The scientific background for identification of selected substances.

Kaja Ilmarinen[a], Merike Nugin[a], Arvo Mere[b], Dzmitry Kananovich[c], Maria Fomitšenko[c], Sandra Kaabel[c], Riina Aav[c].
[a]Department of Chemical Safety, Health Board, Gonsiori 29, 10147 Tallinn, Estonia.
[b]Department of Physics, Faculty of Science, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia.
[c]Department of Chemistry, Faculty of Science, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia.

The purpose of this presentation is to introduce the experimental data of analysing substances, which, in the meaning of REACH Regulation, are chemical elements and its compounds in the natural state or obtained by manufacturing, including any additive necessary to preserve its stability and any impurity deriving from the process used[1].
Based on the analytical characterization of compounds the hazard profile of substances and the potential impacts on human health and the environment are determined and on the ground of that substances are classified in accordance with CLP Regulation[2] requirements. For example the BPR[3] purpose is to improve the free movement of biocidal products within the Union while ensuring a high level of protection of both human and animal health and the environment. REACH Regulation identification requirements are followed to identify active substances of biocidal products and assess them according to the intended use ensuring accurate data on the relevant additives and impurities including constituents of toxicological concern in very low concentrations. Therefore modern sensitive analytical fully validated methods are required for the determination of different compositions facilitating in such a way adequate hazard assessment and ensuring a high level of safety of human health and the environment.

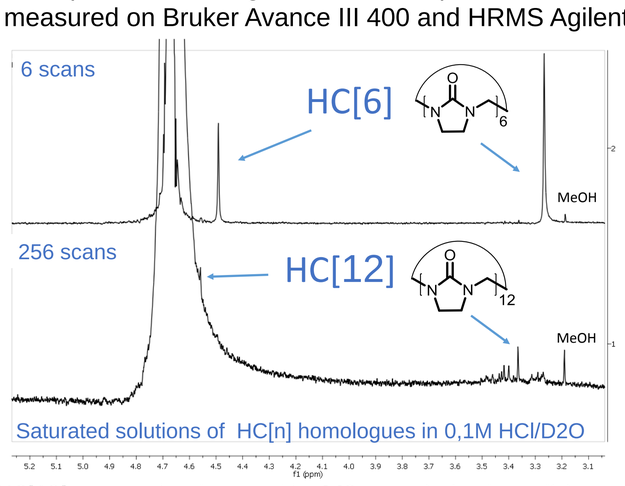
Nuclear magnetic resonance (NMR) spectroscopy is widely used analytical method for determine the molecule structure in academic and industrial research due to the possibility to derive a full uncertainty budget by mathematical equations. Although pursuant to REACH Annex VI (2.3.5)[1] the NMR spectra for substance identification is required, the legal validated standards are not available enough.

X-ray diffraction (XRD) is used for characterisation of solid organic and inorganic substances. For example,if the specific NMR experiments are not enough informative then XRD can solve the chemical composition or spatial structure.

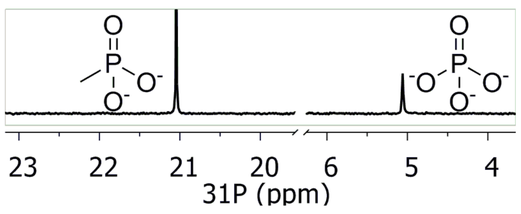
Inorganic substances, Organic substances, Nano-particles[4]

1D and 2D NMR spectroscopy

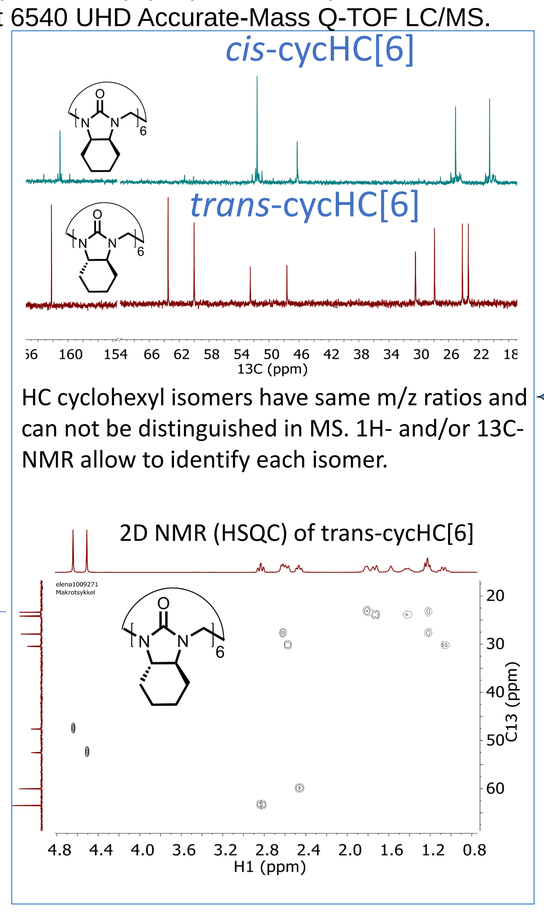
Examples of advantages of NMR compared to mass-spectrometry (MS). All NMR specta have been measured on Bruker Avance III 400 and HRMS Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS.



HC[12] can not be detected by MS at optimized conditions below concentration 0,0025 mg/ml. In this case 1H-NMR is more sensitive technique and signal of HC[12] was detected.



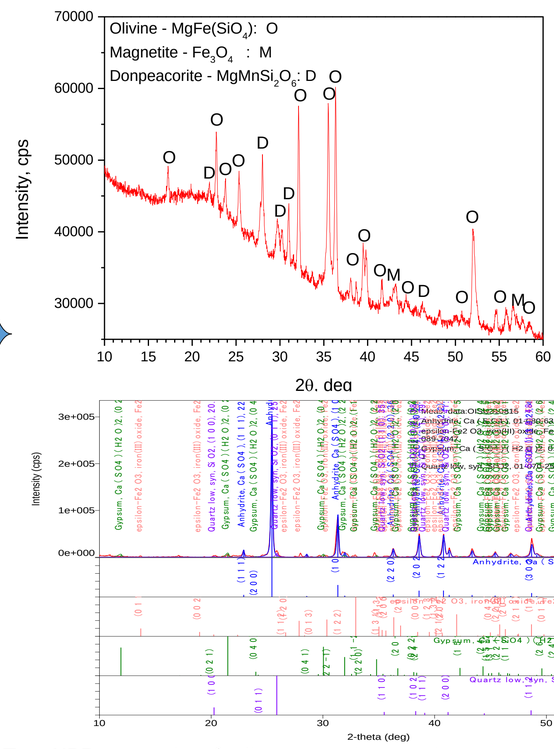
Quantitative analysis of phosphoric acid in Cola-beverage[5] by ^{31}P-NMR



HC cyclohexyl isomers have same m/z ratios and can not be distinguished in MS. 1H- and/or 13C-NMR allow to identify each isomer.

Powder XRD and Single crystal XRD

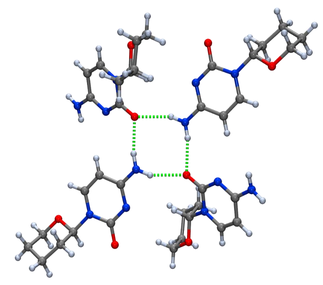
Typical powder XRD diffractogram made by Rigaku Ultima IV diffractometer using fast detector. The sample is the Cheljabinsk meteorite 2015.



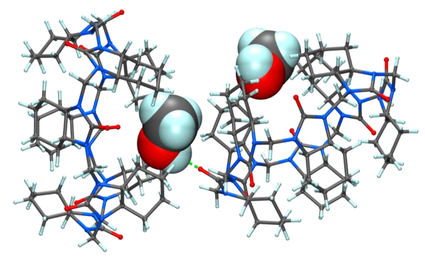
The XRD analysis of oil shale ashes powder, using Rigaku Ultima IV diffractometer. The component proportion analysis is available.

Phase name	Content(%)
Anhydrite	91(7)
epsilon-Fe2 O3, iron(III) oxide	1.9(3)
Gypsum	4.7(11)
Quartz low, syn	2.1(7)

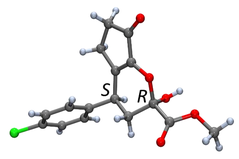
H-bond interactions of a nucleoside derivative[7a]



cycHC[8] methanol solvate[7b]

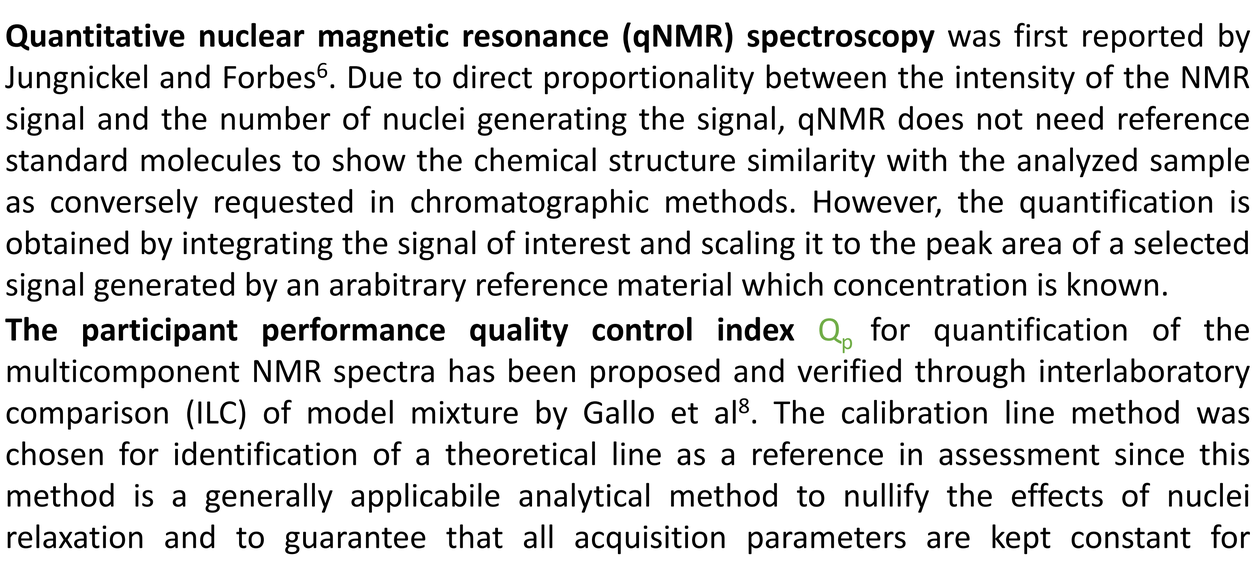


Chiral product of an asymmetric cascade reaction[7c]



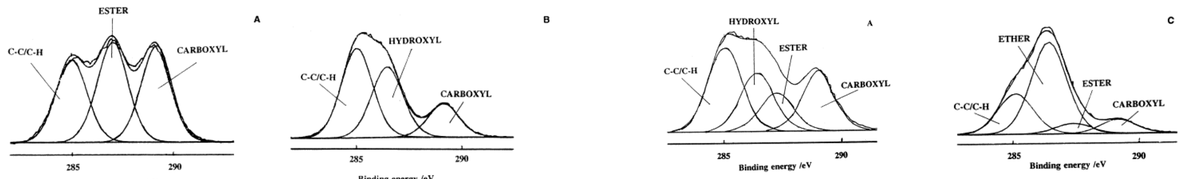
Quantitative nuclear magnetic resonance (qNMR) spectroscopy was first reported by Jungnickel and Forbes[6]. Due to direct proportionality between the intensity of the NMR signal and the number of nuclei generating the signal, qNMR does not need reference standard molecules to show the chemical structure similarity with the analyzed sample as conversely requested in chromatographic methods. However, the quantification is obtained by integrating the signal of interest and scaling it to the peak area of a selected signal generated by an arabitrary reference material which concentration is known.

The participant performance quality control index Q_p for quantification of the multicomponent NMR spectra has been proposed and verified through interlaboratory comparison (ILC) of model mixture by Gallo et al[8]. The calibration line method was chosen for identification of a theoretical line as a reference in assessment since this method is a generally applicabile analytical method to nullify the effects of nuclei relaxation and to guarantee that all acquisition parameters are kept constant for standard and test solution[7].



$Q_p = a_i - \bar{a} / \sigma_{slope}$, where a_i is the slope of the calibration line determined by the i^{th} participant, $\bar{a}$ is the consensus slope value, and σ_{slope} is the interlaboratory standard deviation on slopes, all referred to a single NMR signal.

X-ray photoelectron spectroscopy (XPS) provides quantitative elemental and functional group analysis which elucidated the changes in surface chemistry with formulation procedure for colloidal sub-200 nm PLGA nanospheres stabilised with PVA and PEO-PPO copolymer surfactants.[9]
As reported by Davies et al three specific carbon environments achieved the best fit in the deconvolution of the C1s envelope corresponded to C-C/C-H and chemical shifts at 2.1 and 4.4 eV corresopnding to C-O-CO (ester) and O-C=O (carboxyl) respectively.



Carbon C1s envelopes from XPS analysis of copolymer films: (A) PLGA, (B) PVA (MW 3000)

Carbon C1s envelopes from XPS analysis of PLGA nanosphere: (A) PVA stabilised, cleaned by GPC, (C) Poloxamer 407 stabilised, cleaned by GPC,

However, the XPS data may need additionally specific analysis for surface chemical composition of nanospheres to determine the risk and safe use of nanomaterials due to the nano particles can be observed in the substances in very low concentration as relevant impurities.
To conclude: If chemical composition is determinated by NMR spectra then XRD provides additional information on aggregation and speciation structure as well as surface area and charge of nanoparticles.
Only if all the properties of substances of concern have been identified at high level of accuracy of analysis, hazards from active substances in biocidal products can be evaluated in order to ensure that the products are safe for human health and the environment.

References
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2. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures (CLP).
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