

Empirical Investigation on the Reproducibility of ^{13}C NMR Shift Values

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Received December 16, 1997

Automated NMR spectra interpretation packages are becoming increasingly important especially now that high-throughput synthesis often vastly increases the amount of data to be analyzed in a given time frame. It is vitally important for these interpretation packages to be robust, and this means that it is essential to know how the quality of NMR data varies in real life experiments. A practical test concerning the reproducibility of chemical shift information from recorded ^{13}C NMR spectra was carried out using eight test substances. Two hundred seventy spectra were contributed from both Academia and Industry and have been subjected to a standardized interpretation. For these spectra the confidence limits of their chemical shifts have been calculated. The standard deviations found vary from 0.2 up to about 2.5 ppm, and the range of values for the shifts reported vary from ± 0.7 up to more than ± 7 ppm around the respective average shift. Test substances recorded in more polar solvents generally showed higher standard deviations and also exhibit a dependency of the deviation on the chemical shift value.

1. INTRODUCTION

Laboratory automation and spectroscopic databases have enabled NMR spectroscopists to record and store increasing amounts of spectroscopic information. For permanent storage most original ^{13}C NMR data is still reduced and recorded as peak list data rather than as full spectra. The peak lists contain values for the chemical shift and the intensity of the relevant peaks in a given spectrum. These shift values are used for most purposes, ranging from structure elucidation, documentation as in research reports, patents, and papers, and for storage in electronic databases.

However, there is little evidence in the literature on reproducibility and tolerances of chemical shift values recorded under normal conditions.¹ Some work has been published relating to chemical weapons testing,² polymers,³ and humic substances⁴ although these studies have been limited in the range of substances covered and number of laboratories taking part. This has led to practical difficulties in comparing newly recorded shift values with printed data collections, data from chemical shift increment systems, spectroscopic databases, or other data published in the literature.

Results from a practical test are reported in this paper. The work was carried out in Europe and open to anyone willing to contribute. Data sets were received from both academia and industry. The data enquiry procedure and the results for carbon chemical shifts are discussed.

2. EXPERIMENTAL SECTION

In the literature there have been some investigations on how accurate NMR measurements can be at best.⁵ Usually these measurements involve great care and accuracy during

sample preparation and recording procedure and are followed by carefully controlled processing of the raw data. Final results are often furnished with tolerances and further qualifications when published.

Some practical guidelines have been published by the ASTM.⁶ The paper focused on issues concerning the recording technology in the radio frequency part of the spectrometers. Useful definitions of many terms are given and tetramethylsilane (TMS) is recommended as general reference standard for ^1H and ^{13}C .

2.1. General Data Model. In this work the axiomatic assumption was made that, in principle, everybody is able to record identical digits for the chemical shift of the atoms in a given test substance. However, the recorded values will show deviations introduced by one or more aspects during the following operations: (a) synthesis and purification procedure of the actual substance, (b) sample preparation, (c) recording and data processing procedure, and (d) referencing operation.

These four areas should cover all possible influences on the chemical shift value no matter whether they originate from practical aspects, like various impurities, differing solvents, etc., or deviations caused by software or user errors. The final distribution of shift values found around an average will depend on the sum of deviations in the four categories as applicable to the respective test substance. It can be expected that the actual shape of distributions found will differ depending on the nature of the test substance and its functional groups as well as on the chemical environment of the atom responsible for a given signal.

2.2. Test Substances and Test Procedure. The test substances selected covered a reasonable variety of chemical environments. Eight test substances were selected according to the above data model (see Table 1). Some of them were chosen to meet special requirements, for example, their behavior in different solvents. Furthermore, we did not

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Table 1. List of Test Substances Selected^a

substance	substance name	CAS registry	molecular formula	molecular structure with numbering scheme
1	2-methyl-cyclopentane-1-one	[1120-72-5]	$\text{C}_6\text{H}_{10}\text{O}$	
2	D-gluconic acid sodium salt	[527-07-1]	$\text{C}_6\text{H}_{11}\text{NaO}_7$	
3	4,5-difluorophthalic anhydride	[18959-30-3]	$\text{C}_8\text{H}_2\text{F}_2\text{O}_3$	
4	3-phosphono-propionic acid	[5962-42-5]	$\text{C}_3\text{H}_7\text{O}_5\text{P}$	
5	2-phosphono-propionic acid triethyl ester	[3699-66-9]	$\text{C}_9\text{H}_{19}\text{O}_5\text{P}$	
6	cholesteryl acetate	[604-35-3]	$\text{C}_{29}\text{H}_{48}\text{O}_2$	
7	DL-alanyl-DL-alanine	[2867-20-1]	$\text{C}_6\text{H}_{12}\text{N}_2\text{O}_3$	
8	benzotrifluoride	[98-08-8]	$\text{C}_6\text{H}_5\text{CF}_3$	

^a The substances have been identified to the participants by substance names, CAS registry numbers, and molecular formula.

recommend any particular blend, source, or purity to be met by the compounds used. The variety of samples of different origin was allowed to reflect the fluctuation of probe quality that is met under practical conditions.

The substances were identified to the participants by substance names, CAS registry numbers, and molecular formulas and checked for commercial availability. To cover differences introduced during synthesis and purification arising mainly from impurities, no advice or preference was given on how to obtain the substances.

Participants were instructed to feed the samples into their routine recording procedure without giving evidence that they are part of the test. To ensure a variety for operations participants were recommended to try different instruments, operators, solvents, referencing procedures, probe heads, etc. as would make sense in their laboratory environment. For example, if participants never record e.g. fluorine NMR they were recommended not to do that for this test.

2.3. Performance of the Test and Response. Initial recordings on the test substances were used to produce detailed test instructions which were mailed to about 20 organizations. A total of 14 institutions responded with about 270 spectra. Although the substances were selected to serve different hetero nuclei, only for ^{13}C was the number of contributions sufficient to allow a reasonable statistical analysis. Additionally, some data have been used from the literature as was abstracted into SpecInfo On-line.⁷⁻¹² The overall amount of carbon NMR peak data used is about 2200.

2.4. Standardized Interpretation Procedure. Participants were recommended to return the data in paper form.

A returned data set consisted of a peak list, the plotted spectrum, and a completed form covering all of the accompanying data (e.g. instrument and recording details, solvent, etc.). The data were handled anonymously during the evaluation process.

The registration step implicitly includes signal assignment to atoms. Therefore all the spectra for a given substance have been interpreted in a standardized way. Lines caused from the test substance were localized and numbered consecutively starting at low field. In some cases there were more lines than atoms according to line splitting caused by couplings (substances 3, 4, 5, and 8) or signals for diastereoisomers (substance 7). Lines caused by couplings were assigned to a standard numbering scheme with respect to the underlying multiplet structure. Missing or hidden lines were indicated during this procedure. Relying on the assignment being correct, evaluation programs have extracted shifts according to the numbered lines (see below).

2.5. Evaluation Process. The data were first collected from the original information in the numbered lines to form an extract related to one peak or multiplet in a spectrum. This peak-related extract was processed in order to derive the actual shift values from the information originally provided.

In some cases there was more than one possibility to examine shift values. For example, in a quartet signal it is possible to use the average of inner or outer lines to find the center of the multiplet. Although theoretically all values should be equal in practice there are noticeable differences so the evaluation programs tried to calculate as many

Table 2. Results for All Signals in All Substances^a

substance no.	atom no.	av shift [ppm]	50% coverage	std dev	90% coverage	peaks used	highest value [ppm]	lowest value [ppm]	value range [ppm]
1	1	220.9	0.55	0.81	1.33	19	221.9	219.0	2.9
1	2	43.8	0.32	0.48	0.78	19	44.5	42.7	1.8
1	3	37.4	0.31	0.46	0.75	19	38.0	36.3	1.7
1	4	31.8	0.32	0.48	0.78	19	32.6	30.6	2.0
1	5	20.5	0.32	0.48	0.78	19	21.3	19.4	2.0
1	6	14.0	0.29	0.43	0.71	19	14.5	12.9	1.6
2	1	180.1	1.06	1.57	2.57	14	181.5	176.6	4.9
2	2	75.6	0.87	1.29	2.12	14	76.9	73.1	3.8
2	3	74.2	0.79	1.18	1.93	14	75.4	72.3	3.1
2	4	72.9	0.75	1.11	1.83	14	74.1	71.1	3.0
2	5	72.6	0.78	1.16	1.90	14	73.8	70.6	3.2
2	6	64.3	0.73	1.08	1.77	14	65.5	62.6	2.9
3	1	161.9	1.68	2.49	4.09	10	166.5	159.4	7.1
3	a	154.2	1.41	2.09	3.43	30	156.3	150.2	6.1
3	b	128.7	0.70	1.04	1.71	29	130.4	127.0	3.4
3	c	115.6	1.01	1.50	2.45	27	118.2	113.8	4.4
4	a	177.5	1.52	2.25	3.69	13	179.7	173.5	6.2
4	b	29.2	0.81	1.19	1.96	13	30.8	27.3	3.5
4	c	23.8	0.68	1.00	1.64	13	25.2	21.8	3.4
5	a	169.2	0.64	0.95	1.55	14	170.3	167.3	3.0
5	b	62.3	0.67	0.99	1.63	14	63.8	60.4	3.4
5	c	62.2	0.67	0.99	1.63	14	63.7	60.4	3.3
5	7	60.9	0.65	0.96	1.58	14	62.1	59.0	3.1
5	d	39.0	0.58	0.85	1.40	14	40.0	37.3	2.7
5	e	16.0	0.53	0.79	1.30	14	16.9	14.4	2.5
5	f	16.0	0.54	0.79	1.30	14	16.8	14.4	2.5
5	14	13.6	0.75	1.11	1.82	14	15.0	11.1	3.9
5	g	11.3	0.54	0.80	1.32	14	12.1	9.7	2.5
6	1	170.4	0.21	0.32	0.52	37	172.0	169.8	2.2
6	2	139.7	0.20	0.29	0.48	38	141.2	139.3	1.9
6	3	122.7	0.18	0.27	0.44	38	124.1	122.2	1.9
6	4	74.0	0.20	0.29	0.48	38	75.5	73.4	2.1
6	5	56.8	0.19	0.28	0.46	38	58.2	56.3	1.9
6	6	56.2	0.20	0.29	0.48	38	57.7	55.8	1.9
6	7	50.1	0.19	0.28	0.46	38	51.6	49.7	1.9
6	8	42.4	0.18	0.27	0.45	38	43.8	42.0	1.9
6	9	39.8	0.18	0.27	0.45	37	41.3	39.7	1.6
6	10	39.6	0.19	0.28	0.46	37	41.0	39.1	1.9
6	11	38.2	0.19	0.27	0.45	38	39.7	37.8	1.9
6	12	37.1	0.19	0.28	0.45	38	38.5	36.6	1.9
6	13	36.6	0.18	0.27	0.44	38	38.1	36.2	1.9
6	14	36.3	0.20	0.29	0.48	38	37.7	35.8	1.9
6	15	35.8	0.20	0.30	0.49	38	37.3	35.1	2.2
6	16	31.9	0.19	0.28	0.46	38	33.4	31.5	1.9
6	17	31.9	0.19	0.28	0.46	38	33.4	31.5	1.9
6	18	28.2	0.20	0.30	0.48	38	29.7	27.7	2.0
6	19	28.1	0.20	0.29	0.48	38	29.5	27.6	1.9
6	20	27.9	0.20	0.29	0.48	38	29.3	27.4	1.9
6	21	24.3	0.19	0.28	0.46	38	25.8	23.9	1.9
6	22	23.9	0.21	0.31	0.52	38	25.4	23.3	2.0
6	23	22.9	0.21	0.32	0.52	38	24.3	22.3	2.0
6	24	22.7	0.24	0.36	0.59	38	24.1	22.2	1.9
6	25	21.4	0.19	0.28	0.46	38	22.9	21.0	1.9
6	26	21.1	0.18	0.26	0.43	38	22.6	20.7	1.9
6	27	19.3	0.18	0.26	0.43	38	20.8	18.9	1.9
6	28	18.8	0.18	0.27	0.45	38	20.2	18.3	1.9
6	29	11.9	0.18	0.27	0.44	38	13.4	11.5	1.9
7	1	181.7	0.61	0.91	1.49	14	182.7	180.1	2.7
7	2	181.6	0.61	0.90	1.48	14	182.6	179.9	2.7
7	3	172.0	0.59	0.87	1.43	14	172.9	170.3	2.6
7	4	171.8	0.59	0.88	1.44	14	172.7	170.0	2.6
7	5	53.3	0.59	0.88	1.44	14	54.1	51.5	2.6
7	6	53.2	0.60	0.89	1.46	14	54.1	51.4	2.6
7	7	51.2	0.59	0.87	1.43	14	52.1	49.5	2.6
7	8	51.1	0.59	0.88	1.44	14	51.9	49.3	2.6
7	9	19.1	0.58	0.87	1.42	14	20.0	17.4	2.6
7	10	19.1	0.58	0.87	1.42	14	20.0	17.4	2.6
7	11	18.5	0.59	0.87	1.43	14	19.3	16.7	2.6
7	12	18.4	0.59	0.88	1.44	14	19.2	16.6	2.6
8	1	132.0	0.21	0.31	0.50	9	132.6	131.7	0.9
8	a	131.1	0.24	0.35	0.58	8	131.7	130.7	1.0
8	b	./	./	./	./	./	./	./	./
8	c	124.7	0.33	0.49	0.80	8	125.5	124.1	1.5
8	d	125.4	0.16	0.24	0.40	9	125.9	125.2	0.7

^a Atom numbers consisting of digits refer to shifts directly accessible from the peak lists. Those consisting of characters have been calculated from multiplet analysis. Assignments to the actual atoms within substances are given in Table 1 (except cholesteryl acetate). Evaluation of a separate shift value for atom B in substance 8 was not possible because of overlapping multiplets. The columns 50% coverage and 90% coverage list the deviations from the average values that cover the respective percentage of all signals concerned. The number of peaks used may exceed the number of spectra contributed due to multiple peak combinations used for peaks calculated from multiplet analysis. See text for details.

Table 3. Selected Parameters from the Accompanying Data Have Been Evaluated in a Quantitative Way^a

parameter	units	av value	50% coverage	std dev	90% coverage	peaks used	highest value [ppm]	lowest value [ppm]	value range [ppm]
digital resolution	Hz/pt	0.9	0.16	0.24	0.39	102	1.6	0.4	1.2
line broadening	Hz	1.3	0.58	0.85	1.40	123	5.0	-0.8	5.8
acquisition time	s	1.1	0.22	0.32	0.53	124	1.7	0.6	1.1

^a Averages and deviations are given here for digital resolution (DR), Lorentzian line broadening (LB), and time used for 1 scan (AQ).

combinations as possible with respect to the lines present.

In a few cases it became evident during the evaluation process that contributors had recorded spectra from the wrong compound or that principle (referencing) errors were present in the spectra (see below). These spectra were flagged and not used for further examination.

Finally, the values derived were used to calculate averages, ranges, and distributions for chemical shifts. Selected accompanying data were processed the same way.

3. RESULTS

3.1. Results for Chemical Shifts. The set of substances was selected to contain simple molecules as well as some expected to be sensitive to solvent and recording conditions. The different effects found are monitored mainly by chemical shift ranges and standard deviations. The final values for the chemical shifts are given in Table 2 together with additional information. Standard deviations were found to vary between less than 0.2 and about 2.5 ppm. Chemical shifts range between 0.7 and more than 7 ppm.

More than 85% of the spectra recorded were made using chloroform as solvent. The standard deviation of all the shifts concerned is below 1 ppm and in more than 75% better than 0.5 ppm. The lowest deviations are observable for substance 8, where the aromatic ring additionally attenuates all shift variations. Cholesteryl acetate (substance 6) showed the most homogeneous, low deviations across its spectrum.

3.2. Selected Results for Accompanying Data. Results for selected parameters from the accompanying data are given in Table 3.

The digital resolution found is about 1 Hz per data point. All contributors have applied line broadening on their spectra when processing. Both parameters affect the lower resolution limit for small coupling constants.

It seems that contributors have chosen the acquisition time to be fixed at about 1 s per scan and that other parameters have been adjusted to meet this condition. There was no clear preference concerning the number of scans or the total acquisition time spent on a given sample.

All samples were prepared manually, and only very rarely did contributors have evidence on the concentrations actually used. The data sheet had asked for this value but "only if known" to the participant to make contributors wary of providing concentrations without any real evidence to back up the value.

4. DISCUSSION

4.1. Chemical Shift Ranges. The most obvious single reason to explain the shift ranges found is a dependency on the solvent used. Substances 2, 4, and 7 were preferably recorded in water or methanol. All three substances are acidic themselves and do undergo interaction with protic

Table 4. Average Standard Deviation and Average Shift Range According to Substances^a

substance no.	group	av of std dev per substance	av shift ranges per substance
1	unpolar	0.52	2.0
2	polar	1.23	3.5
3	polar	1.78	5.8
4	polar	1.45	4.4
5	ambiguous	0.91	3.0
6	unpolar	0.29	1.9
7	ambiguous	0.88	2.6
8	unpolar	0.35	1.0

^a Substances may be grouped into a polar group and an unpolar group according to their chemistry and solvents. However, some substances do not fit either of the groups.

solvents. This interaction depends on concentration and actual pH value caused by either the substance itself or acidic and basic impurities in the compound or in the solvent. The difluorophthalic anhydride (substance 3) in most cases was recorded in dimethyl sulfoxide (DMSO) or mixtures containing DMSO. This substance is able to undergo hydrolysis and may form an acid together with traces of water present in the solvent. DMSO is hygroscopic, and it can be expected, under normal conditions, that small amounts of water may eventually contaminate the solvent. This will make the solvent interaction dependent on the condition of the solvent used. Additionally, proton and deuterium shifts of DMSO are known to be very temperature sensitive. This influences the shifts of its deuterium nuclei as well as the nuclei of substances solved. This may also cause locking problems, because locking will be affected depending on the temperature of the sample. However, the reason substance 3 shows the highest deviations and ranges of all recordings is not totally clear. More recordings will be required to clarify this point. It is useful to distinguish between two groups of substances. The "unpolar group" covers ordinary chemicals without basic or acidic functionality. These chemicals are preferably recorded in nonprotic solvents such as chloroform. The standard deviations and shift ranges are small. Substances 1, 6, and 8 clearly would be in this group. The overall standard deviation and the average of the signal ranges are given in Table 4.

The "polar group" may contain acidic or basic functional groups and are preferably recorded in polar protic solvents. The polar group generally shows higher standard deviations and wider shift ranges. Substances 2, 3, and 4 would be members of the polar group. For these substances the averaged standard deviations and signal ranges are also given in Table 4.

More evidence about substances 5 and 7 may be obtained from Figure 1. There is a clear separation of about 0.5 ppm between the symbols indicating the polar and unpolar groups. Additionally, in the unpolar group the standard deviation of

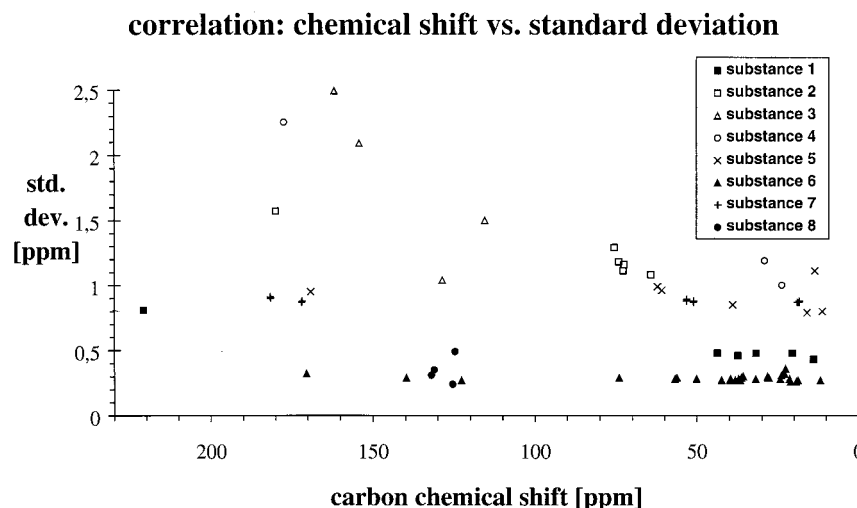


Figure 1. This figure shows the correlation of chemical shift and standard deviation. It shows all peaks of all substances. The full symbols are used to indicate substances of the unpolar group, and open symbols are used for those of the polar group. Symbols “x” and “+” are used for substances of ambiguous character. See text for details.

Table 5. Signals Used for Referencing, Whether TMS or Solvent, Were Investigated Separately^a

group	av value [ppm]	50% coverage	std dev	90% coverage	peaks used	highest value [ppm]	lowest value [ppm]	value range [ppm]
polar	-0.03	0.09	0.13	0.22	28	0.2	-0.6	0.7
unpolar	0.01	0.05	0.07	0.11	47	0.1	-0.4	0.5
all substances	-0.01	0.06	0.09	0.14	97	0.2	-0.6	0.7

^a Surprisingly, the values of neither group actually average to zero. The range of values found for referencing peaks is about ± 0.5 ppm off the actual average value.

the signals does not depend on the chemical shifts. However, in the polar group standard deviation becomes greater as the shifts go to lower field. This is presumably caused by high solvent interaction for functionalized carbons on the left-hand side of the spectrum and low solvent interaction for those carbons situated within plain carbon skeletons. Signals between 0 and 50 ppm show a standard deviation of about 1 ppm, while signals between 150 and 200 ppm vary between 1.5 and 2.5 ppm.

The signals from substances 5 and 7 lie between both groups. Although they are closer to the polar group of substances, they do not show the dependency between shift and standard deviation as was present for the substances in this group.

This would seem to suggest that a spread of shift values of ± 0.5 ppm seems to be reasonable for substances in the “nonpolar group”. Anything beyond 1 ppm in either direction off the average shift should be flagged for inspection. For the “polar group” ± 1 to 1.5 ppm as the expectation range for shifts seems practically achievable, and anything beyond 2.5–3 ppm off the average shift would need inspection.

4.2. Referencing Procedure. The spectra returned have been referenced on either the signal of a reference substance (70%) applied internally or the solvent signal (30%). External referencing was not used at all.

Referencing has been performed in automation using built-in software (55%) as well as manually (45%). Whenever a peak is selected for reference, the actual digital resolution and imperfections in the software resulted in the actual shift value reported for the reference peak being not exactly zero in most cases. The difference between the actual shift value

reported (e.g. 0.018 ppm) and the intended exact value (e.g. 0.000) was registered.

The evaluation results of these data are given in Table 5. The overall average neither for the polar group nor for the unpolar group is exactly zero, and there is a reasonable range of values actually reported in the peak pickings. An average deviation of about 0.1 ppm is introduced to every substance peak just by imperfections of the referencing procedure. Bad cases might introduce a deviation as much as 0.5 ppm.

Another effect was to do with the shifts used for particular reference signals. Somewhere in the referencing process, a digital value has to be assigned to the peak that is selected to become the reference signal. That can be a solvent signal as well as the signal of a reference substance explicitly added to the sample. However, only TMS was assigned to a single value. There was little agreement on the value to be assigned for the other reference signals used (see Table 6). Between 0.1 ppm and more than 0.5 ppm deviation of all signals in a spectrum was introduced in this way, depending on the substance used.

The reference signal for the sodium salt of trimethylsilylpropionic acid (“TMS-salt”) was set by nearly all contributors to 0 ppm. The correct chemical shift of this substance relative to TMS is -1.18 ppm.

4.3. Outliers. Some data sets were submitted with very extreme values reported.

In one spectrum of substance 6 the referencing on solvent chloroform-*d* was run in automation. Obviously the software missed the solvent peaks completely, and the nearest substance signal was set to 77.0 ppm. Consequently, all values in this spectrum were shifted by about 3.5 ppm.

Table 6. For Referencing Purposes Different Shift Values for the Same Reference Substances Have Been Used^a

ref substance	shifts used [ppm]	repetitions
dimethyl ether dioxane	29.4	2
	66.3	2
	67.4	3
	67.56	3
methanol	67.58	1
	48.7	2
	49.0	3
	49.1	1
chloroform-d	76.90	1
	77.0	34
	77.2	6
	78.5	1
DMSO-h ₆	40.46	1
	40.48	3
DMSO-d ₆	39.1	1
	39.5	3
	39.7	2

^a The frequency of use is indicated in the right-most column. The two uses of dimethyl ether are from the same contributor. See text for details.

Another group of spectra from different substances recorded by the same contributor showed shifts approximately 10 ppm off the average calculated from all other contributions. A systematic error in the referencing procedure is held responsible here.

5. CONCLUSIONS

This test has clearly indicated the need of more investigation in this area. The mass of data is great enough to ensure that the results found for shifts can be held to be representative and on a reasonable data platform.

The dependency of chemical shift values on the solvent used for sample preparation is well-known. This test has indicated, that depending on the polar nature of solvent and compound there is a correlation between chemical shift and standard deviation. The magnitude of standard deviations that must be expected for peaks at different shifts has been calculated. This is extremely important for all quality control procedures applied on chemical shift data and might be useful for good laboratory practice (GLP).

The deviations introduced as a result of the referencing practice applied under everyday conditions clearly indicate potential for improvement. Although it is well-known how to reference samples accurately, there is a lack of implementation under normal conditions.

Additional attention has to be drawn to faulty spectrometer and software configurations. Particularly in automation with high sample throughput referencing failures might easily remain undetected.

The authors do not claim that the values reported here do represent "the truth" by any means, but it is a fair representation of what is common laboratory practice.

ACKNOWLEDGMENT

This test would have been impossible without the spectra that have been recorded by all of the participants. The authors like to thank gratefully for all the efforts taken by the following individuals: S. Berger, University of Marburg, Germany; R. Bötzel, University of Düsseldorf, Germany; M. Chippendale, ICI, Manchester, United Kingdom; V. Eiermann, Merck, Darmstadt, Germany; M. Freudenberger, Hitachi Scientific Instruments GmbH, Ratingen, Germany; G. Krack, BASF AG, Ludwigshafen, Germany; J. Lambert, ISAS, Dortmund, Germany; W. Maier, BASF AG, Ludwigshafen, Germany; H.-J. Metternich, Huels AG, Marl, Germany; G. Michl, Schering AG, Berlin, Germany; R. Rieger, WITCO GmbH, Bergkamen, Germany; H. Schouwenaars, DOW Benelux, Terneuzen, The Netherlands; J. Seega, Knoll AG, Ludwigshafen, Germany; H.-V. Siehl, University of Tuebingen, Germany. One author (A.N.D.) would like to thank the Bundesministerium für Bildung Wissenschaft, Forschung, und Technologie, and the Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen, Germany for financial support.

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CI980320Q