A comparative study of the nuclear magnetic resonance spectra of Kalium Bichromicum 12CH manufactured from 3CH and 4CH triturations respectively.

By

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I, Izel Botha, do declare that this dissertation is representative of my own work, both in conception and execution.

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ABSTRACT

The purpose of this study was to compare the Nuclear Magnetic Resonance spectra of Kalium Bichromicum 12CH, which have been potentised using Hahnemannian methods, from initial 3CH trituration or 4CH trituration.

Trituration of base substances, commonly to the 3CH level, is a the cornerstone of the homoeopathic pharmaceutical process. In 1998, Becker and Ehrler unveiled their discovery pertaining to the C4 (or 4CH) trituration level. They claim that trituration to this level gives a new, spiritual dimension to the remedy picture, thus providing a deeper knowledge and understanding of well known remedies (Becker and Ehrler, 1998).

The study sought to establish whether there is any scientific proof in the claim that C4 potencies possess different qualities to the remedies currently employed (derived from a C3 trituration), the researcher must look at the physical and chemical properties of the substances in question. If statistically reliable data provided with Nuclear Magnetic Resonance (NMR) spectroscopy could prove the claims made, then there may be a scientific basis for the hypothesis.

For this study, all potencies were produced by hand according to the German Homoeopathic Pharmacopoeia (GHP) (British Homoeopathic Association, 1985). Five different samples were produced in a volume large enough to accommodate the drawing of 3 600 µl samples from each bottle.

The samples were Kalium Bichromicum 12CH from a soluble Potassium dichromate in 87% alcohol, Kalium Bichromicum 12CH from Potassium dichromate triturated to the 3CH level and then taken up into potency in 87% alcohol, Kalium Bichromicum 12CH from Potassium dichromate triturated to the 4CH level and then taken up into potency in 87% alcohol, Lactose 12CH from lactose triturated to the 3CH level and then taken up into potency in 87% alcohol and Lactose 12CH from lactose triturated to the 4CH level and then taken up into potency in 87% alcohol.

The samples were then analysed using Nuclear Magnetic Resonance Spectroscopy. This was done in the Varian Unity Inova 500MHz Spectrometer. The samples were drawn in a linear fashion. Deuterated acetone (acetone $-d_6$) was used as the external lock and reference substance and was placed in a separate capillary tube within the NMR tube so as not to contaminate the sample. The data collected were transferred onto Microsoft® Excel 2000 spreadsheets and statistically analysed using the SPSS© program performing the Kruskal Wallis and Mann-Whitney tests.

The results indicated a significant difference between the samples produced from a respective 3CH and 4CH trituration level. This was especially prominent in the chemical shift values of all 4 peaks and the relative integration levels of the H_2O, OH and CH_3 peaks when comparing the two Kalium Bichromicum sample groups.

From the results obtained, the researcher assumes that trituration plays a part in the development of physicochemical properties specific to homoeopathic remedies. The higher the level of trituration, the more pronounced the alteration of the physical structure of the active ingredient becomes. The study concludes that 4CH potencies are physico-chemically distinct from 3CH-derived potencies (as currently employed).

Further studies into 4CH (C4) potencies should be conducted to gather more data about the effect of these remedies. A Hahnemannian proving of these potencies is the logical step towards improving the credibility of the theories surrounding 4CH (C4) potencies.

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TABLE OF ABBREVIATIONS

ANOVA	Analysis of Variance
B_o	External or applied magnetic field
CH	Centesimal Hahnemannienne
CH_2	Methylene group
CH_3	Methyl group
FID	Free induction decay
FT	Fourier Transform
g	gram
h	Planck's constant
H_2O	Water
Hz	Hertz; represents the frequency
I	Nuclear spin quantum number
J	Coupling constants
KB 3	Kalium Bichromicum 12CH from a 3CH
KB 4	Kalium Bichromicum 12CH from a 4CH
KB Soluble/KB soln	Kalium Bichromicum 12CH from soluble Potassium Dichromate
ml	Millilitre
NMR	Nuclear Magnetic Resonance
OH	Hydroxyl group
ppm	Parts per million
RF	Radio frequency
S.G.	Specific Gravity
SL 3	Lactose12CH from a 3CH

SL 4	Lactose 12CH from a 4CH
T_1, T_2	Relaxation times
TMS	Trimethylsilane
v	Lamor frequency
α	Significance level of test
δ	Delta; represents the chemical shift
Δ*E*	Energy difference
µl	Microlitre
µs	Microsecond

DEFINITION OF TERMS

Analysis of Variance (ANOVA)

A parametric statistical method used to analyse data testing for significant differences in the means.

Avogadro's number

The number of atoms in exactly 12g of carbon-12. It represents the units contained in 1 mole of a substance equal to $N_A = 6.022 \times 10^{23}$. At a dilution level of 12CH or 24X, not one molecule of the original substance can be found. The concentration of the original substance in a 12CH or 24X dilution is 10^{-24}.

Batch

A specific quantity of medicine, which is uniform in characteristic and quantity within specified limits, and is produced at the same time according to a single specified manufacturing procedure.

Boltzmann Distribution

Links populations of different energy levels to the energy difference between them.

Centesimal

The original concentration scale developed by Hahnemann and the most frequently used in homoeopathic pharmacy. It represents a

dilution level of 1:100 and each succeeding potency thus contains one-hundredth part of the preceding potency. It is denoted by CH or C or it is assumed if no potency scale is indicated.

Chemical shift

Indicates the resonance frequency of the nuclei subjected to the electromagnetic field in relation to a reference standard e.g. Trimethylsilane. It is measured in parts per million (ppm) of the spectrometer's operating frequency.

Fourier transforms

A mathematical operation, which converts functions from time to frequency domains.

Integration

The relative intensity of individual NMR peaks indicating the relative proton distribution within individual groups within a molecule. The area under the respective peaks represents the integration value.

Law of similars

The doctrine that states that if a substance can cause morbid symptoms in a healthy individual, it can cure those exact symptoms in a diseased individual.

Lamor frequency

Measure of the strength of the nucleus' magnetic field in the absence of an external magnetic field.

Magnetic moment (μ)

The intrinsic magnitude of a magnetic dipole, which itself is generated by the overall spin of a charged nucleus along its spin axis.

Magnetogyric ratio *(γ)*

The ratio of a nucleus' magnetic moment to angular momentum, which is a constant for each nucleus and determines its energy dependence on the applied magnetic field.

Materia medica

A reference work listing remedies and their therapeutic actions.

Mean

A statistical term defined as the sum of all the values in a sample divided by the number of values in the sample.

Monograph

A reference book describing the physical and chemical properties of raw materials used to manufacture medicines. It is used to ensure a standard for all medicines produced.

NMR-Spectroscopy

An analytical method employed to measure the interaction of protons within an applied magnetic field in order to obtain information about the structure of organic compounds. The interaction is recorded as a series of peaks known as a spectrum.

Pharmacopoeia

A reference book describing the preparation and use of medicines.

Physical structure

The three-dimensional orientation between different molecules or atoms present in a compound, or mixture, giving it its properties.

Planck's constant (*h*)

A constant representing the quantisation of energy, where vibrational energy is proportional to its frequency. $h = 6.626 \times 10^{-34}$ J.s

Potency

An altered state of a material substance employed as a homoeopathic medicine indicative of the deconcentration level.

Potentisation

Also known as dynamisation. The mechanical and physical process peculiar to homoeopathy used to prepare potencies by means of serial dilution and succussion, or trituration, according to a specific ratio.

Prover

A healthy person who participated in the trial of a homoeopathic remedy. During the course of the trial, (s)he records all the new symptoms experienced as a result of the remedy taken.

Proving

The scientific method through which the remedy picture of a homoeopathic remedy is obtained. The remedy is administered to healthy participants in order to ascertain the type of morbid symptoms it is able to produce. This information can then be collated and recorded in the materia medica.

Relative integration values

Integration represents 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 the integration values of the run. The value is proportional to the number of protons generating each of the peaks.

Remedy

A homoeopathically produced and selected medicine treating the cause of the disease rather than the symptoms.

Specific gravity

Refers to the mass of a substance relative to its volume and which is defined by the following formula:

$$S.G. = \frac{Mass(g)}{Volume(ml)}$$

It may be calculated from a known mass and volume, or measured with the use of a hydrometer.

Spectrum (NMR)

A graphical representation of the peaks generated by a NMR-spectrometer, representing the resonant frequencies of the protons in the compounds in response to their absorption of electromagnetic radiation. Each type of proton has a different resonant frequency, giving rise to different peaks. Ethanol e.g. would have H_2O, OH, CH_2 and CH_3 peaks.

Standard deviation

A measure of dispersion, which uses all the data points in a sample to indicate the variation existing within the data.

Succussion

Involves taking the glass vial of the solution and shaking it by a quick flick of the arm in a downward motion and striking the vial of the solution against a firm, but still flexible surface such as a leather bound book.

Systems organisation

The balance between the static and dynamic aspects of order observable in different solutions. In a gas the dynamic aspects are more strongly developed than the static aspects; in a liquid the two aspects are equally balanced; in a liquid the static aspects are more strongly developed than the dynamic aspects.

In liquids, the systems organisation is influenced by phase boundaries, the inner surfaces of the voids containing the gas molecules ("structure makers") and the solutes in contact with the liquid ("structure breakers"). The structure breakers are solutes, which contract the liquid structure in their immediate surroundings, modifying the entire solution, by changing the oscillation of the gas molecules and the inner surfaces of the voids and perpetuating the oscillating pattern throughout the entire solution structure (Resch and Gutman, 1987).

Trituration

The act of grinding, with a mortar and pestle, lactose and a medicinal source substance together for a prolonged period of time in order to reduce the particle size of the substance, diluting it with the lactose and rendering insoluble substances soluble for the production of homoeopathic potencies.

T-tests

A statistical analytical method used to determine the difference between the means of two groups.

CHAPTER 1 - INTRODUCTION

Throughout Hahnemann's life, he continuously experimented clinically with different methods of preparation of his potencies. Early on in the 18th century, he started employing trituration as a method to render insoluble source material soluble. Initially he triturated to a 2CH (C2) level but his further experiments lead him to look into triturations up to a 12CH (C12) level. Finally, however, he decided that 3CH (C3) was the optimum level up to which a source material should be triturated before converting to a liquid potency. This was also the level he chose for the preparation of his quinquagemillenesimal potencies (Barthel, 1991).

This standard method of preparing homoeopathic potencies was documented in the various pharmacopoeias, for example the German Homoeopathic Pharmacopoeia (GHP) followed in this study.

During 1998, Jűrgen Becker and Witold Ehrler unveiled their discovery pertaining to the C4 (or 4CH) trituration level. They claim that this level gives "a new, spiritual dimension to the remedy picture, thus giving a deeper knowledge and understanding as to the homoeopathic potentisation." (Becker and Ehrler, 1998)

Their revelation was met with mixed responses from homoeopathic circles. Homoeopaths, like Timmermann, were intrigued by the suggestions. She started doing further work on the subject and lecturing on her findings. [2]

Others, such as Dellmour, have objected to both the notion itself, and the scientific basis of the studies. They further questioned the quality of the resulting homoeopathic remedies and remedy pictures obtained using the C4 method of proving. [1]

The C4 provings are done during the trituration process. After every level of trituration has been completed, the prover records all feelings and impressions gathered during the trituration. [1] The trituration of one substance up to the C4 level is usually done over the period of five or six days. At the C1 level, physical symptoms are experienced both during and after the trituration process. The C2 level expresses the sensational and mental information of the substance, whereas the C3 level contains the delusions and dreams as an expression of the psychic/mental level. The C4 level exposes the essence of the remedy, expressing the spiritual, unconscious level of the individual Usually, symptoms experienced on one level disappears on going to the next trituration level. (Brinton and Miller, 2004).

This proving method contrasts to the traditional method where the proving substance is administered, usually orally, to the prover. The prover then records all symptoms experienced during the duration of the proving. This process usually runs over the period of a few weeks. (Hahnemann, 1999)

This study is a step towards providing objective evidence around the validity of the claims that 4CH (C4) potencies possess different qualities to the

remedies currently employed. One must look at the physical and chemical properties of the substances in question. If statistically reliable data provided with Nuclear Magnetic Resonance (NMR) spectroscopy can prove the claims made, then there may be a scientific basis for the hypothesis.

1.1 THE AIM OF THE STUDY

To compare and evaluate the Nuclear Magnetic Resonance spectra of Kalium Bichromicum 12CH, which have been potentised using Hahnemannian methods, from initial 3CH trituration or 4CH trituration

1.2 THE STATEMENT OF OBJECTIVES

1.2.1 The first objective

To compare and evaluate the NMR spectra of Kalium Bichromicum with respect to the chemical shifts and relative integration values of the CH_2, CH_3, H_2O and OH signals, which have been potentised to 12CH potency, from initial 3CH and 4CH trituration, so as to determine differences in their respective physical structure.

1.2.2 The second objective

To compare and evaluate the NMR spectra of the lactose (control) with respect to the chemical shifts and relative integration values of the CH_2, CH_3, H_2O and OH signals, which have been potentised to 12CH potency, from initial 3CH and 4CH trituration, so as to determine differences in their respective physical structure.

1.2.3 The third objective

To compare and evaluate the NMR spectra of Kalium Bichromicum with respect to the chemical shifts and relative integration values of the CH_2, CH_3, H_2O and OH signals, which have been potentised to 12CH potency, from initial soluble form and 4CH trituration, so as to determine differences in their respective physical structure.

1.2.4 The fourth objective

To compare and evaluate the NMR spectra of Kalium Bichromicum and lactose (control) with respect to the chemical shifts and relative integration values of the CH_2, CH_3, H_2O and OH signals, which have been potentised to 12CH potency, from initial 3CH, so as to determine differences in their respective physical structure.

1.2.5 The fifth objective

To compare and evaluate the NMR spectra of Kalium Bichromicum and lactose (control) with respect to the chemical shifts and relative integration values of the CH_2, CH_3, H_2O and OH signals, which have been potentised to 12CH potency, from initial 4CH, so as to determine differences in their respective physical structure.

1.2.6 The sixth objective

To compare and evaluate parallel potencies in terms of substance versus substance, substance versus control and control versus control, so that any differences or similarities between methods in terms of NMR spectra and statistical analysis can be elucidated.

1.3 THE HYPOTHESES

1.3.1 The first hypothesis

It is hypothesized that significant differences exist between the chemical shift (δ) and relative integration values of the CH_2, CH_3, H_2O and OH signals of the 12CH potency level which have been manufactured from a soluble form, a 3CH and a 4CH triturate, and that these indicate differences inherent to each trituration level.

1.3.2 The second hypothesis

It is hypothesized that the level of trituration plays an important part in the development of distinct physicochemical properties specific to homoeopathic remedies. It is therefore hypothesized that statistically significant differences exist between parallel potencies in terms of substance versus substance, substance versus control and control versus control, with regard to chemical shift (δ) and relative integration values of the CH_2, CH_3, H_2O and OH signals of respective trituration levels.

CHAPTER 2 - THE REVIEW OF THE RELATED LITERATURE

2.1 THE INTRODUCTION

Homoeopathy has a reputation of not being scientifically substantiating in its claims. This may be changed by creating a standard for the manufacturing of the medication used in homoeopathic trials. One way to create such a standard is by analyzing all remedies manufactured using NMR spectroscopy and creating a database of spectra for each remedy. Research conducted in the past into homoeopathy using NMR spectroscopy has shown that a significant difference exists between remedies of parallel potencies as well as between individual potencies of the same remedy (As discussed in 2.6).

Different manufacturing processes have also been analysed to show whether differences exist between parallel potencies manufactured by means of different processes. Claims as to the effect of remedies of these different processes can now be linked to the physico-chemical properties of the substance (Davies, 2001, Malan, 2002).

In this review the development of potencies by Hahnemann, and later by Becker, as well as the theory behind Nuclear Magnetic Resonance spectroscopy and its relationship to research into the scientific basis of homoeopathy is explored.

2.2 POTENTISATION DEVELOPMENT BY SAMUEL HAHNEMANN

Samuel Hahnemann (1755-1843) started his career as a Medical practitioner, prescribing medicines in doses that intended to impose specific actions on the body. These doses were very often large e.g. antimony was prescribed in doses of 5-50 grains. [8]

In 1796, after translating Cullen's Materia Medica and proving Peruvian bark, Hahnemann wrote the publication *Essay on a New Principle for Ascertaining the Curative Powers of Drugs, and Some Examinations of Previous Principles*, in which he makes reference to using small doses of the raw substance. [8]

His experiments with his new *Similia* (like cures like) principle lead to the use of even smaller doses and by 1799 the principle of serial dilution was introduced. The first detailed description of this method was given in his booklet *Cure and Prevention of Scarlet Fever*, published in 1801. At this point trituration and succussion were not recognised, but he offered descriptions such as "shaking the whole well" and "shaking it for a minute". He called his reduced doses "weak solutions". [8]

Between 1801 and 1811 he worked with dilutions up to the sixtillionth dilution (10^{-36}), as referred to in the First edition of the *Organon* (1810) and volume 1 of the *Materia Medica Pura* (1811) (Barthel, 1991).

Volume 2 of *Materia Medica Pura* (1816) directs dilution on the centesimal scale ranging from the 3rd potency (10^{-6}) up to the 30th potency(10^{-60}). The method of agitation is still “well shaken” or “accurately shaken” (Barthel, 1991).

In subsequent volumes of his *Materia Medica Pura*, published between 1816 and 1819, there is a great deal of variation in dose and dilution, ranging from 1 drop of the original preparation of *Causticum (ø*), to the 30th dilution (10^{-60}) for *Arsenicum*. [10]

In 1818, in the fourth volume of the *Materia Medica Pura*, Hahnemann introduced the trituration of gold after finding it mentioned in the work of Arabian physicians and started using it in 1CH and 2CH powder forms. In the second edition of the *Materia Medica Pura* (1822-1827), he has Mercury triturated up to a 12CH (10^{-24}). By the third edition of the *Organon* (1824), this was changed and triturations only went as far as the 3CH level (10^{-6}) (Barthel, 1991).

The 3CH level of trituration marks the stage where water insoluble substances become soluble. In the same concentration and with the same number of potentizing stages, the trituration-based medicine proved more powerful. As a result of the medicinal substance and the vehicle being subjected to more intensive mechanical and energetic factors, a more powerful medicine is manufactured (Dellmour, 1994).

In the fifth edition of the *Organon* (1833), Hahnemann provides the first specific instructions on dynamisation: 30 successive serial dilutions of 1/100 dilution, with 2 succussions at each dilutional step to produce the 30CH potency. [8]

Five years later, in the second edition of volume 3 of *Chronic Diseases* (1837), he changed his method again, going back to 10 succussion strokes (Barthel, 1991).

In 1838 Hahnemann developed the LM (Q, Fifty-millesimal) potency scale. This scale utilizes an even greater dilution scale (1/50 000) and uses medicated pillules for the dilutions. There are also more succussions at each level (100). The First level of the LM potency is prepared from a 3CH triturate. This method was set out in the posthumous sixth edition of the *Organon* (1921). [8]

2.3 4CH POTENCIES (C4 POTENCIES)

Becker and Ehrler introduced the idea of C4-trituration, claiming that it brought a new spiritual level to the remedy. They claim that the mechanical friction with lactose created during the trituration process is where the fundamental and substantial form of the homoeopathic potentisation happens, while the succussing with alcohol increases only the frequency of the oscillation level achieved by the friction (Becker and Ehrler, 1998).

They theorise that during the C1 trituration process, the physical aspect of the remedy is revealed to the triturator ('prover'). The C2 trituration process reveals the emotional aspect, while the C3 process reveals the mental or psychic aspect. Trituration of the C4 level brings the spiritual aspect to the fore, giving a new dimension to the remedy. This level, however, is only revealed in C4 remedies manufactured by hand trituration.

Provings of the C4 dimension are not conducted in the traditional manner of testing the substance on a group of healthy provers. It is obtained during the trituration process and is carried out by an individual or a group of triturators. The triturator should also be able to set up a resonance with the substance triturated in order to experience the spiritual level of the remedy.

The aspects revealed at the C5 level is set too high for human perceptions and should only be employed in special cases (Becker and Ehrler, 1998).

Recently, homoeopaths, such as Timmermann, have been looking into triturations up to the 4CH level before converting to liquid potencies. She claims that she has had good results in treating chronically ill patients with 4CH triturated remedies. She conducts numerous provings in Becker's manner at conferences and at the Hahnemann Instituut in the Netherlands.

Her method of trituration, however differs from the GHP method in the time spent triturating and scraping. This is outlined in the following table.

	Trituration (in minutes)	Scraping (in minutes)	Trituration (in minutes)	Scraping (in minutes)
GHP	6	4	6	4
Timmerman [3]	7	3	7	3

Table 2.1 – Comparison between the times allotted for trituration and scraping in the first third of the trituration process

A lot of controversy surrounds the 4CH triturations, for some homoeopaths feel that medicines should only be produced as Hahnemann directed, others disagree.

Dellmour feels that any symptoms obtained by this method of proving should not be included into the materia medica or repertory. He sees them as un-homoeopathic, unclear and not understandable as they are in contradiction to Hahnemann's demands on a proving.[2]

The scientific basis and validity of this method is thus questioned and the origin viewed as esoteric or occult, because Becker does not know where the

images of this spiritual level comes from.[2] A scientific study into these potencies may shed some light onto their validity.

2.4 THE IMPORTANCE OF TRITURATION

There are certain advantages of trituration. Firstly, the resultant medicine has a more powerful action than those with the same number of potentizing stages, but which was not trituration based. The dynamisation is thus more powerful if produced by a 3-hour trituration. The trituration process adds more energy to both the medicinal substance and the vehicle (Dellmour, 1994).

The grinding of solid material leads to a strengthening of the system organization. The strengthened system is in a better position both to integrate influences of the environment and to influence the environment, whereby its chief characteristic is maintained. The greater part of the energy imposed by the grinding is usually passed to the environment, but the small fraction that is retained by the system is usually retained in the highest hierarchic level. This energy is required for the stabilization of the increased surface area. The whole system becomes energetically more differentiated even within the lowest hierarchical level (Resch and Gutman, 1987).

The more advanced the potentisation, the smaller is the influence of the solute-solute and the solute–void interaction in the lattice structure of the system. The void-void interactions are however increased. The total information content of the system does not depend on the presence of the

dissolved molecules, but rather the oscillating pattern of the whole solution as shaped but the molecules present. The information from the original solute has been spread throughout the system, causing a dynamically maintained oscillating pattern. The quality of the information is not disturbed, however, by the quantity of original information in the highly potentised drug. Hence the organism recognizes the information with regard to the uniform, conservative aspects of order (Resch and Gutman, 1987).

Secondly, it allows for the potentisation of substances that are not freely soluble. It also renders them soluble at the 3CH level of trituration, allowing for the liquid preparation of higher potencies.

Thirdly, manufacturing potencies in this way retains more natural constituents. It also allows for a longer shelf life in the case of lower potencies manufactured from fresh plant material (Dellmour, 1994).

Other advantages include an effect on the physical properties of the substance i.e. a reduction in the particle size of the drug, thus allowing an increase in the surface area of the drug which increases the catalytic effect, colloidal properties and absorptive qualities of the drug (Hopkins, 2004:9).

Hahnemann introduced the 3CH level as a standard method and he used and recommended it for all medicines, the exception being very aggressive acids and extremely hygroscopic salts, where trituration is not possible (Dellmour, 1994).

The disadvantages to hand trituration are that it is a cumbersome process with low productivity, that it does now provide a complete inter-mixing of substances which are harder than saccharum lactis e.g. some hard metals and the aldehydic property of saccharum lactis may reduce some substances, especially mercury. It also allows some variables to come into play in the manufacturing process allowing for sample contamination e.g. when the triturator exhales on the sample or the introduction of dust or other particles into the sample (Hopkins, 2004:10).

Becker, however, insists on the manufacture of C4 potencies by hand and such factors are thus unavoidable (Becker and Ehrler, 1998).

2.5 NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

NMR is a process whereby interactions between nuclear magnetic dipoles and electromagnetic radiation are observed (Weingärtner, 1990). It is used to study the properties of matter. The NMR spectrometer measures the spin of protons in a magnetic field. The spin-active nucleus, e.g. a proton, has an electrical charge associated with it and behaves as if it spins around an axis. The charged spinning particle generates a magnetic field around itself, the magnetic moment (μ). In the absence of any external magnetic field these nuclei are spinning at random in their atomic or molecular environments. When placed in a strong external field (B_O) the nuclei orientate themselves with respect to the direction of the magnetic field. They can orientate

themselves in more than one way. For protons there exists two possible alignments, either with the field or against it (Williams, 1986).

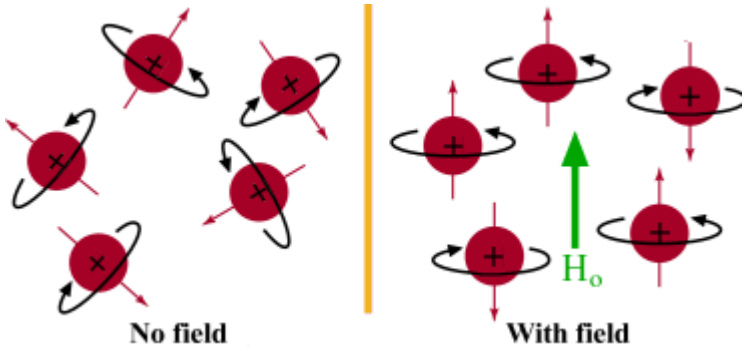

Figure 2.1 – Orientation of the Nuclei in an applied magnetic field 5

The spin quantum numbers are a measure of how many different ways the nuclear magnets can align themselves in a magnetic field. The relationship is given by the expression $2I+1$, where I is the spin quantum number that comes in multiples of ½ and can be – or +. Unpaired protons, electrons and neutrons possess a spin of ½. [4]

When the proton is placed in an external magnetic field, the spin vector of the particle aligns itself with the external field. The particle with a net spin can absorb a photon. It then undergoes a transition between two energy states by the absorption of a photon. If it had a lower energy state and it absorbs a photon, it ends up in an upper energy state. The energy of the photon must exactly match the energy difference between the two states. At room temperature the number of spins in the lower energy level (N^+), slightly outnumbers those in the upper level (N^-). In the NMR experiment the frequency of the photon is in the radio frequency (RF) range. [4] According to

Planck's law, the energy difference (ΔE) between the spin states is directly proportional to the field strength. It is expressed as:

$$\Delta E = hv$$

Where *h* is Planck's constant and *v* is a frequency of electromagnetic radiation, i.e. the frequency of resonance *v*, is directly proportional to the strength of the external magnetic field. The proportionality of constant can be shown to be $\gamma/2\Pi$ where γ is the magnetogyric ratio of the nucleus. The constant is just a measure of how strong the nucleus's magnetic field is. Thus

$$v = \frac{\gamma}{2\Pi} B_o$$

The frequency term, *v*, is usually referred to as the Lamor frequency. For a given magnetic field, different spin-active nuclei have different Lamor frequencies (Williams, 1986).

A NMR spectroscopy of a substance will measure four parameters, namely chemical shifts, coupling constants, relaxation times and integrations.

a) Chemical Shifts (δ)

Chemical shifts are measured in parts per million (ppm) from a standard such as tetramethylsilane (TMS). It indicates the resonance frequency of the nuclei subjected to the electromagnetic field. For an

isolated nucleus, resonance will occur at its Lamor frequency. However, in a molecule, electrons in these bonds and elements influence the local magnetic field around the nucleus in question, resulting in a modification of the size of the external magnetic field (B_O) felt by the nucleus. Electrons from the orbitals shied the nucleus from B_O and are responsible for small magnetic field opposing it. The size of the shielding magnetic field will vary with electron density at a particular nucleus.

The set of resonance absorptions seen in a NMR spectrum corresponds to all the chemically different spin-active nuclei. It is difficult to measure absolute frequencies, thus precise frequency differences between resonances are measured. They are measured in respect to their shift from a standard, which has been added to the sample, usually TMS. This is because the twelve protons in TMS are highly shielded, it is highly volatile and is easily removed from the sample and does not usually interact with samples. Chemical shift is given in terms of the ratio of frequency difference between the resonance and that of TMS

$$\sigma = \frac{v_R - v_{TMS} \times 10^6}{v_{spectrometer}}$$

where v_R is the resonance frequency of the nucleus in question, v_{TMS} is the resonance frequency of TMS, and $v_{spectrometer}$ is the operating

frequency in MHz (Williams, 1986).

b) Coupling constants (J)

Coupling constants are measured in Hertz (Hz) and give information about the number of adjacent spin-active nuclei via the $2nI+1$ rule. Nuclei experiencing the same chemical environment or chemical shift are called equivalent. Those nuclei experiencing different environment or having different chemical shifts are non-equivalent. Nuclei that are close to one another exert an influence on each other's effective magnetic field. This effect shows up in the NMR spectrum when the nuclei are non-equivalent. If the distance between non-equivalent nuclei is less than or equal to three bond lengths, this effect is observable. This effect is called spin-spin coupling or J coupling. [4]

The $2nI+1$ rule helps explain the presence of some patterns of peaks in the NMR spectrum and has nothing to do with the compound being studied. In the formula *n* stands for the number of equivalent nuclei coupling to the nucleus under consideration, while *I* is their spin quantum number (Williams, 1986).

c) Relaxation Times (T_1, T_2)

Relaxation times affect the intensity and shape of NMR signals and involves the loss of spin energy to the molecular system – spin-lattice

relaxation – or transfer of spins between nuclei – spin-spin relaxation. A number of nuclei in a magnetic field will distribute themselves in various energy levels according to Boltzmann Distribution,

$$N_2/N_1 = 1 - \Delta E/kT$$

Where N_2 and N_1 are the populations of higher and lower spin states, ΔE is the energy difference between spin states, k is the Boltzmann constant and T is the absolute temperature of the system in Kelvin. Boltzmann Distribution links populations of different energy levels to the energy difference between them.

Not all the nuclei will relax at the same instant and those in the high spin state decays over a period of time. The rates of these processes were noted to be exponential decays governed by time constants T_1, T_2 (Williams, 1986).

d) Integrations

Integrations or integration ratios of NMR signals give information about the relative number of spin-active nuclei associated with each resonance, as the area under a signal was proportional to the number of spins causing the signal. These integrations are indicated on the spectrum as a step curve across each peak.

There are two main points regarding integrations. Firstly, it involves the measurement of the relative number of each kind of spin-active nucleus and thus indicates the relative proton distribution. Secondly, the peak areas are measured, not the peak heights so the total area can accurately be obtained (Williams, 1986).

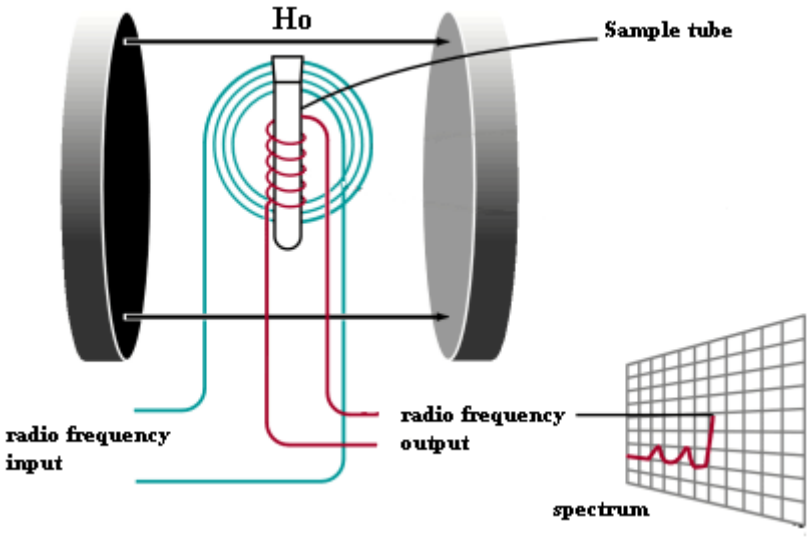

Figure 2.2 – Diagram of the NMR Spectrometer [8]

Signal enhancement is necessary, for as concentration of material in solution decreases, so the NMR signals become weaker. The electronic instrumentation allows for an increase in the sensitivity, but it also leads to an introduction of electronic noise. Electronic filters decrease some of the noise, but the most important signal enhancement method uses a mathematical device called Fourier transform (FT). In FT spectroscopy a sample is given a powerful pulse of radio frequency radiation. The pulse contains a broad band of frequencies and it causes all the spin-active nuclei to resonate at once at their Lamor frequencies. The detector system in the spectrometer senses the

change in magnetisation of the sample and the decay of the magnetisation with respect to time. This decay is called the free induction decay (FID). As a number of frequencies are involved the FID does not have a simple pattern, but is a complex set of interfering wave forms along with a great deal of noise. FID is a spectrum related to time – i.e. a time–domain spectrum. A Fourier transform is an operation, which converts functions from time to frequency domains (Williams, 1986). $F(v)$ is the frequency spectrum and $f(t)$ the time spectrum:

$$F(v) = \int_{-\infty}^{+\infty} f(t) e^{-1(2\Pi)vt} dt$$

$$f(t) = \int_{-\infty}^{+\infty} F(v) e^{1(2\Pi)vt} 2\Pi dv$$

The signal in the NMR spectroscopy thus results from the difference between the energy absorbed by the spin, which make a transition from the lower energy state to the higher energy state, and the energy emitted by the spins, which simultaneously make a transition from the higher state into the lower energy state. The signal is thus proportional to the population difference between the states.[4]

By means of the strong magnetic field, energy levels of the sample molecules are split. The locations of the peaks in a spectrum represent a picture of the

molecular geometry (Weingärtner, 1990). The sample will yield a NMR spectrum (i.e. a graph plotting resonance peaks), illustrating the absorption lines of the CH_3, CH_2 H_2O and OH present in the sample.[4]

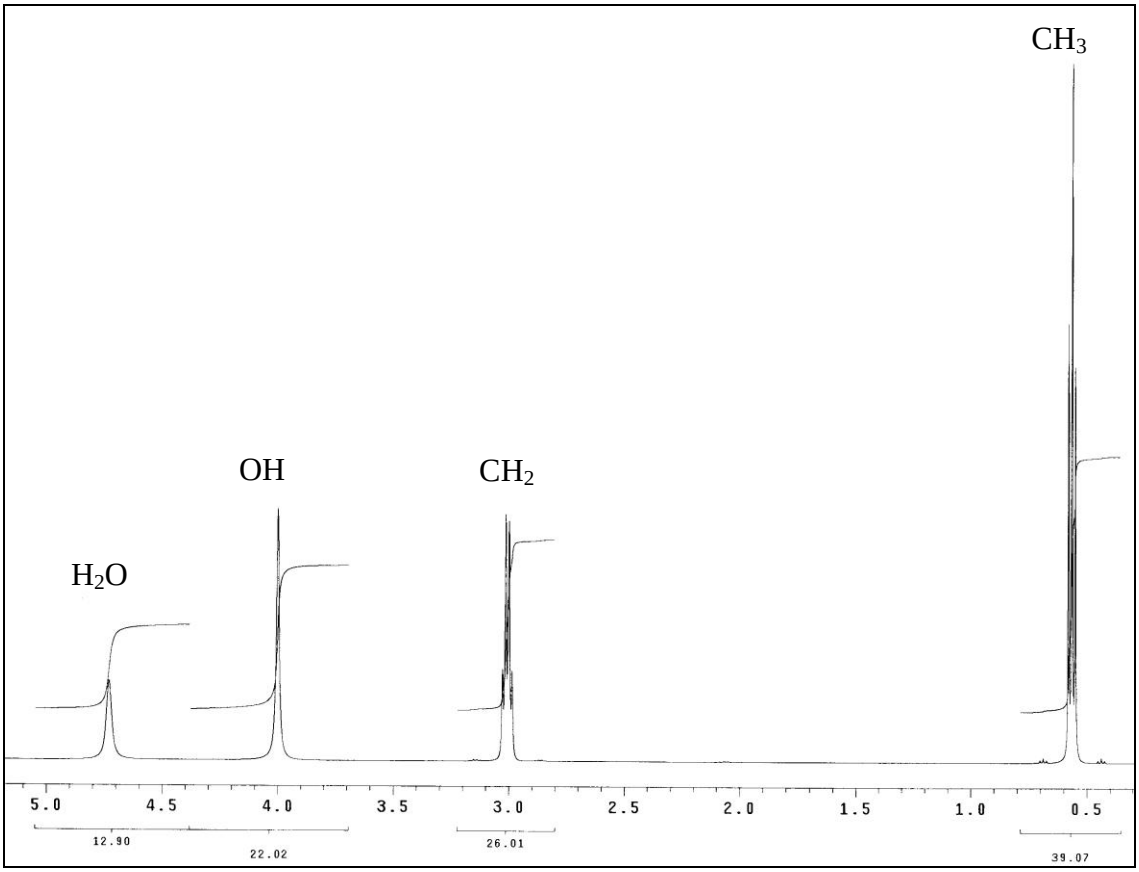

Figure 2.3 – NMR Spectrum example

2.6 NMR RESEARCH IN HOMOEOPATHY

Smith and Boericke started NMR research into homoeopathic remedies in 1966, they studied potentised and non-potentised *Sulphur* D12 in 87% alcohol. They found characteristic differences in the NMR spectra and concluded that potentisation changed the macromolecular structure of the solvent, even if the order of dilution is higher than the Avogadro constant (6.023×10^{-23}) (Endler and Schulte, 1994). The area of the NMR hydroxyl spectrum in 87% ethanol increases when succussed, compared to unsuccussed dilutions containing the same solute. (Smith and Boericke, 1968) Young (1974) extended on the work done by Smith and concluded that the changes observed via NMR methods occur for succussed solutions in glass bottles, but not in succussed solutions in wax-lined glass bottles. He also showed changes in the NMR Spectrum of remedies of different potencies (Endler and Schulte, 1994).

Sacks demonstrated a distinct difference between controls and several homoeopathic remedies in the H_2O and OH-regions of the NMR spectra in 1983. Potentised drugs in higher degrees of dilution were compared with solvents and Lasne *et al*. found that the spin-spin-relaxation times were significantly different. Weingärtner (1990) proved his hypothesis that the NMR spectra of *Sulphur* D23 that has been diluted and succussed and its solvent have statistically different relative intensities of the H_2O and OH signals with respect to the main intensity of the CH_2 signal.

Variations in NMR resonance in the relaxation times T_1 and T_2 in highly diluted solutions of *Silicea* were proven by Demangeat *et al.* in 1992. (Bellavite *et al.* 1995)

Ross (1997) evaluated Hahnemannian quinquagenimillesimal potencies, noting significant differences between the integration values of the CH_2 signals and the chemical shift values of the CH_2, H_2O and OH signals. Power (1999) elaborated on this study by comparing homoeopathic quinquagenimillesimal potencies of *Plumbum metallicum* and *Stannum metallicum*.

T_1 relaxation times of deuterium in potentised and unpotentised ethanol solutions with and without *Nux Vomica* were studied by Sukul *et al.* in 2000. High–resolution NMR instruments were used in this experiment, for the lower resolution instruments were not sensitive enough to the subtle molecular changes in the solution. (Milgrom *et al.* 2001)

Davies (2001) found a significant difference between the chemical shift values for all peak types and potency levels in comparing Hahnemannian and Korsakovian test potencies. The relative integration levels showed no significant differences for any of the peaks or potency levels. Malan (2002) noted significant differences in the relative integration values of the OH peaks when he compared decimal and centesimal potencies of sulphur with equal number of succussions.

The effect of different resolution NMR spectrometry on *Sulphur* 30CH was investigated by Cason (2002), but found no conclusive evidence that the frequency strength is an influential parameter when conducting NMR experiments.

2.7 SUMMARY

It can thus be seen that potency development was an important step in the birth of homoeopathy. Trituration, especially to the 3CH level, developed to form the basis of the later work of Hahnemann while he formulated the principles of homoeopathy. These principles were later further elaborated on by the investigations by Becker and Ehrler, who investigated the deeper levels of the homoeopathic remedy picture with C4 triturations.

It is, however, important to form a scientific basis for homoeopathic potencies. This is especially important when potencies surpasses the level where physical and chemical essays are possible. Here, NMR plays an important role to enable the investigation of the energy fields of the potencies. NMR research in homoeopathy has provided a tool for the standardization of potencies, especially those beyond the molecular level of testing. (Bol, 1997)

CHAPTER 3 - MATERIALS AND METHODS

3.1 PRODUCTION OF SAMPLE POTENCIES

All potencies were produced by hand according to the German Homoeopathic Pharmacopoeia (GHP) (1985). The potencies were manufactured by hand because it allows for comparison with the previous studies conducted, which were all manufactured by hand.

The detailed materials and methodology is given in Appendix A.

Method 6: Trituration by hand

One part, by weight, of the solid Potassium dichromate was triturated together with 99 parts, by weight, of lactose BP. The Potassium dichromate adhered to the standards set out in its monograph. (Appendix B) The lactose vehicle was accurately massed out in three 33 parts, each weighing 3.30 g. After flaming the mortar and pestle using 96% alcohol and allowing it to cool down, one part vehicle was then triturated together with the 0.10 g Potassium dichromate for 6 minutes, followed by 4 minutes of scraping down. This process was then repeated. After the second scraping was completed, the second part of the vehicle was added. It was then triturated and scraped together with the first for 2 sessions of 6 minutes trituration and 4 minutes scraping. The process was repeated after the final part was added. This hour of trituration yielded Kalium Bichromicum 1CH triturate.

The 2CH triturate was manufactured by using 0.10 g of the 1CH triturate and three batches of 3.30 g of lactose. It was then triturated in an identical manner employed to produce the 1CH triturate, only substituting the Kalium Bichromicum with the 1CH triturate. After an hour the 2CH triturate was yielded.

The above process was repeated to manufacture the 3CH triturate and the 4CH triturate respectively. The process is set out in Appendix A (i).

The control trituration was carried out in a similar fashion. The Kalium Bichromicum was substituted with 0.10 g of lactose BP for the manufacture of the 1CH control triturate.

Method 8a – Liquid preparations from triturations

To produce a 6CH potency in a liquid vehicle, 1 part (0.10 g) of the 4CH triturate was dissolved in 99 parts (9.9 ml) distilled water and succussed 10 times. This yielded the 5CH. One part (0.03 ml) of the 5CH and 99 parts (2.97 ml) of 87% alcohol (Specific Gravity 0.826) was then placed in a 5ml bottle and succussed 10 times to yield the 6CH. Although the GHP states the use of 30% alcohol, this ethanol percentage was used to correlate with ethanol/water concentrations used in previous NMR experiments. (Davies 2001, Malan 2002, Cason 2002).

This method used to manufacture the 6CH was then used to manufacture the 7CH to 11CH potencies.

The 12CH potency was manufactured by placing 15.84 ml of 87% alcohol in a 25ml amber glass bottle together with 0.16ml of 11CH. This was then succussed 10 times and yielded the 12CH potency that was taken for analysis. This process was followed for both the 4CH of Kalium Bichromicum and the 4CH of the lactose control. The process is set out in Appendix A (ii) a).

The 3CH was taken up to a 12CH in a similar fashion. This process was followed for both the 3CH of Kalium Bichromicum and the 3CH of the lactose control. The process is set out in Appendix A (ii) b).

Method 5a: Liquid preparations from a soluble salt

A final 12CH potency was manufactured using soluble Potassium dichromate, without any triturations. This functions as a second control. The 1CH potency was manufactured by placing 0.03 g of Potassium dichromate and 2.97g (3.0455 ml) 15 % ethanol into a 5ml screw top bottle and allowing it to dissolve. The bottle was then succussed 10 times thus yielding the Kalium Bichromicum 1CH.

The 2CH to 11CH was manufactured by placing 2.97 ml of 87% alcohol and 0.03 ml of the 1CH (or relevant previous potency) in a 5ml screw top bottle

and succussing it 10 times for each potency level. Although the GHP states the use of 43% alcohol, this ethanol percentage was used to correlate with ethanol/water concentrations used in previous NMR experiments. (Davies 2001, Malan 2002, Cason 2002).

The 12CH potency was manufactured by placing 15.84 ml of 87% alcohol in a 25ml amber glass bottle together with 0.16ml of 11CH. This was then succussed 10 times and yielded the 12CH potency that was taken for analysis. The process is set out in Appendix A (iii).

After manufacture the samples were stored in a temperature-controlled environment, away from any magnetic influences until they were transported for analysis.

3.2 PREPARATION OF SAMPLE POTENCIES FOR ANALYSIS

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

Samples were produced in a volume large enough to accommodate the drawing of three samples from each bottle. The potencies were thus produced in 25ml Amber glass screw top bottles. The amber glass was selected for it protects the remedies from destruction by light. To ensure that the sample had space to be succussed to produce the final potency, the 25ml bottles were only filled two thirds full, thus containing 16ml.

The samples were manufactured in the 12CH potency, for at this level of deconcentration, the concentration of the original substance is 10^{-24}, which is below Avogadro's number (6.022×10^{23}). Thus at this dilution level not one molecule of the original substance can be found.

The five samples were clearly labeled and wrapped in soft tissue paper. They were placed in a thick walled cardboard box and transported to the Chemistry Department of the University of KwaZulu Natal by car. Any stimuli such as noise, vibration, temperature, light or any electromagnetic disturbance was avoided as far as possible. The samples were handed to Mr. C. Gimmer for NMR spectroscopy analysis.

3.3 NMR MEASUREMENT OF SAMPLES

Three samples were drawn from each of the provided volumes using a micropipette and a clean capillary tube. The NMR laboratory technician (Mr. C. Gimmer) drew the samples in a linear fashion and ran the tests. The sample volumes were 600 µl. Deuterated acetone (acetone-d_6) was used as both the external lock and the reference substance, as it provides a very reliable chemical shift value outside the range of other peaks. The Deuterated acetone was placed inside a separate capillary tube within the NMR tube so as not to come in contact with the sample and cause contamination (Grimmer, 2004).

The instrument used was the Varian Unity Inova 500MHz Spectrometer. The following factors were noted during the data acquisition:

^{1}H observe frequency:	499.985 MHz
Acquisition time:	1.892 seconds / transient
Number of complex data points:	38524 / transient
Number of transients:	16
Spectral width:	10180.7 Hz (~20 ppm)
Pulse width:	13.6 µs at 59 dB
Pulse angle:	90°
Relaxation delay:	30 seconds (>5x T1)
Lock nucleus:	^{2}H (acetone-d_6)
Reference:	Internal; capillary; acetone @ 2.05 ppm
Temperature:	25.0 $\pm$ 0.2 °C
Temperature stabilization delay:	20 minutes

To make the data as accurate as possible, the magnet was shimmed before every run to ensure a homogenous field around each sample before it was tested.[3]

The data was recorded in the form of NMR spectra listing the chemical shifts (in units of Hertz) and integration values. These were then transferred onto Microsoft® Excel 2000 spreadsheets.

3.4 STATISTICAL ANALYSIS

Both the chemical shift and integration values were recorded as given on the printout from the NMR spectrometer. For the CH_2 and CH_3 chemical shift values, where the values comprise of more than one peak, the average value was calculated and a single value used.

The integration values were given on the printed data sheet and represent the total area under each peak. The relative integration value for each peak was calculated by dividing the integration values of each peak by the sum of all integration values for the run.

The data was then entered into a Microsoft Excel 2000© spreadsheet and from there transferred into the SPSS© software package for statistical analysis. For analysis, all the samples were compared with each other to determine whether there was a significant difference between any of the samples. Since the sample size per group was small, the comparison was made between the five unpaired groups using the non-parametric *Kruskal-Wallis* test. If a significant difference existed between any of the groups, individual comparisons between groups were made using the non-parametric *Mann-Whitney* test. This was done between the Kalium Bichromicum soluble and Kalium Bichromicum 3CH; Kalium Bichromicum 3CH and Kalium Bichromicum 4CH; Lactose 3CH and Lactose 4CH; Kalium Bichromicum 3CH and Lactose 3CH and Kalium Bichromicum 4CH and Lactose 4CH.

The SPSS© results can be seen in Appendix D.

3.4.1 KRUSKAL WALLIS TEST

This is a non-parametric test that can be run when the assumptions of the one-way ANOVA for independent samples are not met e.g. the samples are not all the same size. The group means are based on ranks, rather than on the raw data. [6]

The Kruskal Wallis statistic can be calculated using the following steps:

- Assemble all the measures from all *k* samples into a single set of size *N*.
- Rank-order the measures from the lowest (rank 1) to the highest (rank n).
- The resulting ranks are then returned to the sample to which they belong and the raw measures are substituted by the ranks.
- The sums of the ranks within each group and their averages are calculated. [6]
- The value of *H* can then be calculated using the following formula:

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

Where n_i represents the sample sizes for each of the k groups (samples) in the data. R_i equal the sum of the ranks for group i. [7]

- The significance (α) is set to 0.05

To this end the hypotheses are stated as follows:

H_0: There is no difference in the trituration levels

H_1: There is a difference in trituration levels.

The hypothesis were accepted or rejected according to the following decision rule:

Accept H_0: if $p \geq \alpha$

Accept H_1: if $p \leq \alpha$

3.4.2 MANN-WHITNEY TEST

This is a non-parametric test used to compare two independent groups of sampled date.[9] This test is used when the distribution of variables do not show a normal distribution or when the samples taken are too small to determine whether or not they are part of a normal distribution.[1]

Mann-Whitney U test statistic can be calculated using the following steps:

- Rank all of the observations regardless of group membership, if two observations are identical, the mean of the two are found and they are given the same rank.

- Calculate the sum of the ranks independently for each group (R_1 and R_2).
- Calculate U_1 and U_2 by the following formulas:

$$U_1 = n_1 n_2 + \left[\frac{n_1(n_1+1)}{2}\right] - \sum R_1$$

$$U_2 = n_1 n_2 - U_2$$

Where n_1 and n_2 are the sample sizes of the two groups and R_1 is the sum of the ranks of one of the samples.[12]

- Use the smaller of U_1 and U_2 (representing the p value) and compare it to the critical value (α) set at 0.05.

To this end the hypotheses are stated as follows:

H_0: There is no difference in the trituration levels

H_1: There is a difference in trituration levels.

The hypothesis were accepted or rejected according to the following decision rule:

Accept H_0: if $p \geq \alpha$

Accept H_1: if $p \leq \alpha$

CHAPTER 4 - THE RESULTS

4.1 CRITERIA GOVERNING THE ADMISSIBILITY OF DATA

Due to the electromagnetic nature of homoeopathic remedies, the utmost care was taken not to expose the samples unnecessarily to anything that may influence their nature.

The samples were prepared in accordance with the methods previously explained in sections 3.1 and 3.2. Only one bottle of each sample was prepared due to the standard method of preparation. This eliminated the additional variables introduced by multiple samples. The three samples drawn from each bottle were drawn in a linear matter, so as to exclude the possibility of contamination. A separate pipette was also used for each bottle. During the preparation and taking of the samples, all the bottles were exposed to the environment for the shortest possible time. All the samples were stored under the same conditions.

The sixteen transients per sample were used to generate the NMR spectra. This raw data was used to obtain the chemical shift (δ) values and to calculate the relative integration values and subjected to the statistical methods outlined in 3.4.

The table in Appendix D outlines the data sent for statistical analysis. The complete data obtained from the statistical analysis can be seen in Appendix E.

4.2 KRUSKAL-WALLIS TEST

4.2.1 COMPARISON OF CHEMICAL SHIFT (δ) VALUES

	H_2O	OH	CH_2	CH_3
α	0.033	0.028	0.017	0.025

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

The comparison of the chemical shift values show a significant difference between all 5 samples in all four peaks. The hypotheses are thus accepted.

4.2.2 COMPARISON OF RELATIVE INTEGRATION VALUES

	H_2O	OH	CH_2	CH_3
α	0.028	0.102	0.022	0.464

Table 4.2 - Kruskal-Wallis Test - Comparison Of Relative Integration Values

The comparison of the relative integration values show a significant difference between the H_2O and CH_2 peaks for all the samples. The hypothesis is thus accepted. An insignificant difference can be observed between the OH and CH_3 peaks. The hypotheses are rejected in the case of these two peaks.

4.3 MANN-WHITNEY TEST

4.3.1) Chemical shift values

a) Kalium Bichromicum soluble and Kalium Bichromicum 3CH

	H_2O	OH	CH_2	CH_3
α	1.000	0.025	0.025	0.025

Table 4.3 - Mann-Whitney Test - Chemical shift values for Kalium Bichromicum soluble and Kalium Bichromicum 3CH

The comparison of the chemical shift values of the Kalium Bichromicum soluble and Kalium Bichromicum 3CH show a significant difference for the OH, CH_2 and CH_3 peaks. The hypotheses are thus accepted. The H_2O peak of the two sample groups are identical, and the hypotheses are rejected in this case.

b) Kalium Bichromicum 3CH and Kalium Bichromicum 4CH

	H_2O	OH	CH_2	CH_3
α	0.025	0.025	0.025	0.025

Table 4.4 - Mann-Whitney Test - Chemical shift values for Kalium Bichromicum 3CH and Kalium Bichromicum 4CH

Comparing the Kalium Bichromicum 3CH and Kalium Bichromicum 4CH groups, the chemical shift values show a significant difference between all the peaks. The hypotheses are thus accepted.

c) Lactose 3CH and Lactose 4CH

	H_2O	OH	CH_2	CH_3
α	0.796	0.317	0.034	0.034

Table 4.5 - Mann-Whitney Test - Chemical shift values for Lactose 3CH and Lactose 4CH

In comparing the chemical shift values of Lactose 3CH and Lactose 4CH a significant difference is seen between the CH_2 and CH_3 peaks. The hypotheses are thus accepted. An insignificant difference can be observed between the H_2O and OH peaks. The hypotheses are rejected in the case of these two peaks.

d) Kalium Bichromicum 3CH and Lactose 3CH

	H_2O	OH	CH_2	CH_3
α	0.480	0.480	0.034	0.371

Table 4.6 - Mann-Whitney Test - Chemical shift values for Kalium Bichromicum 3CH and Lactose 3CH

The comparison of the chemical shift values of Kalium Bichromicum 3CH and Lactose 3CH show a significant difference between the CH_2 peaks of the two groups. The hypotheses are thus accepted for the comparison of the CH_2 peaks. An insignificant difference can be observed between the H_2O, OH and CH_3 peaks. The hypotheses are rejected in the case of these three peaks.

e) Kalium Bichromicum 4CH and Lactose 4CH

	H_2O	OH	CH_2	CH_3
α	0.034	0.025	0.025	0.025

Table 4.7 - Mann-Whitney Test - Chemical shift values for Kalium Bichromicum 4CH and Lactose 4CH

Comparing the Kalium Bichromicum 4CH and Lactose 4CH (control) groups, the chemical shift values show a significant difference between all the peaks. The hypotheses are thus accepted for all four peaks.

4.3.2) Relative Integration values

a) Kalium Bichromicum soluble and Kalium Bichromicum 3CH

	H_2O	OH	CH_2	CH_3
α	0.046	0.046	0.369	0.105

Table 4.8 - Mann-Whitney Test - Relative Integration values for Kalium Bichromicum soluble and Kalium Bichromicum 3CH

When comparing the relative integration values of Kalium Bichromicum soluble and Kalium Bichromicum 3CH, a significant difference is observable between the H_2O and OH peaks. The hypotheses are accepted. An insignificant difference can be observed between the CH_2 and CH_3 peaks. The hypotheses are rejected in the case of these two peaks.

b) Kalium Bichromicum 3CH and Kalium Bichromicum 4CH

	H_2O	OH	CH_2	CH_3
α	0.046	0.046	0.507	0.046

Table 4.9 - Mann-Whitney Test - Relative Integration values for Kalium Bichromicum 3CH and Kalium Bichromicum 4CH

The relative integration values of Kalium Bichromicum 3CH and Kalium Bichromicum 4CH, show a significant difference for the H_2O, OH and CH_3 peaks. The hypotheses are thus accepted. An insignificant difference between these groups can be observed in the CH_2 peak. The hypotheses are rejected for the CH_2 peak.

c) Lactose 3CH and Lactose 4CH

	H_2O	OH	CH_2	CH_3
α	0.825	0.637	0.043	0.246

Table 4.10 - Mann-Whitney Test - Relative Integration values for Lactose 3CH and Lactose 4CH

The comparison of the relative integration values of Lactose 3CH and Lactose 4CH show a significant difference for the CH_2 peak. The hypotheses are thus accepted. An insignificant difference can be observed between the H_2O, OH and CH_3 peaks. The hypotheses are rejected in the case of these three peaks.

d) Kalium Bichromicum 3CH and Lactose 3CH

	H_2O	OH	CH_2	CH_3
α	0.050	0.500	0.072	0.513

Table 4.11 - Mann-Whitney Test - Relative Integration values for Kalium Bichromicum 3CH and Lactose 3CH

In comparing of the relative integration values of Kalium Bichromicum 3CH and Lactose 3CH, a significant difference is observable between the H_2O peaks. The hypotheses are thus accepted in this case. A nearly significant difference is observable between the CH_2 peaks, and an insignificant difference can be observed between the OH and CH_3 peaks. The hypotheses are rejected in the case of these three peaks.

e) Kalium Bichromicum 4CH and Lactose 4CH

	H_2O	OH	CH_2	CH_3
α	0.043	0.822	0.043	0.500

Table 4.12 - Mann-Whitney Test - Relative Integration values for Kalium Bichromicum 4CH and Lactose 4CH

The comparison of the Kalium Bichromicum 4CH and Lactose 4CH groups, with regard to the relative integration values, show a significant difference between the H_2O and CH_2 peaks. The hypotheses are thus accepted. An insignificant difference can be observed between the OH and CH_3 peaks. The hypotheses are rejected in these cases.

CHAPTER 5 - DISCUSSION

The aim of this study was to compare the Nuclear Magnetic Resonance spectra of Kalium Bichromicum 12CH, which have been potentised using Hahnemannian methods, from initial 3CH trituration or 4CH trituration.

This study shows that a significant difference exists between the NMR spectra of Kalium Bichromicum, which have been potentised to 12CH potency, from initial 3CH and 4CH trituration. This difference is observable between all the peaks of the respective samples in both the chemical shift and relative integration values, with the exception of the CH_2 peak when comparing the relative integration values. This peak shows an insignificant difference, however the two samples groups remain different.

From this data, one can then assume that the trituration process causes physical alterations of the substance. These alterations are attenuated through the serial dilution, giving rise to two different substances, even though both were potentised to the 12^{th} centesimal deconcentration level. These results correlate with the findings of Resch and Gutman (1987). They found that the act of grinding has a profound influence on the physical properties of the system. It produces a highly developed systems organization, whereby the energy of the grinding process is absorbed by the system, strengthening the system organization, integrating the solute into the lactose solvent and preserving the characteristics of the solvent even beyond the level at which

any trace of the original molecular substance is present. The quality of the remedy's information is better preserved the greater the attenuation.

When comparing the NMR spectra of the lactose (control) groups, which have been potentised to 12CH potency, from initial 3CH and 4CH trituration, a similar trend is observable. The level of significance of the differences observed is less, however, and the only significant differences can be observed in the chemical shift values of the CH_2 and CH_3 peaks, and the relative integration values of the CH_2 peak.

One can thus theorise, that the trituration process does cause structural changes in the lactose carrier as well, but that the changes are not as marked as those alterations imprinted onto the medicinal source. The structural changes are attributable to the strengthening of the systems organization. The introduction of a solid solution of lactose, when mixed with lactose results in the preservation of the structural information. If another solute is mixed in, e.g. Kalium Bichromicum, the solid modifies the structural framework of the lactose, which accepts and preserves the structural information of the solid. This results in the establishment of a new systems organization with dynamically better developed static aspects of order than the pure lactose, conserving the aspects of order in the highly potentized drug. (Resch and Gutman, 1987)

The NMR spectra of Kalium Bichromicum prepared respectively from an initial soluble form and from a 3CH trituration, show a difference in both the

chemical shift and the relative integration values of the four peaks. A significant difference is observable in the chemical shift values of the OH, CH_2 and CH_3 peaks. The H_20 peaks are however identical. The relative integration values show a significant difference present between the H_2O and OH peaks, and a statistically insignificant difference present in the CH_2 and CH_3 peaks.

This leads the researcher to assume that trituration process alters the structure of the medicinal source and that the two methods of remedy manufacture, i.e. from a soluble substance and through trituration, does give rise to two different substances even when they are potentised to the exact same level. The manufacturing method alters the physical properties of the substance, hence giving rise to different NMR spectra. At the 12CH level, a deconcentration level greater than Avogadro's number is reached. There is thus no molecular trace of the original substance present in the solution. The structural changes are thus present in the systems organisation of the water-ethanol mixture. (Resch and Gutman, 1987)

When comparing the NMR spectra of parallel potencies of Kalium Bichromicum and lactose (control) manufactured from initial 3CH and 4CH triturate respectively, a difference can be observed for all the chemical shift values, but the difference is more marked between those triturated to the 4CH level, where all the differences are statistically significant. The relative integration values show similar trends for both the samples manufactured from an initial 3CH and 4CH level.

The assumption can hence be made that the higher the level of trituration, the more pronounced the alteration of the physical structure of the active ingredient becomes, i.e. a difference that may be present between the lactose and the Kalium Bichromicum at a 3CH trituration level will be accentuated at the 4CH trituration level.

CHAPTER 6 - CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The hypothesis, that significant differences exist between the chemical shift (δ) and relative integration values of the CH_2, CH_3, H_2O and OH signals of the 12CH potency level which have been manufactured from a soluble form, a 3CH and a 4CH triturate, and that these indicate differences inherent to each trituration level, has thus been proven in this trial.

This trial has also accepted the hypothesis that the level of trituration plays an important part in the development of distinct physicochemical properties specific to homoeopathic remedies. There thus exists statistically significant differences between parallel potencies in terms of substance versus substance, substance versus control and control versus control, with regard to chemical shift (δ) and relative integration values of the CH_2, CH_3, H_2O and OH signals of respective trituration levels.

6.2 RECOMMENDATIONS

Due to the nature of homoeopathic remedies, and the number of unknown factors that may influence them, strict scientific methods must be employed in the study of them. If these factors can be minimized, NMR spectroscopy could be a valuable tool in the study of these remedies.

To assist in the formulation of a scientific method, and to help in the analysis of the findings, the following aspects need to be addressed:

1. A standard method of preparation

The same methods must be followed in the preparation of the samples to ensure that the results of separate studies can be compared. Some of the variables during the manufacturing process need to be eliminated, by preparing the remedies under the same conditions, using the same pharmacopoeia. Hand trituration could be substituted by machine trituration to limit the exposure of the substances and to standardize the trituration process e.g. the pressure applied when triturating.

2. Analyse more samples

Analysis of more samples manufactured over a longer period of time can give an average view of the different batches manufactured in different seasons, and even by different people to understand the influence these factors may have on the remedies. Time taken between sampling could also influence the readings and sampling times must be kept to a minimum

to ensure a uniform batch of readings for analysis. This will also ensure better accuracy of the statistical analysis.

3. Further studies into C4 potencies

In view of the results obtained in this study, C4 potencies are an avenue worth exploring. They offer a new way of experiencing existing remedies. A Hahnemannian proving of these potencies may give them more credibility in the world of classical homoeopathy and may offer new insights into remedies.

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APPENDICES

Appendix A: The preparation of Sample Potencies

(Adapted From German Homoeopathic Pharmacopoeia, 1985 and Hahnemann, 1991)

i) Method 6 - Triturations

Aim: To produce, by hand, a 4CH trituration from Kalium Bichromicum.

Apparatus:

Unglazed porcelain pestle and mortar

Steel spatula

Mass balance (accurate and calibrated)

Cigarette lighter

Consumables:

96% alcohol (for flaming)

Clean, empty vials

Filter paper

Labels

Ingredients:

Lactose BP powder

Potassium dichromate B.P. (Appendix B)

Method:

All apparatus and utensils must be clean and odourless

1. Clean the mortar and pestle and spatula with distilled water, and flame with 96% alcohol.

2. Allow mortar and pestle to cool sufficiently before use.

3. Place a new piece of filter paper on the scale and tare it.

4. Mass 0.1 g of Potassium dichromate onto filter paper.

5. Place a new piece of filter paper on the scale and tare it.

6. Mass 3.3g of pure lactose powder onto filter paper.

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 of lactose into mortar and triturate for a short period.

9. Add the 0.1g crude Potassium dichromate into the mortar.

10. Triturate for 6 minutes and scrape down for 4 minutes with a porcelain spatula. Then triturate for 6 minutes and scrape down for 4 minutes. (Trituration time: 2 x 10min = 20min)

11. Add the second portion of 3.3g of lactose powder. Continue as in step 10 above.

12. Finally add the third portion of 3.3g of lactose. Proceed as in step 10 above. (Total trituration time: 20min x 3 = 60min)

13. Place triturate in a vial and label as Kalium Bichromicum 1CH.

14. Repeat steps 1-13 when preparing Kalium Bichromicum 2CH, 3CH and 4CH, replacing crude Kalium Bichromicum with Kalium Bichromicum 1CH, 2CH and 3CH respectively at each dilution level.

ii) Method 8a – Liquid preparations from triturations

a) Aim:

To produce liquid dilutions of Kalium Bichromicum 12CH from the 3CH trituration.

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:

30% alcohol

87% alcohol

Distilled water

Kalium Bichromicum 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 0.1 g of Kalium Bichromicum 3CH on the filter paper. Place it in a 25 ml amber bottle.

3. Add 9.9ml of distilled water and succuss 10 times without stopping. Label as Kalium Bichromicum 4CH.

4. Place 99 parts 87% alcohol in a 5ml clear glass screw top bottle. (99/100 x 3ml = 2.97ml). Add 1 part Kalium Bichromicum 4CH. (1/100 x 3ml = 0.03 ml). Succuss 10 times without stopping. Label as Kalium Bichromicum 5CH.

5. Repeat step 4 to produce Kalium Bichromicum 6CH – 11CH.

6. To prepare Kalium Bichromicum 12CH place 99 parts 87% alcohol in a 25ml amber glass reagent bottle. (99/100 x 16ml = 15.84ml). Add 1 part Kalium Bichromicum 11 CH. Succuss 10 times without stopping. Label as Kalium Bichromicum 12CH.

7. Store Kalium Bichromicum 12CH in a cool environment free from any electromagnetic disturbance until it can be transported for NMR Spectroscopy.

b) Aim:

To produce liquid dilutions of Kalium Bichromicum 12CH from the 4CH trituration.

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:

30% alcohol

87% alcohol

Distilled water

Kalium Bichromicum 4CH 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 0.1 g of Kalium Bichromicum 4CH on the filter paper. Place it in a 25 ml amber bottle.

3. Add 9.9ml of distilled water and succuss 10 times without stopping. Label as Kalium Bichromicum 5CH.

4. Place 99 parts 87% alcohol in a 5ml clear glass screw top bottle. (99/100 x 3ml = 2.97ml). Add 1 part Kalium Bichromicum 5CH. (1/100 x 3ml = 0.03 ml). Succuss 10 times without stopping. Label as Kalium Bichromicum 6CH.

5. Repeat step 4 to produce Kalium Bichromicum 7CH – 11CH.

6. To prepare Kalium Bichromicum 12CH place 99 parts 87% alcohol in a 25ml amber glass reagent bottle. (99/100 x 16ml = 15.84ml). Add 1 part Kalium Bichromicum 11 CH. Succuss 10 times without stopping. Label as Kalium Bichromicum 12CH.

7. Store Kalium Bichromicum 12CH in a cool environment free from any electromagnetic disturbance until it can be transported for NMR Spectroscopy.

iii) Method 5 - Soluble

Aim:

To prepare Kalium Bichromicum 12CH from soluble Kalium Bichromicum.

Apparatus:

Spatula

Mass balance (accurate and calibrated)

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

Rubber dropper bulbs

Consumables:

5ml screw top bottles

25ml amber bottles

Paper towelling

Labels and pens

Pasteur pipettes

Filter paper

Ingredients:

Potassium dichromate B.P. (Appendix B)

Purified water

15% ethanol

87% ethanol

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 Potassium dichromate using mass balance and place into first 5ml screw top bottle. Place 2.97g (3.0455 ml) 15 % ethanol into first 5ml screw top bottle using 5ml pipette.

4. Succuss ten times and label “Kalium Bichromicum 1CH”.

5. Place 2.97ml 87% ethanol into second new 5ml screw top bottle. Add 0.03ml of Kalium Bichromicum 1CH into second screw top bottle using a clean Pasteur pipette.

6. Succuss ten times and label Kalium Bichromicum 2CH.

7. Repeat the above procedure 5-6 up to 11CH.

8. Place 15.84ml 87% ethanol into 25ml amber dropper bottle using 5ml pipette, 2ml pipette, and 10ml measuring cylinder.

9. Place 0,16ml of 11CH into amber 25ml bottle using 2ml pipette.

10. Succuss ten times and label Kalium Bichromicum 12CH.

11. Store Kalium Bichromicum 12CH in a cool environment free from any electromagnetic disturbance until it can be transported for NMR Spectroscopy.

Appendix B: Kalium Bichromicum Monograph

Adapted from Varma & Vaid, 2002

KALI BICHROMICUM

(Bichromate of potash)

$K_2Cr_2O_7$

Mol. wt.: 294.19

Synonyms:

English: Potassium bichromate.

French: Bichromate de potasse.

German: Kaliumbichromat.

Description: Large, orange-red, transparent, crystals or crystalline powder, odourless, taste metallic, stable in air, soluble in water, insoluble in alcohol, commonly prepared from chrome iron ore. Contains not less than 99.0 per cent of $K_2Cr_2O_7$ with reference to the substance dried to constant weight at 105°.

Reaction:

5 per cent aqueous solution is acidic to litmus.

Identification:

(i) To 5 ml of 5 per cent solution add lead acetate solution, a yellow precipitate is formed.

(ii) To 5 ml of above solution add silver nitrate solution, a red precipitate is obtained.

(iii) To 5 ml add 20 ml water and 5 ml hydrochloric acid and gradually 1 ml alcohol, the solution turns green.

Aluminium and calcium:

2 g dissolved in 20 ml of water shows no turbidity on making distinctively alkaline with solution of ammonia and adding ammonium oxalate solution.

Chloride:

2 g complies with the limit test for chlorides.

Sulphate:

1 g complies with the limit test for sulphates.

Assay:

Dissolve about 0.2g accurately weighed in 25ml freshly boiled and cooled water in a glass stoppered flask, add 2 g of potassium iodide and 10 ml of hydrochloric acid and allow to stand in the dark for ten minutes. Add about 200 ml of freshly boiled and cooled water and titrate with 0.1 N sodium

thiosulphate, using starch as indicator. Each ml of 0.1 N sodium thiosulphate is equivalent to 0.004904 9 of $K_2Cr_2O_7$.

Preparation:

(a) Mother Solution Ø Drug strength 1/10

Kali Bichromicum 100 g

Purified Water in sufficient quantity to make one liter of the Mother Solution.

(b) Potencies: 2x and 3x to be prepared in Purified Water. 4x and higher with Dispensing Alcohol.

Storage:

Below 3x fresh preparation of this salt should be used. Should be discarded if there is discolouration, sedimentation or visible particles.

Prescribed dose:

Third Trituration, also thirtieth attenuation and higher. The lower preparations of this salt should not be kept too long

INDEX	FREQUENCY	PPM	HEIGHT
1	2364.661	4.729	11.6
2	2000.842	4.002	34.7
3	1514.612	3.029	19.3
4	1507.777	3.016	53.0
5	1500.631	3.001	51.6
6	1493.795	2.988	19.7
7	290.180	0.580	83.6
8	286.763	0.574	11.5
9	283.034	0.566	135.0
10	275.889	0.552	76.4

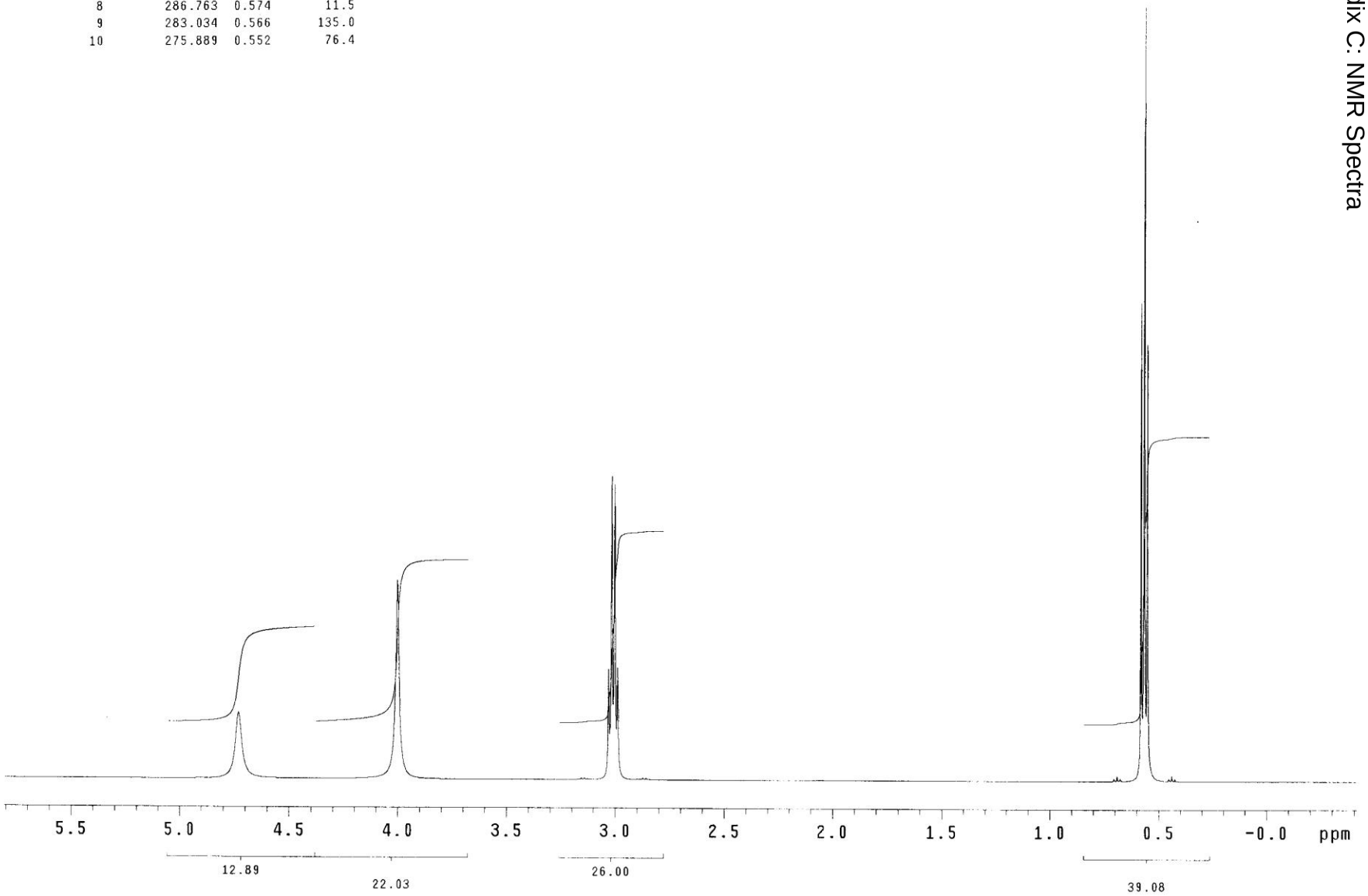


Figure 1: NMR Spectra of Kalium Bichromicum 12CH from Soluble Potassium Dichromate – Run 1

INDEX	FREQUENCY	PPM	HEIGHT
1	2364.661	4.729	11.7
2	2000.842	4.002	34.7
3	1514.612	3.029	19.3
4	1507.777	3.016	53.0
5	1500.631	3.001	51.6
6	1493.795	2.988	19.6
7	289.870	0.580	83.6
8	286.763	0.574	11.5
9	283.034	0.566	135.0
10	275.889	0.552	76.3

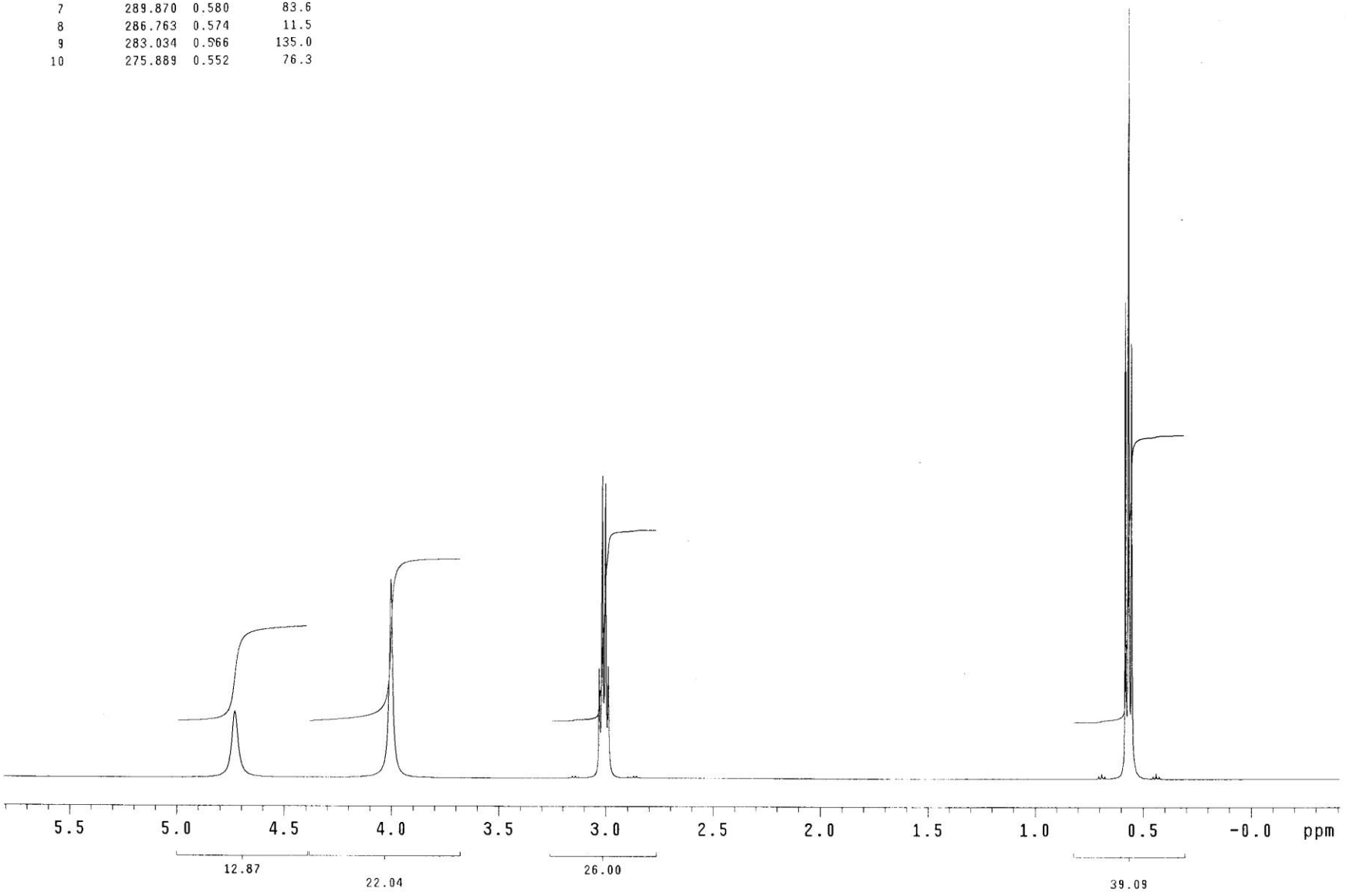


Figure 2: NMR Spectra of Kalium Bichromicum 12CH from Soluble Potassium Dichromate – Run 2

INDEX	FREQUENCY	PPM	HEIGHT
1	2364.661	4.729	11.6
2	2000.842	4.002	34.7
3	1514.612	3.029	19.3
4	1507.777	3.016	52.9
5	1500.631	3.001	51.5
6	1493.795	2.988	19.6
7	290.180	0.580	83.5
8	286.763	0.574	11.5
9	283.034	0.566	135.0
10	275.889	0.552	76.2

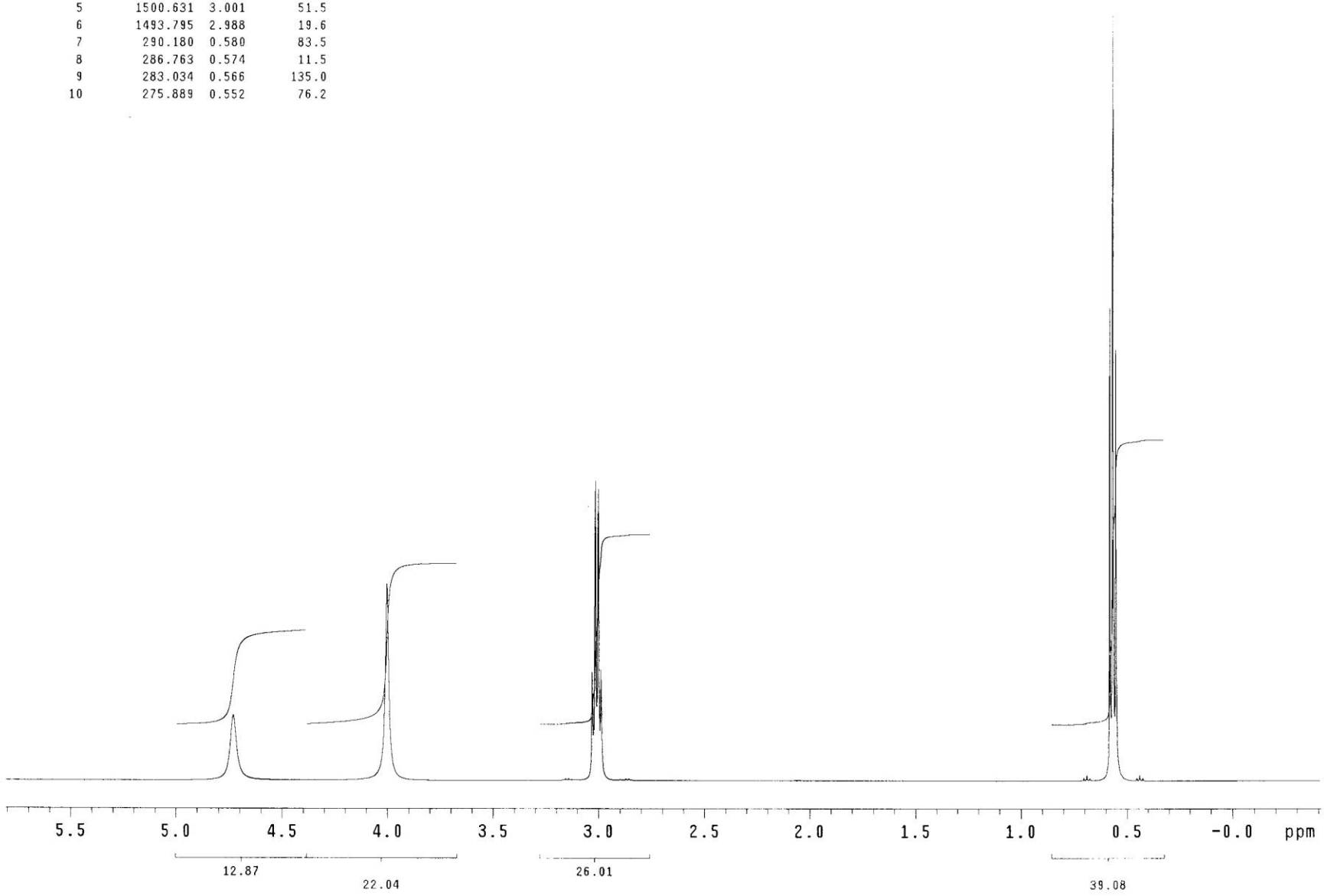


Figure 3: NMR Spectra of Kalium Bichromicum 12CH from Soluble Potassium Dichromate - Run 3

INDEX	FREQUENCY	PPM	HEIGHT
1	2364.350	4.729	15.5
2	2000.221	4.001	49.1
3	1513.369	3.027	18.0
4	1506.534	3.013	48.2
5	1499.699	3.000	46.9
6	1492.553	2.985	17.7
7	288.627	0.577	85.9
8	282.102	0.564	136.7
9	274.646	0.549	77.4

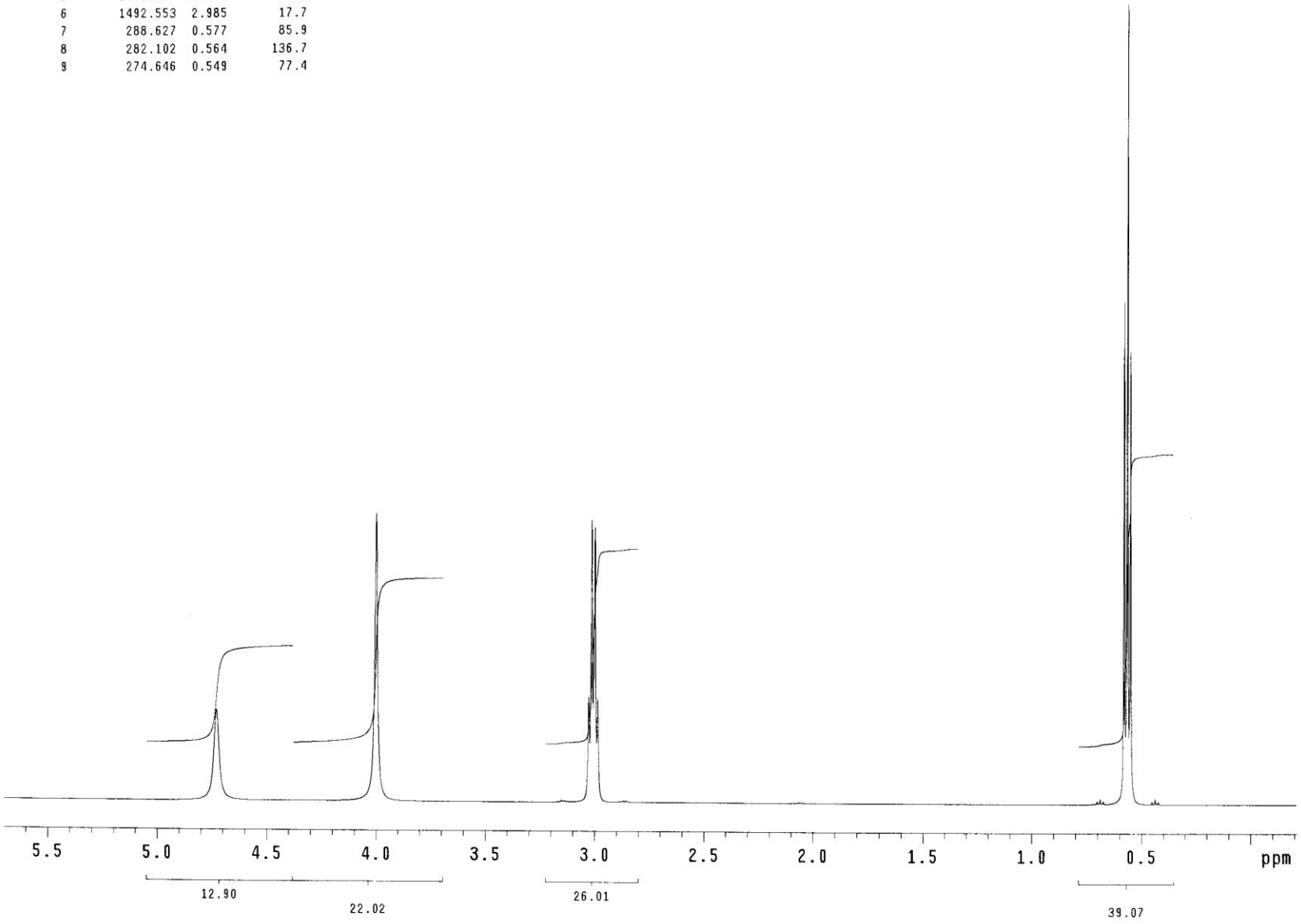


Figure 4: NMR Spectra of Kalium Bichromicum 12CH from 3CH trituration – Run 1

INDEX	FREQUENCY	PPM	HEIGHT
1	2364.350	4.729	15.6
2	2000.221	4.001	49.5
3	1513.369	3.027	18.1
4	1506.534	3.013	48.4
5	1499.699	3.000	47.0
6	1492.553	2.985	17.7
7	288.627	0.577	88.0
8	281.792	0.564	138.0
9	274.646	0.549	79.0

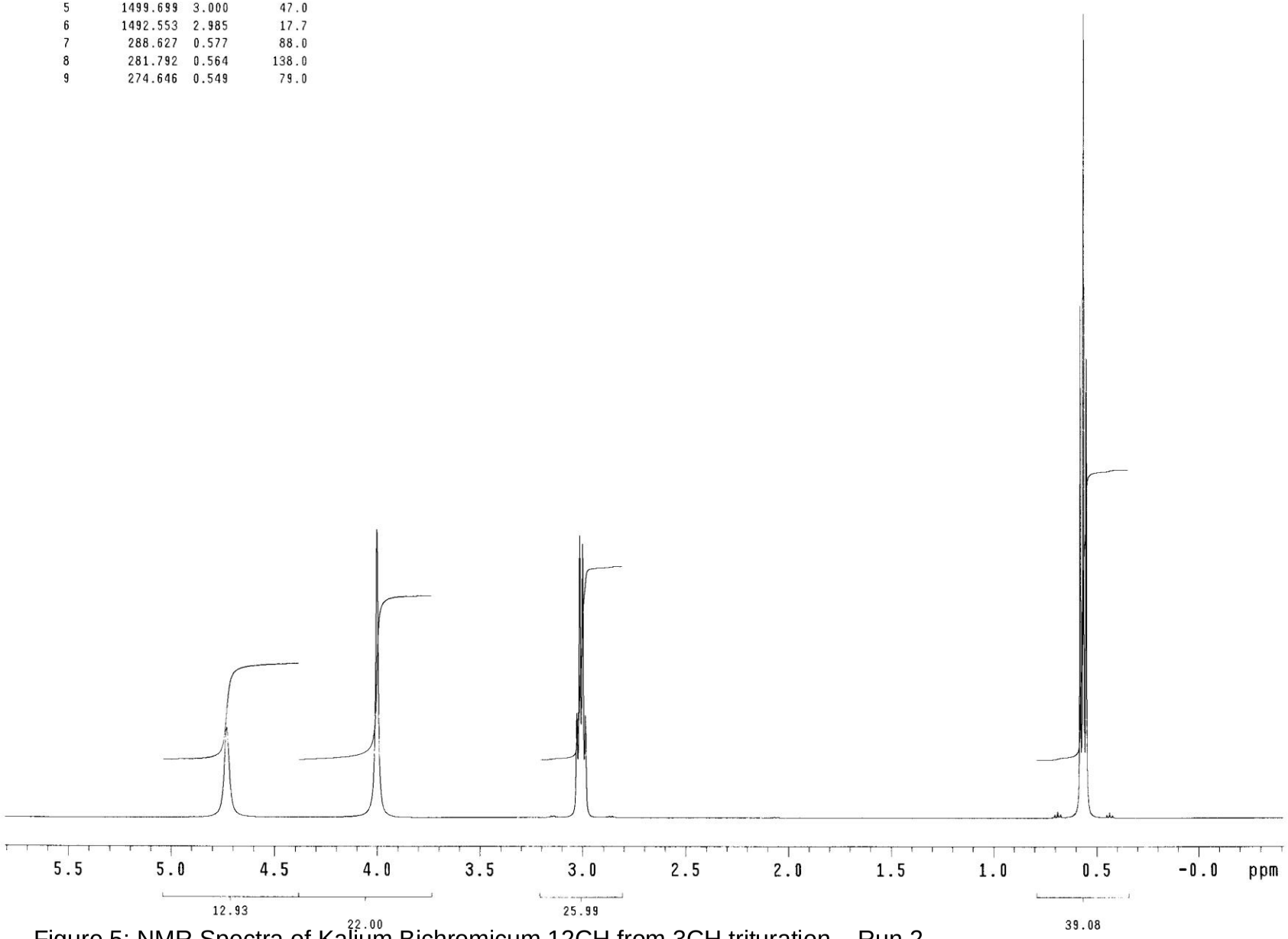


Figure 5: NMR Spectra of Kalium Bichromicum 12CH from 3CH trituration – Run 2

INDEX	FREQUENCY	PPM	HEIGHT
1	2364.350	4.729	15.2
2	2000.221	4.001	48.3
3	1513.369	3.027	17.6
4	1506.534	3.013	47.1
5	1499.699	3.000	45.7
6	1492.553	2.985	17.2
7	288.627	0.577	86.2
8	281.792	0.564	135.0
9	274.646	0.549	77.4

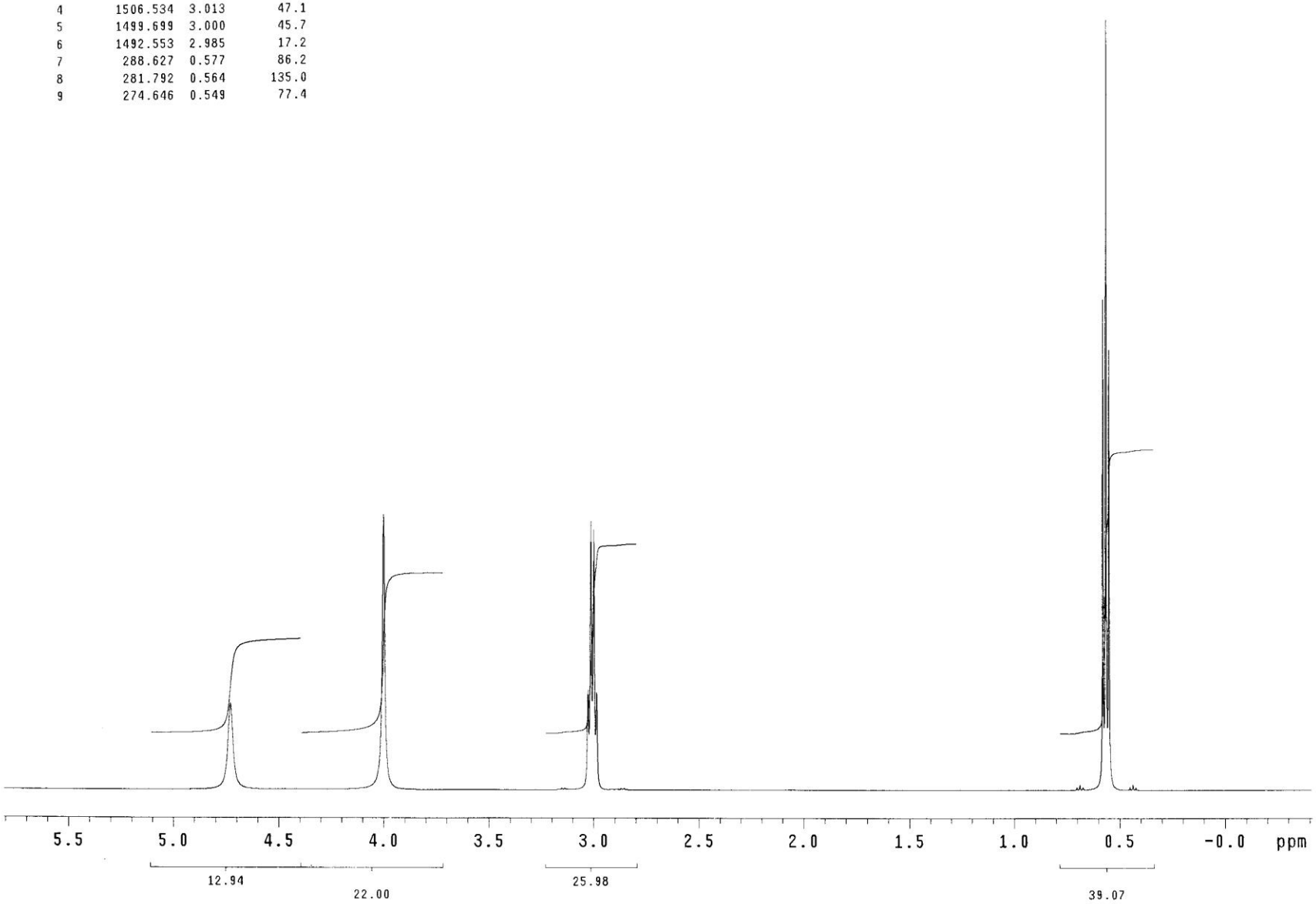


Figure 6: NMR Spectra of Kalium Bichromicum 12CH from 3CH trituration – Run 3

INDEX	FREQUENCY	PPM	HEIGHT
1	2365.282	4.731	18.2
2	2001.153	4.002	59.1
3	1514.612	3.029	17.6
4	1507.466	3.015	46.6
5	1500.631	3.001	45.5
6	1493.485	2.987	16.9
7	289.559	0.579	84.1
8	282.413	0.565	135.0
9	275.267	0.551	76.2

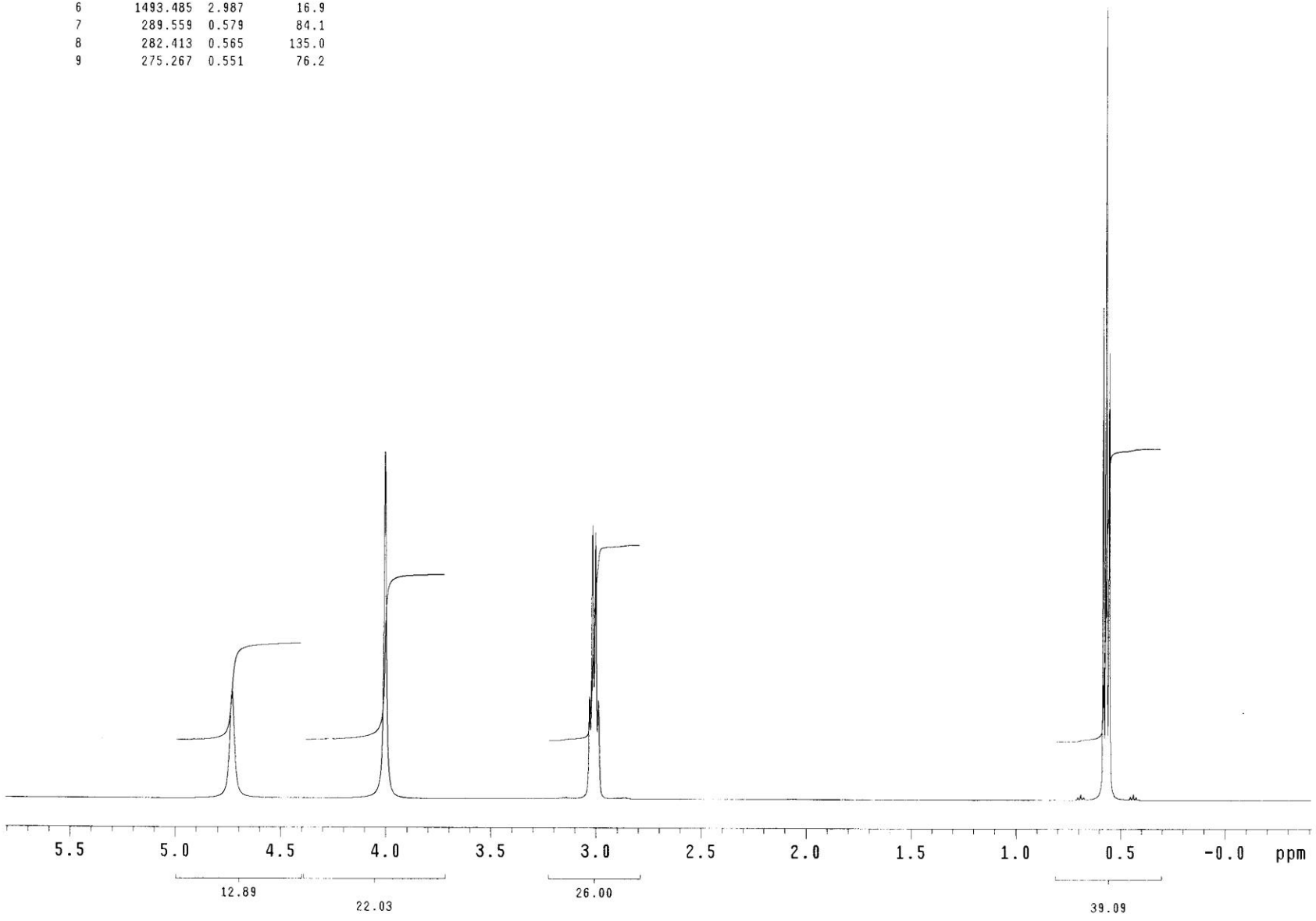


Figure 7: NMR Spectra of Kalium Bichromicum 12CH from 4CH trituration – Run 1

INDEX	FREQUENCY	PPM	HEIGHT
1	2365.282	4.731	18.3
2	2001.153	4.002	59.1
3	1514.612	3.029	17.6
4	1507.466	3.015	46.7
5	1500.631	3.001	45.7
6	1493.485	2.987	16.9
7	289.559	0.579	84.2
8	282.413	0.565	135.0
9	275.267	0.551	76.2

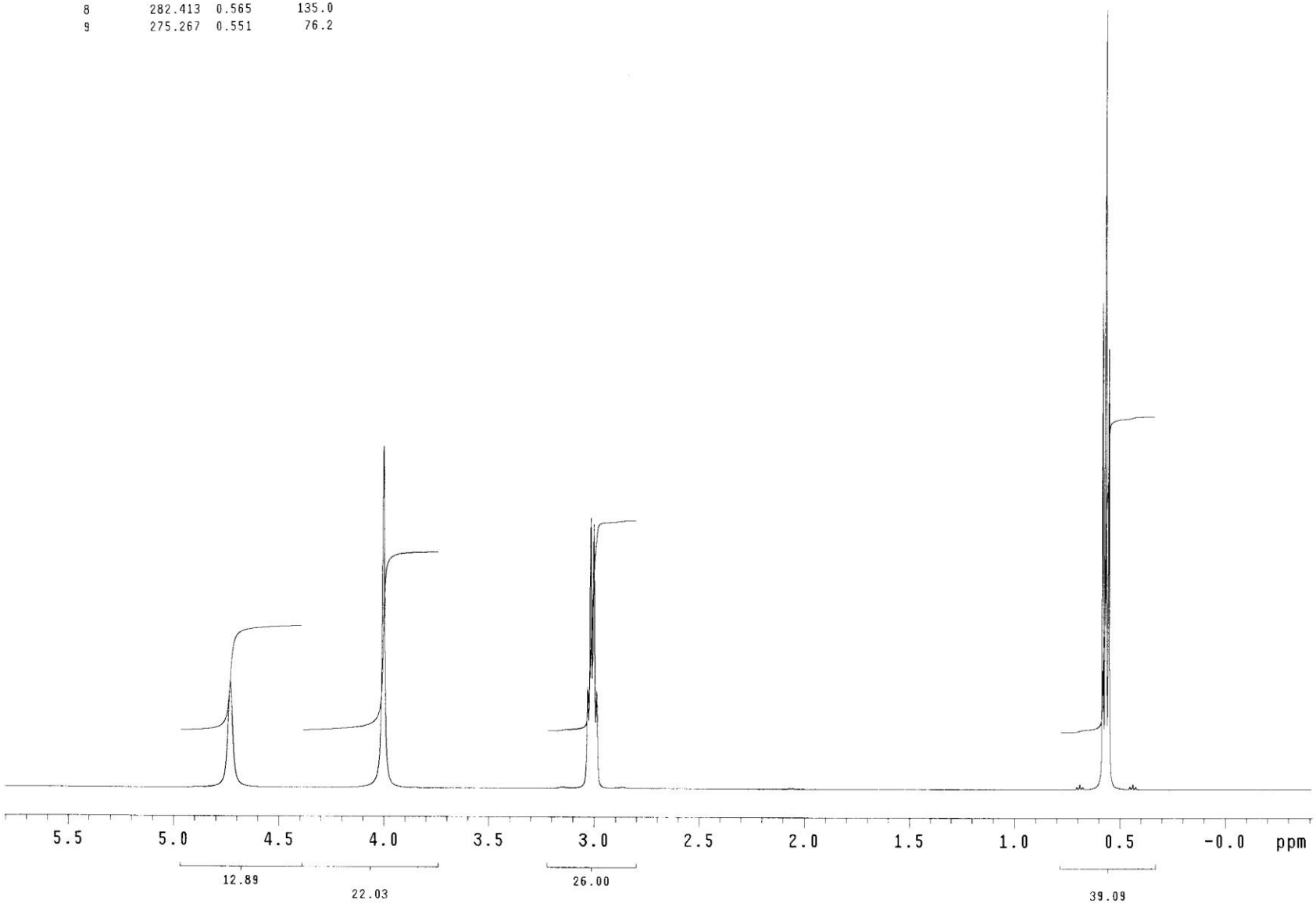


Figure 8: NMR Spectra of Kalium Bichromicum 12CH from 4CH trituration – Run 2

INDEX	FREQUENCY	PPM	HEIGHT
1	2365.282	4.731	18.2
2	2001.153	4.002	58.8
3	1514.612	3.029	17.6
4	1507.466	3.015	46.7
5	1500.631	3.001	45.6
6	1493.485	2.987	16.9
7	289.559	0.579	84.1
8	282.413	0.565	135.0
9	275.267	0.551	76.3

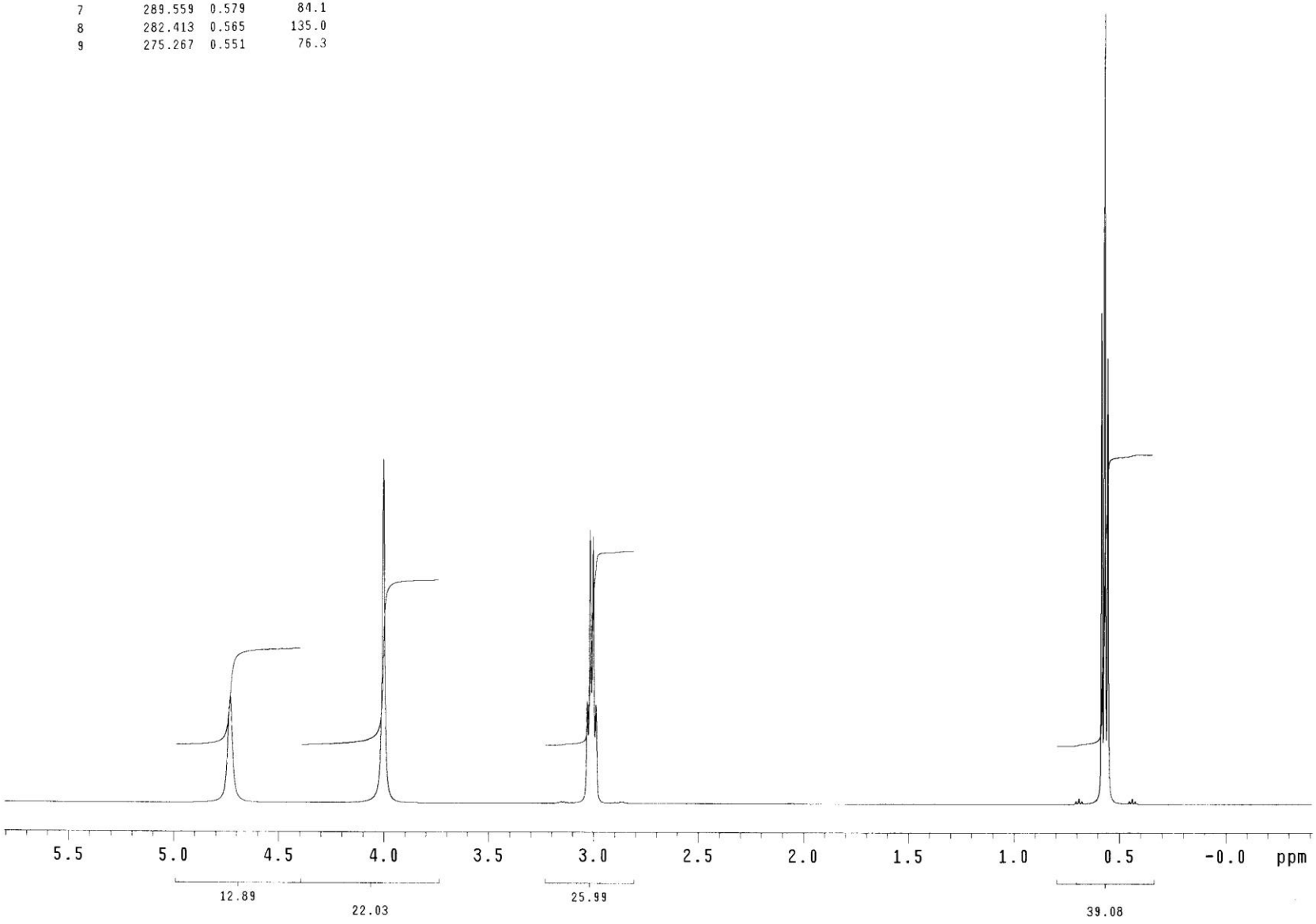


Figure 9: NMR Spectra of Kalium Bichromicum 12CH from 4CH trituration – Run 3

INDEX	FREQUENCY	PPM	HEIGHT
1	2363.418	4.727	9.9
2	1999.600	3.999	30.0
3	1513.680	3.027	15.5
4	1506.844	3.014	43.9
5	1499.699	3.000	43.3
6	1492.553	2.985	16.5
7	289.248	0.579	86.0
8	282.102	0.564	135.0
9	274.956	0.550	78.2

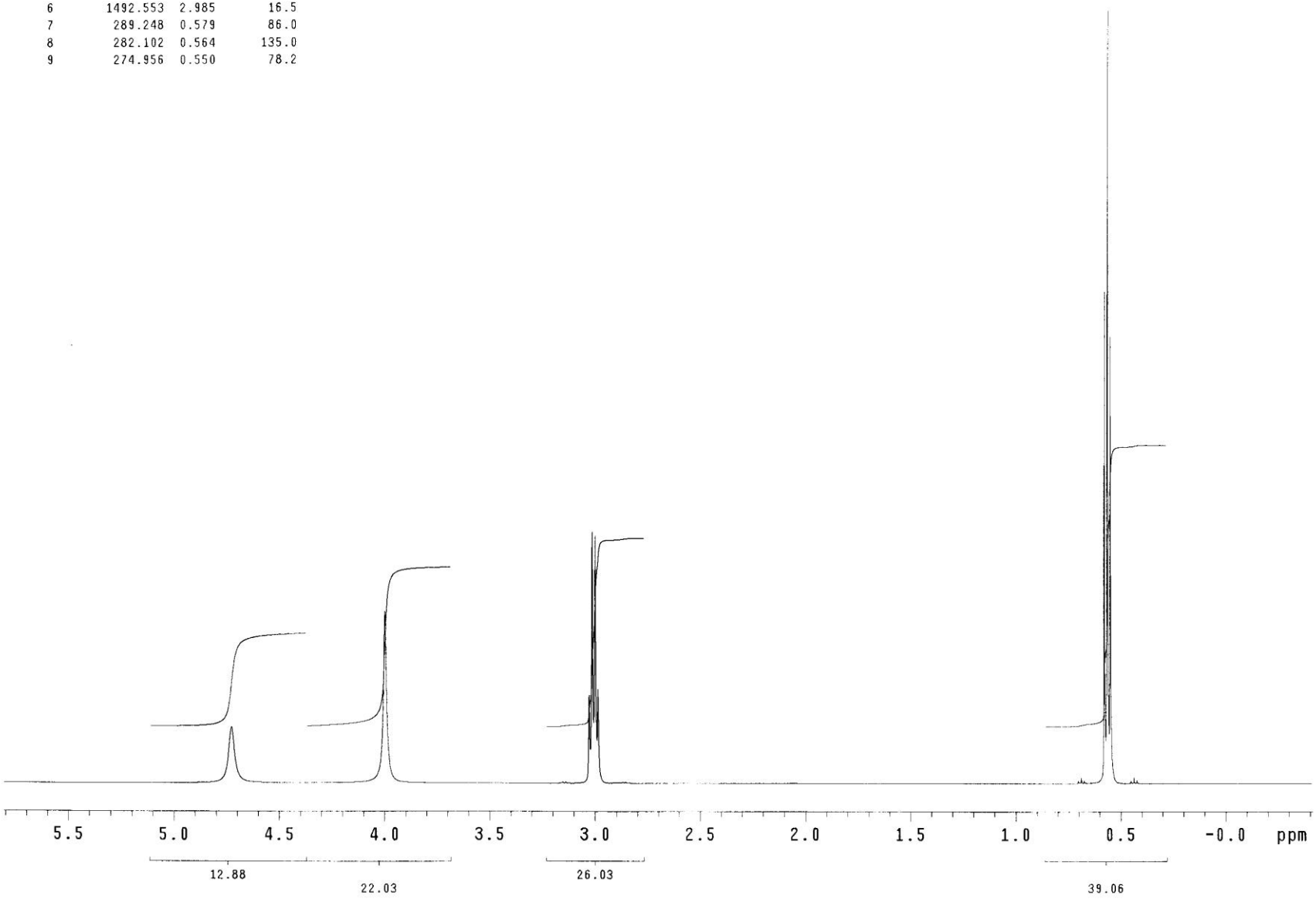


Figure 10: NMR Spectra of lactose 12CH from 3CH trituration – Run 1

INDEX	FREQUENCY	PPM	HEIGHT
1	2363.418	4.727	10.3
2	1999.600	3.999	31.1
3	1513.680	3.027	16.0
4	1506.844	3.014	45.2
5	1499.699	3.000	44.6
6	1492.553	2.985	17.0
7	289.248	0.579	86.2
8	282.102	0.564	135.0
9	274.956	0.550	78.0

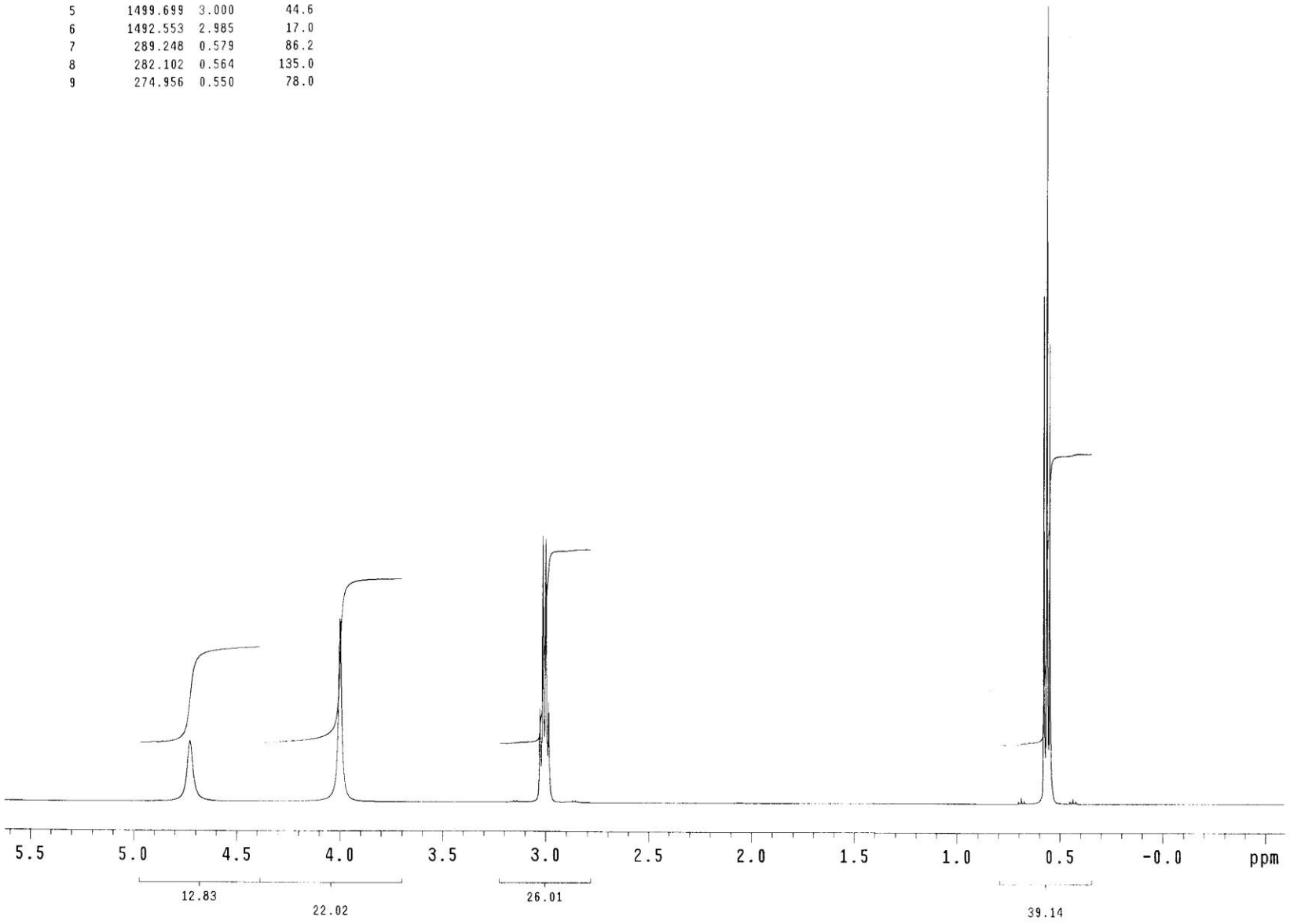


Figure 11: NMR Spectra of lactose 12CH from 3CH trituration – Run 2

INDEX	FREQUENCY	PPM	HEIGHT
1	2364.922	4.730	10.3
2	2001.103	4.002	31.2
3	1515.183	3.030	15.9
4	1508.348	3.017	44.9
5	1501.202	3.003	44.4
6	1494.056	2.988	17.0
7	290.752	0.582	86.6
8	283.606	0.567	135.0
9	276.460	0.553	78.0

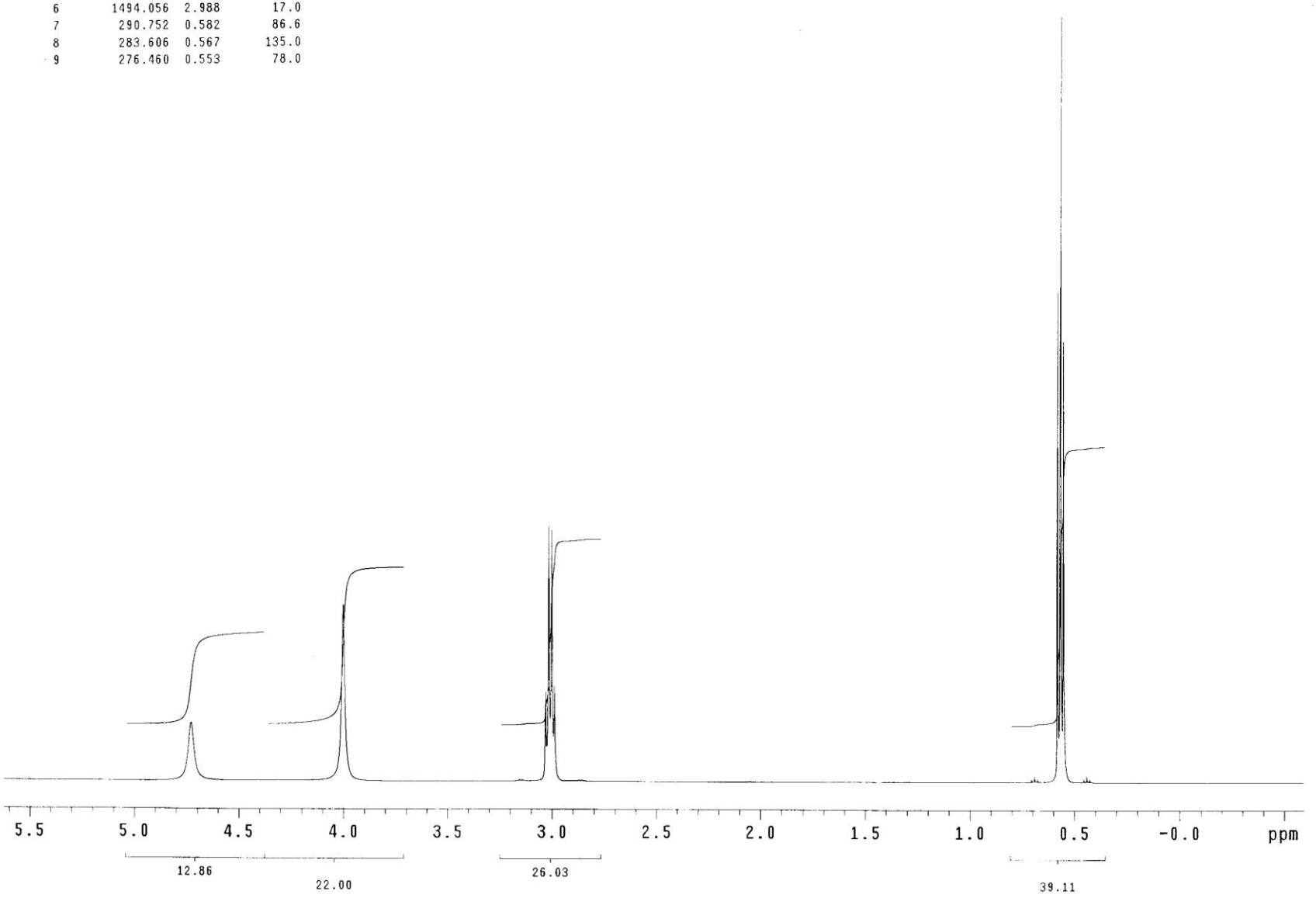


Figure 12: NMR Spectra of lactose 12CH from 3CH trituration – Run 3

INDEX	FREQUENCY	PPM	HEIGHT
1	2363.418	4.727	15.7
2	1999.600	3.999	49.9
3	1513.058	3.026	17.0
4	1505.912	3.012	46.1
5	1499.077	2.998	45.2
6	1491.931	2.984	17.1
7	288.316	0.577	88.8
8	281.170	0.562	140.0
9	274.024	0.548	78.8

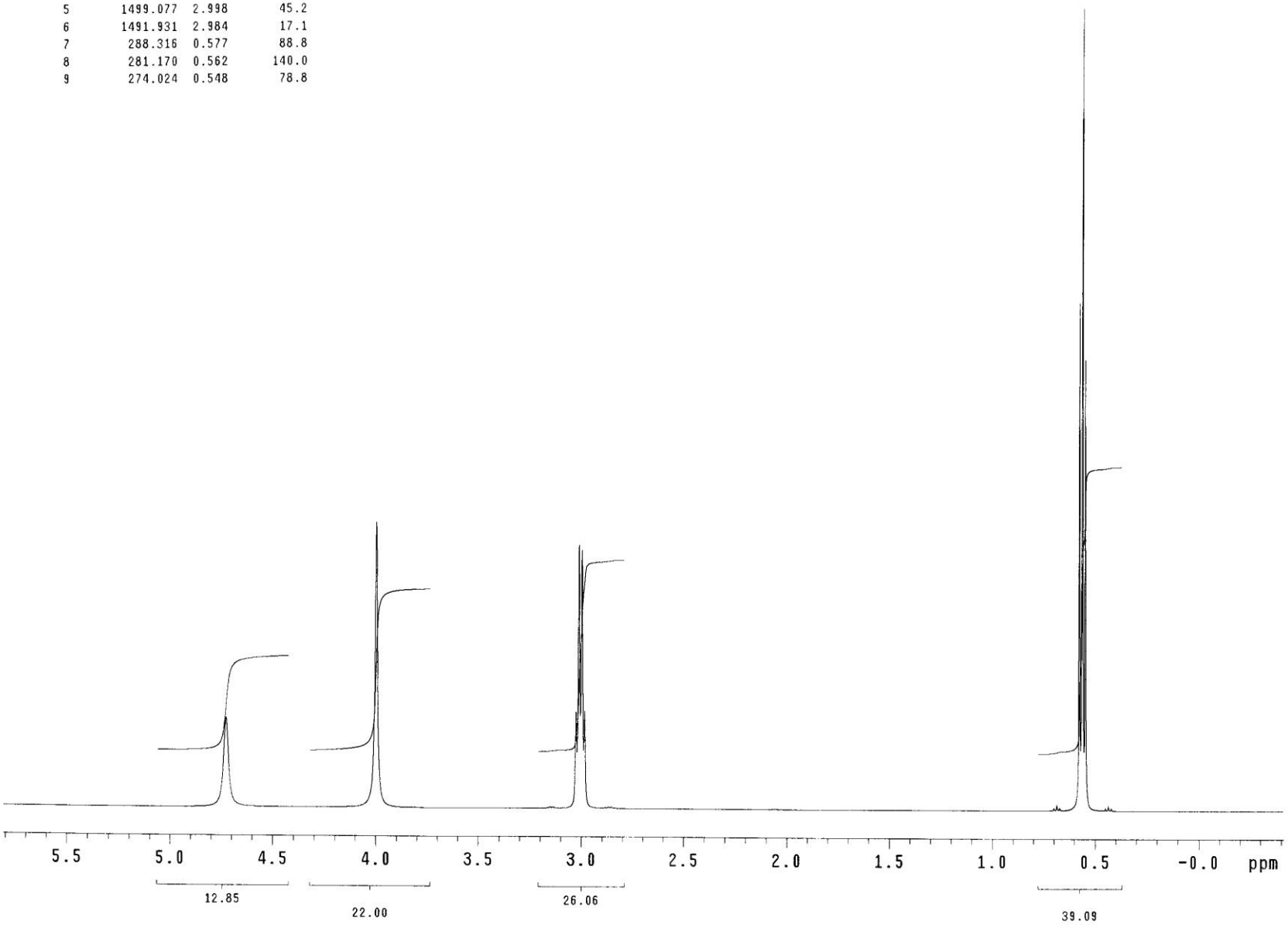


Figure 13: NMR Spectra of lactose 12CH from 4CH trituration – Run 1

INDEX	FREQUENCY	PPM	HEIGHT
1	2363.418	4.727	15.7
2	1999.600	3.999	50.0
3	1513.058	3.026	16.7
4	1505.912	3.012	45.4
5	1499.077	2.998	44.5
6	1491.931	2.984	16.8
7	288.316	0.577	89.3
8	281.170	0.562	140.0
9	274.024	0.548	78.8

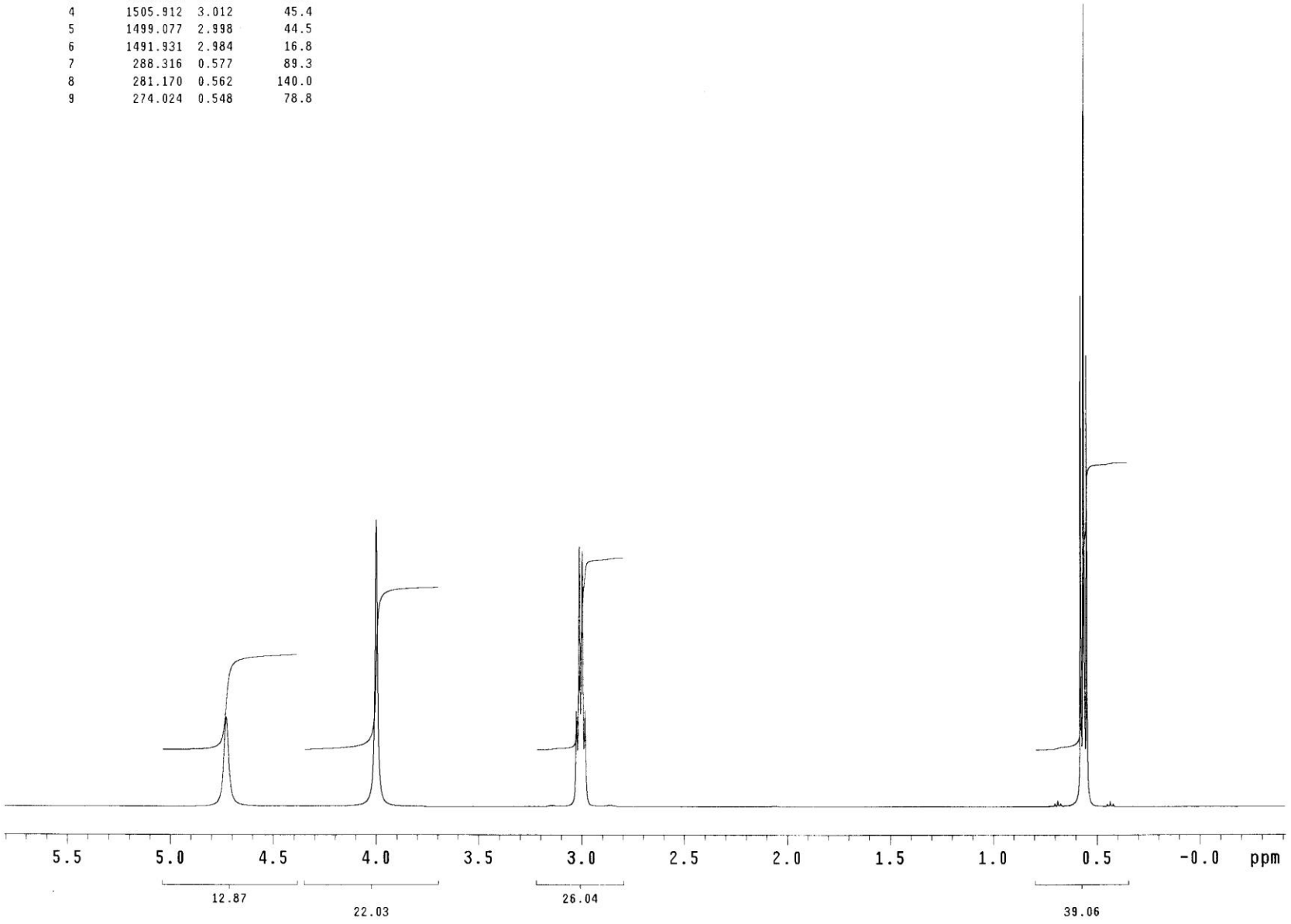
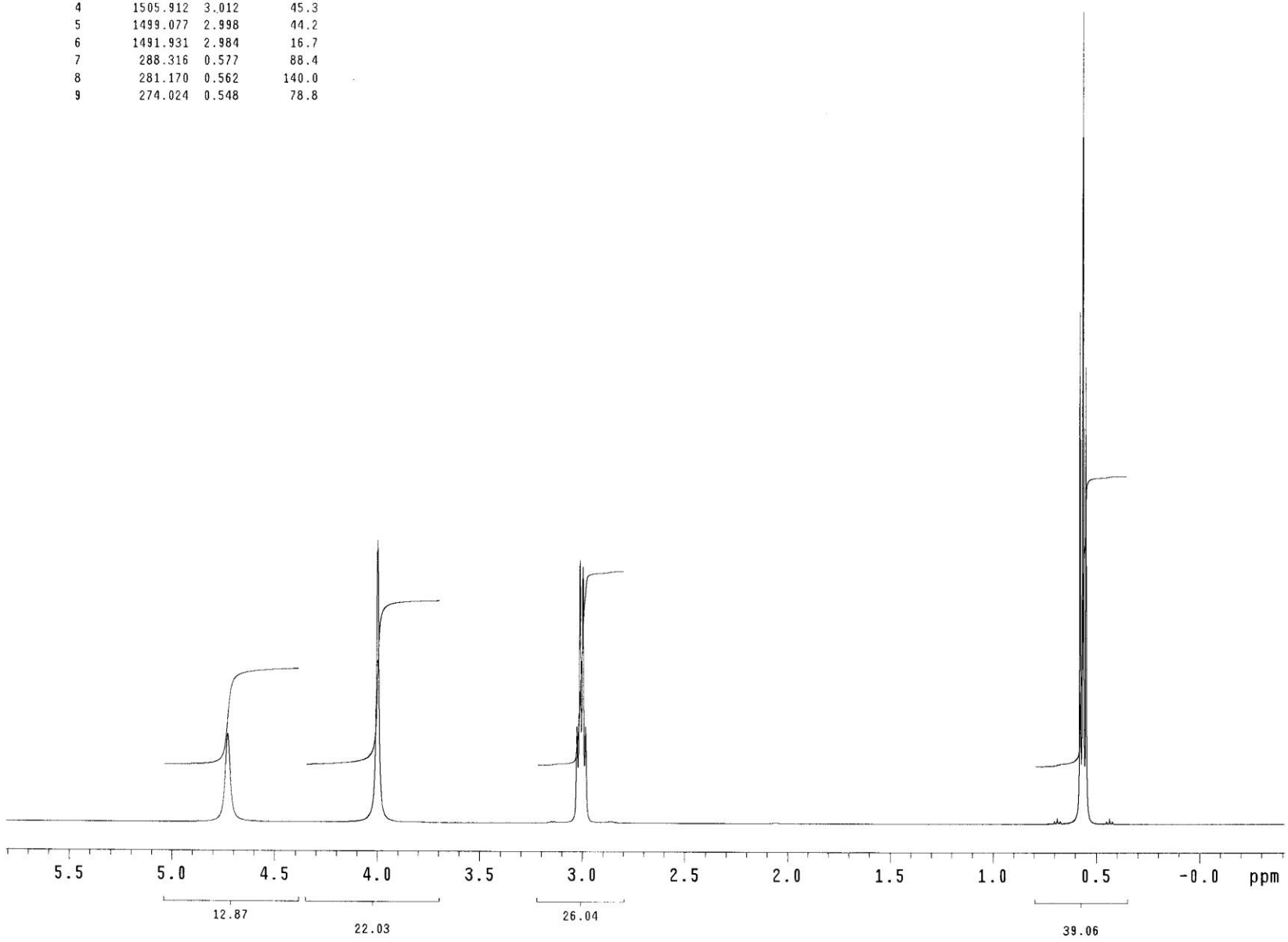


Figure 14: NMR Spectra of lactose 12CH from 4CH trituration – Run 2

INDEX	FREQUENCY	PPM	HEIGHT
1	2363.729	4.728	15.3
2	1999.599	3.999	48.6
3	1512.747	3.026	16.7
4	1505.912	3.012	45.3
5	1499.077	2.998	44.2
6	1491.931	2.984	16.7
7	288.316	0.577	88.4
8	281.170	0.562	140.0
9	274.024	0.548	78.8



Appendix D: Summary of the data obtained from the NMR spectroscopy

		Chemical Shift			Relative Integration		
	Run	1	2	3	1	2	3
H2O	Kalium Bichromicum Soluble	4.729	4.729	4.729	0.128900	0.128700	0.128700
	Kalium Bichromicum From a 3CH	4.729	4.729	4.729	0.129000	0.129300	0.129413
	Kalium Bichromicum From a 4CH	4.731	4.731	4.731	0.128887	0.128887	0.128913
	Lactose from a 3CH	4.727	4.727	4.730	0.128800	0.128600	0.128300
	Lactose from a 4CH	4.727	4.727	4.728	0.128500	0.128700	0.128700
OH	Kalium Bichromicum Soluble	4.002	4.002	4.002	0.220300	0.220400	0.220400
	Kalium Bichromicum From a 3CH	4.001	4.001	4.001	0.220200	0.220000	0.220022
	Kalium Bichromicum From a 4CH	4.002	4.002	4.002	0.220278	0.220278	0.220322
	Lactose from a 3CH	3.999	3.999	4.002	0.220300	0.220000	0.220200
	Lactose from a 4CH	3.999	3.999	3.999	0.220000	0.220300	0.220300
CH2	Kalium Bichromicum Soluble	3.0085	3.0085	3.0085	0.260000	0.260000	0.260100
	Kalium Bichromicum From a 3CH	3.0063	3.0063	3.0063	0.260100	0.259900	0.259826
	Kalium Bichromicum From a 4CH	3.0080	3.0080	3.0080	0.259974	0.259974	0.259926
	Lactose from a 3CH	3.0065	3.0065	3.0095	0.260300	0.260300	0.260100
	Lactose from a 4CH	3.0050	3.0050	3.0050	0.260600	0.260400	0.260400

CH3	Kalium Bichromicum Soluble	0.566	0.566	0.566	0.390800	0.390900	0.390800
	Kalium Bichromicum From a 3CH	0.564	0.564	0.564	0.390700	0.390800	0.390739
	Kalium Bichromicum From a 4CH	0.565	0.565	0.565	0.390861	0.390861	0.390839
	Lactose from a 3CH	0.564	0.564	0.567	0.390600	0.391100	0.391400
	Lactose from a 4CH	0.562	0.562	0.562	0.390900	0.390600	0.390600

Appendix E: Results Obtained From Statistical Analysis

1. KRUSKAL-WALLIS TEST

a) Chemical Shift values

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum	Percentiles		
						25th	50th (Median)	75th
H2O	15	4.72887	.001457	4.727	4.731	4.72700	4.72900	4.73000
OH	15	4.00080	.001373	3.999	4.002	3.99900	4.00100	4.00200
CH2	15	3.00720	.001656	3.005	3.010	3.00600	3.00700	3.00900
CH3	15	.56440	.001549	.562	.567	.56400	.56400	.56600
SAMPLE#	15	3.00	1.464	1	5	2.00	3.00	4.00

Ranks

	SAMPLE#	N	Mean Rank
H2O	1	3	8.50
	2	3	8.50
	3	3	14.00
	4	3	5.67
	5	3	3.33
	Total	15	
OH	1	3	12.00
	2	3	7.00
	3	3	12.00
	4	3	6.00
	5	3	3.00
	Total	15	
CH2	1	3	13.00
	2	3	5.00
	3	3	10.00
	4	3	10.00
	5	3	2.00
	Total	15	
CH3	1	3	13.00
	2	3	6.00
	3	3	10.00
	4	3	9.00
	5	3	2.00
	Total	15	

Test Statistics[a,b]

	H2O	OH	CH2	CH3
Chi-Square	10.475	10.850	12.066	11.136
df	4	4	4	4
Asymp. Sig.	.033	.028	.017	.025

a. Kruskal Wallis Test

b. Grouping Variable: SAMPLE#

b) Relative Integration Values

Ranks

	SAMPLE#	N	Mean Rank
H2O	1	3	7.33
	2	3	14.00
	3	3	10.33
	4	3	4.00
	5	3	4.33
	Total	15	
OH	1	3	13.17
	2	3	3.83
	3	3	9.33
	4	3	6.00
	5	3	7.67
	Total	15	
CH2	1	3	7.33
	2	3	4.00
	3	3	4.00
	4	3	10.67
	5	3	14.00
	Total	15	
CH3	1	3	8.83
	2	3	5.33
	3	3	10.00
	4	3	10.33
	5	3	5.50
	Total	15	

Test Statistics[a,b]

	H2O	OH	CH2	CH3
Chi-Square	10.914	7.726	11.498	3.589
df	4	4	4	4
Asymp. Sig.	.028	.102	.022	.464

a. Kruskal Wallis Test

b. Grouping Variable: SAMPLE#

2. MANN-WHITNEY TEST

a) Chemical Shift Values

1) Kalium Bichromicum Soluble and Kalium Bichromicum 3CH

Ranks

	SAMPLE#	N	Mean Rank	Sum of Ranks
H2O	1	3	3.50	10.50
	2	3	3.50	10.50
	Total	6		
OH	1	3	5.00	15.00
	2	3	2.00	6.00
	Total	6		
CH2	1	3	5.00	15.00
	2	3	2.00	6.00
	Total	6		
CH3	1	3	5.00	15.00
	2	3	2.00	6.00
	Total	6		

Test Statistics[b]

	H2O	OH	CH2	CH3
Mann-Whitney U	4.500	.000	.000	.000
Wilcoxon W	10.500	6.000	6.000	6.000
Z	.000	-2.236	-2.236	-2.236
Asymp. Sig. (2-tailed)	1.000	.025	.025	.025
Exact Sig. [2*(1-tailed Sig.)]	1.000[a]	.100[a]	.100[a]	.100[a]

a. Not corrected for ties.

b. Grouping Variable: SAMPLE#

2) Kalium Bichromicum 3CH and Kalium Bichromicum 4CH

Ranks

	SAMPLE#	N	Mean Rank	Sum of Ranks
H2O	2	3	2.00	6.00
	3	3	5.00	15.00
	Total	6		
OH	2	3	2.00	6.00
	3	3	5.00	15.00
	Total	6		
CH2	2	3	2.00	6.00
	3	3	5.00	15.00
	Total	6		
CH3	2	3	2.00	6.00
	3	3	5.00	15.00
	Total	6		

Test Statistics[b]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	.000	.000	.000
Wilcoxon W	6.000	6.000	6.000	6.000
Z	-2.236	-2.236	-2.236	-2.236
Asymp. Sig. (2-tailed)	.025	.025	.025	.025
Exact Sig. [2*(1-tailed Sig.)]	.100[a]	.100[a]	.100[a]	.100[a]

a. Not corrected for ties.

b. Grouping Variable: SAMPLE#

3) Kalium Bichromicum 3CH and Lactose 3CH

Ranks

	SAMPLE#	N	Mean Rank	Sum of Ranks
H2O	2	3	4.00	12.00
	4	3	3.00	9.00
	Total	6		
OH	2	3	4.00	12.00
	4	3	3.00	9.00
	Total	6		
CH2	2	3	2.00	6.00
	4	3	5.00	15.00
	Total	6		
CH3	2	3	3.00	9.00
	4	3	4.00	12.00
	Total	6		

Test Statistics[b]

	H2O	OH	CH2	CH3
Mann-Whitney U	3.000	3.000	.000	3.000
Wilcoxon W	9.000	9.000	6.000	9.000
Z	-.707	-.707	-2.121	-1.000
Asymp. Sig. (2-tailed)	.480	.480	.034	.317
Exact Sig. [2*(1-tailed Sig.)]	.700[a]	.700[a]	.100[a]	.700[a]

a. Not corrected for ties.

b. Grouping Variable: SAMPLE#

4) Kalium Bichromicum 4CH and Lactose 4CH

Ranks

	SAMPLE#	N	Mean Rank	Sum of Ranks
H2O	3	3	5.00	15.00
	5	3	2.00	6.00
	Total	6		
OH	3	3	5.00	15.00
	5	3	2.00	6.00
	Total	6		
CH2	3	3	5.00	15.00
	5	3	2.00	6.00
	Total	6		
CH3	3	3	5.00	15.00
	5	3	2.00	6.00
	Total	6		

Test Statistics[b]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	.000	.000	.000
Wilcoxon W	6.000	6.000	6.000	6.000
Z	-2.121	-2.236	-2.236	-2.236
Asymp. Sig. (2-tailed)	.034	.025	.025	.025
Exact Sig. [2*(1-tailed Sig.)]	.100[a]	.100[a]	.100[a]	.100[a]

a. Not corrected for ties.

b. Grouping Variable: SAMPLE#

5) Lactose 3CH and Lactose 4CH

Ranks

	SAMPLE#	N	Mean Rank	Sum of Ranks
H2O	4	3	3.67	11.00
	5	3	3.33	10.00
	Total	6		
OH	4	3	4.00	12.00
	5	3	3.00	9.00
	Total	6		
CH2	4	3	5.00	15.00
	5	3	2.00	6.00
	Total	6		
CH3	4	3	5.00	15.00
	5	3	2.00	6.00
	Total	6		

Test Statistics[b]

	H2O	OH	CH2	CH3
Mann-Whitney U	4.000	3.000	.000	.000
Wilcoxon W	10.000	9.000	6.000	6.000
Z	-.258	-1.000	-2.121	-2.121
Asymp. Sig. (2-tailed)	.796	.317	.034	.034
Exact Sig. [2*(1-tailed Sig.)]	1.000[a]	.700[a]	.100[a]	.100[a]

a. Not corrected for ties.

b. Grouping Variable: SAMPLE#

b) Relative Integration Values

1) Kalium Bichromicum soluble and Kalium Bichromicum 3CH

Ranks

	SAMPLE#	N	Mean Rank	Sum of Ranks
H2O	1	3	2.00	6.00
	2	3	5.00	15.00
	Total	6		
OH	1	3	5.00	15.00
	2	3	2.00	6.00
	Total	6		
CH2	1	3	4.17	12.50
	2	3	2.83	8.50
	Total	6		
CH3	1	3	4.67	14.00
	2	3	2.33	7.00
	Total	6		

Test Statistics[b]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	.000	2.500	1.000
Wilcoxon W	6.000	6.000	8.500	7.000
Z	-1.993	-1.993	-.899	-1.623
Asymp. Sig. (2-tailed)	.046	.046	.369	.105
Exact Sig. [2*(1-tailed Sig.)]	.100[a]	.100[a]	.400[a]	.200[a]

a. Not corrected for ties.

b. Grouping Variable: SAMPLE#

2) Kalium Bichromicum 3CH and Kalium Bichromicum 4CH

Ranks

	SAMPLE#	N	Mean Rank	Sum of Ranks
H2O	2	3	5.00	15.00
	3	3	2.00	6.00
	Total	6		
OH	2	3	2.00	6.00
	3	3	5.00	15.00
	Total	6		
CH2	2	3	3.00	9.00
	3	3	4.00	12.00
	Total	6		
CH3	2	3	2.00	6.00
	3	3	5.00	15.00
	Total	6		

Test Statistics[b]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	.000	3.000	.000
Wilcoxon W	6.000	6.000	9.000	6.000
Z	-1.993	-1.993	-.664	-1.993
Asymp. Sig. (2-tailed)	.046	.046	.507	.046
Exact Sig. [2*(1-tailed Sig.)]	.100[a]	.100[a]	.700[a]	.100[a]

a. Not corrected for ties.

b. Grouping Variable: SAMPLE#

3) Kalium Bichromicum 3CH and Lactose 3CH

Ranks

	SAMPLE#	N	Mean Rank	Sum of Ranks
H2O	2	3	5.00	15.00
	4	3	2.00	6.00
	Total	6		
OH	2	3	3.00	9.00
	4	3	4.00	12.00
	Total	6		
CH2	2	3	2.17	6.50
	4	3	4.83	14.50
	Total	6		
CH3	2	3	3.00	9.00
	4	3	4.00	12.00
	Total	6		

Test Statistics[b]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	3.000	.500	3.000
Wilcoxon W	6.000	9.000	6.500	9.000
Z	-1.964	-.674	-1.798	-.655
Asymp. Sig. (2-tailed)	.050	.500	.072	.513
Exact Sig. [2*(1-tailed Sig.)]	.100[a]	.700[a]	.100[a]	.700[a]

a. Not corrected for ties.

b. Grouping Variable: SAMPLE#

4) Kalium Bichromicum 4CH and Lactose 4CH

Ranks

	SAMPLE#	N	Mean Rank	Sum of Ranks
H2O	3	3	5.00	15.00
	5	3	2.00	6.00
	Total	6		
OH	3	3	3.67	11.00
	5	3	3.33	10.00
	Total	6		
CH2	3	3	2.00	6.00
	5	3	5.00	15.00
	Total	6		
CH3	3	3	4.00	12.00
	5	3	3.00	9.00
	Total	6		

Test Statistics[b]

	H2O	OH	CH2	CH3
Mann-Whitney U	.000	4.000	.000	3.000
Wilcoxon W	6.000	10.000	6.000	9.000
Z	-2.023	-.225	-2.023	-.674
Asymp. Sig. (2-tailed)	.043	.822	.043	.500
Exact Sig. [2*(1-tailed Sig.)]	.100[a]	1.000[a]	.100[a]	.700[a]

a. Not corrected for ties.

b. Grouping Variable: SAMPLE#

5) Lactose 3CH and Lactose 4CH

Ranks

	SAMPLE#	N	Mean Rank	Sum of Ranks
H2O	4	3	3.33	10.00
	5	3	3.67	11.00
	Total	6		
OH	4	3	3.17	9.50
	5	3	3.83	11.50
	Total	6		
CH2	4	3	2.00	6.00
	5	3	5.00	15.00
	Total	6		
CH3	4	3	4.33	13.00
	5	3	2.67	8.00
	Total	6		

Test Statistics[b]

	H2O	OH	CH2	CH3
Mann-Whitney U	4.000	3.500	.000	2.000
Wilcoxon W	10.000	9.500	6.000	8.000
Z	-.221	-.471	-2.023	-1.159
Asymp. Sig. (2-tailed)	.825	.637	.043	.246
Exact Sig. [2*(1-tailed Sig.)]	1.000[a]	.700[a]	.100[a]	.400[a]

a. Not corrected for ties.

b. Grouping Variable: SAMPLE#