H + Unag	Chemical Shift	0.009	0.008	0.005	0.007
S + Unag	Chemical Shift	0.008	0.006	0.009	0.031
Both + Unag	Chemical Shift	0.009	0.008	0.008	0.006
H + SVR	Chemical Shift	0.007	0.009	0.005	0.006
S + SVR	Chemical Shift	0.009	0.007	0.008	0.006
Both + SVR	Chemical Shift	0.009	0.008	0.008	0.006
SVR + Unag	Chemical Shift	0.007	0.008	0.008	0.006

4.4.4 Relative integration values of all the Mann-Whitney sample pairs

Table 4.32: Mann-Whitney analysis of relative integration values.

	Measure	H_20	OH	CH_2	CH_3
H + S	Relative integ	0.009	0.142	0.175	0.016
H + Both	Relative integ	0.016	0.675	0.009	0.009
S + Both	Relative integ	0.009	0.117	0.116	0.009
H + Unag	Relative integ	0.175	0.009	0.009	0.753
S + Unag	Relative integ	0.016	0.016	0.059	0.076
Both + Unag	Relative integ	0.917	0.009	0.009	0.009
H + SVR	Relative integ	0.009	0.175	0.602	0.009
S + SVR	Relative integ	0.251	0.117	0.465	0.175
Both + SVR	Relative integ	0.028	0.251	0.600	0.009
SVR + Unag	Relative integ	0.076	0.465	0.009	0.009

CHAPTER 5: DISCUSSION

The aim of this study was to ascertain whether or not sonication used as an agitation technique during potentisation would produce remedies that were similar to those produced by Hahnemannian succussion in terms of the respective NMR spectra. Analysis of the data revealed that the samples produced by the Hahnemannian method, sonication, and both Hahnemannian and sonication (both) were dissimilar across more peak values in terms of chemical shift and relative integration than they were similar. At face value this might suggest that the samples are ultimately different. An interesting trend arose, however, when only the chemical shift values were considered.

All the samples, controls included, had statistically significant differences between each other in terms of the chemical shift values of all of the peaks. The exceptions to this, however, were the Hahnemannian, sonicated and Hahnemannian and sonication (both) samples, which were all similar in terms of the CH_2 chemical shift values (see Table 4.31). This may suggest that the application of an agitating process, be it Hahnemannian succussion, sonication or both, produces similarities between remedies in terms of the CH_2 chemical shift values.

Chemical shift values are determined by the electromagnetic environment, the nature of the bonds with neighbouring nuclei, and the degree of shielding that a nucleus possesses (Robinson *et al.* 2005). Thus, the three agitated sample groups (Sulphur 12c Hahnemannian, Sulphur 12c sonicated, and Sulphur 12c both) must have had very similar structural properties surrounding the CH_2 groups for the CH_2 chemical shift values to all be similar. Given that the controls (Lactose 12c and Sulphur 12c unagitated) did not undergo an agitation process, the structural similarities of the agitated samples can possibly be attributed to the agitation process. Furthermore, it demonstrates a similarity in the effect of Hahnemannian succussion and sonication on liquid remedy structure.

The liquid structure-forming effect of succussion and other agitating techniques such as sonication are congruent with Resch and Gutmann's (1991) theory of 'super molecular system' development within homoeopathic remedies. They proposed that dissolved contaminants and gases help to shape an ordered

lattice structure within the liquid solvent; ‘solvation spheres’ develop around hydrophilic solutes and ‘inner surface’ molecules form around hydrophobic solutes. They postulate that with the aid of agitation such as succussion, a substance or solute will modify the water structure and thereby impart information and medicinal effect.

Demangeat (2015) further developed the liquid solvent structure theory using the presence of nanobubbles to explain how structure is formed within a liquid. According to the nanobubble theory, vigorous shaking such as Hahnemannian succussion supersaturates the liquid solvent with gases, thus favouring the formation of nanobubbles. Cavitation that occurs from the shearing forces created by succussion also creates nanobubbles, and favours bulk nanobubble formation, i.e. clusters of nanobubbles.

Sonication is one of the most effective ways of inducing cavitation in a liquid, and would thus also result in mass nanobubble formation. Thus both Hahnemannian succussion and sonication used independently will produce liquids that both have well-developed bulk nanoparticle superstructures. In theory, the combination of both Hahnemannian succussion and sonication would produce a liquid with the most highly developed bulk nanoparticle superstructure due to the high gas saturation induced by succession, and the particularly effective cavitation induced by sonication.

Therefore the nanobubble superstructure developed by the respective agitation techniques together with the presence of the same hydrophobic starting substance, sulphur, may explain why the chemical shift values of the CH_2 peaks of the agitated samples were similar. If the medicinal message of these sample remedies is carried by this particular nanobubble superstructure arrangement that results in similar chemical shift values for the CH_2 groups of the solvent, then it could be possible that sonication and Hahnemannian succussion, while not producing remedies that are physicochemically identical, still produce remedies that have the same medicinal effect.

The structural similarities around the chemical shift values for the CH_2 peaks of the agitated samples can also be explained within the framework of the low-dose nanoparticle theory. The theory proposes that modified nanoparticles

present in the solvent convey the medicinal message (Bell and Koithan 2012). Nanoparticles originate from the starting medicinal substance as well as silica particles leached from the glassware used in the preparation of the remedies. Agitation such as succussion and sonication are both particularly effective at producing nanoparticles, thus the agitated samples would have all had significantly higher nanoparticle contents than the unagitated samples.

The silica, lactose and other (especially metallic) nanoparticles are modified by the medicinal starting substance, thereby developing unique three dimensional structure. When transferred to the next potency level, these modified nanoparticles induce ‘nanoseeding’ or self-assembly of silica nanostructures in higher potencies, thereby conveying the medicinal message of the starting substance (Bell and Koithan 2012). The presence of such nanoparticles in the agitated samples may also explain why the three sample groups were similar structurally in terms of the CH_2 chemical shift values, while distinct from the unagitated sample groups in the same regard.

The trituration process employed in the manufacture of the samples will have also contributed to the nanoparticle content of the samples. Ball milling is a process employed in the production of nanoparticles in modern science, and resembles manual grinding by mortar and pestle (Bell and Koithan 2012). Thus, trituration may actually be a crude method of ‘top down’ nanoparticle production of the starting material.

In terms of the relative integration values of the agitated samples (Hahnemannian, sonication, and both), significant differences existed across all the H_2O and CH_3 peaks. All three samples were similar in terms of the relative integration values of the OH groups. Generally, the sample groups were dissimilar on more peaks than similar and no clear trends could be identified.

When comparing the relative integration values of the agitated samples and the controls, there were similarities and dissimilarities between the groups in varying amounts on varying peak values. There appeared to be no consistency, or any distinct trend emerging from the results, and no clear conclusions could be drawn. The effect on the relative integration values may have to be studied over a number of parallel potencies that extend into the 15c to 30c dilution

range before any distinct trends become clear, as studies by Demangeat (2015) have revealed.

The fact that no clear trends arose from the analysis of the relative integration values does not necessarily disprove the hypothesis. Relative integration values indicate the quantity of spin active nuclei present in a sample (Williams 1986). In terms of the nanobubble superstructure theory the structure of the solvent, as well as the quantity and arrangement of nanobubbles is key, as opposed to the quantity of spin active nuclei present. Therefore two samples in theory may contain differing quantities of spin active nuclei, but could still support similar nanobubble superstructure arrangements.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The hypothesis that insignificant differences exist between the Sulphur 12c Hahnemannian, Sulphur 12c sonicated and Sulphur 12c (both) groups was supported in terms of the chemical shift values of the CH_2 peaks, revealing that the three samples must have had structural similarities. In terms of the chemical shift values for H_2O, OH and CH_3 peaks, the hypothesis was not supported. The relative integration values revealed no clear trends that either supported or disproved the hypothesis.

The study revealed that the hypothesis that statistically significant differences existed between the Sulphur 12c Hahnemannian, Sulphur 12c sonicated and Sulphur 12c (both) groups, and the control sample groups Lactose 12c and Sulphur 12c unagitated, was completely supported in terms of all of the chemical shift values. In terms of the relative integration values, however, no definite conclusions could be drawn as the relative integration values showed no clear trends in favour of or against the hypothesis.

6.2 RECOMMENDATIONS

Due to the sensitivity of homoeopathic remedies, the many influential factors in the manufacturing process, and the extreme sensitivity and delicacy of NMR spectroscopy, it is of paramount importance that NMR studies carried out in the realm of homoeopharmaceutics be particularly strict when applying scientific methods.

Recommendations for further NMR research involving sonicated potencies are:

1. Increase in sample sizes
   For the sake of accuracy, reproducibility and generalisability, the sample sizes should be larger.

2. Inclusion of parallel potencies at a number of dilution levels, especially within the ultradilution range.

   In order to observe clear trends, especially with regard to relative integration values, the range of potencies should be extended. Inclusion of lower potencies (below 12c), but more importantly the inclusion of potencies within the ultradilution range (15c to 30c specifically), may reveal more distinct trends.

3. Inclusion of additional controls

   The addition of a third, agitated control may prove beneficial in discerning which outcomes may be attributed to the starting substance, and which can be attributed to the agitation process.

4. Inclusion of NMR relaxation times

   While chemical shift and relative integration values reveal a lot about the properties of high dilution samples, the inclusion of relaxation times may prove beneficial in exposing trends of structure development within samples produced by different agitation techniques.

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APPENDICES

Appendix A: The preparation of sample potencies

i) Method 6 – Triturations

Aim: To produce a 3CH trituration from Sulphur powder.

Apparatus:

Unglazed porcelain mortar and pestle

Steel spatula

Mass balance (accurate and calibrated)

Cigarette lighter

Consumables:

96% alcohol for flaming

Clean empty vials

Filter paper

Labels

Ingredients:

Lactose BP powder (lactose monohydrate)(Charge/lot: 1033011)

Sulphur powder

Method:

All apparatus and utensils were thoroughly cleaned and odourless.

1. Clean the mortar, pestle and spatula with distilled water, and flame with 96% alcohol.
2. Allow mortar, pestle and spatula to cool sufficiently before use.
3. Place a new piece of filter paper on the scale and tare it.
4. Mass 0.1 gram of Sulphur powder onto the filter paper.
5. Place a new piece of filter paper on the scale and tare it.

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

ii) Method 6 – Triturations

Aim: To produce a 3CH trituration from Lactose powder.

Apparatus:

Unglazed porcelain mortar and pestle

Steel spatula

Mass balance(accurate and calibrated)

Cigarette lighter

Consumables:

96% alcohol for flaming

Clean empty vials

Filter paper

Labels

Ingredients:

Lactose BP powder (lactose monohydrate) (Charge/lot: 1033011).

Method:

All apparatus and utensils were thoroughly cleaned and odourless.

1. Clean the mortar, pestle and spatula with distilled water, and flame with 96% alcohol.
2. Allow mortar, pestle and spatula to cool sufficiently before use.
3. Place a new piece of filter paper on the scale and tare it.
4. Mass 0.1 gram of Lactose powder onto the filter paper.
5. Place a new piece of filter paper on the scale and tare it.
6. Mass 3.3 grams of pure lactose powder onto filter paper.
7. Repeat step 6 twice more. (Total lactose powder mass: 3 x 3.3grams = 9.9grams, therefore the drug-substance to vehicle ratio = 0.1grams:9.9grams is 1:100.)
8. Place 3.3 grams of lactose into the mortar and triturate for a short period.
9. Add the 0.1 gram of lactose powder into the mortar.
10. Triturate for 6 minutes and scrape down for 4 minutes with a metal spatula. Then triturate for 6 minutes and scrape down for 4 minutes. (Trituration time: 2 x 10 minutes = 20 minutes.)
11. Add the second portion of 3.3 grams of lactose powder. Continue as in step 10 above.
12. Finally add the third portion of 3.3 grams of lactose. Proceed as in step 10 above. (Total trituration time: 20 minutes x 3 = 60 minutes.)
13. Place triturate in a vial and label as Lactose 1CH.
14. Repeat steps 1-13 when preparing Lactose 2CH and 3CH, replacing the pure lactose powder with Lactose 1CH and 2CH respectively and each dilution level.

iii) Method 8a – liquid preparations from triturations

a) Aim: To produce liquid dilutions of Sulphur 12CH from the 3CH triturate.

Apparatus:

Mass balance (accurate and calibrated)

Rubber dropper bulbs

5ml and 2ml pipettes, and 10ml measuring cylinder

Consumables:

5ml clear glass pipettes

25ml amber glass dropper bottles

5ml clear glass screw top bottles

Filter paper

Pasteur pipettes

Labels

Ingredients:

Distilled water

87% alcohol

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 0.03 grams of Sulphur 3CH on the filter paper. Place it in a 5ml screw top bottle.
3. Add 2.97ml of distilled water and success 10 times without stopping. Label as Sulphur 4CH.
4. Place 99 parts 87% alcohol in a 5ml clear glass screw top bottle. (99/100 x 3ml = 2.97ml). Add 1 part Sulphur 4CH. (1/100 x 3ml = 0.03ml). Succuss 10 times without stopping. Label as Sulphur 5CH.

5. Repeat step 4 to produce Sulphur 6CH to 11CH.
6. To prepare Sulphur 12CH place 99 parts 87% alcohol in a 25ml amber glass bottle. (99/100 x 16ml = 15.84ml). Add 1 part Sulphur 11CH. Succuss 10 times without stopping. Label as Sulphur 12CH.
7. Store Sulphur 12CH in a cool environment free of electromagnetic disturbance until it can be transported for NMR Spectroscopy.

b) Aim: To produce liquid dilutions of Sulphur 12c sonicated from the 3CH triturate.

Apparatus:

Mass balance (accurate and calibrated)

Rubber dropper bulbs

5ml and 2ml pipettes, and 10ml measuring cylinder

40Hz Sonicator (UMC20, Ultrasonic Manufacturing Company (PTY) LTD, P.O.Box 3157, Krugersdorp. Serial number: 38031810)

Consumables:

5ml clear glass pipettes

25ml amber glass dropper bottles

5ml clear glass screw top bottles

Filter paper

Pasteur pipettes

Labels

Ingredients:

Distilled water

87% alcohol

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 0.03 grams of Sulphur 3CH on the filter paper. Place it in a 5ml screw top bottle.
3. Add 2.97ml of distilled water, seal with a screw cap then place the sample in the sonicator and sonicate for 30 seconds. Label as Sulphur 4CS.
4. Place 99 parts 87% alcohol in a 5ml clear glass screw top bottle. (99/100 x 3ml = 2.97ml). Add 1 part Sulphur 4CS. (1/100 x 3ml = 0.03ml). Sonicate for 30 seconds. Label as Sulphur 5CS.
5. Repeat step 4 to produce Sulphur 6CS to 11CS.
6. To prepare Sulphur 12CS place 99 parts 87% alcohol in a 25ml amber glass bottle. (99/100 x 16ml = 15.84ml). Add 1 part Sulphur 11CS. Sonicate for 30 seconds. Label as Sulphur 12CH.
7. Store Sulphur 12CS in a cool environment free of electromagnetic disturbance until it can be transported for NMR Spectroscopy.

c) Aim: To produce liquid dilutions of Sulphur 12C with sonication and Hahnemannian succussion from the 3CH triturate.

Apparatus:

Mass balance (accurate and calibrated)

Rubber dropper bulbs

5ml and 2ml pipettes, and 10ml measuring cylinder

40Hz Sonicator (UMC20, Ultrasonic Manufacturing Company (PTY) LTD, P.O.Box 3157, Krugersdorp. Serial number: 38031810)

Consumables:

5ml clear glass pipettes

25ml amber glass dropper bottles

5ml clear glass screw top bottles

Filter paper

Pasteur pipettes

Labels

Ingredients:

Distilled water

87% alcohol

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 0.03 grams of Sulphur 3CH on the filter paper. Place it in a 5ml screw top bottle.
3. Add 2.97ml of distilled water and succuss 10 times without stopping. Then place the sample in the sonicator bath and sonicate for 30 seconds. Label as Sulphur 4CH+S.
4. Place 99 parts 87% alcohol in a 5ml clear glass screw top bottle. (99/100 x 3ml = 2.97ml). Add 1 part Sulphur 4CH+S. (1/100 x 3ml = 0.03ml). Succuss 10 times without stopping, then sonicate for 30 seconds. Label as Sulphur 5CH+S.
5. Repeat step 4 to produce Sulphur 6CH+S to 11CH+S.
6. To prepare Sulphur 12CH+S place 99 parts 87% alcohol in a 25ml amber glass bottle. (99/100 x 16ml = 15.84ml). Add 1 part Sulphur 11CH+S. Succuss 10 times without stopping, then sonicate for 30 seconds. Label as Sulphur 12CH+S.
7. Store Sulphur 12CH+S in a cool environment free of electromagnetic disturbance until it can be transported for NMR Spectroscopy.

d) Aim: To produce liquid dilutions of Sulphur 12c unagitated from the 3CH triturate.

Apparatus:

Mass balance (accurate and calibrated)

Rubber dropper bulbs

5ml and 2ml pipettes, and 10ml measuring cylinder

Consumables:

5ml clear glass pipettes

25ml amber glass dropper bottles

5ml clear glass screw top bottles

Filter paper

Pasteur pipettes

Labels

Ingredients:

Distilled water

87% alcohol

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 0.03 grams of Sulphur 3CH on the filter paper. Place it in a 5ml screw top bottle.
3. Add 2.97ml of distilled water and gently swirl until the triturate has dissolved. Do not succuss. Label as Sulphur 4C Unagitated.
4. Place 99 parts 87% alcohol in a 5ml clear glass screw top bottle. (99/100 x 3ml = 2.97ml). Add 1 part Sulphur 4C Unagitated. (1/100 x 3ml = 0.03ml). Do not agitate. Label as Sulphur 5C Unagitated.
5. Repeat step 4 to produce Sulphur 6C Unagitated to 11C Unagitated.
6. To prepare Sulphur 12C Unagitated place 99 parts 87% alcohol in a 25ml amber glass bottle. (99/100 x 16ml = 15.84ml). Add 1 part Sulphur 11C Unagitated. Do not agitate. Label as Sulphur 12C Unagitated.
7. Store Sulphur 12C Unagitated in a cool environment free of electromagnetic disturbance until it can be transported for NMR Spectroscopy.

e) Aim: To produce liquid dilutions of Lactose 12C Control from the 3CH triturate.

Apparatus:

Mass balance (accurate and calibrated)

Rubber dropper bulbs

5ml and 2ml pipettes, and 10ml measuring cylinder

Consumables:

5ml clear glass pipettes

25ml amber glass dropper bottles

5ml clear glass screw top bottles

Filter paper

Pasteur pipettes

Labels

Ingredients:

Distilled water

87% alcohol

Lactose 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 0.03 grams of Lactose 3CH triturate on the filter paper. Place it in a 5ml screw top bottle.
3. Add 2.97ml of distilled water and swirl gently until triturate dissolves. Label as Lactose 4C Control.
4. Place 99 parts 87% alcohol in a 5ml clear glass screw top bottle. (99/100 x 3ml = 2.97ml). Add 1 part Lactose 4C Control. (1/100 x 3ml = 0.03ml). Do not agitate. Label as Lactose 5C Control.
5. Repeat step 4 to produce Lactose 6C Control to 11C Control.

6. To prepare Lactose 12C Control place 99 parts 87% alcohol in a 25ml amber glass bottle. (99/100 x 16ml = 15.84ml). Add 1 part Lactose 11C Control. Do not agitate. Label as Sulphur 12CH.
7. Store Lactose 12C Control in a cool environment free of electromagnetic disturbance until it can be transported for NMR Spectroscopy.

Appendix B: NMR Spectra

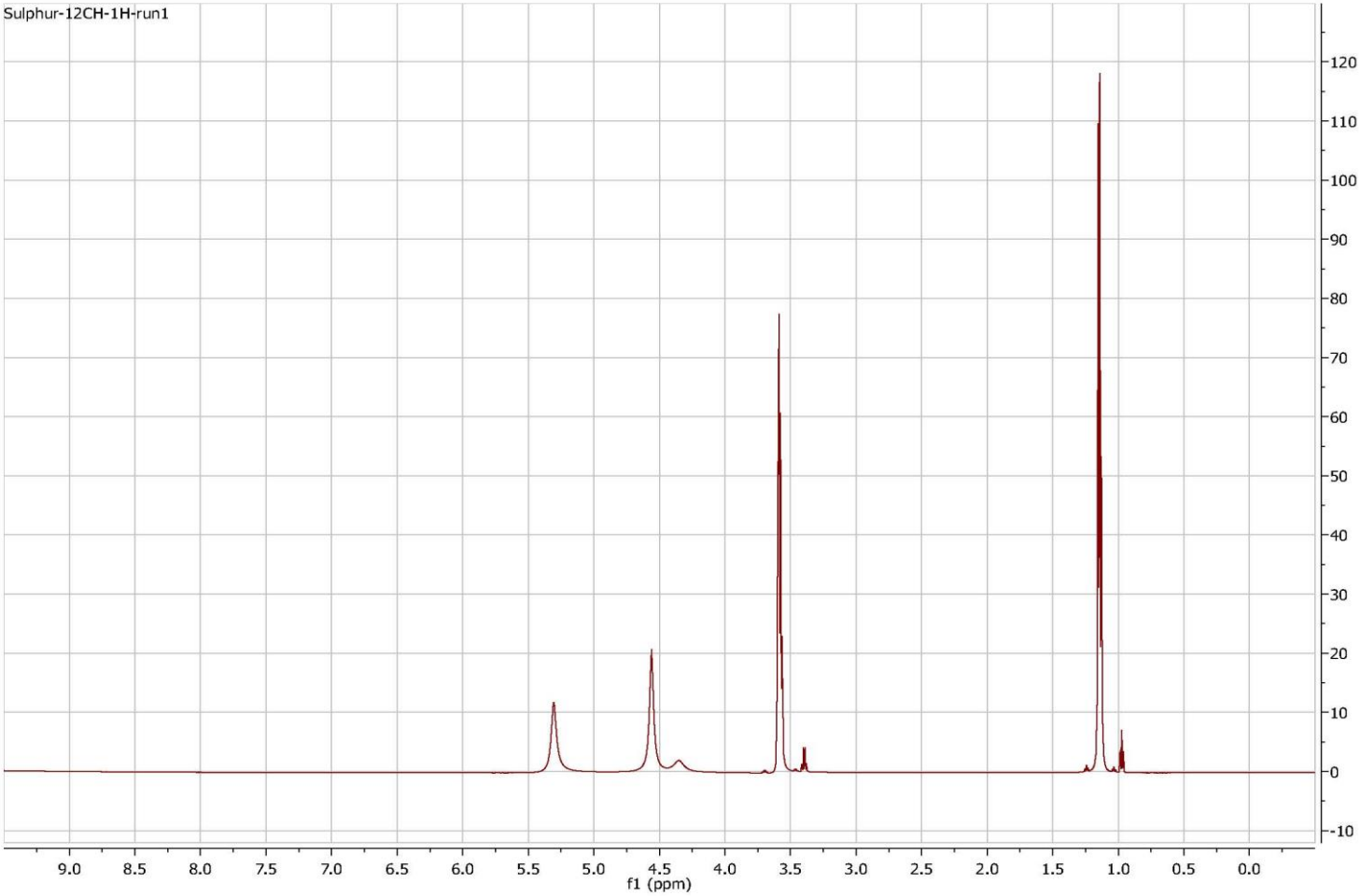

Figure 1: NMR Spectra, Sulphur 12c Hahnemannian- run 1

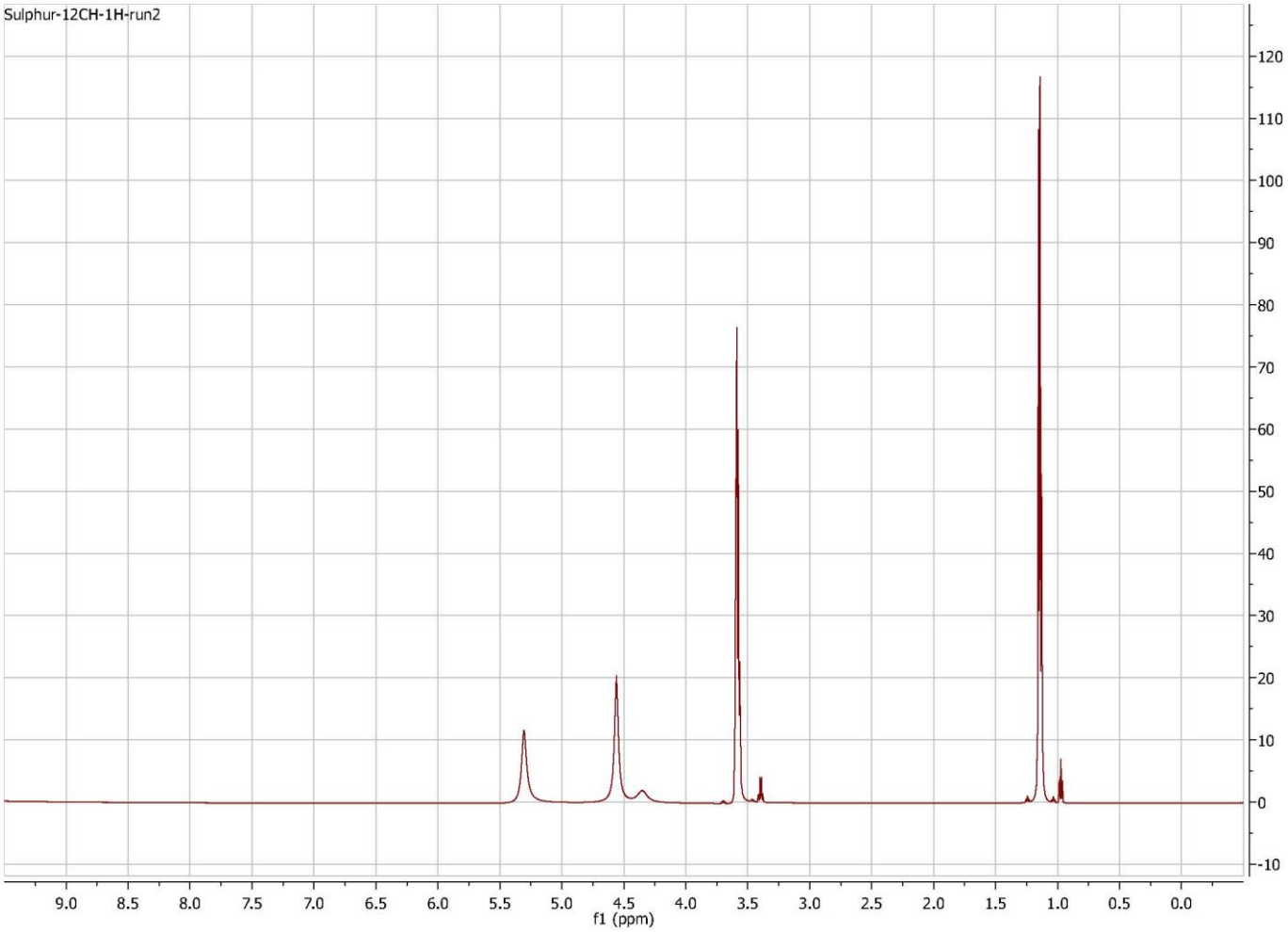

Figure 2: NMR Spectrum, Sulphur 12c Hahnemannian- run 2

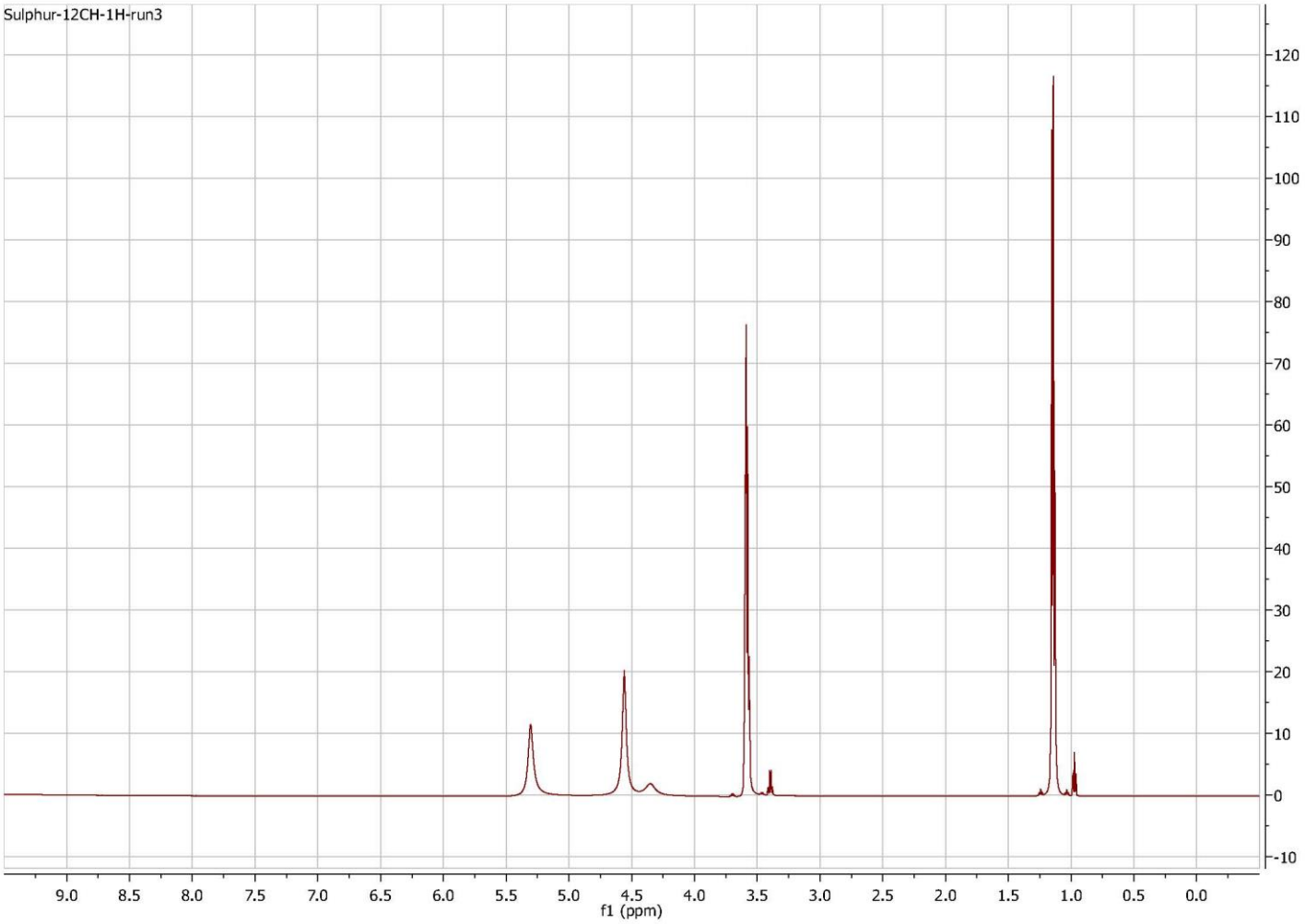

Figure 3: NMR Spectrum, Sulphur 12c Hahnemannian- run 3

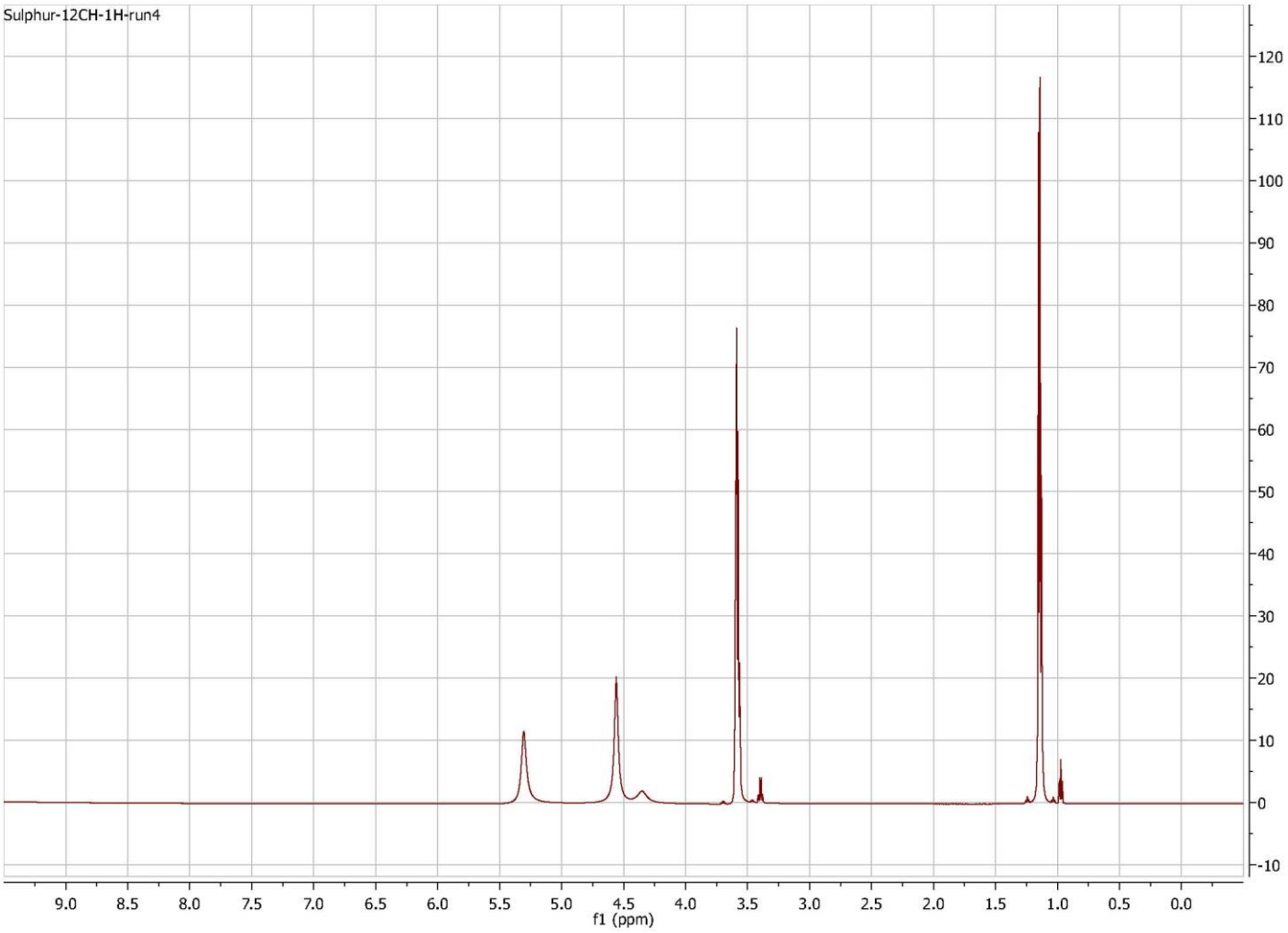

Figure 4: NMR Spectrum, Sulphur 12c Hahnemannian- run 4

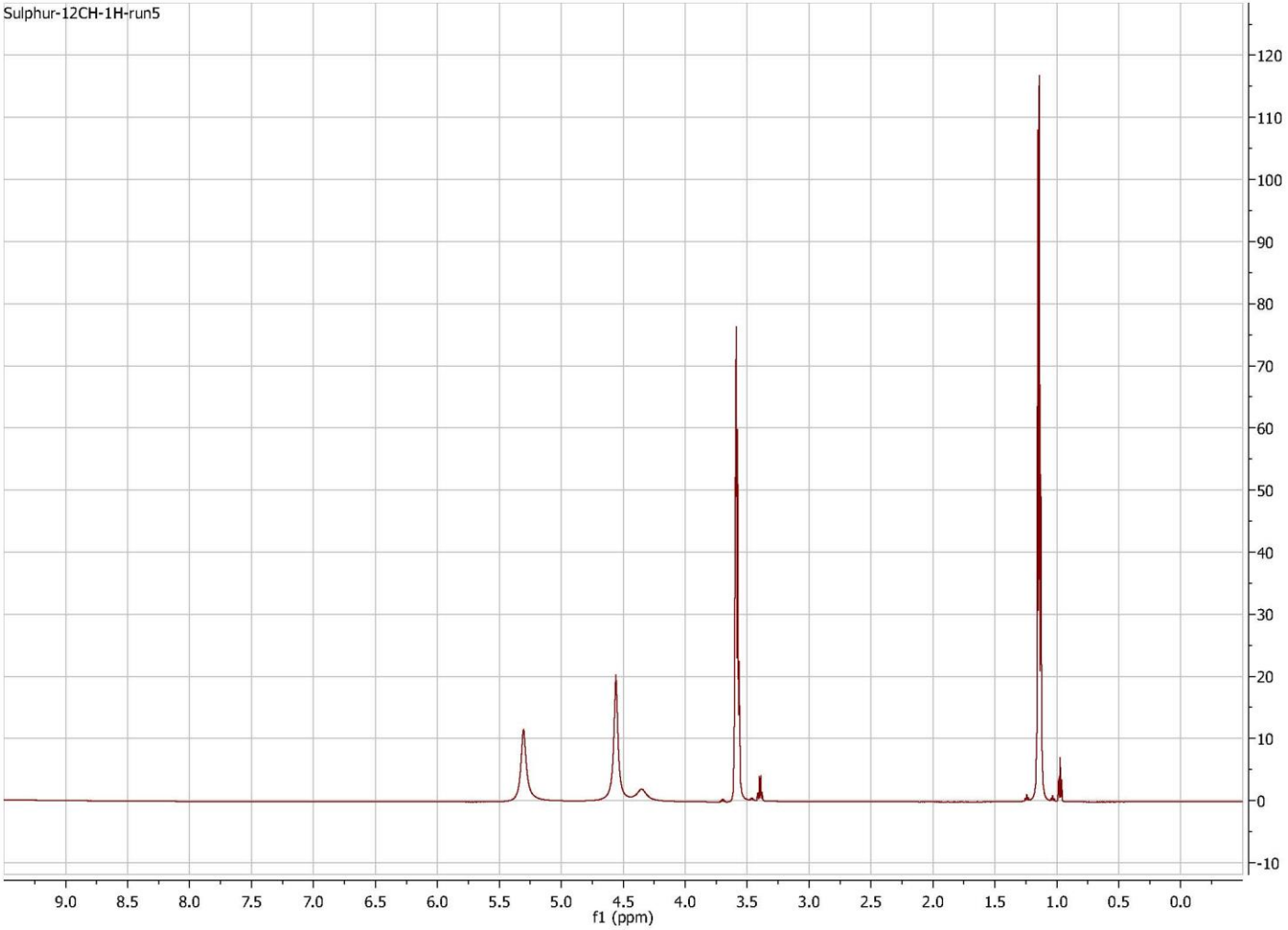

Figure 5: NMR spectrum, Sulphur 12c Hahnemannian- run 5

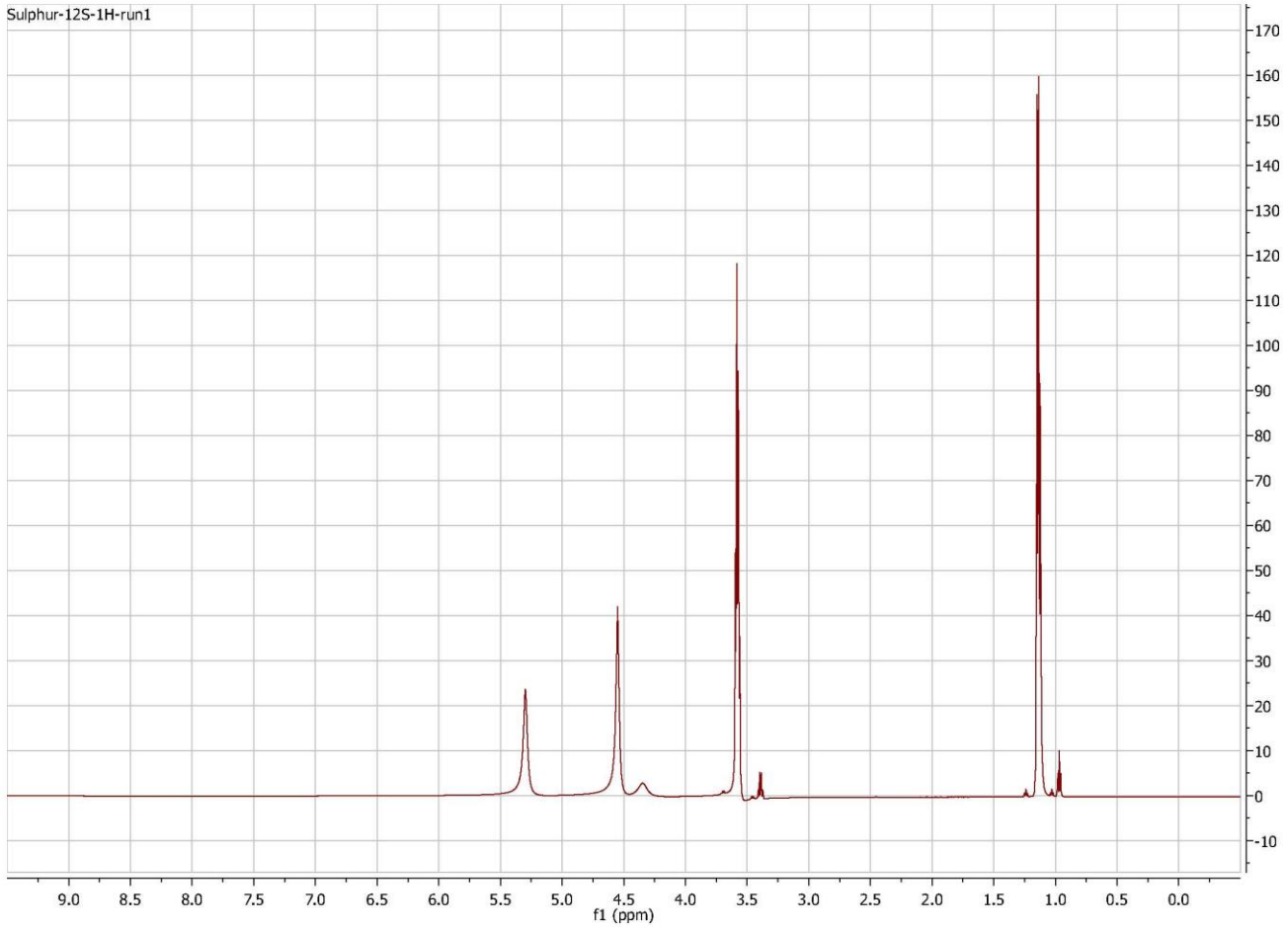

Figure 6: NMR spectrum, Sulphur 12c sonicated- run 1

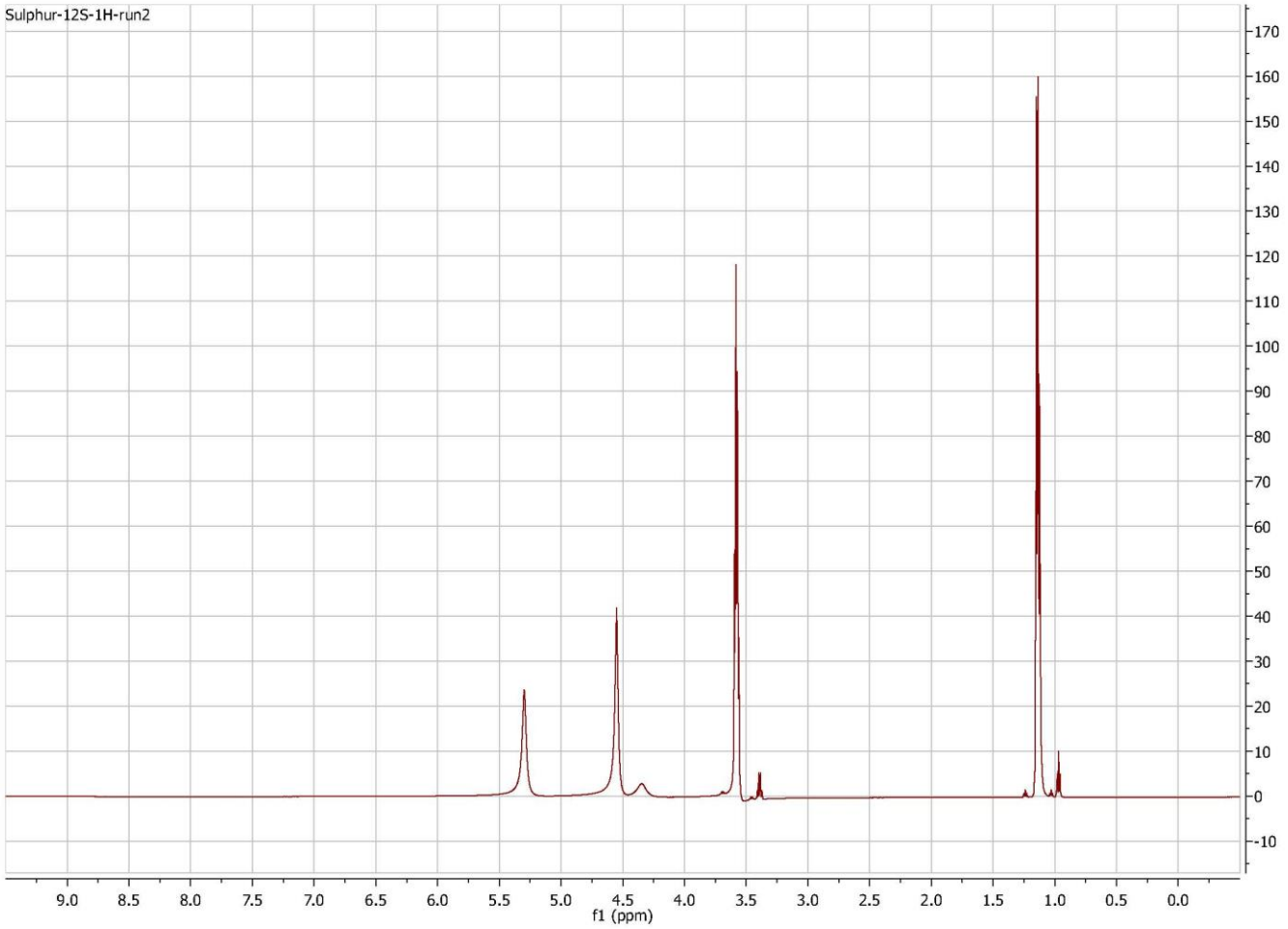

Figure 7: NMR spectrum, Sulphur 12c sonicated- run 2

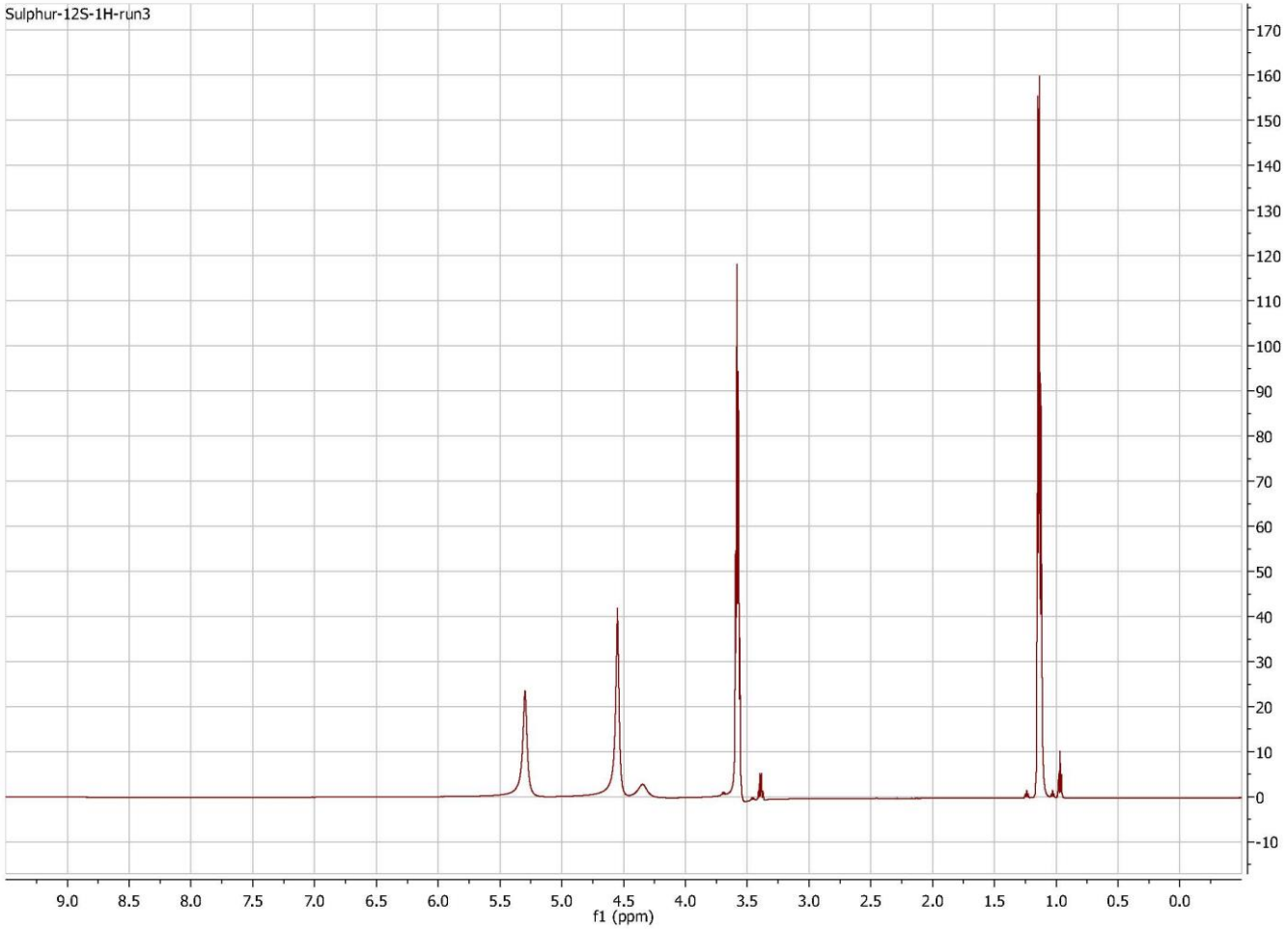

Figure 8: NMR spectrum, Sulphur 12c sonicated- run 3

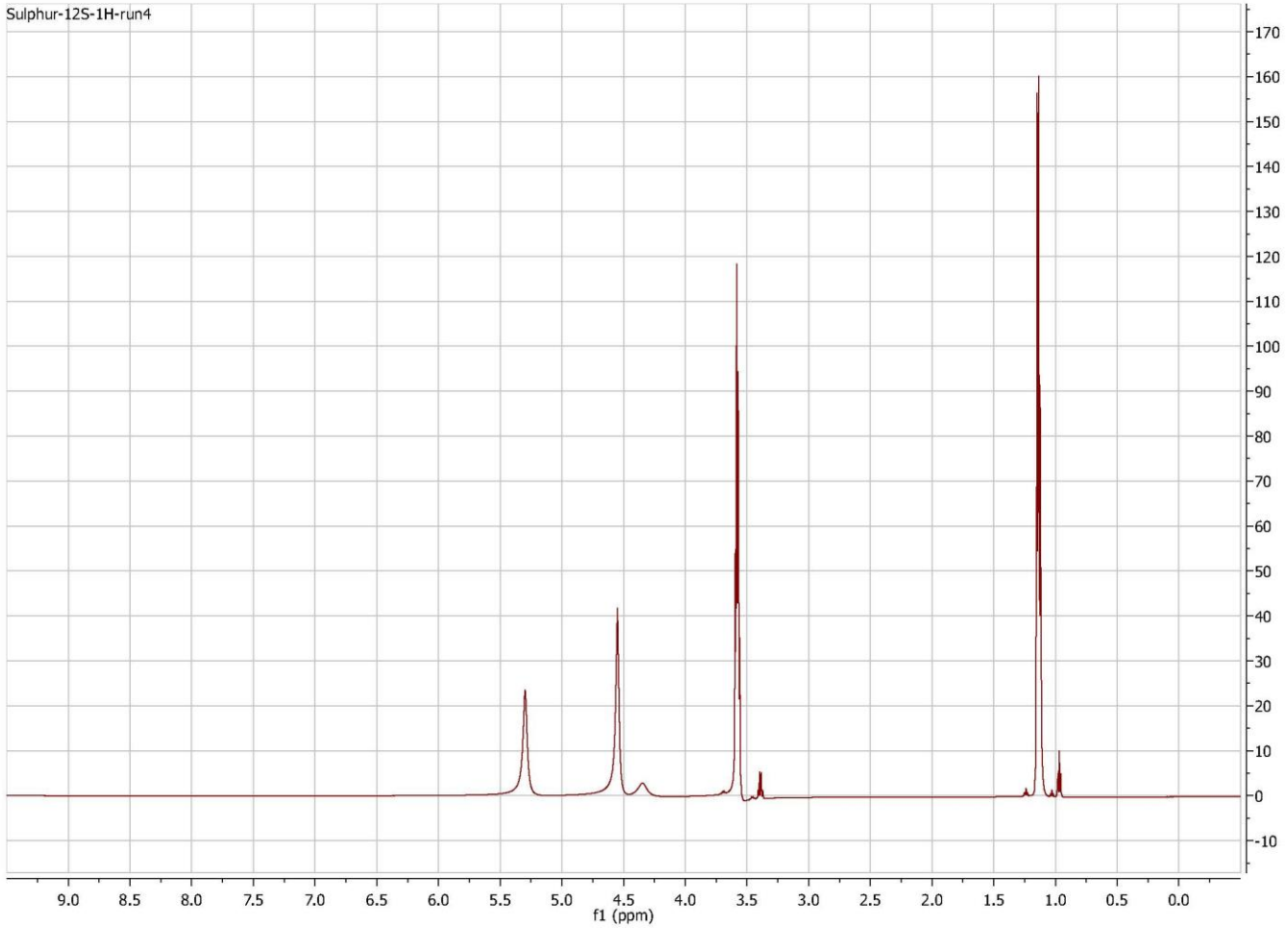

Figure 9: NMR spectrum, Sulphur 12c sonicated- run 4

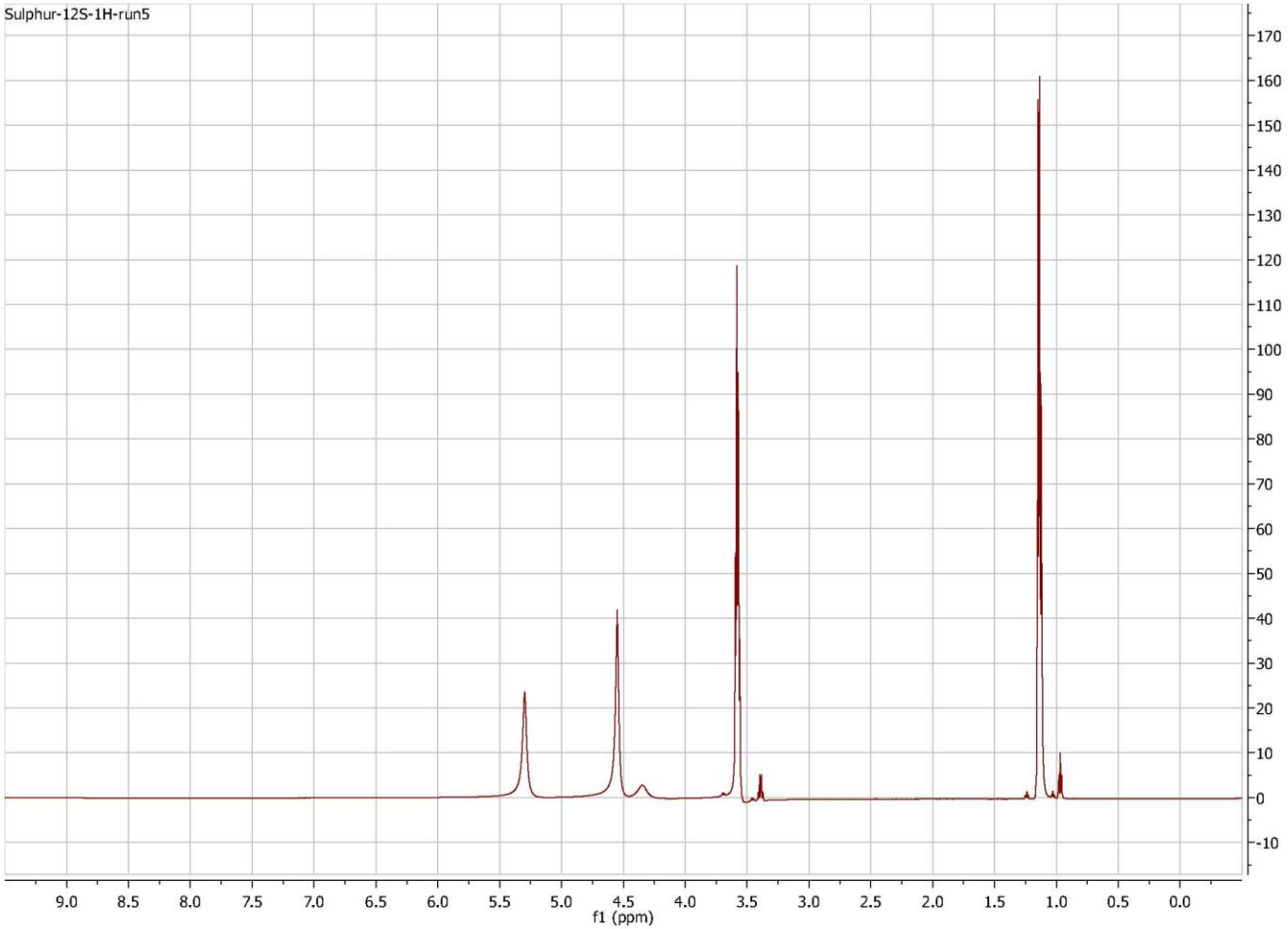

Figure 10: NMR spectrum, Sulphur 12c sonicated- run 5

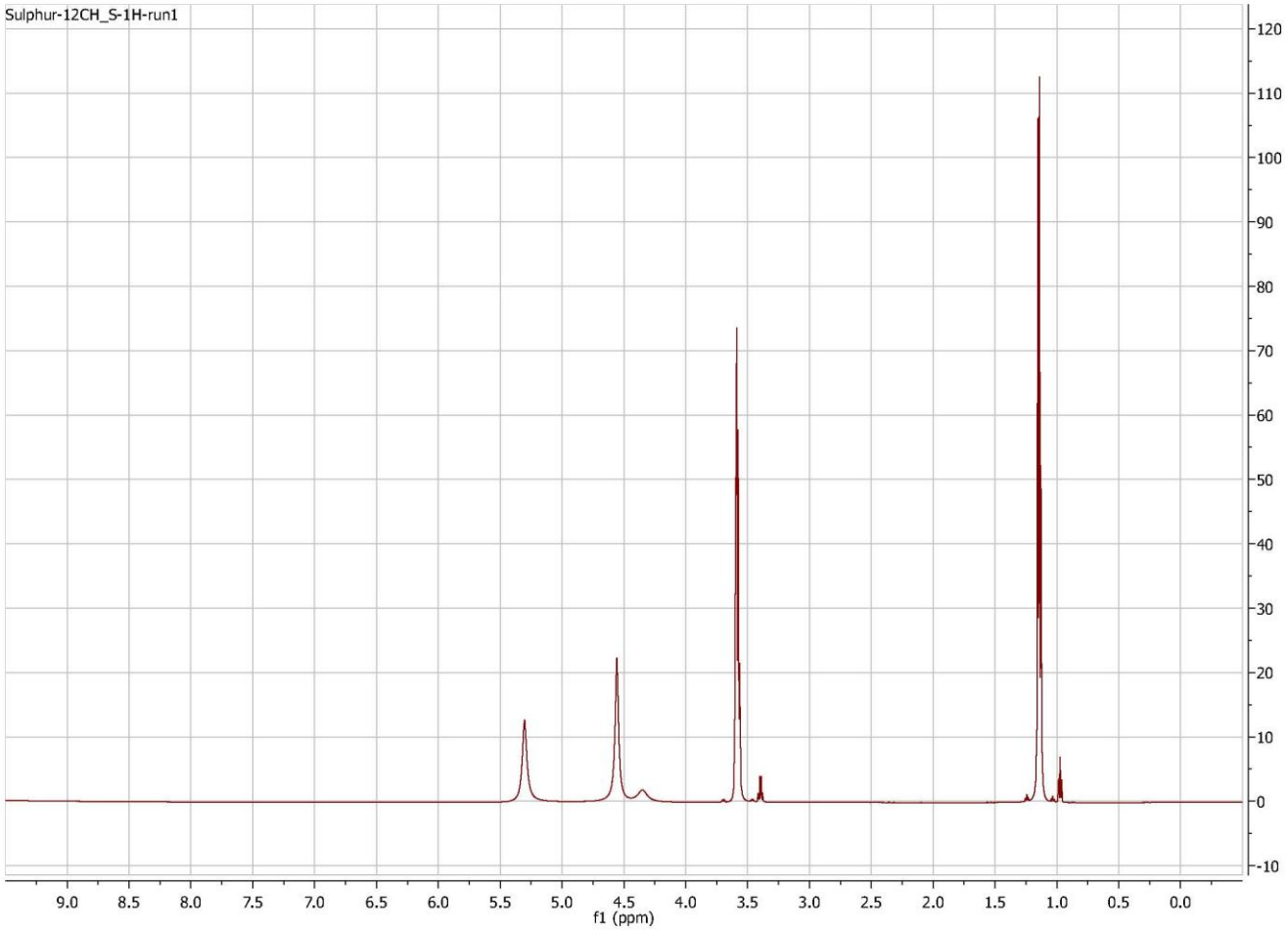

Figure 11: NMR spectrum, Sulphur 12c both- run 1

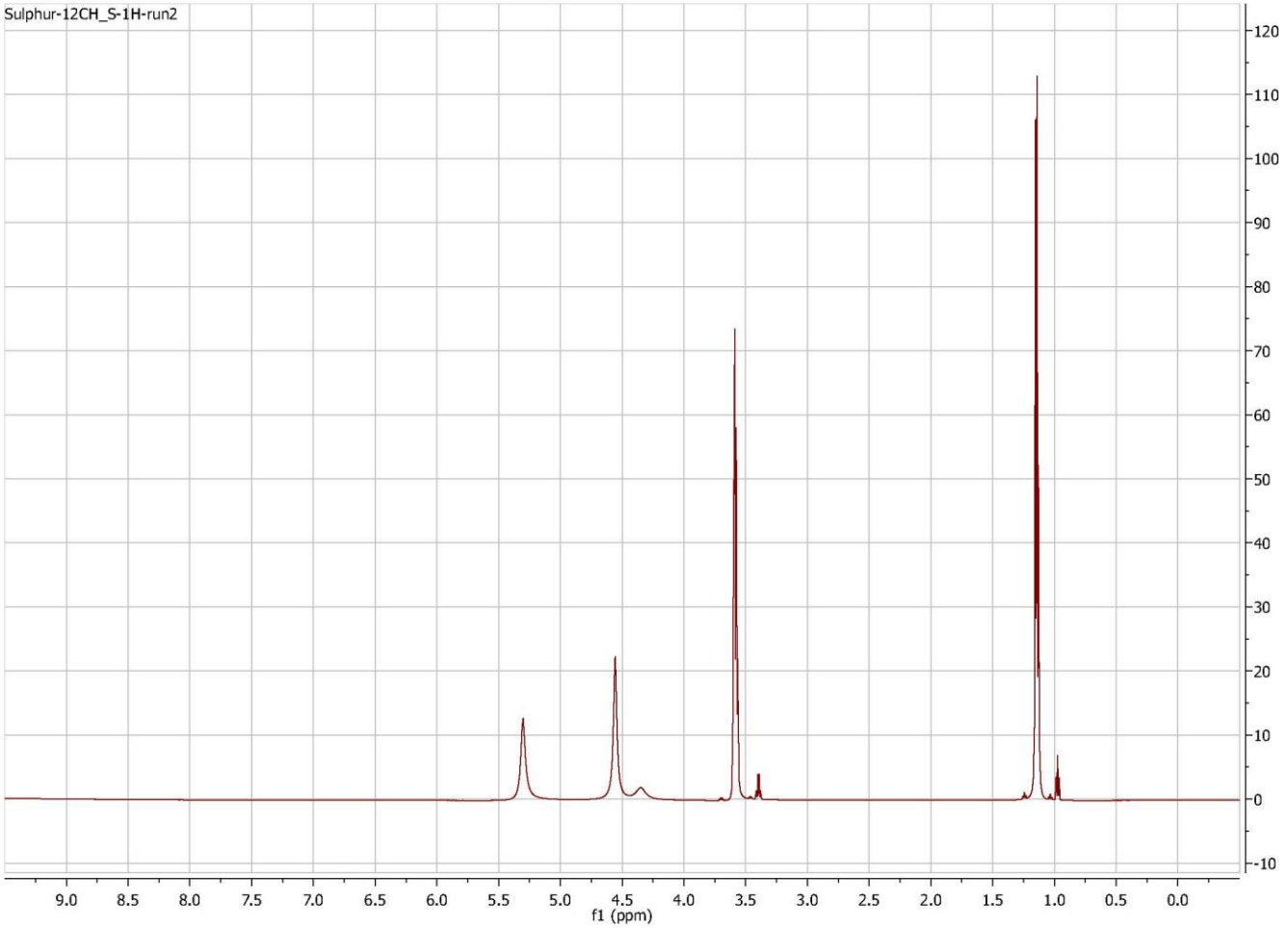

Figure 12: NMR spectrum, Sulphur 12c both- run 2

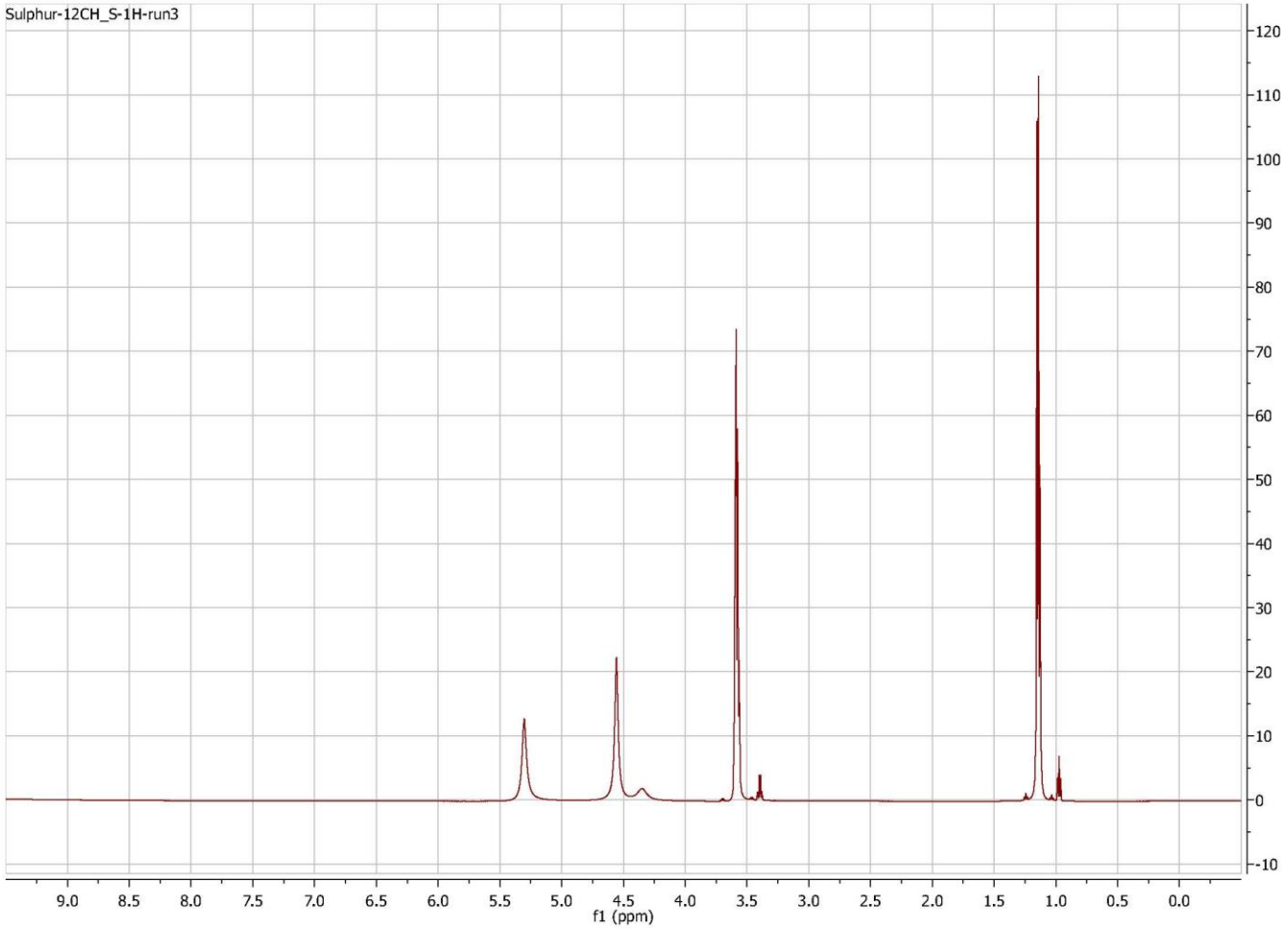

Figure 13: NMR spectrum, Sulphur 12c both- run 3

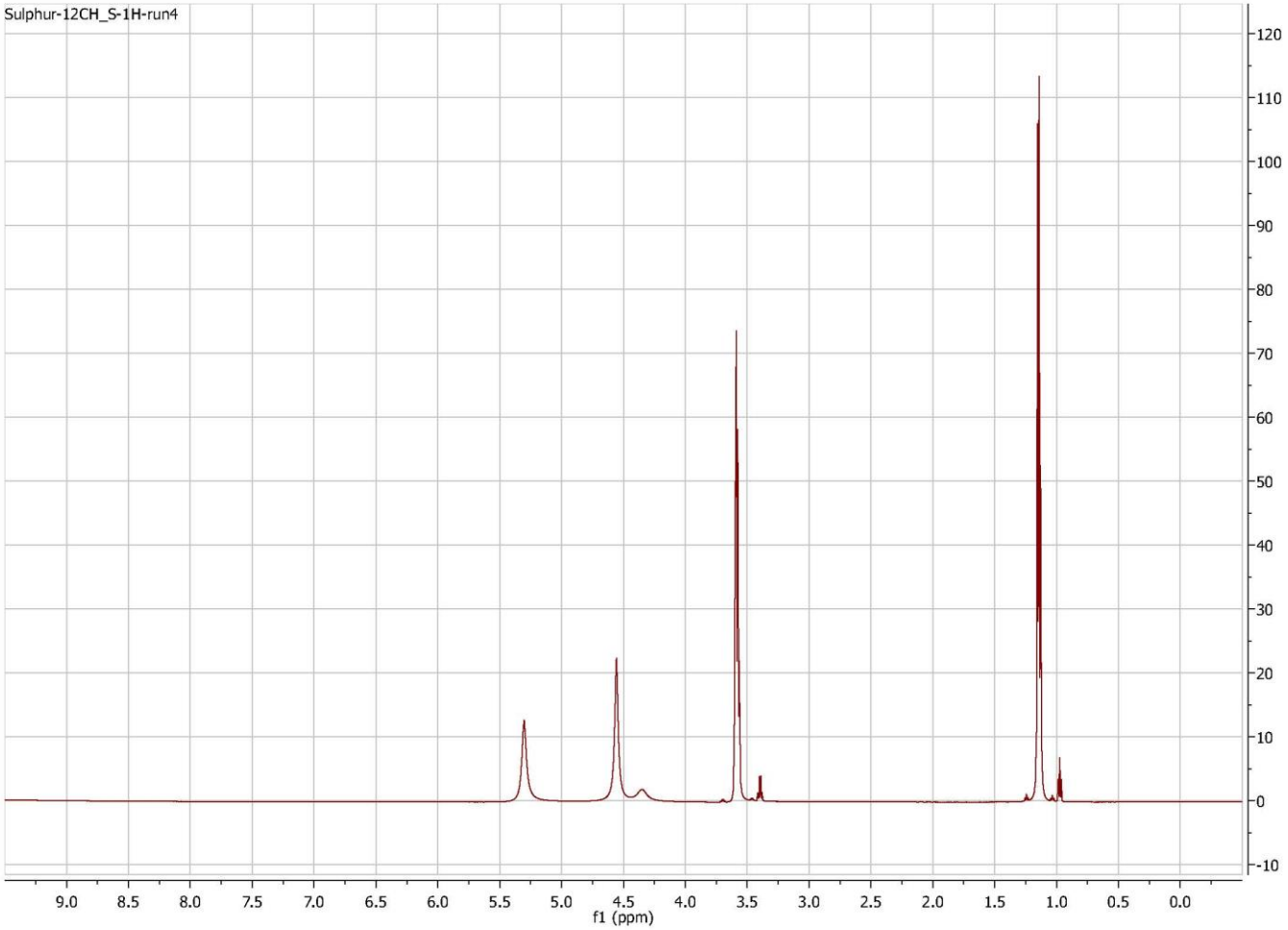

Figure 14: NMR spectrum, Sulphur 12c both- run 4

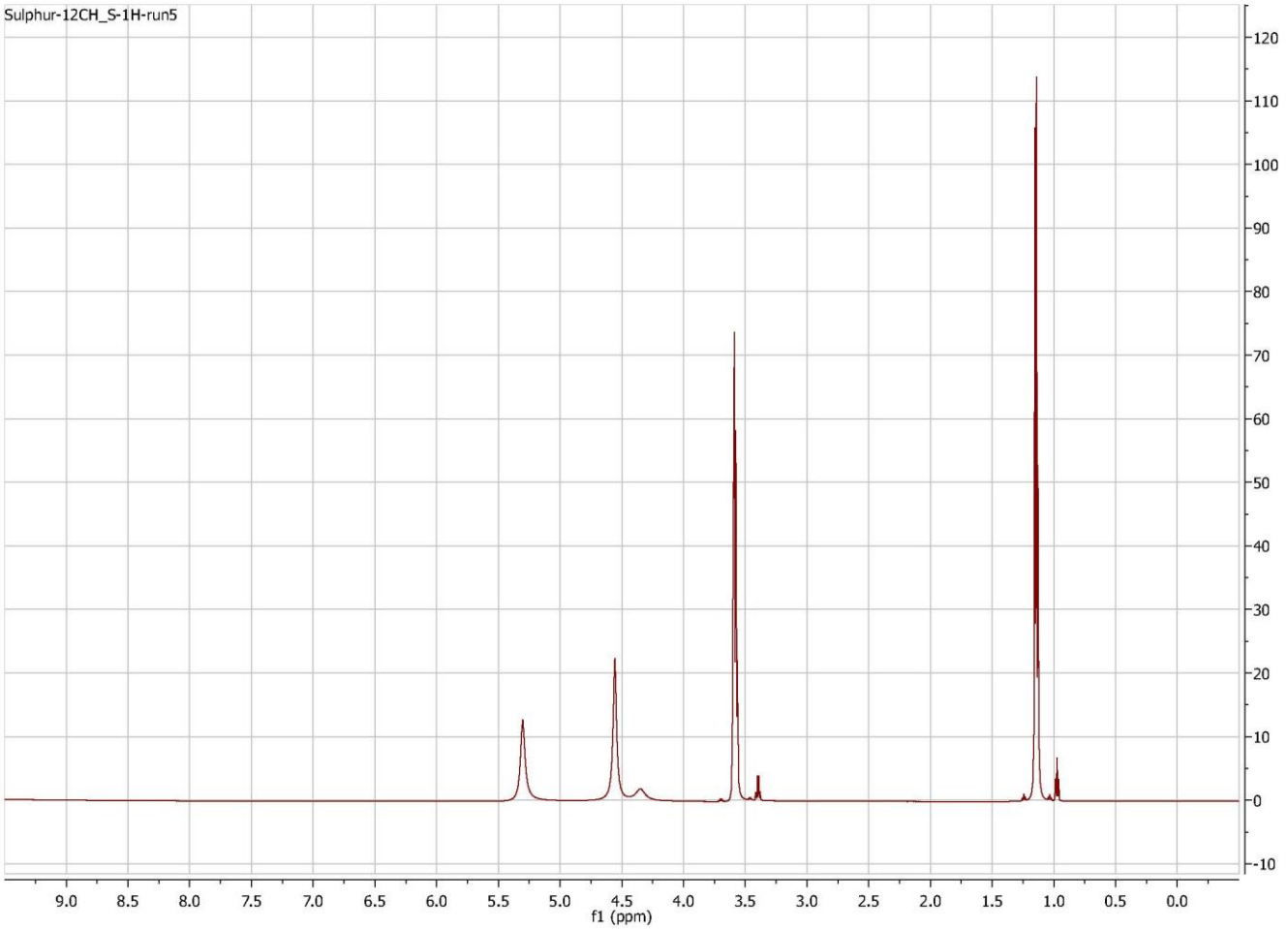

Figure 15: NMR spectrum, Sulphur 12c both- run 5

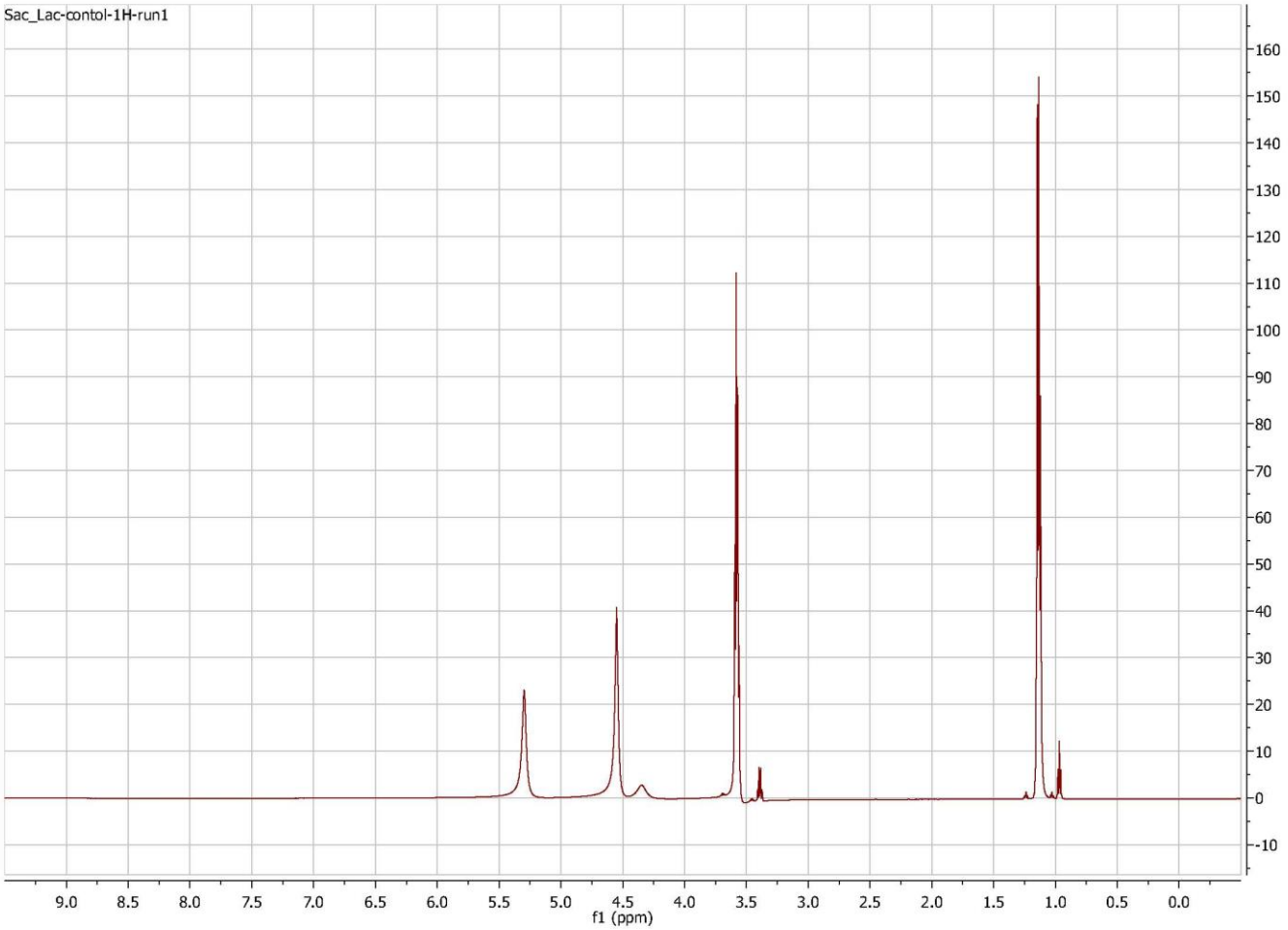

Figure 16: NMR spectrum, Lactose 12c control unagitated- run 1

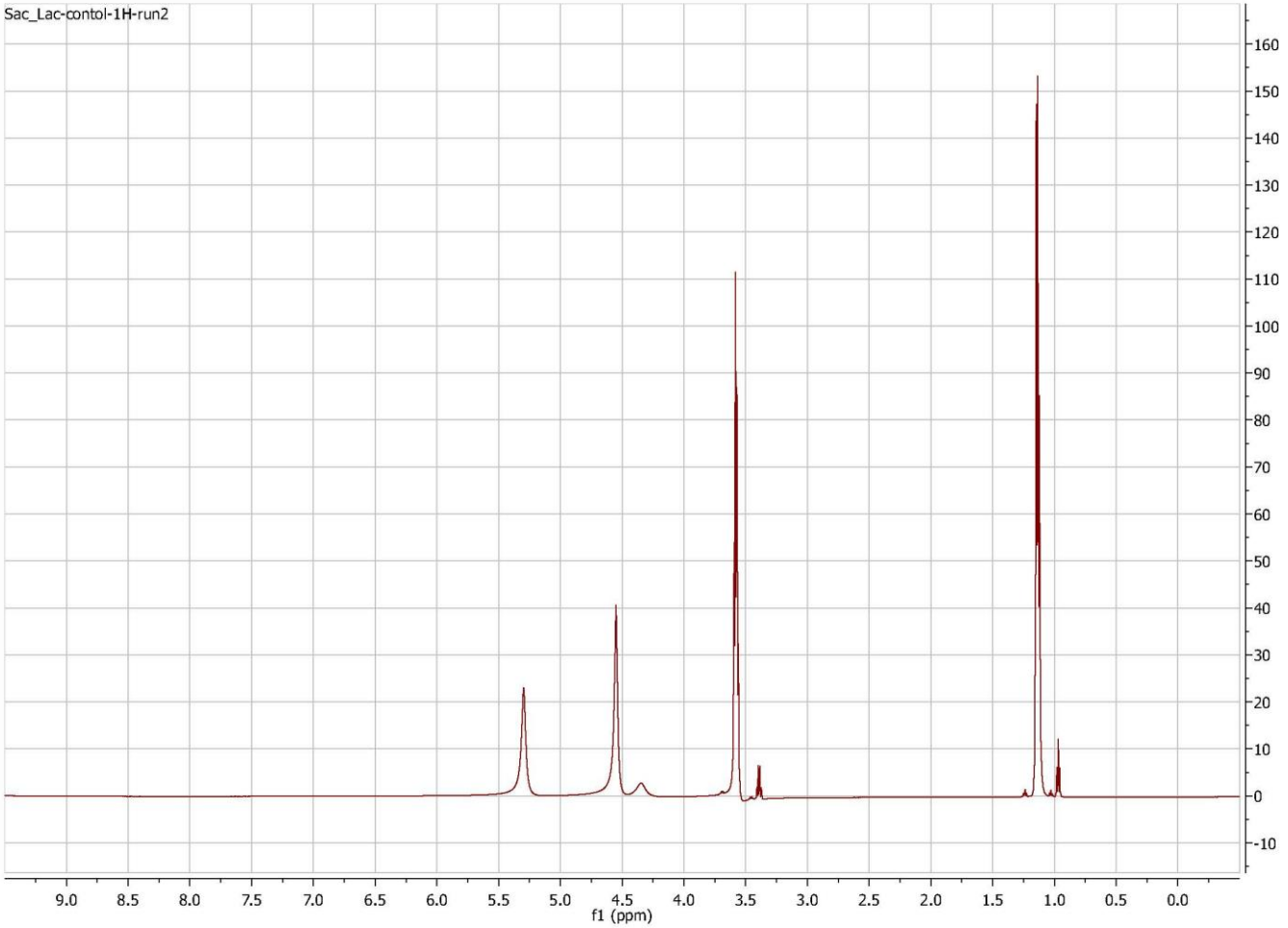

Figure 17: NMR spectrum, Lactose 12c control unagitated- run 2

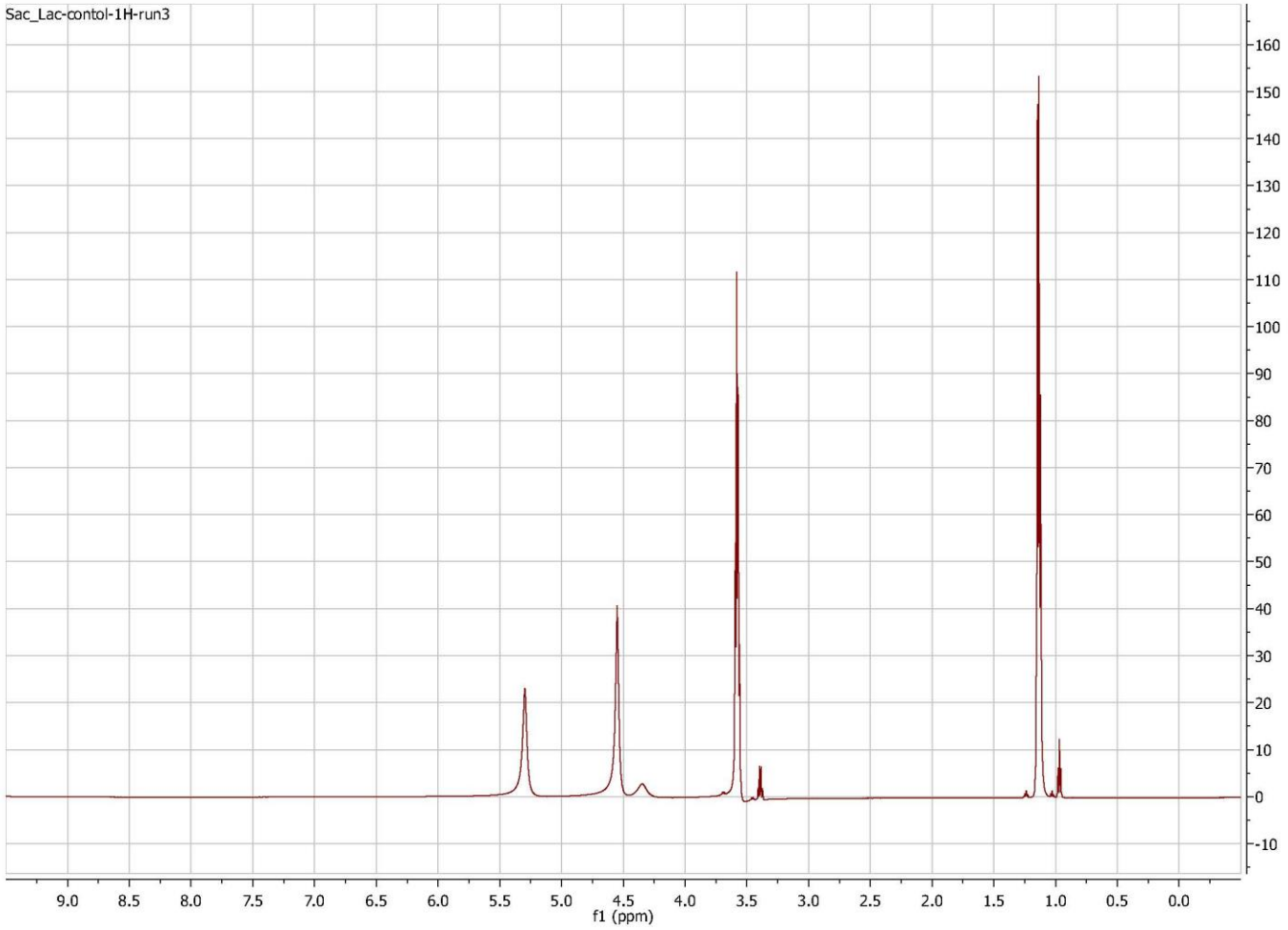

Figure 18, NMR spectrum, Lactose 12c control unagitated- run 3

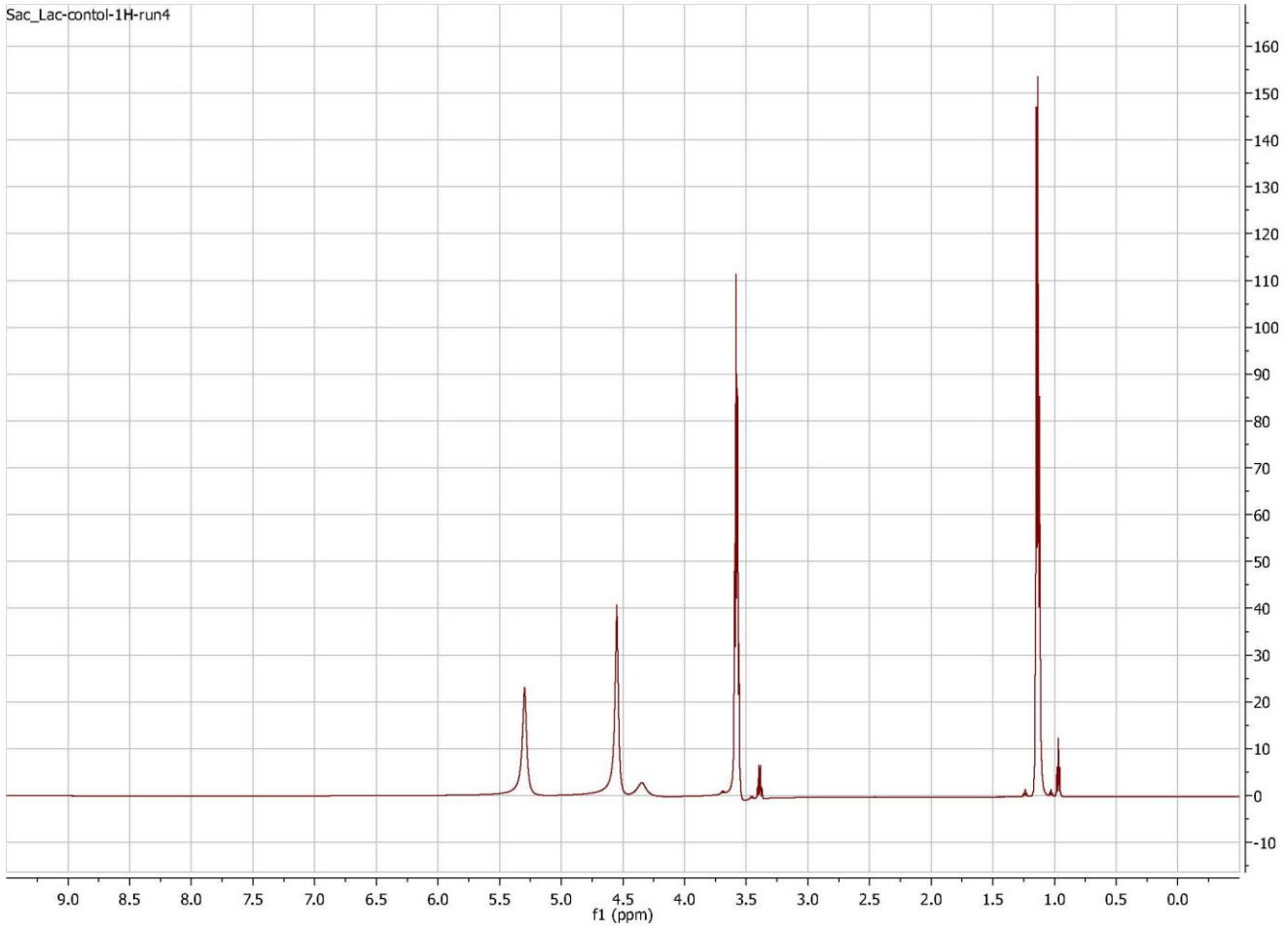

Figure 19: NMR spectrum, Lactose 12c control unagitated- run 4

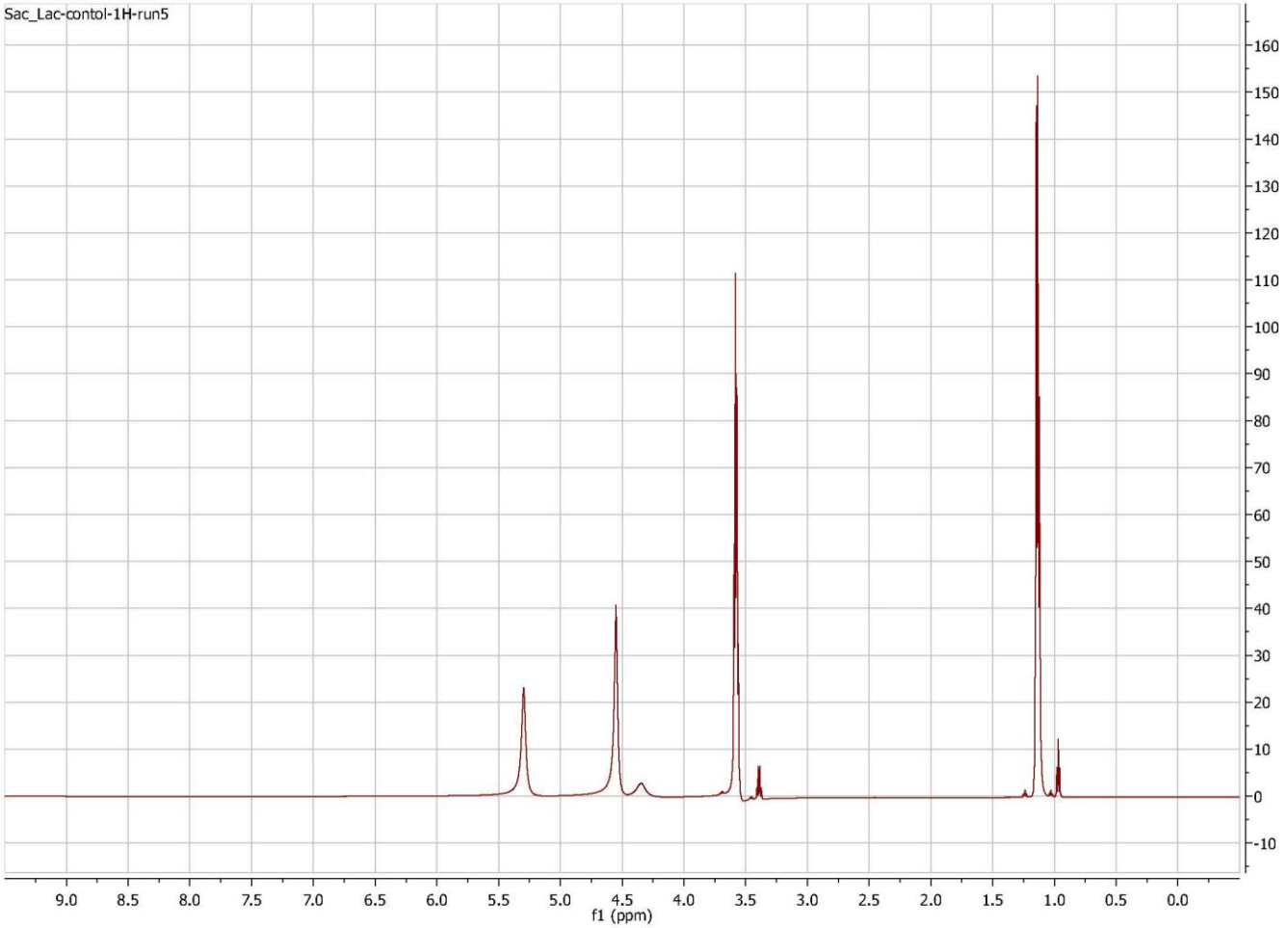

Figure 20: NMR spectrum, Lactose 12c control unagitated- run 5

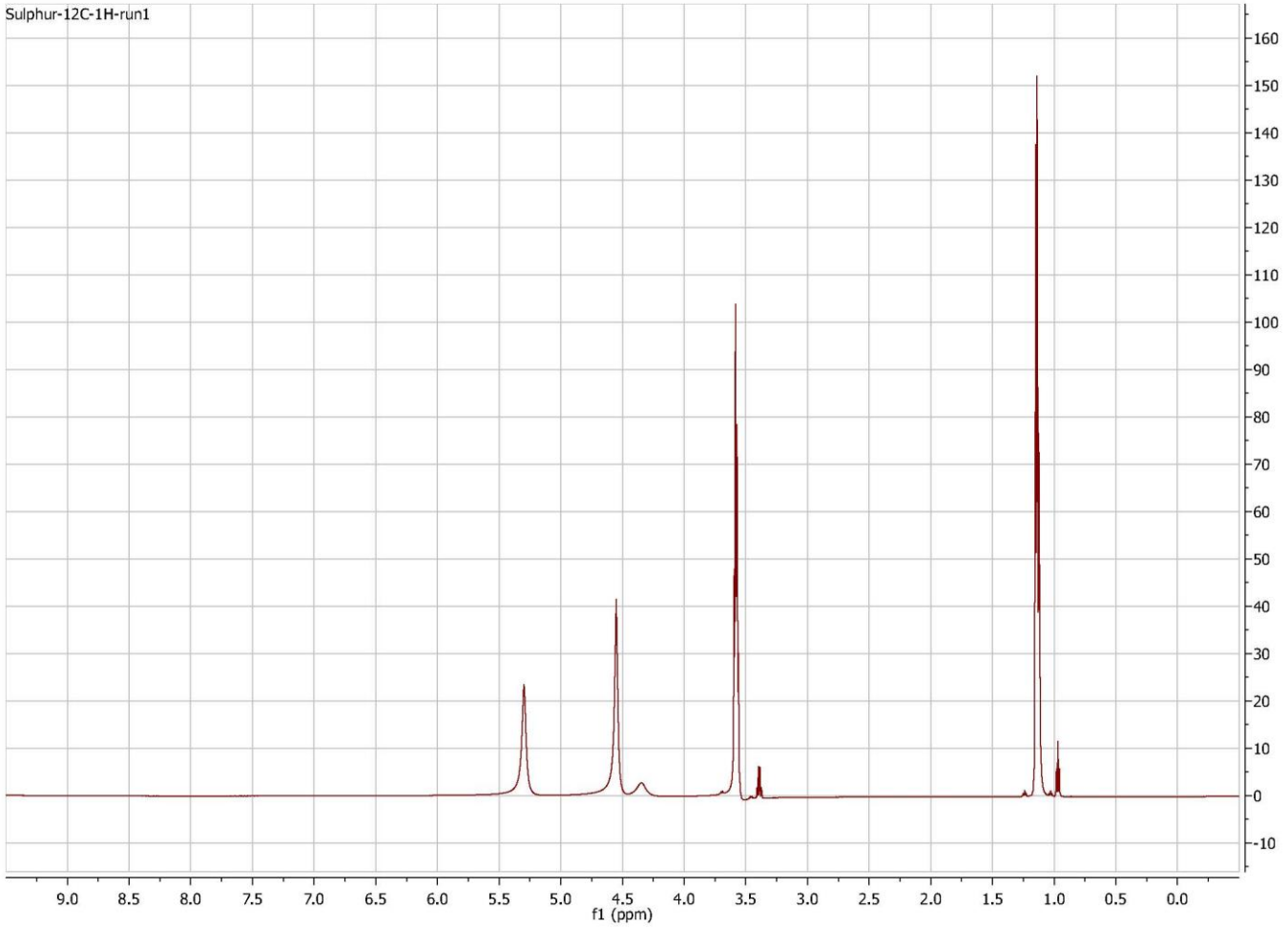

Figure 21: NMR spectrum, Sulphur 12c unagitated- run 1

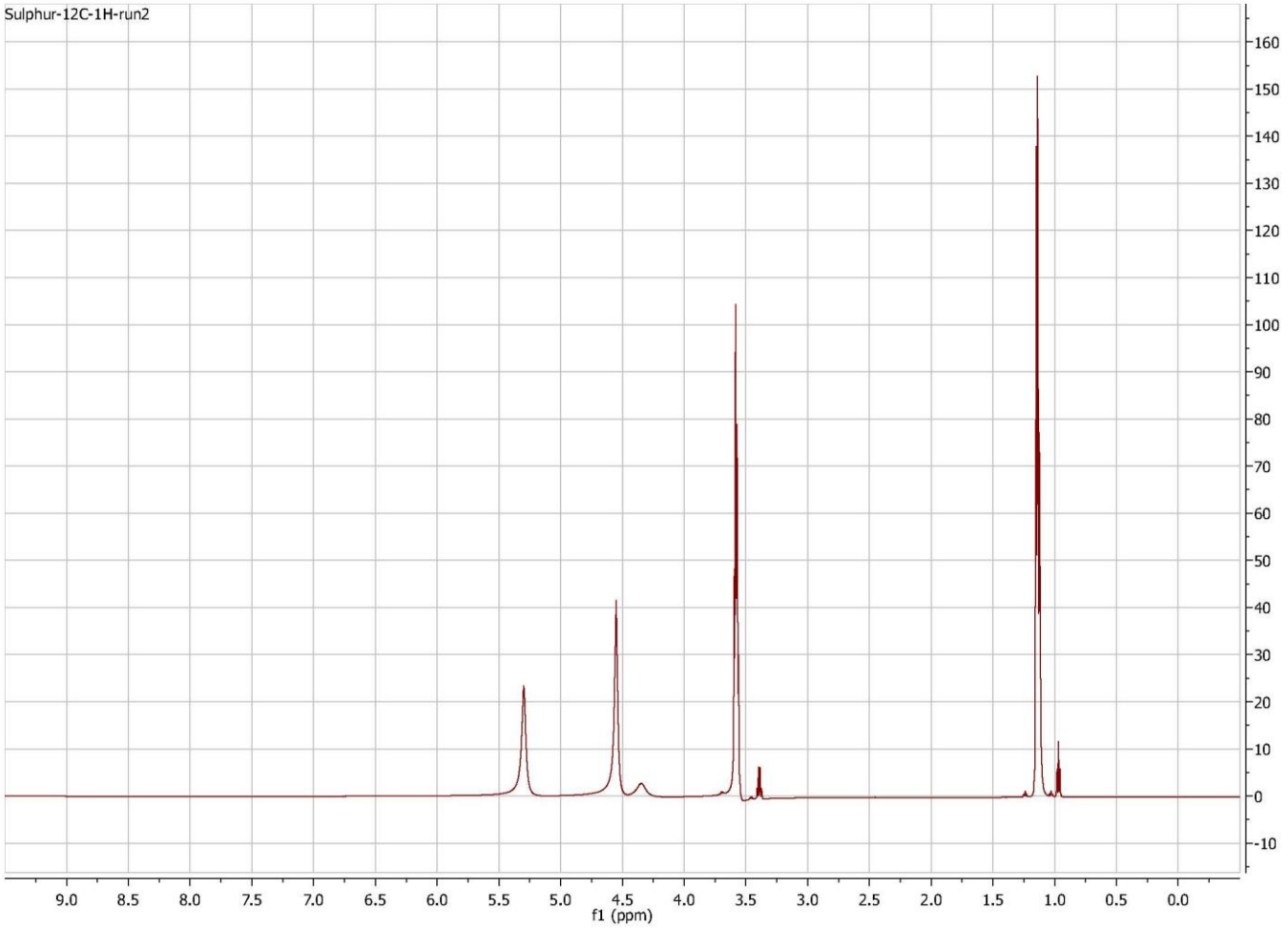

Figure 22: NMR spectrum, Sulphur 12c unagitated- run 2

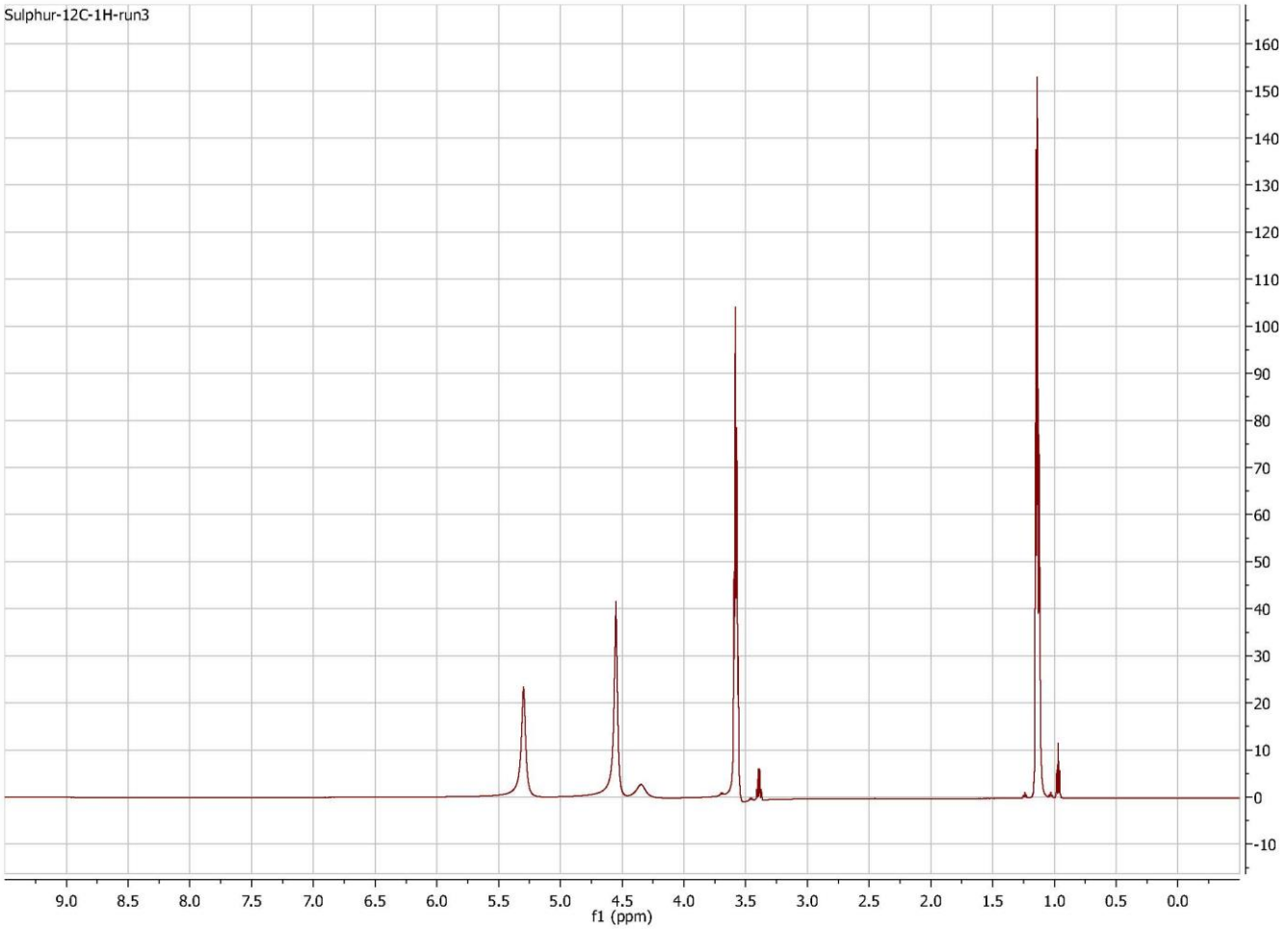

Figure 23: NMR spectrum, Sulphur 12c unagitated- run 3

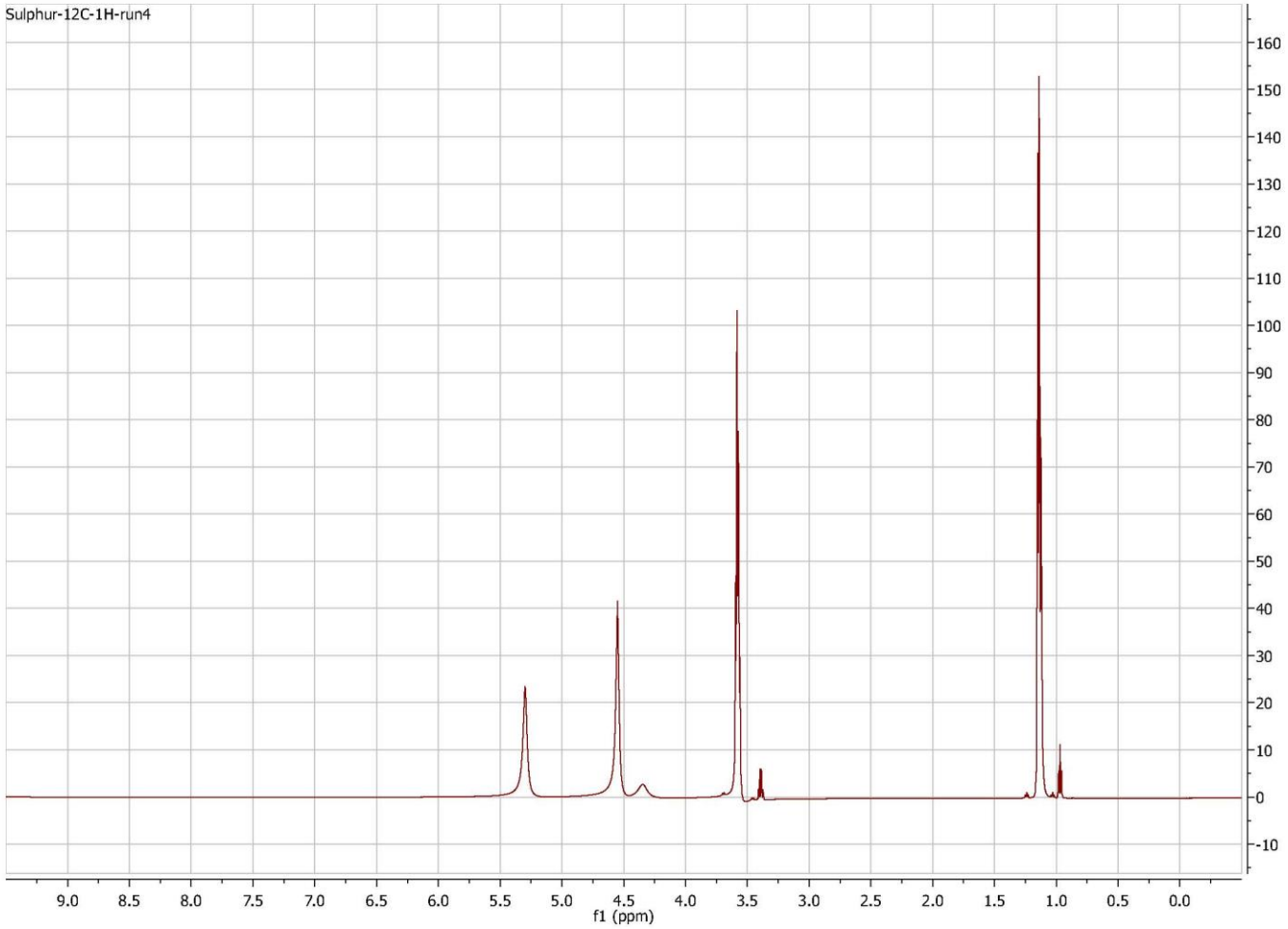

Figure 24: NMR spectrum, Sulphur 12c unagitated- run 4

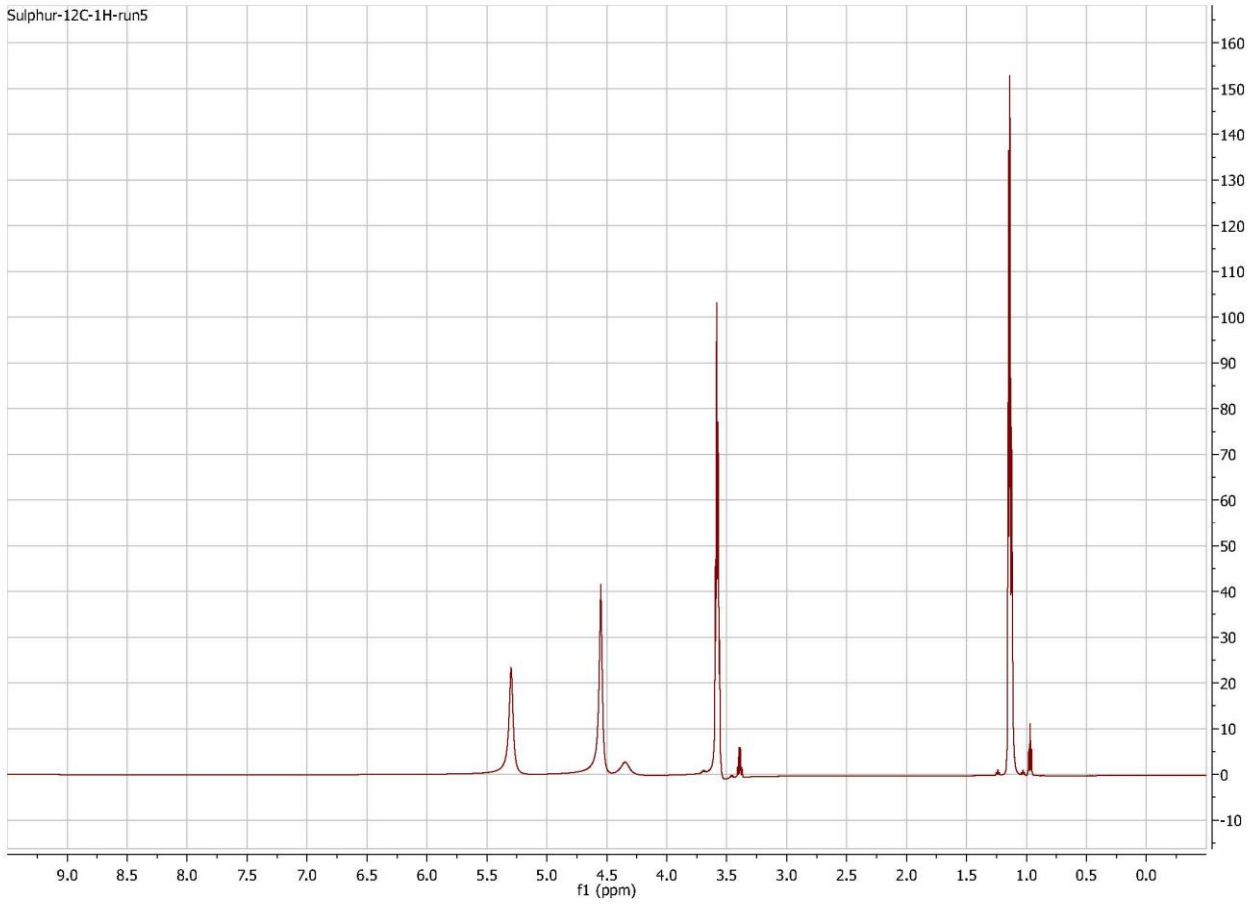

Figure 25: NMR spectrum, Sulphur 12c unagitated- run 5

Appendix C: Summary of data obtained from NMR spectroscopy

a) Chemical Shift values

			H2O	OH	CH2	CH3
Sulphur 12C - Hahnemann	Run 1		5,3058	4,5595	3,5818	1,1414
	Run 2		5,3055	4,5595	3,5818	1,1416
	Run 3		5,3052	4,5592	3,5818	1,1417
	Run 4		5,3051	4,5591	3,5818	1,1416
	Run 5		5,3049	4,5588	3,5818	1,1416
Sulphur 12C - Sonication	Run 1		5,2975	4,5496	3,5936	1,1361
	Run 2		5,2975	4,5496	3,5988	1,1362
	Run 3		5,2973	4,5495	3,5924	1,1362
	Run 4		5,2974	4,5496	3,5981	1,1362
	Run 5		5,2975	4,5496	3,5816	1,1361
Sulphur 12C - Both	Run 1		5,3033	4,5567	3,5818	1,1414
	Run 2		5,3027	4,5563	3,5817	1,1414
	Run 3		5,3030	4,5566	3,5818	1,1414
	Run 4		5,3030	4,5564	3,5817	1,1413
	Run 5		5,3027	4,5562	3,5817	1,1414
86% SVR			5.29			
	Run 1	72		4,5492	3.5763	1,1356
	Run 2		5.2972	4,5491	3,5762	1,1356
	Run 3		5,2972	4.5493	3,5763	1,1356
	Run 4		5,2972	4,5494	3,5763	1,1356
	Run 5		5,2973	4,5494	3,5762	1,1355
Sulphur 12C - Unagitated	Run 1		5,2979	4,5502	3,5770	1,1363
	Run 2		5,2980	4,5502	3,5771	1,1363
	Run 3		5,2981	4,5502	3,5771	1,1363
	Run 4		5,2978	4,5500	3,5772	1,1362
	Run 5		5,2977	4,5500	3,5772	1,1362

b) Relative integration values

		H2O	OH	CH2	CH3
Sulphur 12C - Hahnemann	Run 1	0,139100	0,174900	0,252300	0,357400
	Run 2	0,137800	0,179200	0,249100	0,357600
	Run 3	0,139300	0,177900	0,251200	0,353800
	Run 4	0,138500	0,177300	0,250400	0,357400
	Run 5	0,138900	0,178000	0,250700	0,357600
Sulphur 12C - Sonication	Run 1	0,124800	0,171100	0,251600	0,339800
	Run 2	0,127700	0,173600	0,254200	0,341000
	Run 3	0,129600	0,174000	0,261300	0,345100
	Run 4	0,131100	0,174900	0,264500	0,349400
	Run 5	0,132800	0,181600	0,230100	0,357300
Sulphur 12C - Both	Run 1	0,135600	0,178400	0,247300	0,368200
	Run 2	0,136500	0,178000	0,245500	0,366300
	Run 3	0,138300	0,175900	0,244500	0,368000
	Run 4	0,136700	0,179600	0,246100	0,365300
	Run 5	0,137400	0,177100	0,247300	0,364900
86% SVR	Run 1	0,132300	0,183700	0,243800	0,339600
	Run 2	0,136100	0,187700	0,240700	0,345700
	Run 3	0,136000	0,188500	0,241400	0,347900
	Run 4	0,130000	0,178100	0,256900	0,328600
	Run 5	0,126600	0,173100	0,255500	0,325500
Unagitated	Run 1	0,135800	0,184000	0,232200	0,352100
	Run 2	0,139200	0,184100	0,236400	0,355200
	Run 3	0,138700	0,189900	0,236900	0,362300
	Run 4	0,132500	0,180400	0,226400	0,348600
	Run 5	0,136600	0,183900	0,230100	0,357800

Appendix D: Statistical analysis output

1. Kruskal-Wallis Test

a) Chemical Shift values

Ranks

	Method	N	Mean Rank
H2O	Hahnemann	5	23.00
	Sonication	5	7.90
	Both	5	18.00
	SVR_Control	5	3.10
	Unagitated	5	13.00
	Total	25	
OH	Hahnemann	5	23.00
	Sonication	5	8.00
	Both	5	18.00
	SVR_Control	5	3.00
	Unagitated	5	13.00
	Total	25	
CH2	Hahnemann	5	18.00
	Sonication	5	21.00
	Both	5	15.00
	SVR_Control	5	3.00
	Unagitated	5	8.00
	Total	25	
CH3	Hahnemann	5	22.60
	Sonication	5	8.60
	Both	5	18.40
	SVR_Control	5	3.00
	Unagitated	5	12.40
	Total	25	

Test Statistics[a,b]

	H2O	OH	CH2	CH3
Chi-Square	23.138	23.229	20.655	22.766
df	4	4	4	4
Asymp. Sig.	.000	.000	.000	.000

a. Kruskal Wallis Test

b. Grouping Variable: Method

b) Relative integration

Ranks

	Method	N	Mean Rank
H20	Hahnemann	5	21.60
	Sonication	5	4.60
	Both	5	15.20
	SVR control	5	7.80
	Unagitated	5	15.80
	Total	25	
OH	Hahnemann	5	10.20
	Sonication	5	6.30
	Both	5	11.30
	SVR control	5	16.20
	Unagitated	5	21.00
	Total	25	
CH2	Hahnemann	5	17.20
	Sonication	5	18.30
	Both	5	12.00
	SVR control	5	13.80
	Unagitated	5	3.70
	Total	25	
CH3	Hahnemann	5	15.60
	Sonication	5	7.80
	Both	5	23.00
	SVR control	5	4.20
	Unagitated	5	14.40
	Total	25	

Test Statistics[a,b]

	H20	OH	CH2	CH3
Chi-Square	17.007	11.996	12.366	19.695
df	4	4	4	4
Asymp. Sig.	.002	.017	.015	.001

a. Kruskal Wallis Test

b. Grouping Variable: Method

2. Mann-Whitney test

a) Chemical shift values

1) Sulphur 12C Hahnemannian and Sulphur 12C sonicated

Ranks

	Method	N	Mean Rank	Sum of Ranks
H2O	Hahnemann	5	8.00	40.00
	Sonication	5	3.00	15.00
	Total	10		
OH	Hahnemann	5	8.00	40.00
	Sonication	5	3.00	15.00
	Total	10		
CH2	Hahnemann	5	4.00	20.00
	Sonication	5	7.00	35.00
	Total	10		
CH3	Hahnemann	5	8.00	40.00
	Sonication	5	3.00	15.00
	Total	10		

Test Statistics[a]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	.000	5.000	.000
Wilcoxon W	15.000	15.000	20.000	15.000
Z	-2.643	-2.703	-1.671	-2.685
Asymp. Sig. (2-tailed)	.008	.007	.095	.007
Exact Sig. [2*(1-tailed Sig.)]	.008[b]	.008[b]	.151[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.

2) Sulphur 12C Hahnemannian and Sulphur 12C Hahnemannian and sonication

Ranks

	Method	N	Mean Rank	Sum of Ranks
H2O	Hahnemann	5	8.00	40.00
	Both	5	3.00	15.00
	Total	10		
OH	Hahnemann	5	8.00	40.00
	Both	5	3.00	15.00
	Total	10		
CH2	Hahnemann	5	7.00	35.00
	Both	5	4.00	20.00
	Total	10		
CH3	Hahnemann	5	7.60	38.00
	Both	5	3.40	17.00
	Total	10		

Test Statistics[a]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	.000	5.000	2.000
Wilcoxon W	15.000	15.000	20.000	17.000
Z	-2.627	-2.619	-1.964	-2.373
Asymp. Sig. (2-tailed)	.009	.009	.050	.018
Exact Sig. [2*(1-tailed Sig.)]	.008[b]	.008[b]	.151[b]	.032[b]

a. Grouping Variable: Method

b. Not corrected for ties.

3) Sulphur 12C Hahnemannian and Sac Lac 12C control

Ranks

	Method	N	Mean Rank	Sum of Ranks
H2O	Hahnemann	5	8.00	40.00
	SVR_Control	5	3.00	15.00
	Total	10		
OH	Hahnemann	5	8.00	40.00
	SVR_Control	5	3.00	15.00
	Total	10		
CH2	Hahnemann	5	8.00	40.00
	SVR_Control	5	3.00	15.00
	Total	10		
CH3	Hahnemann	5	8.00	40.00
	SVR_Control	5	3.00	15.00
	Total	10		

Test Statistics[a]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	.000	.000	.000
Wilcoxon W	15.000	15.000	15.000	15.000
Z	-2.694	-2.627	-2.835	-2.730
Asymp. Sig. (2-tailed)	.007	.009	.005	.006
Exact Sig. [2*(1-tailed Sig.)]	.008[b]	.008[b]	.008[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.

4) Sulphur 12C Hahnemannian and Sulphur 12C unagitated

Ranks

	Method	N	Mean Rank	Sum of Ranks
H2O	Hahnemann	5	8.00	40.00
	Unagitated	5	3.00	15.00
	Total	10		
OH	Hahnemann	5	8.00	40.00
	Unagitated	5	3.00	15.00
	Total	10		
CH2	Hahnemann	5	8.00	40.00
	Unagitated	5	3.00	15.00
	Total	10		
CH3	Hahnemann	5	8.00	40.00
	Unagitated	5	3.00	15.00
	Total	10		

Test Statistics[a]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	.000	.000	.000
Wilcoxon W	15.000	15.000	15.000	15.000
Z	-2.611	-2.660	-2.805	-2.685
Asymp. Sig. (2-tailed)	.009	.008	.005	.007
Exact Sig. [2*(1-tailed Sig.)]	.008[b]	.008[b]	.008[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.

5) Sulphur 12C sonicated and Sulphur 12C Hahnemannian and sonication

Ranks

	Method	N	Mean Rank	Sum of Ranks
H2O	Sonication	5	3.00	15.00
	Both	5	8.00	40.00
	Total	10		
OH	Sonication	5	3.00	15.00
	Both	5	8.00	40.00
	Total	10		
CH2	Sonication	5	7.00	35.00
	Both	5	4.00	20.00
	Total	10		
CH3	Sonication	5	3.00	15.00
	Both	5	8.00	40.00
	Total	10		

Test Statistics[a]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	.000	5.000	.000
Wilcoxon W	15.000	15.000	20.000	15.000
Z	-2.660	-2.694	-1.591	-2.739
Asymp. Sig. (2-tailed)	.008	.007	.112	.006
Exact Sig. [2*(1-tailed Sig.)]	.008[b]	.008[b]	.151[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.

6) Sulphur 12C sonicated and Sac Lac 12C control

Ranks

	Method	N	Mean Rank	Sum of Ranks
H2O	Sonication	5	7.90	39.50
	SVR_Control	5	3.10	15.50
	Total	10		
OH	Sonication	5	8.00	40.00
	SVR_Control	5	3.00	15.00
	Total	10		
CH2	Sonication	5	8.00	40.00
	SVR_Control	5	3.00	15.00
	Total	10		
CH3	Sonication	5	8.00	40.00
	SVR_Control	5	3.00	15.00
	Total	10		

Test Statistics[a]

	H2O	OH	CH2	CH3
Mann-Whitney U	.500	.000	.000	.000
Wilcoxon W	15.500	15.000	15.000	15.000
Z	-2.629	-2.703	-2.652	-2.739
Asymp. Sig. (2-tailed)	.009	.007	.008	.006
Exact Sig. [2*(1-tailed Sig.)]	.008[b]	.008[b]	.008[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.

7) Sulphur 12C sonicated and Sulphur 12C unagitated

Ranks

	Method	N	Mean Rank	Sum of Ranks
H2O	Sonication	5	3.00	15.00
	Unagitated	5	8.00	40.00
	Total	10		
OH	Sonication	5	3.00	15.00
	Unagitated	5	8.00	40.00
	Total	10		
CH2	Sonication	5	8.00	40.00
	Unagitated	5	3.00	15.00
	Total	10		
CH3	Sonication	5	3.60	18.00
	Unagitated	5	7.40	37.00
	Total	10		

Test Statistics[a]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	.000	.000	3.000
Wilcoxon W	15.000	15.000	15.000	18.000
Z	-2.643	-2.739	-2.627	-2.154
Asymp. Sig. (2-tailed)	.008	.006	.009	.031
Exact Sig. [2*(1-tailed Sig.)]	.008[b]	.008[b]	.008[b]	.056[b]

a. Grouping Variable: Method

b. Not corrected for ties.

8) Sulphur 12C Hahnemannian and sonication and Sac Lac 12C control

Ranks

	Method	N	Mean Rank	Sum of Ranks
H2O	Both	5	8.00	40.00
	SVR_Control	5	3.00	15.00
	Total	10		
OH	Both	5	8.00	40.00
	SVR_Control	5	3.00	15.00
	Total	10		
CH2	Both	5	8.00	40.00
	SVR_Control	5	3.00	15.00
	Total	10		
CH3	Both	5	8.00	40.00
	SVR_Control	5	3.00	15.00
	Total	10		

Test Statistics[a]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	.000	.000	.000
Wilcoxon W	15.000	15.000	15.000	15.000
Z	-2.712	-2.619	-2.694	-2.785
Asymp. Sig. (2-tailed)	.007	.009	.007	.005
Exact Sig. [2*(1-tailed Sig.)]	.008[b]	.008[b]	.008[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.

9) Sulphur 12C Hahnemannian and sonication and Sulphur 12C unagitated

Ranks

	Method	N	Mean Rank	Sum of Ranks
H2O	Both	5	8.00	40.00
	Unagitated	5	3.00	15.00
	Total	10		
OH	Both	5	8.00	40.00
	Unagitated	5	3.00	15.00
	Total	10		
CH2	Both	5	8.00	40.00
	Unagitated	5	3.00	15.00
	Total	10		
CH3	Both	5	8.00	40.00
	Unagitated	5	3.00	15.00
	Total	10		

Test Statistics[a]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	.000	.000	.000
Wilcoxon W	15.000	15.000	15.000	15.000
Z	-2.627	-2.652	-2.668	-2.739
Asymp. Sig. (2-tailed)	.009	.008	.008	.006
Exact Sig. [2*(1-tailed Sig.)]	.008[b]	.008[b]	.008[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.

10) Sac Lac 12C control and Sulphur 12C unagitated

Ranks

	Method	N	Mean Rank	Sum of Ranks
H2O	SVR_Control	5	3.00	15.00
	Unagitated	5	8.00	40.00
	Total	10		
OH	SVR_Control	5	3.00	15.00
	Unagitated	5	8.00	40.00
	Total	10		
CH2	SVR_Control	5	3.00	15.00
	Unagitated	5	8.00	40.00
	Total	10		
CH3	SVR_Control	5	3.00	15.00
	Unagitated	5	8.00	40.00
	Total	10		

Test Statistics[a]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	.000	.000	.000
Wilcoxon W	15.000	15.000	15.000	15.000
Z	-2.694	-2.660	-2.668	-2.739
Asymp. Sig. (2-tailed)	.007	.008	.008	.006
Exact Sig. [2*(1-tailed Sig.)]	.008[b]	.008[b]	.008[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.

b) Relative integration values

1) Sulphur 12C Hahnemannian and Sulphur 12C sonicated

Ranks

	Method	N	Mean Rank	Sum of Ranks
H20	Hahnemann	5	8.00	40.00
	Sonication	5	3.00	15.00
	Total	10		
OH	Hahnemann	5	6.90	34.50
	Sonication	5	4.10	20.50
	Total	10		
CH2	Hahnemann	5	4.20	21.00
	Sonication	5	6.80	34.00
	Total	10		
CH3	Hahnemann	5	7.80	39.00
	Sonication	5	3.20	16.00
	Total	10		

Test Statistics[a]

	H20	OH	CH2	CH3
Mann-Whitney U	.000	5.500	6.000	1.000
Wilcoxon W	15.000	20.500	21.000	16.000
Z	-2.611	-1.467	-1.358	-2.417
Asymp. Sig. (2-tailed)	.009	.142	.175	.016
Exact Sig. [2*(1-tailed Sig.)]	.008[b]	.151[b]	.222[b]	.016[b]

a. Grouping Variable: Method

b. Not corrected for ties.

2) Sulphur 12C Hahnemannian and Sulphur 12C Hahnemannian and sonication

Ranks

	Method	N	Mean Rank	Sum of Ranks
H20	Hahnemann	5	7.80	39.00
	Both	5	3.20	16.00
	Total	10		
OH	Hahnemann	5	5.10	25.50
	Both	5	5.90	29.50
	Total	10		
CH2	Hahnemann	5	8.00	40.00
	Both	5	3.00	15.00
	Total	10		
CH3	Hahnemann	5	3.00	15.00
	Both	5	8.00	40.00
	Total	10		

Test Statistics[a]

	H20	OH	CH2	CH3
Mann-Whitney U	1.000	10.500	.000	.000
Wilcoxon W	16.000	25.500	15.000	15.000
Z	-2.402	-.419	-2.619	-2.627
Asymp. Sig. (2-tailed)	.016	.675	.009	.009
Exact Sig. [2*(1-tailed Sig.)]	.016[b]	.690[b]	.008[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.

3) Sulphur 12C Hahnemannian and Sac Lac 12C control

Ranks

	Method	N	Mean Rank	Sum of Ranks
H20	Hahnemann	5	8.00	40.00
	SVR control	5	3.00	15.00
	Total	10		
OH	Hahnemann	5	4.20	21.00
	SVR control	5	6.80	34.00
	Total	10		
CH2	Hahnemann	5	6.00	30.00
	SVR control	5	5.00	25.00
	Total	10		
CH3	Hahnemann	5	8.00	40.00
	SVR control	5	3.00	15.00
	Total	10		

Test Statistics[a]

	H20	OH	CH2	CH3
Mann-Whitney U	.000	6.000	10.000	.000
Wilcoxon W	15.000	21.000	25.000	15.000
Z	-2.611	-1.358	-.522	-2.627
Asymp. Sig. (2-tailed)	.009	.175	.602	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[b]	.222[b]	.690[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.

4) Sulphur 12C Hahnemannian and Sulphur 12C unagitated

Ranks

	Method	N	Mean Rank	Sum of Ranks
H20	Hahnemann	5	6.80	34.00
	Unagitated	5	4.20	21.00
	Total	10		
OH	Hahnemann	5	3.00	15.00
	Unagitated	5	8.00	40.00
	Total	10		
CH2	Hahnemann	5	8.00	40.00
	Unagitated	5	3.00	15.00
	Total	10		
CH3	Hahnemann	5	5.80	29.00
	Unagitated	5	5.20	26.00
	Total	10		

Test Statistics[a]

	H20	OH	CH2	CH3
Mann-Whitney U	6.000	.000	.000	11.000
Wilcoxon W	21.000	15.000	15.000	26.000
Z	-1.358	-2.611	-2.611	-.315
Asymp. Sig. (2-tailed)	.175	.009	.009	.753
Exact Sig. [2*(1-tailed Sig.)]	.222[b]	.008[b]	.008[b]	.841[b]

a. Grouping Variable: Method

b. Not corrected for ties.

5) Sulphur 12C sonicated and Sulphur 12C Hahnemannian and sonication

Ranks

	Method	N	Mean Rank	Sum of Ranks
H20	Sonication	5	3.00	15.00
	Both	5	8.00	40.00
	Total	10		
OH	Sonication	5	4.00	20.00
	Both	5	7.00	35.00
	Total	10		
CH2	Sonication	5	7.00	35.00
	Both	5	4.00	20.00
	Total	10		
CH3	Sonication	5	3.00	15.00
	Both	5	8.00	40.00
	Total	10		

Test Statistics[a]

	H20	OH	CH2	CH3
Mann-Whitney U	.000	5.000	5.000	.000
Wilcoxon W	15.000	20.000	20.000	15.000
Z	-2.611	-1.567	-1.571	-2.611
Asymp. Sig. (2-tailed)	.009	.117	.116	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[b]	.151[b]	.151[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.

6) Sulphur 12C sonicated and Sac Lac 12C control

Ranks

	Method	N	Mean Rank	Sum of Ranks
H20	Sonication	5	4.40	22.00
	SVR control	5	6.60	33.00
	Total	10		
OH	Sonication	5	4.00	20.00
	SVR control	5	7.00	35.00
	Total	10		
CH2	Sonication	5	6.20	31.00
	SVR control	5	4.80	24.00
	Total	10		
CH3	Sonication	5	6.80	34.00
	SVR control	5	4.20	21.00
	Total	10		

Test Statistics[a]

	H20	OH	CH2	CH3
Mann-Whitney U	7.000	5.000	9.000	6.000
Wilcoxon W	22.000	20.000	24.000	21.000
Z	-1.149	-1.567	-.731	-1.358
Asymp. Sig. (2-tailed)	.251	.117	.465	.175
Exact Sig. [2*(1-tailed Sig.)]	.310[b]	.151[b]	.548[b]	.222[b]

a. Grouping Variable: Method

b. Not corrected for ties.

7) Sulphur 12C sonicated and Sulphur 12C unagitated

Ranks

	Method	N	Mean Rank	Sum of Ranks
H20	Sonication	5	3.20	16.00
	Unagitated	5	7.80	39.00
	Total	10		
OH	Sonication	5	3.20	16.00
	Unagitated	5	7.80	39.00
	Total	10		
CH2	Sonication	5	7.30	36.50
	Unagitated	5	3.70	18.50
	Total	10		
CH3	Sonication	5	3.80	19.00
	Unagitated	5	7.20	36.00
	Total	10		

Test Statistics[a]

		H20	OH	CH2	CH3
Mann-Whitney U		1.000	1.000	3.500	4.000
Wilcoxon W		16.000	16.000	18.500	19.000
Z		-2.402	-2.402	-1.886	-1.776
Asymp. Sig. (2-tailed)		.016	.016	.059	.076
Exact Sig. [2*(1-tailed Sig.)]		.016[b]	.016[b]	.056[b]	.095[b]

a. Grouping Variable: Method

b. Not corrected for ties.

8) Sulphur 12C Hahnemannian and sonication and Sac Lac 12C control

Ranks

	Method	N	Mean Rank	Sum of Ranks
H20	Both	5	7.60	38.00
	SVR control	5	3.40	17.00
	Total	10		
OH	Both	5	4.40	22.00
	SVR control	5	6.60	33.00
	Total	10		
CH2	Both	5	6.00	30.00
	SVR control	5	5.00	25.00
	Total	10		
CH3	Both	5	8.00	40.00
	SVR control	5	3.00	15.00
	Total	10		

Test Statistics[a]

	H20	OH	CH2	CH3
Mann-Whitney U	2.000	7.000	10.000	.000
Wilcoxon W	17.000	22.000	25.000	15.000
Z	-2.193	-1.149	-.524	-2.611
Asymp. Sig. (2-tailed)	.028	.251	.600	.009
Exact Sig. [2*(1-tailed Sig.)]	.032[b]	.310[b]	.690[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.

9) Sulphur 12C Hahnemannian and sonication and Sulphur 12C unagitated

Ranks

	Method	N	Mean Rank	Sum of Ranks
H20	Both	5	5.40	27.00
	Unagitated	5	5.60	28.00
	Total	10		
OH	Both	5	3.00	15.00
	Unagitated	5	8.00	40.00
	Total	10		
CH2	Both	5	8.00	40.00
	Unagitated	5	3.00	15.00
	Total	10		
CH3	Both	5	8.00	40.00
	Unagitated	5	3.00	15.00
	Total	10		

Test Statistics[a]

	H20	OH	CH2	CH3
Mann-Whitney U	12.000	.000	.000	.000
Wilcoxon W	27.000	15.000	15.000	15.000
Z	-.104	-2.611	-2.619	-2.611
Asymp. Sig. (2-tailed)	.917	.009	.009	.009
Exact Sig. [2*(1-tailed Sig.)]	1.000[b]	.008[b]	.008[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.

10) Sac Lac 12C control and Sulphur 12C unagitated

Ranks

	Method	N	Mean Rank	Sum of Ranks
H20	SVR control	5	3.80	19.00
	Unagitated	5	7.20	36.00
	Total	10		
OH	SVR control	5	4.80	24.00
	Unagitated	5	6.20	31.00
	Total	10		
CH2	SVR control	5	8.00	40.00
	Unagitated	5	3.00	15.00
	Total	10		
CH3	SVR control	5	3.00	15.00
	Unagitated	5	8.00	40.00
	Total	10		

Test Statistics[a]

	H20	OH	CH2	CH3
Mann-Whitney U	4.000	9.000	.000	.000
Wilcoxon W	19.000	24.000	15.000	15.000
Z	-1.776	-.731	-2.611	-2.611
Asymp. Sig. (2-tailed)	.076	.465	.009	.009
Exact Sig. [2*(1-tailed Sig.)]	.095[b]	.548[b]	.008[b]	.008[b]

a. Grouping Variable: Method

b. Not corrected for ties.