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NMR interlaboratory comparisons

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Validation of NMR fingerprinting methods: effects of processing on measure reproducibility and laboratory performance assessment

by
Innovative Solutions S.r.l.
Spin off company
of the Technical University of Bari

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1. Introduction

Since its first application as quantitative technique,^[1] NMR spectroscopy has been successfully used for many applications involving several kind of matrixes (small molecules, pharmaceuticals and natural products in both simple and complex mixtures).

One of the major advantages of quantitative NMR (qNMR) is its primary analytical characteristic. In principle, it can be applied in the quantitative estimation of purity of compounds without using any specific reference standard. Moreover, the recent progress in the development of high sensitive instruments allowed for reduction of the detection limits, thus making the technique appealing for analysis of molecules in very low concentrations. A comprehensive recent review aiming to extend awareness of experimental protocols for accurate NMR quantification of analytes is available in “Trends in Analytical Chemistry”.^[2]

Despite the advantages deriving from the use of qNMR and despite the plethora of well documented validation processes (e.g., precision, accuracy, linearity, reproducibility, robustness, selectivity, and specificity), very few official NMR based quantification methods are known.

With the aim to extend the use of qNMR in official methods, Innovative Solutions organizes NMR inter-laboratory comparisons (ILCs) according to ISO/IEC 17043:2010^[3] and reference normative therein. ILCs provide objective standards for individual laboratories, permit them to compare analytical results from different laboratories and represent a way to check the quality and the accuracy of the analytical job.

Validation by ILCs is the starting point for official recognition of the analytical methods.

The ILCs are organized by Innovative Solutions either for its own account or on behalf of third parties. The first ILC was organized under the auspices of RETELAB, the network of the laboratories of the Italian Chambers of Commerce, and of SAMER (the laboratory of the Chamber of Commerce of Bari). It was aimed to assess the performances of the NMR laboratories in the quantification of analytes. A five-component model mixture was considered and the calibration line approach was used for quantification.^[4] Among the quantification approaches available for NMR spectroscopy,^[2] the calibration line method was chosen because of its general applicability in analytical chemistry and because it has the advantage to nullify the effects of nuclei relaxation on quantitative accuracy, provided that all the acquisition parameters are kept constant for standard and test solutions.^[2] Moreover, it minimized systematic errors deriving from hardware features or from the set of acquisition parameters.

Re-elaboration of the data produced during the first ILC allowed for identification of a theoretical line to be taken as reference in performance assessment. Thus, the performance assessment was carried out by means of the parameter (z-score) usually considered as performance index in single component quantifications as well as by means of a new parameter, named Q_p -score, better suited for performance assessment in multi-component and fingerprinting analyses. In addition, a third index (NR), specific for each NMR signal, was introduced to gain insights into the possible effects of the acquisition parameters on signal intensities.^[5] NR represents an index of the specific response of the various nuclei submitted to a definite NMR experiment. Q_p -score and NR are parameters accounting for statistical equivalence of NMR

spectra of model mixtures. In order to ascertain statistical equivalence of NMR spectra of real samples, a second inter-laboratory comparison was organized. It consisted in the analysis of aqueous extracts of wheat (2 samples) and flours (2 samples). 780 NMR spectra were produced by 32 participants using 39 different NMR spectrometers. Seven NMR signals were selected for elaboration by univariate internationally agreed statistics typically applied in performance assessment of ILC participants.^[6]

Some unsuccessful performances of a limited number of participants prompted us to reconsider spectra processing. Thus, the 780 NMR spectra were submitted to 3 additional processing sessions differing from the first session^[6] for number of operators, processing procedure, software and integration mode.

2. Aims of this interlaboratory comparison

The aim of this interlaboratory comparison is the evaluation of the effects of processing on measure reproducibility and laboratory performance assessment of a fingerprinting NMR method for classification of wheat and flours.

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