acceptable with the 15-20 rule. The widest beta-expectation intervals despite fulfilled 15-20 rule were 38.1 to 55.5% at low concentrations (talinolol, assay V), 40.9 to 38.1% at medium concentrations (esmolol, assay V), and 13.0 to 41.4% at high concentrations (9-HO-risperidone, assay IV).

Conclusion: Even in bioanalytical methods fulfilling the widely used 15-20 rule single measurements considerably deviating from the target value and hence causing problems in routine QC are not unlikely, especially if precision and bias values are close to the respective acceptance limits. Using the beta-expectation interval approach with a tolerance interval corresponding to routine QC limits, methods associated with a high risk of causing problems in routine QC can be more effectively identified during validation than with the 15-20 rule.

Keywords: beta-expectation interval, tolerance interval, bias, precision, validation

O75. Total error for the validation of bioanalytical methods

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Introduction: Consistent and efficient use of any analytical procedure requires the knowledge of its reliability prior to its use. It is therefore necessary for each laboratory to validate their analytical methods. Validation is not only required by regulatory authorities [ICH, FDA, GxP] or in order to access accreditation [ISO 17025], but is also the ultimate phase before the routine use of the method. Analytical method validation must bring confidence to the laboratories in the results that will be generated since they are used to make critical decision. However, very little information is included about the process and rules for making a decision – *i.e.* to reject or to accept an analytical method – with respect to its ability to achieve reliable results.

Aim: An innovative universal strategy using Total Error is thus proposed to decide about methods' validity that controls the risk of accepting an unsuitable assay together with the ability to predict the reliability of future results. Several examples of applications of this validation methodology to various types of assays [LC-MS, ELISA, Bio-Assays] will be presented.

Method: Total error is the simultaneous combination of systematic (bias) and random (imprecision) error of analytical methods. Using validation standards both types of error are combined through the use of a prediction interval (β-expectation tolerance interval). Finally, an accuracy profile is built by connecting, on one hand all the upper tolerance limits, and on the other hand all the lower tolerance limits. This profile combined with pre-specified acceptance limits (*e.g.* 30% of bioanalysis) allows to evaluate the validity of any quantitative analytical method.

Results: The accuracy profile determines a region of results where a defined proportion of future measurements will be included inside the acceptance limits. If the analyst is willing to take, for example, a risk of 5%, this approach can give the laboratory as well as the regulatory authorities the guarantee that 95 times out of 100 the future measurements of unknown samples using the validated method will be included within the acceptance limits assessed according to the requirements. The accuracy profile is used to select the most appropriate standard curve, to estimate the limit(s) of quantification, to evaluate a potential matrix effect and, nonetheless provide estimates of measurement uncertainty.

Conclusions: This validation methodology approach allows the analysts as well as the regulatory bodies to know the risk to obtain future results out of the specified acceptance limit. Validation criteria such as the selection of the adequate standard curve and definition of the lower and upper limits of quantitation are straightforward and fit perfectly to their respective definition. Using the proposed approach, each analyst can predict the quality of the results that he will provide and thus earn confidence in the subsequent critical decisions made.

Keywords: validation, total error, accuracy profile, risk

O76. Use of accuracy profile for the validation of a gas chromatography-negative chemical ionization tandem mass spectrometry method: quantification of ethyl glucuronide in hair

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Introduction: Ethylglucuronide (EtG) is a direct and specific metabolite of ethanol. Its determination in hair is of increasing interest for detecting and monitoring alcohol abuse. The quantification of EtG in hair requires analytical methods showing highest sensitivity and specificity. We present a fully validated method based on gas chromatography-negative chemical ionization tandem mass spectrometry (GC-NCI-MS/MS). The method was validated using French Society of Pharmaceutical Sciences and Techniques (SFSTP) guidelines which are based on the determination of the total measurement error and accuracy profiles.

Methods: Washed and powdered hair is extracted in water using an ultrasonic incubation. After purification by Oasis MAX solid phase extraction, the derivatized EtG is detected and quantified by GC-NCI-MS/MS method in the selected reaction monitoring mode. The transitions m/z 347 / 163 and m/z 347 / 119 were used for the quantification and identification of EtG. Four quality controls (QC) prepared with hair samples taken post mortem from 2 subjects with a known history of alcoholism were used. A proficiency test with 7 participating laboratories was first run to validate the EtG concentration of each QC sample. Considering the results of this test, these samples were then used as internal controls for validation of the method.

Results: The mean EtG concentrations measured in the 4 QC were 259.4, 130.4, 40.8, and 8.4 pg/mg hair. Method validation has shown linearity between 8.4 and 259.4 pg/mg hair ($r2 > 0.999$). The lower limit of quantification was set up at 8.4 pg/mg. Repeatability and intermediate precision were found less than 13.2% for all concentrations tested.

Conclusion: The method proved to be suitable for routine analysis of EtG in hair. GC-NCI-MS/MS method was then successfully applied to the analysis of EtG in hair samples collected from different alcohol consumers.

Keywords: ethyl glucuronide, hair, accuracy profile, alcohol markers

O77. Screening for pharmaco/toxicologically relevant compounds in biosamples using high mass resolution. A metabolomic approach

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Introduction: The screening for pharmaco/toxicologically relevant compounds (PTRC) in biosamples has benefited a lot from MS techniques. The library search approach has enabled the development of effective identification methods based on comparison of unknown and reference spectra. However, a downside of this approach is the limited number of reference mass spectra, particularly in the case of LC-MS where in-house/commercial databases typically include not more than one thousand compounds. High mass resolution (HRMS) enables the identification of a molecular formula (MF) through the accurate measurement of mass and isotopic pattern. However, the identification of an unknown compound starting from MF requires additional tools: (a) a database associating MFs to compound names, and (b) a way to discriminate between compounds with identical MF.