Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 2015, **25**, 454–456

Mendeleev
Communications

How sensitive and accurate are routine NMR and MS measurements?

**Andrey M. Tsedilin, Artem N. Fakhrutdinov, Dmitry B. Eremin, Sergey S. Zalesskiy,
Alexander O. Chizhov, Natalya G. Kolotyrkina and Valentine P. Ananikov***

*N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation.
Fax: +7 499 135 5328; e-mail: val@ioc.ac.ru*

DOI: 10.1016/j.mencom.2015.11.019

The necessary prerequisites to carry out efficient NMR/MS studies and the important points required to avoid inconsistent measurements are discussed. A comparative assessment of the sensitivity and accuracy of NMR, EI-MS and ESI-MS measurements was carried out to evaluate typical laboratory research performance. Accurate NMR measurements are possible in the 10^{-1}–10^{-3} M concentration range, with spectral studies still being possible at concentrations of approximately 10^{-4}–10^{-5} M. EI-MS is more sensitive and can operate at concentrations of 10^{-6} M, while commonly available ESI-MS can be efficient up to a concentration of 10^{-18} M.

The rapid development of analytical methods in the 20th century has opened a new era of fascinating discoveries in the chemical sciences. Nuclear magnetic resonance spectroscopy[1] (NMR) and mass spectrometry[2] (MS) are nowadays ubiquitously used in all fields of chemistry. The application of these methods makes it possible not only to reliably elucidate the structure (including conformation)[3] of an individual compound, but also to analyze complex mixtures, especially when combined with gas (GC) and high-performance liquid (HPLC) chromatography or capillary electrophoresis.[4]

The study of reaction mechanisms is necessary for understanding molecular complexity and gaining insight into the fundamental nature of chemical transformations.[5] The detection of highly reactive species, the characterization of intermediates and the direct monitoring of chemical reactions are the key advantages of NMR and MS techniques.[5,6] Complementary analyses of gas phase, liquid state and solid state samples by NMR and MS,[7] as well as joint NMR/MS studies,[8] are integrated into modern laboratory practices.

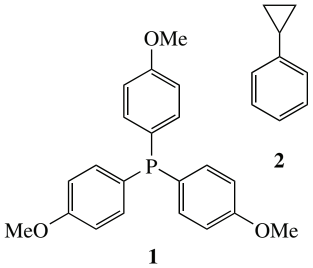
The outstanding evolution of NMR and MS equipment, as well as fabulous progress in software development, has made it possible for researchers at all levels – from highly qualified experts to undergraduate students – to operate these complicated instruments. In spite of obvious advantages, it is the widespread application of NMR and MS in everyday laboratory life that frequently raises some questions.

In the present study, we compare the sensitivity and accuracy of NMR and MS measurements for different samples in varying concentration ranges. The aim of this comparison is to depict a typical laboratory scenario that researchers are faced with in everyday chemistry research. Careful use of these powerful techniques can prevent many inconsistences and misinterpretations that have become increasingly common in modern literature.

We are certainly aware of the high-end development of analytical methods that are targeted, like carrying out NMR measurements on a nanoliter of sample[9] and MS detection of a single molecule.[10] However, these techniques are not routinely utilized in chemistry labs at the moment and thus will not be considered here. Our goal is to illustrate the accuracy and sensitivity of the spectral analyses that researchers in organic chemistry, catalysis, biochemistry and other fields may typically perform.

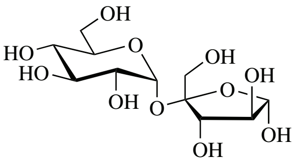
In this work, we provide a comparative assessment of three key techniques for the analysis of organic compounds: ^{1}H/^{13}C NMR, EI-MS and ESI-MS. The NMR method[1] is based on the resonant interaction of electromagnetic energy with a substance, which contains nuclei with nonzero spin, subjected to external magnetic field. In the electron impact (EI) ionization technique,[2,11] low energy electrons produced by thermionic emission excite the electron shell of neutral molecules in the gas phase to produce ions. The electrospray ionization (ESI) technique[2,12] generates gas-phase ions from a sample solution by spraying it from a small diameter capillary with an applied high voltage, followed by rapid solvent evaporation due to charge-induced droplet disintegration.

Tris(*p*-methoxyphenyl)phosphine **1**, cyclopropylbenzene **2** and sucrose **3** were chosen as model compounds because they belong to different classes of molecules and exhibit different behaviors in NMR and MS studies. Measurements were carried out on solutions of these compounds in DMSO-d_6 and acetonitrile at concentrations ranging from 10^{-1} to 10^{-18} M. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX 500 MHz and Bruker Avance II 600 MHz instruments, ESI-MS spectra were measured on Bruker micrOTOF II and Bruker maXis time-of-flight high-resolution spectrometers, and EI-MS spectra were recorded on an Agilent 5977A single quadrupole spectrometer with sample injection *via* an Agilent 7890B gas chromatograph.



1

2



3

Signal-to-noise ratios (S/N) were used as criteria for the comparative assessment of the spectral data (Figure 1). The results were divided into 3 groups – high, good and poor – based on the quality of the measured data from NMR, EI-MS and ESI-MS (see Online Supplementary Materials for details).[†] High quality and, in most cases, good quality spectral data indicate reliable measurements. Poor quality data indicate that the measurements are possible, although the interpretation of the results requires

---

[†] This should be considered as a rough estimation since the results of the measurements may depend on hardware and samples.

Table 1 A comparison of the performance of the spectral measurements from NMR, EI-MS and ESI-MS for the model compounds **1**–**3** (ND – not detected).

Method	Compound	Concentration/M									
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}–10^{-10}	10^{-11}–10^{-13}	10^{-14}–10^{-18}
^{1}H NMR	**1**	high	high	good	good	poor (good)[a]	ND	ND	ND	ND	ND
	2	high	high	good	poor	ND (poor)[a]	ND	ND	ND	ND	ND
	3	high	high	good	poor	ND (poor)[a]	ND	ND	ND	ND	ND
^{13}C NMR	**1**	good	poor	poor	ND	ND	ND	ND	ND	ND	ND
	2	good	poor	ND	ND	ND	ND	ND	ND	ND	ND
	3	good	poor	ND	ND	ND	ND	ND	ND	ND	ND
EI-MS	**1**	—[b]	—[b]	high	good	ND (poor)[c]	ND (poor)[c]	ND	ND	ND	ND
	2	—[b]	—[b]	high	good	poor (high)[c]	ND (good)[c]	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ESI-MS	**1**	—[b]	—[b]	—[b]	—[b]	high	high	good	good	poor	poor
	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3	—[b]	—[b]	—[b]	—[b]	high	high	good	good	poor	ND

[a] The results in parenthesis were obtained with 3200 scans. [b] Direct measurements at such high concentrations may be harmful for the equipment; these samples should be diluted before measuring. [c] The results in parentheses were obtained in SIM mode; the performance also depends on Split Mode.

more attention. A brief summary of the comparative study is shown in Table 1.

As expected, NMR has the lowest sensitivity among the studied methods (Table 1). Routine ^{1}H spectra can be obtained for a concentration range of 10^{-1}–10^{-3} M resulting in high and good quality data within a short period of time (about 5 min for a routine spectra registration). A 10^{-4} M concentration is still sufficient for performing a ^{1}H study, although an experiment will be considerably longer if a high-quality spectrum with good S/N is desirable. ^{1}H NMR signals for 10^{-5} M concentrations were obtained after more than 3000 scans, which took approximately 9 h. The registration of ^{13}C NMR spectra required concentrations of approximately 10^{-2} M and higher. For routine measurements both 500 and 600 MHz instruments have shown a similar performance, and the difference between the instruments may become more pronounced at the lower concentration limit. With NMR, all of the model compounds **1**, **2** and **3** were efficiently detected with several characteristic signals for each molecule.

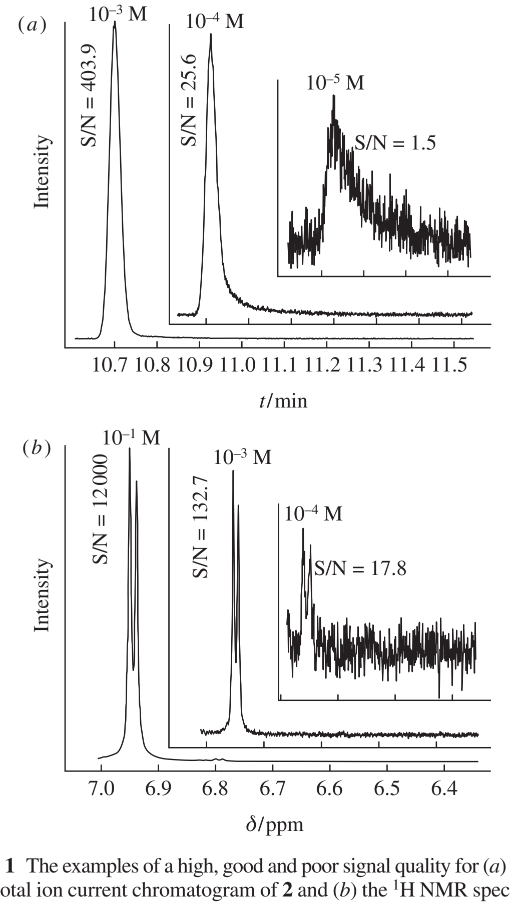


Figure 1 The examples of a high, good and poor signal quality for (*a*) the GC-EI-MS total ion current chromatogram of **2** and (*b*) the ^{1}H NMR spectra of **1**.

For EI-MS in full-scan mode, the detection limit for cyclopropylbenzene **2** was 10^{-5} M, whereas for tris(*p*-methoxyphenyl)phosphine **1** it was one order of magnitude lower (Table 1). This results from the difference between the ionization cross sections of these compounds as well as the high retention time of phosphine, which lies within an area of increased noise caused by the thermal degradation of the stationary phase. The use of the Selected Ion Monitoring (SIM) mode improved the detection limit for both compounds up to 10^{-6} M. Sample must be transferred to the gaseous phase to be ionized by EI, which was not possible for sucrose **3** using GC injection due to its low volatility, and thus its EI mass spectra could not be obtained.

A high sensitivity of ESI-MS was observed for tris(*p*-methoxyphenyl)phosphine **1**,[‡] where the measurements were classified as high and good quality ones at a concentration range up to 10^{-7} M (Table 1). Impressively, the signal was still detectable at very low concentrations ranging from 10^{-14}–10^{-18} M. In fact, this concentration range corresponds to the detection of less than a few hundred molecules per milliliter of sample on routine equipment.

To avoid system contamination and the capillary ‘memory’ effect,[13] the measurements were started from the lowest concentration, involved rigorous system cleanings before each measurement on a different concentration, and were repeated on both mass spectrometers. Nevertheless, even with all the precautions taken, the slow release of compounds accumulated inside the machine cannot be completely excluded. Tris(*p*-methoxyphenyl)phosphine **1** provides a good example of very high sensitivity of ESI-MS and the level of plausible impurities effect.

Due to the low solubility of carbohydrates in pure acetonitrile, the experiments with sucrose **3** were carried out in a MeCN–water (1:1) mixture. High and good quality ESI-MS data were observed in a concentration range up to 10^{-10} M with a detection limit of ~10^{-13} M for sucrose (Table 1). Cyclopropylbenzene **2** cannot be ionized under the given conditions; therefore, no ESI-MS spectra were obtained. The time period needed for the registration of a single mass-spectrum was 1 min for ESI and 10–30 min for EI.[§]

[‡] Note that compound **1** may be oxidized during the measurements and detected as corresponding oxide $(MeOC_6H_4)_3PO$.

Within the acceptable concentration range, the time needed for MS analysis does not depend on the concentration. In contrast, the time needed for NMR analysis strongly depends on the sample concentration (*i.e.*, for ^{1}H NMR: 5 min for 10^{-3} M and 9 h for 10^{-5} M).

The necessity to significantly dilute a sample prior to the MS measurement is worth mentioning (measurements at high concentration may be harmful for MS equipment, Table 1). For reactive compounds, dilution may lead to a complete disappearance of the signals. Organometallic compounds may be considered to be a representative example: while NMR measurements are usually possible, MS measurements may not detect the species of interest due to their rapid decomposition (*i.e.*, hydrolysis with traces of water, *etc*.). MS studies of such reactive organometallic species are indeed possible and can be quite informative, however, the measurements may require special procedures.[14–16]

To summarize, accurate NMR measurements are possible in the 10^{-1}–10^{-3} M concentration range, and studies can be carried out at concentrations of 10^{-4}–10^{-5} M. EI-MS is more sensitive down to a concentration of 10^{-6} M, and ESI-MS could be remarkably sensitive down to a concentration of 10^{-18} M. However, there is a limitation of both MS methods in terms of the nature of the sample because many compounds do not yield detectable signals even at high concentrations, whereas, in the majority of cases, corresponding signals for all analyzed compounds are present in the NMR spectra.

It is not uncommon case in the modern literature, when different results have been obtained with NMR and MS and they have been interpreted as new mechanistic findings. A joint NMR/MS study is a technically simple and yet very powerful combination in mechanistic studies; nevertheless, it requires a careful interpretation of the results.

It is important to realize that NMR and MS operate at different concentration ranges. Most often it is not possible to study the same sample on NMR and MS without changing the concentration (which may noticeably affect the studied sample if reactive species or dynamic systems are concerned). Moreover, the sensitivity and accuracy of the measurements strongly depend on the nature of the sample, which should always be taken into account.

The next important point concerns the measurements of reaction selectivity and the purity of the synthesized compounds. The absence of signal(s) of certain compound(s) is often interpreted as presenting a high selectivity and good purity. It should be emphasized that if the signal of a certain compound is missing in an NMR or MS spectrum, it does not necessarily mean that this compound is absent in the studied sample. It may actually indicate an insufficient concentration/solubility below the detection limit (mostly in NMR), inefficient ionization (for MS) or the decomposition of the sample during sample preparation (sometimes for MS and seldom for NMR).

As a necessary comment, it should be noted that the performance of routine spectral studies will strongly depend on several factors,[¶] and the data discussed here should be considered to be an estimate only. Assessment of the sensitivity and accuracy is suggested for a local analytical instrument if accurate data are required.

This sudy was supported by the Russian Science Foundation (grant no. 14-50-00126).

[§] For EI-GC-MS, chromatography is a time-limiting factor rather than mass spectrometry. Though in this particular case a routine chromatography method was used, analysis time can be significantly changed depending on sample volatility and mixture composition.

[¶] Including type and technical configuration of equipment, experimental conditions, software used for spectra processing, nature of the sample, *etc.*

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2015.11.019.

References

1 (*a*) A. Bax, *Two-dimensional NMR in Liquids*, Delf University Press, Delf, 1982; (*b*) R. R. Ernst, G. Bodenhausen and A. Wokaun, *Principles of NMR in One and Two Dimensions*, Clarendon Press, Oxford, 1987; (*c*) G. E. Martin and A. S. Zektzer, *Two-Dimensional NMR Methods for Establishing Molecular Connectivity*, Wiley-VCH, 1988; (*d*) H. Friebolin, *Basic One- and Two-Dimensional NMR Spectroscopy*, Wiley-VCH, Weinheim, 2005.

2 (*a*) S. Maher, F. P. M. Jjunju and S. Taylor, *Rev. Mod. Phys.*, 2015, **87**, 113; (*b*) H. J. Gross, *Mass Spectrometry: A Textbook*, Springer, Berlin, 2004; (*c*) A. G. Marshall and C. L. Hendrickson, *Ann. Rev. Anal. Chem.*, 2008, **1**, 579.

3 E. E. Kwan and S. G. Huang, *Eur. J. Org. Chem.*, 2008, 2671.

4 (*a*) *On-Line LC-NMR and Related Techniques*, ed. K. Albert, John Wiley & Sons, New York, 2002; (*b*) M. McMaster and C. McMaster, *GC/MS: A Practical User's Guide*, Wiley-VCH, New York, 1998; (*c*) M. V. Silva Elippe, *Anal. Chim. Acta*, 2003, **1**, 497; (*d*) J. A. Olivares, N. T. Nguyen, C. R. Yonker and R. D. Smith, *Anal. Chem.*, 1987, **59**, 1230.

5 (*a*) S. S. Zalesskiy, E. Danieli, B. Blumich and V. P. Ananikov, *Chem. Rev.*, 2014, **114**, 5641; (*b*) V. P. Ananikov, L. L. Khemchyan, Yu. V. Ivanova, V. I. Bukhtiyarov, A. M. Sorokin, I. P. Prosvirin, S. Z. Vatsadze, A. V. Medved'ko, V. N. Nuriev, A. D. Dilman, V. V. Levin, I. V. Koptyug, K. V. Kovtunov, V. V. Zhivonitko, V. A. Likholobov, A. V. Romanenko, P. A. Simonov, V. G. Nenajdenko, O. I. Shmatova, V. M. Muzalevskiy, M. S. Nechaev, A. F. Asachenko, O. S. Morozov, P. B. Dzhevakov, S. N. Osipov, D. V. Vorobyeva, M. A. Topchiy, M. A. Zotova, S. A. Ponomarenko, O. V. Borshchev, Y. N. Luponosov, A. A. Rempel, A. A. Valeeva, A. Yu. Stakheev, O. V. Turova, I. S. Mashkovsky, S. V. Sysolyatin, V. V. Malykhin, G. A. Bukhtiyarova, A. O. Terent'ev and I. B. Krylov, *Russ. Chem. Rev.*, 2014, **83**, 885.

6 (*a*) K. L. Vikse, Z. Ahmandi, C. C. Manning, D. A. Harrington and S. J. McIndoe, *Angew. Chem. Int. Ed.*, 2011, **50**, 8304; (*b*) K. L. Vikse, Z. Ahmadi and S. J. McIndoe, *Coord. Chem. Rev.*, 2014, **279**, 96; (*c*) L. P. E. Yunker, R. L. Stoddard and S. J. McIndoe, *Mass Spectrom.*, 2014, **49**, 1; (*d*) X. Yan, E. Sokol, X. Li, G. Li, S. Xu and G. R. Cooks, *Angew. Chem. Int. Ed.*, 2014, **53**, 5931.

7 V. V. Kachala, L. L. Khemchyan, A. S. Kashin, N. V. Orlov, A. A. Grachev, S. S. Zalesskiy and V. P. Ananikov, *Russ. Chem. Rev.*, 2013, **82**, 648.

8 P. A. Belyakov, V. I. Kadentsev, A. O. Chizhov, N. G. Kolotyrkina, A. S. Shashkov and V. P. Ananikov, *Mendeleev Commun.*, 2010, **20**, 125.

9 (*a*) P. Hemmer, *Science*, 2013, **339**, 529; (*b*) H. J. Mamin, M. Kim, M. H. Sherwood, C. T. Rettner, K. Ohno, D. D. Awschalom and D. Rugar, *Science*, 2013, **339**, 557; (*c*) T. Staudacher, F. Shi, S. Pezzagna, J. Meijer, J. Du, C. A. Meriles, F. Reinhard and J. Wrachtrup, *Science*, 2013, **339**, 561.

10 (*a*) A. K. Naik, M. S. Hanay, W. K. Hiebert, X. L. Feng and M. L. Roukes, *Nat. Nanotechnol.*, 2009, **4**, 445; (*b*) D. Twerenbold, D. Gerber, D. Gritti, Y. Gonin, A. Netuschill, F. Rossel and J.-L. Vuilleumier, *Proteomics*, 2001, **1**, 66; (*c*) D. Twerenbold, *Cryogenic Detectors: Detection of Single Molecules*, in eLS, John Wiley & Sons, 2006; (*d*) M. S. Hanay, S. Kelber, A. K. Naik, D. Chi, S. Hentz, E. C. Bullard, E. Colinet, L. Duraffourg and M. L. Roukes, *Nat. Nanotechnol.*, 2012, **7**, 602; (*e*) J. W. F. Robertson, C. G. Rodrigues, V. M. Stanford, K. A. Robinson, O. V. Krasilnikov and J. J. Kasianowicz, *Proc. Natl. Acad. Sci. USA*, 2007, **104**, 8207.

11 F. H. Field and J. L. Franklin, *Electron Impact Phenomena and the Properties of Gaseous Ions*, Academic Press, Waltham, 2013.

12 (*a*) J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Science*, 1989, **246**, 64; (*b*) J. B. Fenn, *Angew. Chem. Int. Ed.*, 2003, **42**, 3871.

13 H. Rütters, T. Möhring, J. Rullkötter, J. Griep-Raming and J. O. Metzger, *Rapid Commun. Mass Spectrom.*, 2000, **14**, 122.

14 M. D. Eelman, J. M. Blacquiere, M. M. Moriarty and D. E. Fogg, *Angew. Chem.*, 2008, **120**, 309.

15 (*a*) A. Putau, M. Wilken and K. Koszinowski, *Chem. Eur. J.*, 2013, **19**, 10992; (*b*) A. Putau, H. Brand and K. Koszinowski, *J. Am. Chem. Soc.*, 2012, **134**, 613.

16 D. Agrawal and D. Schröder, *Organometallics*, 2011, **30**, 32.

Received: 29th October 2015; Com. 15/4763