A Nuclear Magnetic Resonance study of potencies of Natrum muriaticum 15CH prepared by trituration and succussion versus Natrum muriaticum 15CH prepared by succussion alone

By

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Dissertation submitted in partial compliance with the requirements for the Master's Degree in Technology: Homoeopathy in the Department of Homoeopathy at
Durban Institute of Technology.

I, Dorita Hofmeyr, do hereby declare that this dissertation is representative of my own work.

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Dedicated to my beloved family.

With special thanks to my grandparents,
Nico and Elizabeth Ann Hofmeyr,
For their unfailing love and encouragement.

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ABSTRACT

The purpose of this investigation was to analyse and compare the Nuclear Magnetic Resonance (NMR) spectra of potencies of Natrum muriaticum 15CH prepared by trituration and succussion, and Natrum muriaticum 15CH prepared by succussion alone. It was hypothesized that in terms of the effect of trituration (or not) of these substances that significant differences exist between the chemical shift and relative integration values of the CH_2, CH_3, H_2O and OH signals of these homoeopathic substances. It was further hypothesized that the process of trituration plays an integral part in the development of distinct physicochemical identities in the potencies mentioned above.

The investigation was designed as a scientific experiment whereby centesimal potencies were prepared according to the directions of Hahnemann to the 15CH level. Volumes of 15ml of the final liquid potencies (87% ethanol) of each group were prepared and sent for analysis.

NMR spectroscopy was conducted on three samples of each sample group. The samples were drawn into coaxial sample tubes making use of acetone as an external lock and using ethanol as the reference. The samples were drawn by the resident NMR-technician in the Department of Chemistry, University of KwaZulu-Natal, Pietermaritzburg. The NMR spectrometer used was a Varian 500MHz INOVA having a 5mm broadband switchable probe and

a 5mm inverse detection probe. The pulse angle was set at 90^{o} and the emperature was maintained at a constant value of 298.1 K (25.0^{o}C).

The data was recorded and expressed in the form of NMR spectra giving the chemical shift value and integration values of the peaks. The chemical shift and relative integration values of the CH_2, CH_3, H_2O and OH signals were subjected to a process of statistical analysis using two main steps. The two sample groups were statistically compared by applying the t-test to the chemical shifts and the ANOVA method to the relative integrations. The level of significance was set at $\alpha = 0.05$ for all test comparisons.

Statistically significant differences were noted in the t-test comparison of the chemical shift values for the H_2O and OH signals. No statistically significant differences were noted for CH_2 or CH_3 signals. For the ANOVA method of comparison of relative integration statistically significant differences were found to exist for CH_2, H_2O and OH signals. There were no statistically significant differences observed for CH_3 signals.

The result of the study did not allow a conclusive explanation of the specific structures responsible for homoeopathic remedy action. However the results did serve to support the hypothesis that the effect of trituration (or not) on the substance render ethanol samples that are distinct from each other in terms of their identities measured by NMR spectroscopy. It also serves to support the use of NMR spectroscopy as a tool in the investigation of the nature of homoeopathic potencies.

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TABLE OF ABBREVIATIONS

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

THE DEFINITION OF TERMS

Analyse: For the purpose of this investigation, the term analyse refers to the statistical manipulation of the recorded chemical shift-values and integration values of CH_2, CH_3, H_2O and OH signals.

Analysis of Variance: a method of statistical analysis used for the analysis of data.

Batch: a specific quantity of a medicine which has a uniform characteristic and quantity within specified limits, and is produced according to a single preparation procedure during the same cycle of manufacture.

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

Chemical shift: indicates the resonance frequency of nuclei subjected to an electromagnetic forcefield. The resonance frequency of individual nuclei is affected by the molecular environment and is an indicator of three-dimensional structure within molecules.

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

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

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

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

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

Mean (x): the most commonly used measure of central tendency defined as the sum of the values in the sample divided by the sample size.

NMR-spectroscopy: an analytical method most frequently employed to obtain information about the structure of organic compounds, by measuring the interaction of protons within a magnetic field. Such interaction is recorded as a series of peaks known as a spectrum (see below).

Physical structure: the three-dimensional geometry existing between individual atoms and /or radicals, within molecules, and that existing between molecules of a compound, or within a mixture.

Potency: a state of altered remedial activity to which a drug is taken by means of a measured process of deconcentration and the introduction of kinetic energy through succession or trituration (see below). Three rates of deconcentration are used in preparation o homoeopathic potencies.

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

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

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

Standard deviation (S_x): a frequently used measure of dispersion, which uses all of the data points in a sample to indicate the variation existing within the data set.

Succussion: the action of shaking up vigorously a liquid dilution of a homoeopathic medicine in its vial/bottle, where each stroke ends with a jolt; usually effected by pounding the hand engaged in the shaking against the palm of the opposite hand.

Trituration: the act of prolonged grinding with a mortar and pestle to reduce an insoluble homoeopathic drug to a fine powder while amalgamating it thoroughly with lactose by rubbing together under the pestle.

T-tests: parametric hypothesis tests applied to the means of two normal distributions of differences. They are of two types: paired and unpaired.

CHAPTER ONE: INTRODUCTION

1.1 THE AIM OF THE STUDY

The aim of this study is to analyse the Nuclear Magnetic Resonance spectra of potencies of Natrum muriaticum 15CH prepared by trituration and succussion, as opposed to Natrum muriaticum 15CH prepared by succussion alone, in order to evaluate the effect of the trituration method in homoeopathic pharmacy. All samples are identical in substance and level of deconcentration, and differ only in the method of preparation.

1.2 THE STATEMENT OF THE OBJECTIVES

1.2.1 The first objective

The first objective is to establish the NMR spectra of Natrum muriaticum 15CH with respect to the chemical shifts and integration values of the CH_2, CH_3, H_2O and OH signals, which has been prepared by trituration and succussion.

1.2.2 The second objective

The second objective is to establish the NMR spectra of the Natrum muriaticum 15CH with respect to the chemical shifts and integration values of the CH_2, CH_3, H_2O and OH signals, which has been prepared by succussion alone.

1.2.3 The third objective

The third objective is to compare and evaluate the NMR spectra of the two potencies of Natrum muriaticum 15CH with respect to the chemical shifts and relative integration values of the CH_2, CH_3, H_2O and OH signals, which have been prepared by trituration and succussion, and by succussion alone.

1.3 THE HYPOTHESIS

It is hypothesised that significant differences exist between the chemical shifts and relative integration values of CH_2, CH_3, H_2O and OH signals of the two Natrum muriaticum 15CH potencies, and that these indicate differences in the physical structure of the respective potencies.

CHAPTER TWO: THE REVIEW OF THE RELATED LITERATURE

2.1 THE INTRODUCTION

Traditional medicine attempts to deny the veracity of almost 200 years of homoeopathic investigation by thousands of researchers and practitioners, many of whom themselves were conventionally trained. While homoeopathic practitioners know that their medicine works, there is little available scientific data to explain how it works. A discussion of "vital forces" leaves much to be desired in the minds of most scientific investigators, and the hypotheses of energy medicine that are being explored in the far reaches of modern physics have yet to penetrate medical science.

Homoeopathy still faces the problem of explaining the therapeutic action of medicines diluted beyond the theoretical Avogadro limit of molecular presence. The remedy is diluted to such an extent that there is theoretically none of the original solute remaining. One of the apparently implausible bases of homoeopathy is the law of infinitesimals: the greater the dilution of a remedy, the greater its effect. This holds true only if the remedy is dynamized by succussion or trituration. As unscientific as it may seem, these substances are observed clinically to have an effect. This raises an interesting question: What effect does the dynamization process (succussion or trituration) have on the remedy? More specifically, what is the effect of trituration in the manufacturing process?

This question is the foundation out of which this investigation is built. Does the process of trituration produce a remedy that is distinctly different from one that has not been triturated? How does one best measure these differences if they do exist? Research has been conducted with a view to addressing these questions, a brief review of which follows.

2.2 TRITURATION

Medicinal substances often have to be processed before they can be potentized. This may be done by producing a solution or a mother tincture, or by triturating the substance with lactose. According to Hahnemann the 3 methods are not interchangeable, because they produce medicines with different levels of homoeopathic activity. The process of trituration, by mortar and pestle, is essential to homoeopathic pharmacy, as many solid or insoluble substances have to be triturated to a certain deconcentration level before they can be dissolved. Trituration results in a quantitative reduction in the drug substance by a qualitative increase in its medicinal or therapeutic property. It is reasonable to hypothesize that trituration, which is the variable factor in the proposed experiment, does, in some way, change the physical structure of the solvent even when the order of dilution is beyond the Avogadro limit of molecular presence. Dellmour conducted a chronological study of Hahnemann's writings on the importance of the 3CH trituration in the manufacture of homoeopathic medicines. From his research it is evident that Hahnemann used and recommended trituration to the 3CH (before he continued the further potentizing with alcohol) for all medicines (Dellmour, 1994).

Until 1818, Hahnemann had used gold only in solution. He found a passage on the trituration of gold in the work of Arabian physicians and took to using the 1CH and 2CH in powder form. Since the anionic acid part in these compounds changed the qualities of the metal too much he tried to find a way to preserve the medicinal properties of the pure metal. For this purpose he triturated gold leaf in 1818 with lactose and found an effective medicine in the 1CH potency which several times turned out to be successful in the

therapy of severe suicidal depressions. As a result he triturated Aurum to higher potency degrees and introduced trituration as a common preparation method into homoeopathy (Hahnemann, 1822:253).

In the early years, Hahnemann produced plant-based homoeopathic medicines from mother tinctures. He used the trituration method only for those medicinal plants that were available as dry substances. Later he used this method also for fresh plants. Through this he learned that triturated juices unfold more medicinal power through potentizing than those that are produced from mother tinctures: "The fresh juices thus seem to acquire more of dynamization, as experience teaches me, than when the juice without any preparation by triturating is merely diluted in thirty vials of alcohol and potentized each time with (two) successive strokes,'' (Hahnemann, 1828:147). Therefore he started to triturate plants. The Organon still contains instructions for the preparation of tinctures from juice, but the trituration of fresh plants is definitely favoured now: "If the physician prepares his homoeopathic medicines himself, as he should reasonably do in order to save men from sickness, he may use the fresh plant itself, as but little of the crude article is required, if he does not need the expressed juice perhaps for purposes of healing," (Hahnemann, 1921:242). Although dissolving a substance in water or a mixture of ethanol and water is the simplest method of preparation, Hahnemann soon ceased to use it, however, having discovered that triturated medicines have more powerful actions than medicines produced by potentizing solutions.

After 1835, he made trituration the general processing method in homoeopathic pharmacy. He no longer used mother tinctures or solutions even of soluble substances, but instead triturated them up to the 3CH (Dellmour, 1994).

Hahnemann stated that with using trituration the evolution of medicinal power was 'much more complete' than with solution-based potencies. Dynamization is more powerful when produced by 3-hour trituration, with the medicinal substance and the vehicle subjected to more intensive mechanical and energy factors (Hahnemann, 1828:147-152).

This change has important advantages because the entire crude drug is passed over to the remedy. On the other hand mother tinctures contain only water- and alcohol-soluble parts while all other components are lost. Compared to medicines produced from mother tinctures and solutions, the trituration method offered the advantages of more powerful action, more constituents retained and guaranteed shelf life (Dellmour, 1994).

From 1835 on, Hahnemann triturated practically all drugs including water-soluble substances, metals and juices and fresh plants to 3CH before he continued the further potentizing with alcohol. The finality of this development was fixed when the beginning of LM-potentizing was set up in 3CH-trituration (Dellmour, 1994).

2.3 THEORIES OF REMEDY MECHANISMS

Homoeopathy still faces the problem of explaining the therapeutic action of medicines diluted beyond the theoretical Avogadro limit of molecular presence. Presently the greatest challenge to those working on homoeopathy is to develop a proper theoretical context for their observations.

According to Gaier (1991) the experimental evidence indicates the existence of a "physicochemical force field" in the potencies. This so-called "force-field" is said to be

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responsible for carrying forward information of the medicine into stages of ultra molecular dynamization. This hypothesis emphasizes the need to adopt a biophysical paradigm to explain the action of these ultra molecular dilutions in order to understand the nature of the homoeopathic potency.

Anagnostatos et al (1991) provided a clathrate model that describes the formation of complex structures within water through a large amount of hydrogen bonding. These clathrates are hollow grid-like arrangements around an interior cavity and the water molecules assume pentagonal or hexagonal forms due to their hydrogen bonding. It is proposed that during the process of potentization, it is possible for the interior solute molecules to be expelled from the clathrates. The “empty” clathrates would then serve as nuclei for new clathrates and thus the initial base solute maintains its original pattern with increasing dilution and succussion stages. It is suggested that resonance between a coherence pattern of the solution and a frequency pattern of the organism would result in an interaction with a biological system.

Resch and Guttman (1991) propose a system whereby there exists a hierarchic system organization of a liquid. Within this system there exists the presence of hydrophobic molecules, such as dissolved gas molecules. It is proposed that these molecules strongly influence the oscillating pattern of the whole liquid by taking over structural information from the solution and dynamically preserving it within this oscillating behaviour. A level of hydrophilic solutes with surrounding water molecules exists in a subordinate level in the liquid. The hydrophilic solutes form “hydration shells” that influence the whole solution structure. The oscillation pattern is modified and the information pertaining to the

dissolved solutes is spread throughout the solution. In the process of dilution an interface forms between the solute and the solvent which allows for the integration of the structural information from the solute into the more diluted solution. With subsequent dilution the ratio of starting solute is decreased but the informational content is retained. The 'structure makers' (gases) then maintain these changes via the effect of their oscillating pattern. It is further concluded that the system organization of the new solution is improved by shaking, which has the effect of maintaining the integral configuration and functionality of the new solution.

The theory of organization in liquids does not, however, explain how potency information is retained in lactose during the process of trituration. Resch and Guttman (1987) propose that the flexible three-dimensional network of lactose monohydrate (milk sugar) with its many loose hydrogen bonds and surrounding water molecules, contribute to the dynamic maintenance of the structural features in the presence of solutes. During the process of trituration, the actions of diluting and grinding the lactose results in an entirely new system that is increasingly differentiated by the grinding. Simultaneously, the static aspects of order of the solid solution help in the retention of the structural information of the new solution, and hence favour the retention of some form of informational content of the original solute. It is hypothesized by Resch and Guttman that so-called "void-lattices" (vacancies) develop in the crystalline lactose that serve to preserve the static framework of the solid material and thus in doing so retain the informational content of the dissolved solute.

2.4 METHODS OF MEASUREMENT

It is becoming more apparent that molecular theory offers nothing but conceptual limitation for this field of inquiry, and that an alternative that goes beyond it must be found (Rubik, 1994). It follows that research in the field of chemico-physical properties of the remedies is urgently needed.

The measurement of electrical conductivity, relative permittivity and surface tension have been employed to study the properties of homoeopathic potencies. Raman laser spectroscopes, UV spectroscopes, light polarizers and Nuclear Magnetic Resonance (NMR) spectroscopy have been conducted as well (Lessell, 1994:37). In this respect, Nuclear Magnetic Resonance (NMR) spectroscopy appears to be a method of choice (Bol, 1997). Both Smith (1989: 113) and Gaier (1991: 446) estimate NMR spectroscopy as a valuable tool to measure structural changes in homoeopathic solutions.

2.5 ~~RUBIK, B. British Homoeopathic Journal. Vol 83, Numer 3, July 1994.~~ NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Nuclear Magnetic Resonance (NMR) spectroscopy is concerned with the measurement of the magnetic properties of certain atomic nuclei. Studying a molecule by NMR spectroscopy enables us to record differences in the magnetic properties of the various nuclei present, and to deduce what the positions of these nuclei are within the molecule. This is directly influenced by the environment of a proton, and thus enables predictions concerning the structure of a liquid.

2.5.1 THE NMR PHENOMENON

2.5.1.1 The Spinning Nucleus

The nucleus of the hydrogen atom (the proton) behaves as a tiny spinning bar magnet, and it does so because it possesses both electric charge and mechanical spin. The spinning charged body will generate a magnetic field.

2.5.1.2 The Effect of an External Magnetic Field

Like a bar magnet, the proton will respond to the influence of an external magnetic field, and will tend to align itself with that field. The proton adopts one of two orientations with respect to an external magnetic field – either align with the field (the lower energy state) or opposed to the field (the higher energy state).

2.5.1.3 Precessional Motion

Because the proton is behaving as a spinning magnet, it will move in a characteristic way under the influence of the external magnet. This is precessional motion. The precession arises from the interaction of spin with the earth's gravity acting vertically downwards.

2.5.1.4 Precessional Frequency

The spinning frequency of the nucleus does not change, but the speed of precession does. The precessional frequency is directly proportional to the strength of the external field.

2.5.1.5 Energy Transitions

If a proton is precessing in the aligned orientation, it can absorb energy and pass into the opposed orientation; subsequently it can lose this extra energy and relax back into the aligned position. If the precessing nuclei is irradiated with a beam of radiofrequency energy of the correct frequency, the low-energy nuclei may absorb this energy and move

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to a higher energy state. The precessing proton will only absorb energy from the radiofrequency source if the precessing frequency is the same as the frequency of the

radiofrequency beam; when this occurs, the nucleus and the radiofrequency beam are said to be in resonance; hence the term nuclear magnetic resonance. The simplest NMR experiment consists of exposing the protons in an organic molecule to a powerful external magnetic field. The protons will precess, although they may not all precess at the same frequency. These precessing protons are irradiated with radiofrequency energy of the appropriate frequencies, and promote protons from the low-energy (aligned) state to the high-energy (opposed) state. This absorption of energy is recorded in the form of an MNR spectrum.

2.5.1 THEORY OF NUCLEAR MAGNETIC RESONANCE

The only nuclei that exhibit the NMR phenomenon are those for which the spin quantum number l is greater than 0. The spin quantum number l is associated with the mass number and atomic number of the nuclei. The nucleus of ^{1}H, the proton, has $l = ½$, whereas ^{12}C and ^{16}O have $l = 0$ and are therefore nonmagnetic. If ^{12}C and ^{16}O had been magnetic, the NMR spectra of organic molecules would have been much more complex. Under the influence of an external magnetic field, a magnetic nucleus can take up different orientations with respect to that field; so that for nuclei with spin ½ only two orientations are allowed.

In an applied magnetic field, magnetic nuclei like the proton precess at a frequency v, which is proportional to the strength of the applied field. The exact frequency is given by

$$v = \frac{uB_N B_0}{hl}$$

where $B_0 =$ strength of the applied external field experienced by the proton,

$I =$ spin quantum number

$h =$ Planck's constant

$u =$ magnetic moment of the particular nucleus,

$B_N =$ nuclear magneton constant.

The strength of the signal, and hence the sensitivity of the NMR experiment for a particular nucleus is related to the magnitude of the magnetic moment μ. The magnetic moments of ^{1}H and ^{19}F are relatively large, and detection of NMR with these nuclei is fairly sensitive. The magnetic moment for ^{13}C is about one quarter that of ^{1}H, and thus these nuclei are less sensitively detected in NMR.

When protons absorb radiofrequency energy, nuclei in the lower energy state undergo transitions to the higher energy state. The populations of the two states may approach equality, and if this arises no further net absorption of energy can occur, and the observed resonance signal will fade out. This is called *saturation* of the signal. In the recording of a normal NMR spectrum, however, the populations in the two spin states do not become equal, because higher-energy nuclei are constantly returning to the lower energy spin state. The high-energy nucleus can undergo energy loss (or *relaxation*) by transferring energy difference to some electromagnetic vector present in the surrounding environment.

2.5.3 CHEMICAL SHIFT AND ITS MEASUREMENT

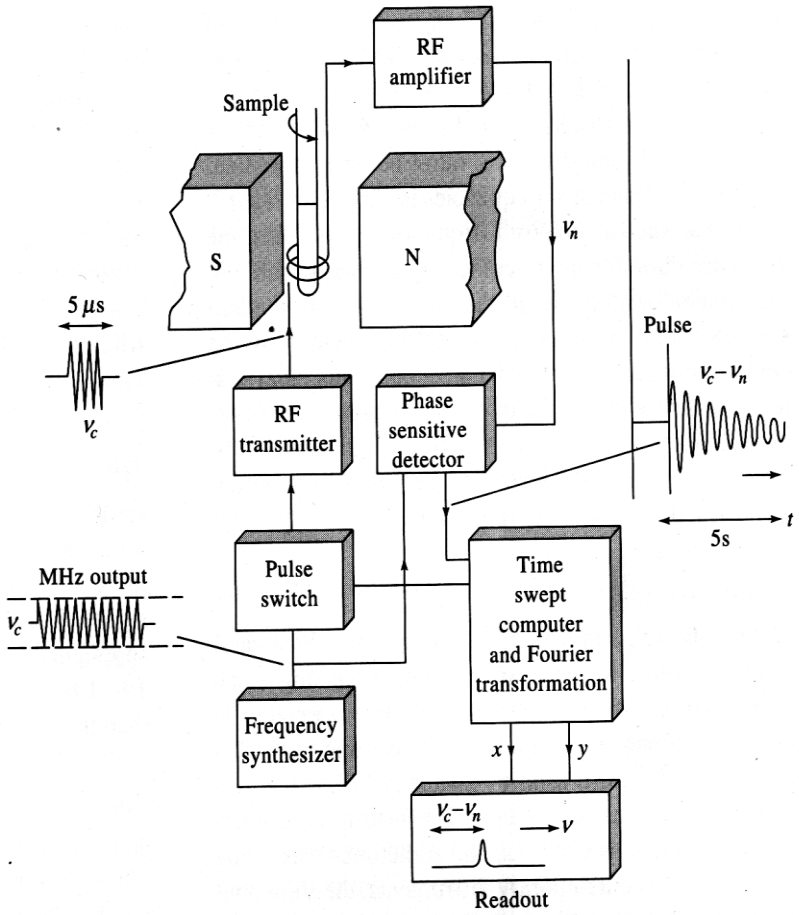
The precessional frequency of all protons in the same external applied field is not, however, the same, and the precise value for any one proton depends on a number of factors. Historically, this was first observed by Packard in 1951 (Kemp, 1987:91). He was able to detect three different values for the precessional frequencies of the protons in ethanol, and the realization that these corresponded to the three different chemical environments for the protons in ethanol (CH_3, CH_2 and OH) marked the beginning of NMR as a tool of the organic chemist. Because the shift in frequency depended on chemical environment, this gave rise to the term *chemical shift* (Kemp, 1987:91).

To measure the precessional frequency of a group of nuclei in absolute frequency units is extremely difficult and rarely required. More commonly the differences in frequency are measured with respect to some reference group of nuclei.

2.5.3.1 Measurement of Chemical Shift – the NMR spectrometer

The basic features of the instrumentation needed to record an NMR spectrum are a magnet, a radiofrequency source and a detection system to indicate that energy is being transferred from the radiofrequency beam to the nucleus.

Figure 2.1 Block Diagram of a Fourier NMR Spectrometer (Williams and Fleming, 1980:76).



The sample is placed in a glass tube between the pole faces of a magnet. A radiofrequency source feeds energy into a coil wound around the sample tube, and the radiofrequency detector is tuned to the same energy frequency. If the nuclei in the sample do not resonate with the source, the detector will only record a weak signal coming directly

from the source coil to the detector coil. An increased signal will be detected if the nuclei in the sample resonate with the source, since energy will be transferred from the source, via the nuclei, to the detector coil.

2.6 NMR RESEARCH IN HOMOEOPATHY

NMR has become a frequently used measuring technique in homoeopathic research (Schulte, 1999).

In a recent study, Cason (2001) analyzed the NMR spectra of homoeopathic Sulphur 30CH at three different nuclear magnetic frequencies (80MHz, 200MHz and 500MHz respectively), using three different NMR spectrometers. Cason analyzed the differences between the spectra derived from the three spectrometer frequencies for Sulphur and a water-ethanol control. Comparison of the spectra at parallel frequency strengths revealed no significant differences between the Sulphur and control. The author postulates that differences in chemical shift values are unlikely to be a reflection of chemico-physical differences present prior to measurement, but are rather a result of an aspect of the analytical procedure. A variation in chemical shift values is likely to be the result of a change in temperature during the NMR spectroscopy analysis.

The most significant difference between the NMR spectrometers, besides their frequencies, is the fluctuation in temperature during measurements. Modern instruments with superior technology will ensure more accurate chemical shift and relative integration values, and more stable temperature control. Therefore, the more modern instruments are recommended to analyze homoeopathic remedies. There is a need for accuracy and

standardization in NMR research conducted on homoeopathic dilutions, in order that results may be meaningful.

NMR studies in homoeopathy have focused on the effects of potentization and dilution on the solvents and solutes. In as early as 1968, Smith and Boericke were utilizing NMR spectroscopy to study the effects of succussion on the NMR hydroxyl (OH) spectrum of bradykinin triacetate compared to identical unsuccussed dilutions of the same solute. They found that the hydroxyl areas became large and small in cyclical order up to the 60X dilution. This was found when comparing the succussed to identical unsuccussed dilutions of the test substance. The CH_2 and CH_3 spectra did not change in this study. The area under the hydroxyl curve is supplied by the water component of the mixed solvent when one component is ethanol. This finding further emphasizes that NMR is a valid method to detect changes in the physicochemical identity of the solute/solvent.

Davies (2001) compared NaCl Hahnemannian and Korsakovian potencies (9CH, 30CH and 200CH) and their respective controls, by means of a NMR analysis. Statistically significant differences were found with relation to the chemical shift values between these two different methods of preparation. Insignificant results were found when comparing the relative integration values.

In his paper presented at The American Institute of Homeopathy Convention, in 1974, Young states that there are noticeable changes in various solutions of water, alcohol and Sulphur after they have been succussed, rotated or diluted, as demonstrated by NMR spectra (Young, 1974). NMR spectra showed an increased area under the hydroxyl part

of the curve for the succussed Sulphur samples, thus demonstrating that succussion does change the physical structure of the solvent.

Ross (19971977) investigated the properties of quinquagenimillesimal (LM) potencies using NMR spectroscopy. Differences between the chemical shift and integration values of the CH_2, H_2O and OH signals of parallel LM potencies (LM2, 6, 10) of Sulphur and a lactose-based control were observed. The most significant differences were noted between LM10 and the control. At this level, the chemical shift values of all three signals were significantly different. The integration values of the CH_2 and H_2O were also significant. These findings are anomalous when considering that the deconcentration of an LM10 potency far exceeds Avogadro's constant. Ross suggested that higher LM potencies be investigated.

Thus, Power (1999), analyzed LM6, LM14 and LM22 of Tin and Lead. Potentized as well as unpotentized controls were used. Both chemical shift and relative integration values showed significant differences between substance and control and substance versus substance. No differences were found within the control groups or between the control groups.

2.7 SUMMARY

The available literature clearly indicates that differences can be detected in homoeopathic potencies and dilutions, and that NMR-spectroscopy is the an effective method of investigation.

In the past, NMR research in homoeopathy has focused on the different scales of dilution (Malan, 2002), the effect of succussion on the medicines (Smith and Boericke, 1968) and Hahnemann's LM potency (Ross, 1997. Powers, 1999). The purpose of this investigation was therefore to analyze and compare the NMR-spectra of two remedies manufactured according to the same basic methodology, the Hahnemannian method, and having the same primary substance, same level of deconcentration, and succussion employed for a substantial portion of the manufacturing process. The only variable in this experiment was the employment (or not) of trituration in the first three stages of potentization (i.e. base substance to 3CH), in order to evaluate this method of preparation in terms of the production of a distinct physico-chemical entity. As has been suggested, such investigation is all the more important because of the use of trituration in homoeopathic pharmacy.

CHAPTER THREE: MATERIALS AND METHODS

3.1 PRODUCTION OF SAMPLE POTENCIES

The two potencies of Natrum muriaticum 15CH were prepared by the laboratory technician at the Department of Homoeopathy, Durban Institute of Technology. The potencies were prepared by hand under laminar flow, according to the German Homoeopathic Pharmacopoeia, as per method 5a, 6, 7 and 8a (refer to Appendix A).

To prepare Natrum muriaticum 15CH from both trituration and succussion, one part B.P. standard Rock Salt was triturated with 99 parts of pure lactose powder to produce the 1CH dilution of Natrum muriaticum. Trituration is continued up to the 3CH potency. One part of the dry, triturated 3CH potency is diluted in 99 parts of distilled water, followed by 10 succussions, thus converting to a liquid potency. Hereafter, potentization up to the 15CH is continued by succussion and dilution (in ethanol).

To prepare Natrum muriaticum 15CH by succussion alone, one part B.P. standard Rock Salt is diluted in 99 parts ethanol, followed by 10 succussions. This method of dilution and succussion is continued up to the 15CH potency.Thus preparing two 15CH potencies of Natrum muriaticum; one prepared by trituration and succussion, the other prepared by succussion alone.

All the potencies were prepared from a single container/batch of 87% ethanol, distilled water and lactose. This was to prevent the introduction of extraneous variables into the

process of manufacture. In addition to this precaution, a parallel process of production was employed to minimize differences in the conditions surrounding the manufacture of the potencies. That is, after the completion of a step of production of one substance the remaining three substances were brought up to the same level of manufacture before proceeding to next level. The substances were all prepared under laminar flow, using an accurately calibrated Labaire unit at a constant air pressure of 150 Pascals. Precautions were followed to eliminate the possibility of any contaminants in the samples, which could alter NMR spectra readings. This was done according to the standards in the British Pharmacopoeia for sterilization of glass apparatus (BP:A208). The 5ml clear glass bottles used in the preparation of the Natrum muriaticum potencies were rinsed in distilled water and underwent dry heat sterilization at 180^{o}C for 35 minutes. All the bottles were left to cool before manufacturing commences.

The Natrum muriaticum 15CH potencies were manufactured to a final volume of 15ml each. On completion of the potentization process both homoeopathic potencies were stored in amber bottles to reduce possible influence of sunlight during storage and transportation. The final potencies were packed, stored and transported in insulated containers to minimize temperature changes and unnecessary movement within the samples.

3.2 THE MEASUREMENT OF SAMPLES

The research was conducted at the Department of Chemistry, University of KwaZulu-Natal, Pietermaritzburg. The running and operation of the machine was performed by the resident NMR-technician, Mr Craig Grimmer. The NMR-spectrometer employed was a Varian 500MHz INOVA having a 5mm broadband switchable probe, and a 5mm inverse detection probe. The pulse angle was set at 90^{o} and the temperature was thermostatically controlled, remaining constant at $25^{o}C$.

1.75 ml of each sample was drawn into a coaxial tube by means of micropipette, using an unused capillary tube for each sample. The experiment was conducted using acetone as an external lock and ethanol as he reference.

Three samples of each were drawn from the provided volumes and MNR-spectra recorded for each sample. Readings were repeated sixteen times for every sample to eliminate inconsistencies and inaccuracies. Therefore each spectrum is the result of sixteen measurements.

Data were recorded in the form of NMR-spectra listing the chemical shift (Hz) and integration values (See Appendix B). The NMR-spectroscopy laboratory of the Department of Chemistry, University of KwaZulu-Natal, printed the spectra. The data were transferred into Microsoft Word.

3.3 STATISTICAL ANALYSIS

Chemical shift and relative integration values of CH_2, CH_3, H_2O and OH signals were recorded. CH_2, CH_3, H_2O and OH values were used for subsequent statistical analysis. The chemical shift values for both samples were measured for reliability, using Cronbach's alpha. Data was tested for normality by using the Kolmogorov-Smirnov test and the Shapiro-Wilk test.

The unpaired t-test was used to evaluate chemical shift values for both samples. The integration values for both samples were evaluated by means of the one-way analysis of variance (ANOVA) method.

The software package used to facilitate the statistical analysis was SPSS.

3.3.1 CRONBACH'S ALPHA

Cronbach's alpha measures how well a set of items (or variables) measures a single unidimensional latent construct. When data have a multidimensional structure, Cronbach's alpha will usually be low. Technically speaking, Cronbach's alpha is not a statistical test – it is a coefficient of reliability (or consistency).

Cronbach's alpha can be written as a function of the number of test items and the average inter-correlation among the items. Below, for conceptual purposes, we show the formula for the standardized Cronbach's alpha:

$$\alpha = \frac{N.\bar{r}}{1+(N-1).\bar{r}}$$

Here N is equal to the number of items and $\bar{r}$ is the average inter-item correlation among the items. One can see from this formula that if you increase the number of items, you increase Cronbach's alpha. Additionally, if the average inter-item correlation is low, alpha will be low. As the average inter-item correlation increases, Cronbach's alpha increases as well.

This makes sense intuitively – if the inter-item correlations are high, then there is evidence that the items are measuring the same underlying construct. This is really what is meant when someone says they have "high" or "good" reliability. They are referring to how well their items measure a single unidimensional latent construct.

3.3.2 TESTS OF NORMALITY OF A DISTRIBUTION

Normal distribution is a theoretical frequency distribution for a set of variable data, usually represented by a bell-shaped curve symmetrical about the mean. Normality of data is assumed in many statistical tests, including the normal test for means, the t-test, and the analysis of variance. A user who is not sure that the assumption of normality is justified is on firmer ground by testing the sampling distribution(s) for normality before deciding on the appropriate test, although we must understand a limitation of this use. A significant outcome implies the conclusion that the distribution is not normal, whereas, like all tests of hypotheses, a nonsignificant outcome implies only that no deviation from normality has been demonstrated (not that it does not exist). However, a negative result is some evidence of normality and all the evidence we have, which leads us to carry out the analysis as if normality had been demonstrated.

The choice of the test to use depends on both the sample size and whether the user would rather err on the side of being too conservative or the opposite. The two tests of normality used for this statistical analysis was the Kolmogorov-Smirnov test and the Shapiro-Wilk

test. The Shapiro-Wilk test tends to reject the null hypothesis more readily than one would wish; whereas the Kolmogorov-Smirnov test is too conservative, retaining the null hypothesis too often.

3.3.3 THE TWO-SAMPLE UNPAIRED T -TEST

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

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

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

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

$\alpha =$ level of significance of test $= 0.05$

The H_0 is rejected if the absolute value of the calculated t-statistic (t_{cal}) is greater than the tabulated t-value (t_{tab}). If the absolute value of t_{cal} is less than or equal to t_{tab} the H_0 is accepted $(i.e. \mu_1 = \mu_2)$.

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

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

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

= the pooled error variance;

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

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

α= level of significance of the test

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

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

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

where L is the margin of error as given below:

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

If the 95% confidence interval for $\mu_1 - \mu_2$ contained 0, and / or the p-value > α [0.05] the H_0 was accepted (i.e. $\mu_1 = \mu_2$). Otherwise H_0 was rejected at the same level (Ross, 1997).

3.3.4 THE ONE-WAY ANALYSIS OF VARIANCE (ANOVA) METHOD

Analysis of Variance (ANOVA) is a statistical procedure used to evaluate the mean difference between two or more (usually more) groups. ANOVA, like z tests and t tests, uses sample data to draw inferences about the population from which they are drawn. That is, ANOVA is an inferential procedure that is based on the concepts that underlie t-tests.

Z-tests can be used with only one sample, and only when you know the population mean and standard deviation. T tests can be used with two samples, and it is not necessary to know either the population mean or standard deviation. But t tests can be used with only one dependent variable at a time. There are times when researchers desire to use more than two samples at a time.

ANOVA is used to determine if the differences amongst the Means are significantly different than you would expect to find simply by chance. ANOVA compares the variance found between samples with the variance found within samples. The ANOVA score is noted by the capital F.

Thus, a significant result allows the investigator to conclude a difference, but a nonsignificant result does not allow the investigator to conclude no difference; it may only be said that significance has not been shown.

CHAPTER FOUR: THE RESULTS

4.1 THE CRITERIA GOVERNING THE ADMISSIBILITY OF THE DATA

Due to the sensitive nature of this experiment, it was vital that absolute care was taken during every stage of the experiment. As is evident in the manufacturing process, storage and transport of samples every possible precaution was taken to exclude external factors that may affect the nature of the Homoeopathic medicine [see Appendix A (i),(ii) and 3.1]. Similarly, this same high degree of caution was exercised during every other step of the experiment, i.e. the drawing of samples and subsequent measurement (see 3.2). Samples for analysis were drawn from the same respective bottle by random means determined by the NMR technician at the University of KwaZulu-Natal. Pipettes were not used between samples and all sample bottles were kept under the same conditions at all times.

NMR spectra were generated from sixteen transients of each sample, thus each sample was analyzed sixteen times. The initial chemical shift values of the four peaks CH_2, CH_3, H_2O and OH were recorded and the subsequent relative integration values of the data determined. Crude data were subjected to statistical analysis as set out in 3.3.

4.2 STATISTICAL RELIABILITY OF THE DATA

Cronbach's alpha was used to test the reliability of the data (see **3.3.1**).

Reliability does not necessarily influence validity. With reliability, one measurement of a variable on a group of subjects is compared with another measurement of the same variable on the same subjects. With validity, one measurement is for the variable of interest (which is usually some practical variable), whilst the second is for a variable that gives values as close as can be achieved to the true values of what is being tried to measure.

4.2.1 CH_2

Cronbach's Alpha	N of Items
.996	2

Table 4.1.1 Reliability Statistics for CH_2 data.

The alpha value indicates that the reliability of the relevant data is acceptable.

4.2.2 CH_3

Cronbach's Alpha	N of Items
.830	2

Table 4.1.2 Reliability Statistics for CH_3 data.

The alpha value (.830) indicates that the reliability of the relevant data is acceptable.

4.2.3 H_2O

Cronbach's Alpha	N of Items
.571	2

Table 4.1.3 Reliability Statistics for H_2O data.

The alpha value (.571) indicates that the reliability of the relevant data is acceptable.

4.2.4 OH

Cronbach's Alpha	N of Items
-1.770	2

Table 4.1.4 Reliability Statistics for OH data.

The value is negative due to a negative average covariance among items. This violates reliability model assumptions. The reliability of the data is rejected. However, reliability does not necessarily influence validity (Singh, 2004).

4.3 COMPARISON OF CHEMICAL SHIFT VALUES

Chemical shifts were statistically compared by using the unpaired t-test.

In order to do the t-test we had to satisfy:

i) the tests for normality (significance values > α)

ii) Levene's test for homogeneity (accepted homogeneity since sample sizes are equal.

4.3.1 CH_2

4.3.1.1 Tests for Normality

α = level of significance of test = 0.05

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	Df	Sig.
Succussion only	.321	12	.001	.689	12	.001
Trituration and Succussion	.323	12	.001	.677	12	.001

Table 4.2.1 Tests of normality for CH_2 chemical shifts.

For both tests and both groups there is a significant deviation from normality. Levene's criteria for homogeneity have been satisfied.

4.3.1.2 Comparison of the mean values:

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

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

$\alpha =$ level of significance of test $= 0.05$

	Succussion Alone	Succussion and Trituration
Mean	41.94167	36.8
Variance	402.1827	308.8727
Observations	12	12
Hypothesized Mean Difference	0	
df	22	
t Stat	0.667948	
P(T<=t) one-tail	0.255557	
Critical one-tail	1.717144	
P(T<=t) two-tail	0.511114	
t Critical two-tail	2.073875	

Table 4.2.2 T-Test Comparison of CH_2 Chemical Shifts.

The P-value = 0.511

0.511 > 0.05 (α = level of significance of test)

Therefore the null hypothesis is accepted. This indicates that statistically, there is no significant difference in the mean values of the chemical shifts of the CH_2 for the two samples.

4.3.2 CH_3

4.3.2.1 Tests for Normality

α = level of significance of test = 0.05

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	Df	Sig.
Succussion Only	.346	9	.003	.727	9	.003
Trituration and Succussion	.206	9	.200(*)	.854	9	.082

Table 4.2.3 Tests of Normality for CH_3 chemical shifts.

There is no deviation from normality as demonstrated by both tests for Trituration and Succussion only. However, the significance-values of both tests imply that distribution is slightly skewed.

4.3.2.2 Comparison of the mean values:

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

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

α = level of significance of test = 0.05

	Succussion Alone	Succussion and Trituration
Mean	102.5333	82.92222222
Variance	809.095	1964.626944
Observations	9	9
Hypothesized Mean Difference	0	
df	14	
t Stat	1.1171	
P(T<=t) one-tail	0.141378	
t Critical one-tail	1.761309	
P(T<=t) two-tail	0.282757	
t Critical two-tail	2.144789	

Table 4.2.4 T-Test Comparison of CH_3 Chemical Shifts.

The P-value = 0.282

0.282 > 0.05 (α = level of significance of test)

Therefore the null hypothesis is accepted. This indicates that there is no statistically significant difference for the mean values for the chemical shifts of the CH_3 for the two samples.

4.3.3 H_2O

4.3.3.1 Tests for Normality

α = level of significance of test = 0.05

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	Df	Sig.
Succussion Only	.219	3	.000	.987	3	.780
Trituration and Succussion	.385	3	.000	.750	3	.000

Table 4.2.5 Tests of Normality for H_2O chemical shifts.

The Shapiro-Wilk test demonstrates no deviation from normality for Trituration and Succussion. Levene's test for homogeneity is accepted for both methods.

4.3.3.2 Comparison of the mean values:

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

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

α = level of significance of test = 0.05

	Succussion Alone	Succussion and Trituration
Mean	10.86666667	12.66666667
Variance	0.063333333	0.003333333
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
T Stat	-12.07476708	
P(T<=t) one-tail	0.003394472	
T Critical one-tail	2.91998731	
P(T<=t) two-tail	0.006788945	
T Critical two-tail	4.302655725	

Table 4.2.6 T-Test Comparison of H_2O chemical shifts.

The P-value = 0.006

0.006 < 0.05 (α = level of significance of test)

Therefore the null hypothesis is rejected and the alternative hypothesis is accepted (P=0.006). This indicates that there is a statistically significant difference in the mean values for the chemical shifts of the H_2O for the two samples.

4.3.4 OH

4.3.4.1 Tests for Normality

α = level of significance of test = 0.05

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	df	Sig.
Succussion Only	.175	3	.000	1.000	3	1.000
Trituration and Succussion	.253	3	.000	.964	3	.637

Table 4.2.7 Tests of Normality for OH chemical shifts.

The Shapiro-Wilk test demonstrates that both distributions follow a normal distribution pattern.

4.3.4.2 Comparison of the mean values:

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

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

α = level of significance of test = 0.05

	Succussion Alone	Trituration and Succussion
Mean	22.4	26.93333
Variance	0.36	0.023333
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
T Stat	-12.68206539	
P(T<=t) one-tail	0.003080084	
T Critical one-tail	2.91998731	
P(T<=t) two-tail	0.006160168	
T Critical two-tail	4.302655725	

Table 4.2.8 T-Test Comparison of OH Chemical Shifts.

The P-value = 0.006

0.006 < 0.05 (α = level of significance of test)

Therefore the null hypothesis is rejected and the alternative hypothesis is accepted. This indicates that there is a statistically significant difference in the chemical shifts of the OH for the two samples.

4.4 COMPARISON OF RELATIVE INTEGRATION

4.4.1 CH_2

Groups	Count	Sum	Average	Variance
Succussion Alone	3	81.04	27.01333	0.000133
Trituration and Succussion	3	80.88	26.96	2.27E-13

Table 4.3.1 Summary of Comparison of Relative Integration for CH_2.

Comparison of the mean values:

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

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

α = level of significance of test = 0.05

Source of Variation	SS	df	MS	F	P-value	F crit
Between groups	0.004267	1	0.004267	64	0.001324	7.70865
Within groups	0.000267	4	6.67E-05			
Total	0.004533	5				

Table 4.3.2 ANOVA Comparison of Relative Integration for CH_2.

The P-value = 0.001

0.001 < 0.05 (α = level of significance of test)

The null hypothesis is rejected and the alternative hypothesis is accepted. This indicates that there is a statistically significant difference in the relative integration of the CH_2 for the two samples.

4.4.2 CH_3

Groups	Count	Sum	Average	Variance
Succussion Alone	3	121.65	40.55	0.0007
Trituration and Succussion	3	121.78	40.59333	3.33E-05

Table 4.3.3 Summary of Comparison of Relative Integration for CH_3.

Comparison of the mean values:

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

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

α = level of significance of test = 0.05

Source of Variation	SS	df	MS	F	P-value	F crit
Between groups	0.002817	1	0.002817	7.681818	0.050248	7.70865
Within groups	0.001467	4	0.000367			
Total	0.004283	5				

Table 4.3.4 ANOVA Comparison of Relative Integration for CH_3.

The P-value = 0.05

0.0502 > 0.05 (α = level of significance of test)

Therefore the null hypothesis is accepted. This indicates that there is no statistically significant difference in the relative integration mean values of the CH_3 for the two samples.

4.4.3 H_2O

Groups	Count	Sum	Average	Variance
Succussion Alone	3	40.23	13.41	0
Trituration and Succussion	3	40.19	13.39667	3.33E-05

Table 4.3.5 Summary of Comparison of Relative Integration for H_2O.

Comparison of the mean values:

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

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

α = level of significance of test = 0.05

Source of Variation	SS	df	MS	F	P-value	F crit
Between groups	0.000266667	1	0.000267	16	0.01613	7.70865
Within groups	6.66667E-05	4	1.67E-05			
Total	0.000333333	5				

Table 4.3.6 ANOVA Comparison of Relative Integration for H_2O.

The P-value = 0.016

0.016 < 0.05 (α = level of significance of test)

Therefore the null hypothesis is rejected and the alternative hypothesis is accepted. This indicates that there is a statistically significant difference in the mean values for the relative integration of the H_2O for the two samples.

4.4.4 OH

Groups	Count	Sum	Average	Variance
Succussion Alone	3	57.09	19.03	0.0001
Trituration and Succussion	3	57.16	19.05333	3.33E-05

Table 4.3.7 Summary of Comparison of Relative Integration for OH.

Comparison of the mean values:

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

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

α = level of significance of test = 0.05

Source of Variation	SS	df	MS	F	P-value	F crit
Between groups	0.000817	1	0.000817	12.25	0.024896	7.70865
Within groups	0.000267	4	6.67E-05			
Total	0.001083	5				

Table 4.3.8 ANOVA Comparison of Relative Integration for OH.

The P-value = 0.02

0.02 < 0.05 (α = level of significance of test)

Therefore the null hypothesis is rejected and the alternative hypothesis is accepted. This indicates that there is a statistically significant difference in the relative integration of the OH for the two samples.

4.5 SUMMARY OF STATISTICAL ANALYSES

The chemical shift and relative integration values of the CH_2, CH_3, H_2O and OH peaks in the NMR analysis of the sample groups prepared by succussion only and by trituration and succussion were used as input to the unpaired t-test (for comparison of chemical shifts of the two sample groups) and the ANOVA test (for comparison of relative integration of the two sample groups).

The level of significance for all tests was set at $\alpha = 0.05$ ($p \leq 0.05$).

	T-test Comparison of Chemical Shifts	**ANOVA Comparison of Relative Integration**
CH_2	No statistically significant difference	**Statistically significant difference**
CH_3	No statistically significant difference	No statistically significant difference
H_2O	**Statistically significant difference**	**Statistically significant difference**
OH	**Statistically significant difference**	**Statistically significant difference**

Table 4.4 Summary of Statistical Results.

Final conclusions drawn from the T-Test:

- No statistically significant differences were found to exist for the chemical shifts of CH_2 or CH_3 of the two sample groups.
- Statistically significant differences were found to exist for the chemical shifts of H_2O and OH of the two sample groups.

Final conclusions drawn from the ANOVA test:

- Statistically significant differences were found to exist for the relative integration of CH_2, H_2O and OH of the two sample groups.

- No statistically significant differences were found to exist for the relative integration of CH_3 of the two sample groups.

CHAPTER FIVE: DISCUSSION

The results of this study suggest that statistically significant differences exist between two remedies manufactured according to the same basic methodology and having the same primary substance, same level of deconcentration, and succussion employed for a substantial portion of the manufacturing process. The only variable employed (or not) was trituration in the first three stages of potentization (i.e. base substance to 3CH).

- Statistically significant differences were found to exist for both relative integration and chemical shifts of H_2O and OH.
- No statistically significant differences were found to exist for either relative integration or chemical shifts of CH_3.
- Statistically significant differences were found to exist for relative integration of CH_2, but not for chemical shifts.

These results are in keeping with previous research in this field. The observations suggest that the trituration process lays down some sort of structure, independent of the starting substance or solute, which is reflected in the significant differences observed for the relative integration values.

The most accurate NMR spectra are obtained from one sample with sufficient transients per run (in this instance 16). Therefore each spectrum is the result of 16 measurements (Grimmer, 2004). The NMR spectrum relevant to this study consists of four peaks. Each peak consists of one or more peak indices. As an example, the NMR spectrum of Sulphur

D6 (figure **5.1**), exhibits four peaks, each made up of their respective peak indices. The peak is a graphical representation of chemical shift (as discussed in **2.5.2** and **2.5.3**). In more complicated molecules such spectra contain much chemical information and can help in the determination of unknown molecular structures.

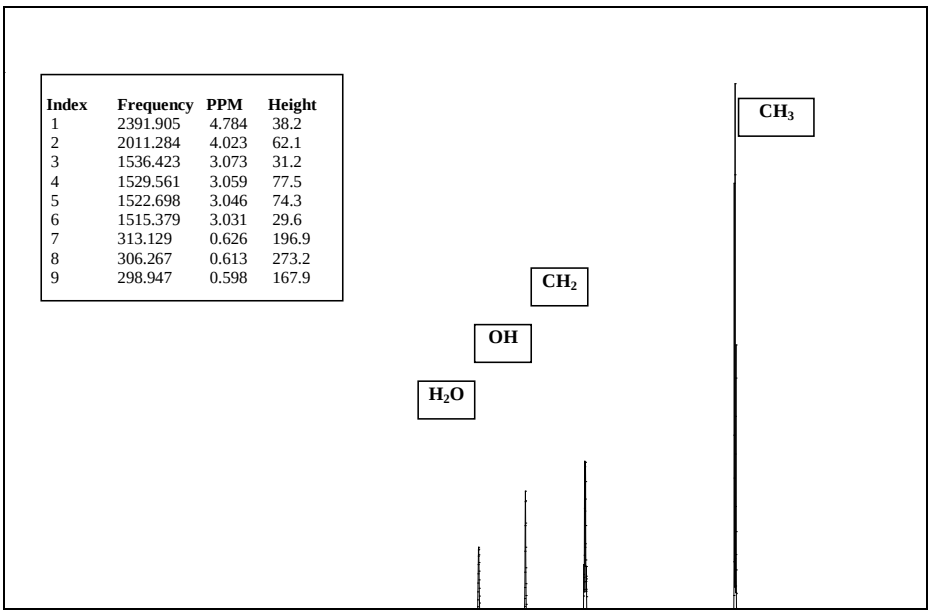


Figure 5.1 Example of the NMR Spectrum of Sulphur D6.

Peaks from left to right:

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

PPM values = chemical shift.

Calculation of chemical shift per peak: (Sum of the peak's PPM indices) ÷ (number of indices for the peak).

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

Table 5.1 Table of Peak Indices for the NMR Spectrum.

The chemical shift of a peak is calculated by dividing the sum of that peak's indices by the number of indices for that peak. Chemical shift values are primarily used for the identification of the type of substance analyzed. In this analysis, it is clear that all the substances tested are water-ethanol as far as the chemical shift values and the spin-spin splitting of the peaks are concerned.

However, the relative integration values that do show a significant difference, indicate that there is a structural difference in the basic water-ethanol. Relative integration is calculated from the integration values that are indicated below each peak on the laboratory printed NMR spectra. The relative integration values were derived from the integral values. This value is proportional to the number of protons generating a peak at a specific chemical shift value.

Hydrogen nuclei are surrounded by electron density which to some extent shields the nucleus from the influence of the applied NMR field, and to bring a proton to resonance the magnetic flux must overcome this shielding effect. In a magnetic field, the electrons around the proton are induced to orbit and in doing so they generate a small secondary

magnetic field, which opposes the applied field. The greater the electron density orbiting around the proton, the greater the induced opposing field, and the greater the external field required to overcome the shielding effect. Therefore, it is clear that a difference in relative integration indicates a difference in the electro-magnetic environment surrounding the respective molecules.

These findings do not provide enough evidence to comment on any of the current theories of remedy mechanisms or any of the models of the meta-structure of water (as discussed in **2.2**). The results do give some indication, however of a substance and dilution related change in the molecular and proton environment of the solvent molecules.

It needs to be pointed out that the choice of the 15CH potency for the two samples in this study was deliberate since this is the level of deconcentration at which it is reasonable to assume that the Avogadro's limit of molecular presence for the lactose is exceeded. The results of this study do however confirm that more work needs to be done into the physical structure of ultra high dilutions in homoeopathy. A more complete investigation in particular, needs to be done into the structure and ability of lactose to retain informational content of base solutes. The urgency of this work is evident as lactose is used extensively in homoeopathic pharmacy for dispensing and manufacturing purposes.

Finally, the use of NMR spectroscopy has served to confirm the existence of physico-chemical processes within homoeopathic remedies. This study provides further support for the employment of NMR spectroscopy in research about the physical nature of homoeopathic remedies. Further study requiring comparison of a large number of different

substances and potencies is required in order for more convincing hypotheses to be put forward concerning the nature of homoeopathic remedies.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

It is apparent from the results of this investigation that the preparation of a remedy by the process of trituration yields a solution that is distinct in its physicochemical identity in terms of the NMR spectra recorded. The study found that statistically significant differences exist in the t-test comparison of the chemical shift values. The ANOVA comparison of the relative integration values also revealed statistically significant variation. It is thus clear that these findings are supportive of the hypothesis relating to the influence of trituration (or not) on the creation of distinct physicochemical identities in the different test solutions. However, a clear conclusion cannot be drawn regarding the influence of the action of trituration in this study. This does not preclude the possibility that this part of the pharmaceutical procedure is important in the production of homoeopathic potencies. It thus contributes further to the standardization of homoeopathic practice and manufacture. This study adds to the scientific data available for the assessment of homoeopathy.

6.2 RECOMMENDATIONS

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

1. Trituration to higher potencies

This study should be repeated using higher potencies of the triturated component. It is hypothesized that the NMR spectra of higher potencies may show greater differences and thereby provide clearer information regarding the possible effects of trituration on the samples.

2. Repetition of experiments

It is necessary for all experiments to be repeated several times. The scientific method requires that a certain type of reproducibility be present, whereby a particular result must be obtained in a significant number of the overall cases. Time and funding is necessary in order to do this.

3. Control of external factors

It should be attempted to replicate the potentization process as is done in practice as closely as possible, with as few variations (variables) as possible. Standards for temperature and pressure variation need to be set in scientific research.

4. Standardisation of the pharmaceutical process

Strict standardisation of the potentization process is required for worthwhile scientific evaluation between researchers. Human error is an unavoidable fact of remedy preparation. Succussion force may therefore require control with calibrated machines.

5. The inclusion of a control

It is recommended that further studies include a control. This may prove useful for comparisons when all the tests have been completed.

6. Greater number of samples

Results will be scientifically sound and more valuable if more samples are prepared and evaluated by this method. This would make statistical analysis more meaningful. Funding is necessary for this.

7. Compare hand and machine triturated remedies

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

8. Compare samples triturated for different lengths of time

Most homoeopaths believe that the longer a remedy is triturated, the stronger the remedy becomes. This belief has not been scientifically verified. One could compare the difference for remedy samples triturated for different time intervals.

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APPENDIX A: METHODOLOGY OF REMEDY PREPARATION

Method 1:

Aim: To prepare a Hahnemannian Natrum muriaticum 3CH trituration, as per GHP methods 6 and 7.

Apparatus:
- Unglazed pestle and mortar
- 96% alcohol (for flaming)
- Cigarette lighter
- Porcelain spatula
- Steel spatula
- Pure lactose powder
- B.P. standard Rock Salt
- Mass balance (accurate and calibrated)
- Filter paper
- Clean, empty vials
- Labels and pens

Method:
1. All utensils should be clean and odorless.
2. Clean the mortar and pestle with water, flame with 96% alcohol.
3. Flame the spatula.
4. Allow mortar and pestle to cool sufficiently before use.

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

Method 2:

Aim: To prepare a liquid potency of Hahnemannian Natrum muriaticum 15CH from 3CH trituration, as per GHP method 8a.

Apparatus:
25ml amber glass bottles
5ml clear glass screw top bottles
87% ethanol
Distilled water
5ml clear glass pipettes
Rubber dropper bulbs
Natrum muriaticum 3CH triturate
Mass balance (accurate and calibrated)
Filter paper
Micropipettes

Method:
1. Clean all apparatus.
2. Place a piece of filter paper on the scale. Tare the scale. Mass 0,1g of Natrum muriaticum 3CH and place it in 25ml amber bottle.
3. Add 9,9ml of aqua distillata and succuss 10 times without stopping. Label as Natrum muriaticum 4CH.
4. Place 99 parts 87% ethanol in a clear glass screw top bottle and add 1 part Natrum muriaticum 4CH. Succuss 10 times without stopping. Label as Natrum muriaticum 5CH.

5. To prepare Natrum muriaticum 6CH–14CH repeat step (4.) adding 1 part of the previous potency to 99 parts 87% ethanol at each dilution level. Label appropriately.
6. To prepare 15ml of Natrum muriaticum 15CH place 99 parts 87% ethanol in a 25ml amber glass bottle. Add 1 part Natrum muriaticum 14CH. Succuss 10 times without stopping. Label as “Natrum muriaticum 15CH. Prepared by trituration and succussion”.
7. Label the required potency for NMR spectroscopy appropriately, and store in a cool environment free from electromagnetic disturbance.

Method 3:

Aim: To prepare Hahnemannian Natrum muriaticum 15CH from soluble Salt Rock, as per GHP method 5a.

Apparatus: B.P. standard Rock Salt
5ml screw top bottles
Distilled water
15% ethanol
87% ethanol
Filter paper
25ml amber bottles
Labels
Mass balance (calibrated and accurate)
Spatula
5ml and 2ml pipettes, and measuring cylinder
Micropipettes
Rubber dropper bulbs

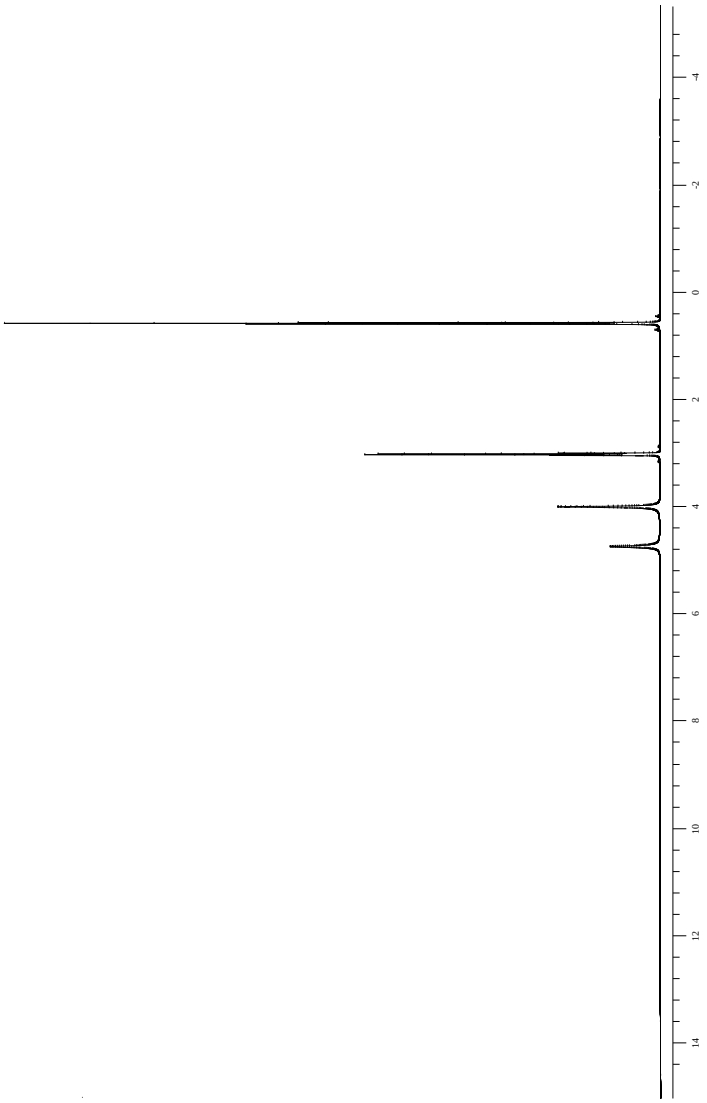
Method:
1. Rinse and autoclave equipment, allowing to cool.
2. Place single sheet of paper on chemical balance and tare.
3. Weigh out 0,03g of Rock Salt using mass balance and place into first 5ml screw top bottle.

4. Place 2,97g 15% ethanol into first 5ml screw top bottle using 5ml pipette. Succuss 10 times without stopping. Label as 1CH.
5. Place 2,97g 87% ethanol into second new 5ml screw top bottle.
6. Add 0,03g of 1CH into second screw top bottle using clean micropipette. Succuss 10 times without stopping. Label as 2CH.
7. Repeat the above procedure 5-6 up to 14CH.
8. To prepare 15ml of Natrum muriaticum 15CH, place 99 parts 87% ethanol into 25ml amber dropper bottle. Add 1 part of 14CH into the bottle. Succuss 10 times without stopping. Label as “Natrum muriaticum 15CH. Prepared by succussion alone.”
9. Label the required potency for NMR Spectroscopy appropriately, and store in cool environment free from any electromagnetic disturbance.

APPENDIX B: NMR SPECTRA

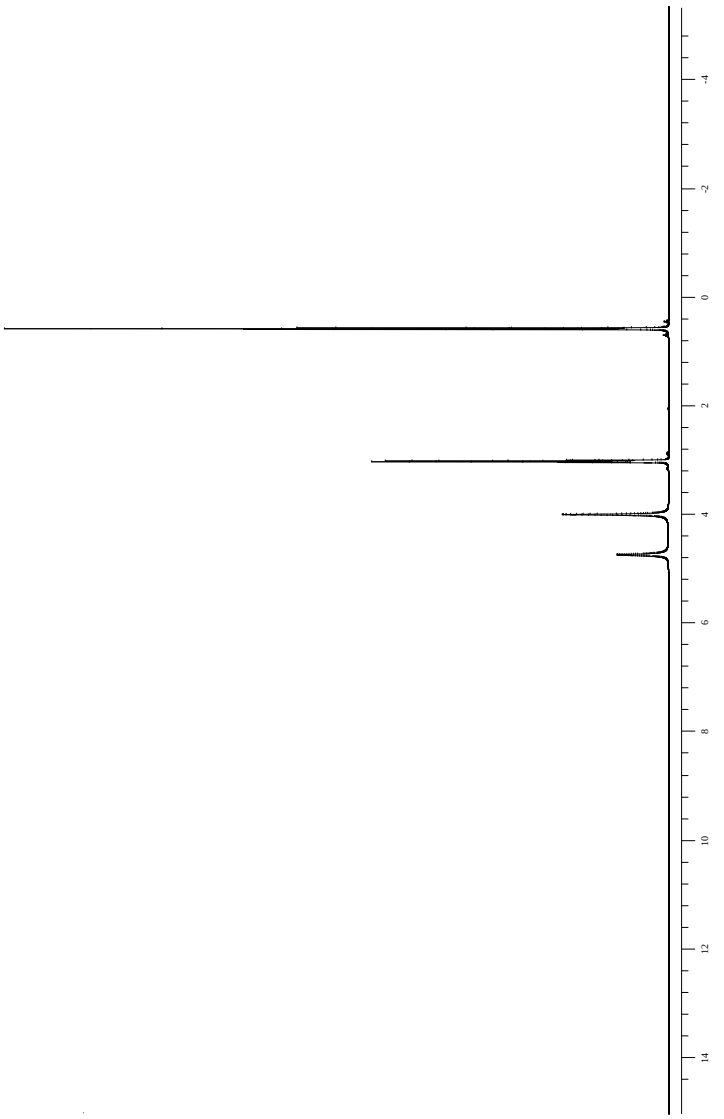
Trituration and Succussion
1st acquisition

INDEX	FREQUENCY	PPM	HEIGHT
1	2369.632	4.739	10.6
2	1998.357	3.997	21.8
3	1518.961	3.038	23.4
4	1511.815	3.024	62.6
5	1504.670	3.009	60.0
6	1467.834	2.996	22.2
7	294.841	0.590	87.7
8	287.695	0.575	139.3
9	280.549	0.561	78.2



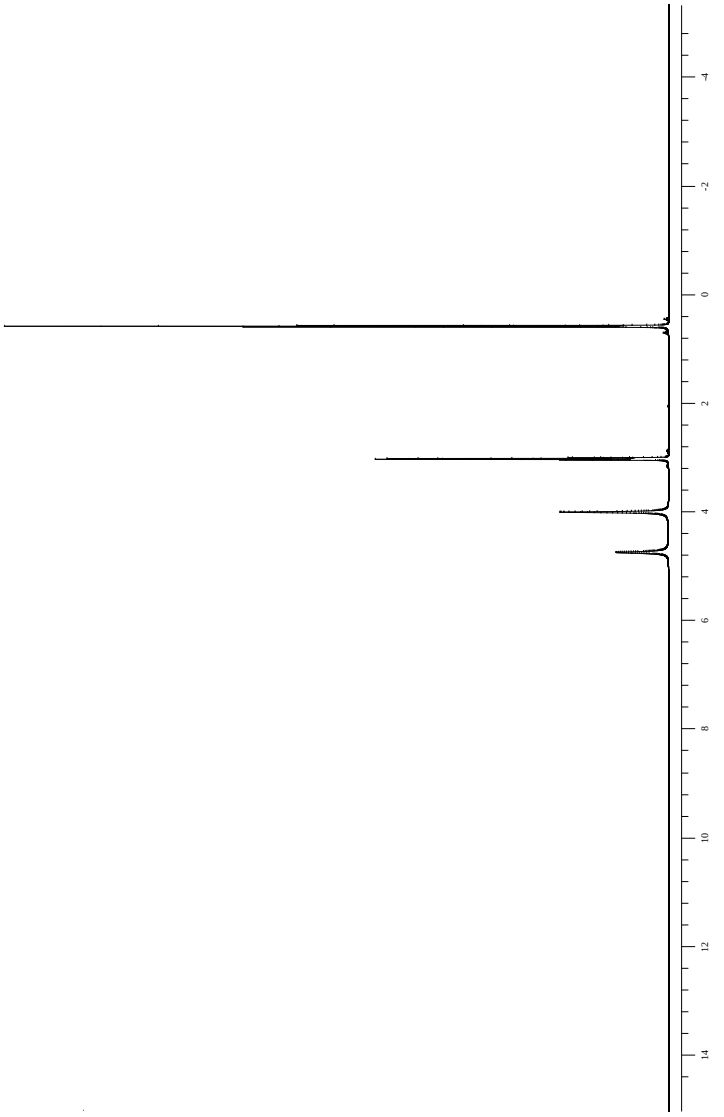
Trituration and Succussion
2nd acquisition

INDEX	FREQUENCY	PPM	HEIGHT
1.	2369.632	4.739	10.9
2	1998.357	3.997	22.4
3	1518.961	3.038	23.5
4	1511.815	3.024	62.5
5	1504.670	3.009	59.9
6	1497.834	2.996	22.1
7	294.530	0.589	88.9
8	287.695	0.575	140.1
9	280.549	0.561	78.9



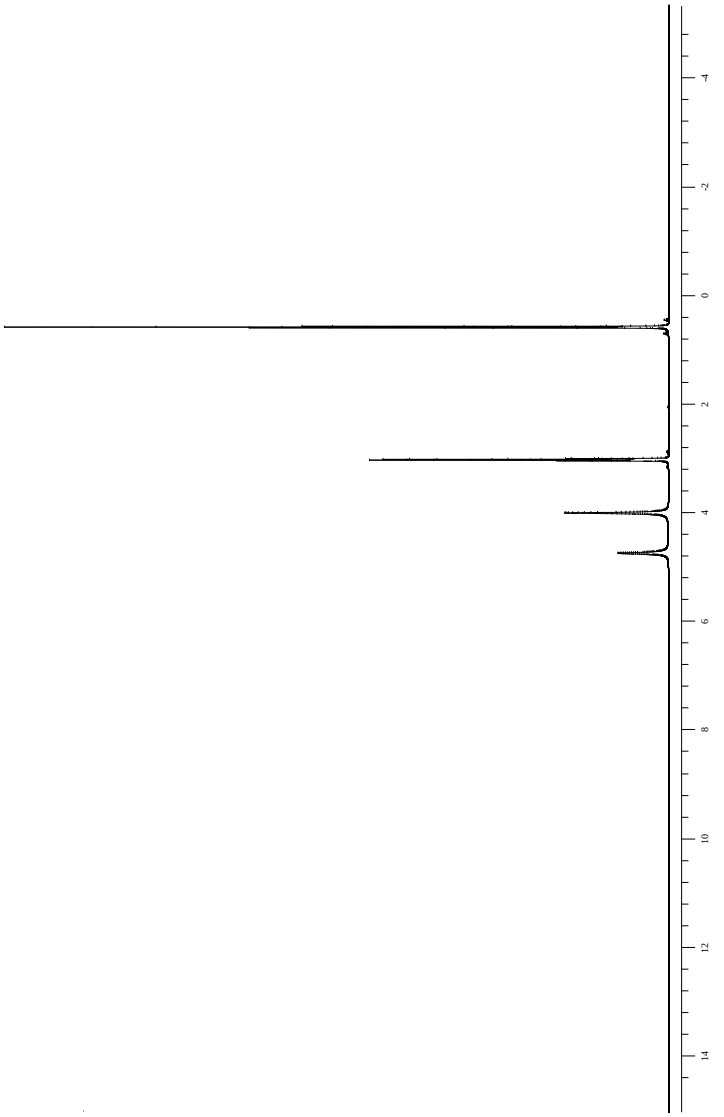
Trituration and Succussion
3rd acquisition

INDEX	FREQUENCY	PPM	HEIGHT
1	2369.633	4.739	11.1
2	1998.357	3.997	23.0
3	1518.962	3.038	23.2
4	1511.816	3.024	62.0
5	1504.981	3.010	59.7
6	1497.835	2.996	22.2
7	294.530	0.589	89.3
8	287.695	0.575	140.7
9	280.549	0.561	79.7



Trituration only
1st acquisition

INDEX	FREQUENCY	PPM	HEIGHT
1	2375.846	4.752	12.6
2	2004.260	4.009	26.8
3	1524.864	3.050	20.5
4	1518.029	3.036	54.4
5	1510.883	3.022	53.1
6	1503.738	3.008	19.5
7	300.744	0.602	83.7
8	293.598	0.587	132.9
9	286.452	0.573	75.1



Trituration only
2nd acquisition

INDEX	FREQUENCY	PPM	HEIGHT
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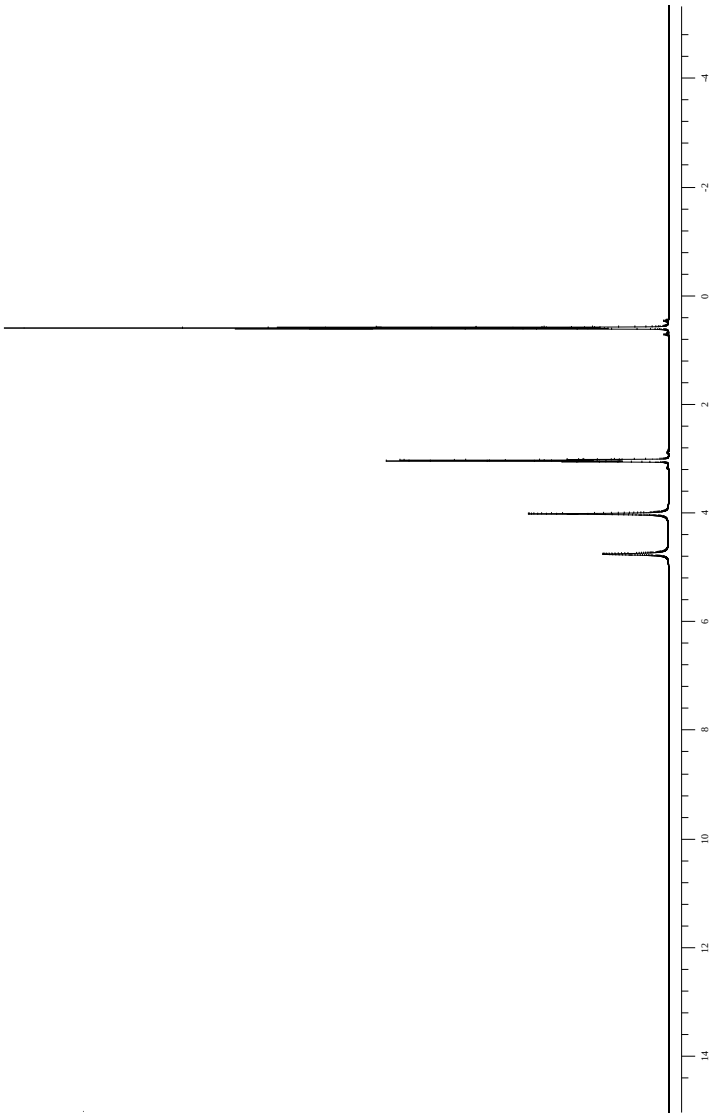
4	1518.029	3.036	54.2
5	1510.883	3.022	52.9
6	1503.738	3.008	19.5
7	300.744	0.602	83.4
8	293.598	0.587	132.9
9	286.452	0.573	75.0



Trituration only
3rd acquisition

INDEX	FREQUENCY	PPM	HEIGHT
1	2375.846	4.752	12.7
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3	1524.864	3.050	20.4
4	1518.029	3.036	54.2
5	1510.883	3.022	52.9
6	1503.738	3.008	19.5
7	300.744	0.602	83.7
8	293.598	0.587	132.8
9	286.452	0.573	74.9