Formalization of validation procedures of quantitative LC-MS methods.

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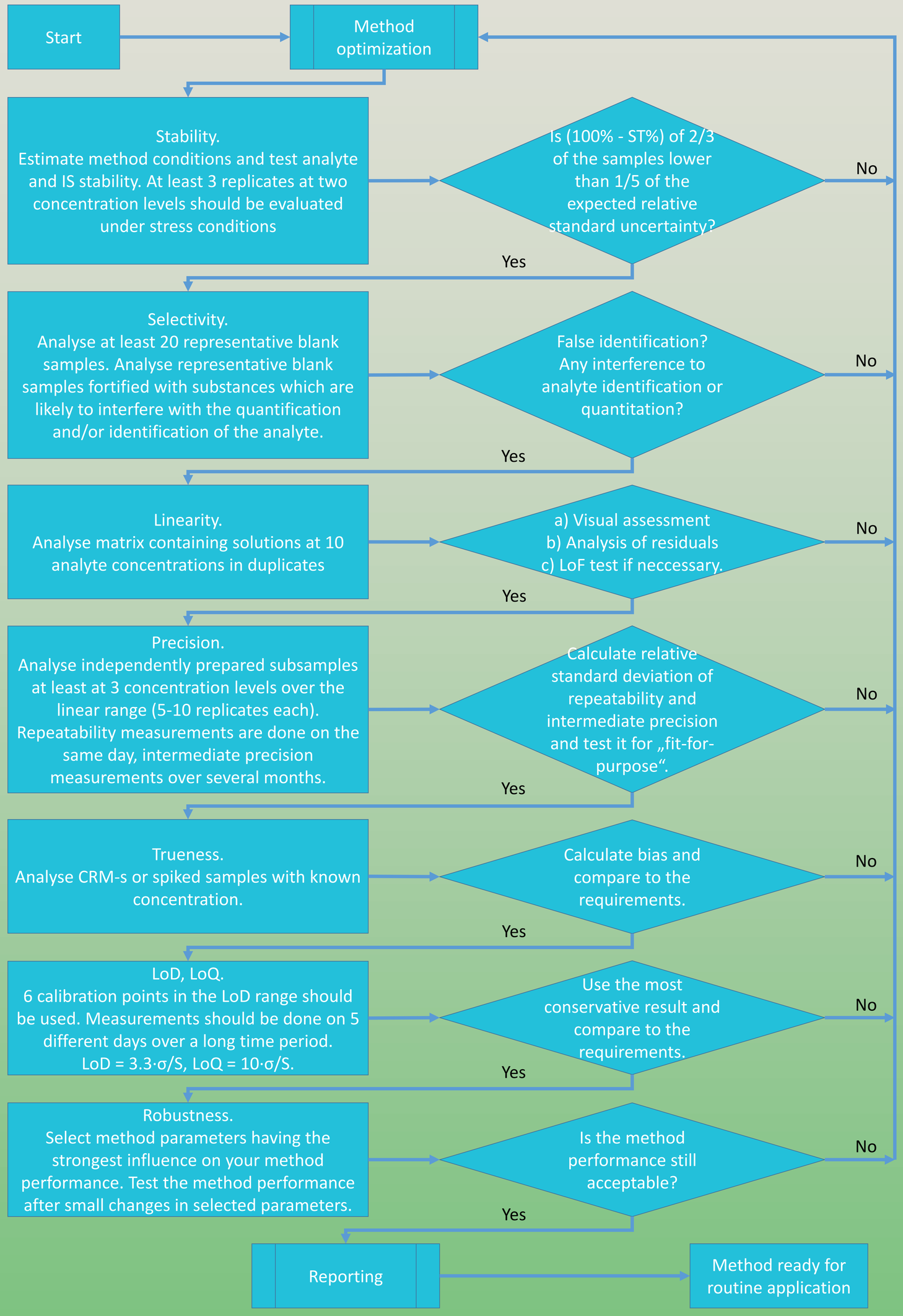
Introduction

All analytical methods have to be fit for the foreseen purpose, i.e. the methods have to be validated. Numerous validation guides have been issued by different organizations. Some of these are very general and can be applied to virtually any analytical technique, others also aadress specific techniques. In this work validation guides are reviewed from the point of view of LC-MS.

Review of validation guides

Validation guides issued by diferent organizations (e.g. SANCO, EMA, FDA, IUPAC, ICH) were reviewed. Out of nine validation guides studied only SANCO guide and European Commission Decision 2002/657/EC specifically address LC-MS as technique. Workflow for validation of LC-MS methods is proposed.



Stability

Long- and short-term stability, freeze and thaw stability, processed sample stability, stability of pure standard substance and standard solutions. Possible factors: temperatuure, pH, light. Stability is unrelated to LC detector.

Selectivity

Most guides rely solely on chromatographic resolution (RS) neglecting the detector-side selectivity of LC-MS. LC-MS (especially MS/MS, HRMS) enable selectivity orthogonal to chromatographic separation, which should be acknowledged while validating the method.

Linearity

As the sample matrix components affect ionization efficiency of analyte, linearity should be carried out using solutions containig sample matrix (matrix-matched).

Precision

Performance of LC-MS changes in time due to ageing, contamination/cleaning, replacements made, etc. As a result, precision of LC-MS is relatively low and repeatability study should extend over several months.

Trueness

Matrix effect (ionization suppression or enhancement due to coeluting compounds) is an infamous problem of LC-MS which may compromise method trueness.

LoD, LoQ

LoD is the lowest concentration of analyte in sample, which can be reliably detected and identified. Hence, both qualitative and quantitative transition must be of adequate S/N.

Robustness

LC-MS is a complex instrument which can not be regarded robust. For example, ion source condition changes due to aging and contamination brings about variation in ionization efficiency. As a result, robustness testing of LC-MS method must include more variables.

Formalization of validation guides and development of VaLChrom

Validation guides issued by Eurachem, ICH, IUPAC, AOAC, EMA, FDA and NordVal were formalized and on-line software tool VaLChrom was created.[2] VaLChrom guides user through planning of validation experiments, calculates validation characteristics and compares to set criteria. In addition, VaLChrom enables estimation of measurement uncertainty.

References

1. Anneli Kruve, Riin Rebane, Karin Kipper, Maarja-Liisa Oldekop, Hanno Evard, Koit Herodes, Pekka Ravio, Ivo Leito. Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part I and II Analytica Chimica Acta 870 (2015) 8-44
2. VaLChrom webpage: http://www.valchrom.ut.ee

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VaLChrom

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