New certified reference materials (CRMs) for reliable measurements of oxytetracycline in milk

Reinhard Zeleny, Heinz Schimmel

INTRODUCTION

• Oxytetracycline (OTC) is an authorized broad-spectrum antibiotic. It is applied to the treatment of enteritis, pneumonia, and diphtheria in lactating and non-lactating dairy cattle
• Potential issue: epimerisation at C4-atom; matrix- and method-dependent
• Maximum residue limit (MRL) in milk: 100 µg/kg
• LC-MS/MS and LC-UV are the predominant methods to analyse OTC in milk
• CRM is an important tool to establish reliable measurements (method validation, method performance verification)

EXPERIMENTAL

Materials and processing
• Raw materials: partially skimmed milk (fat content 1.5 w/w %); OTC crystalline substance (SIGMA-Aldrich)
• Processing of spiked material (ERM-BB492): spiking of milk with OTC solution (target concentration: MRL), pre-concentration, spray-drying, powder milling, bottling under nitrogen atmosphere.
• Processing of blank material (ERM-BB493): pre-concentration, spray-drying, powder milling, bottling under nitrogen atmosphere.

Calibrant
• Common calibrant (crystalline OTC) provided by IRMM – purity assessed by qNMR, LC-DAD, Karl-Fischer titration, TG-FTIR, total ash, LC-TOF, and LC-MS/MS. Purity 94.34 ± 0.61 g/g % (mean ± SD, n=12, qNMR results)

Homogeneity
• 15 units, triplicate measurements, LC-MS/MS, technical and statistical scrutiny, ANOVA, calculation of between-bottle variation

Stability: isochronous studies
• Short-term study (4 weeks; 4, 18, and 60 ºC, reference temperature -70 ºC) to establish suitable dispatch conditions
• Long-term study (12 months; 4 and -20 ºC, reference temperature -70 ºC) to establish suitable storage conditions
• LC-MS/MS, technical and statistical scrutiny, linear regression analysis, calculation of uncertainty contributions

Characterisation intercomparison (12 laboratories)

Laboratory selection and study protocol
• Validated LC-MS/MS or LC-DAD method (compulsory), ISO 17025 accreditation (asset)
• Successful participation in recent ring test/proficiency testing round
• Methods with base-line separation of OTC and its 4-epimer, and methods validated LC-MS/MS or LC-DAD method (compulsory), ISO 17025

Value assignment
• Technical and statistical data evaluation
• Certified values as mean of accepted laboratory means
• Uncertainty includes contributions from homogeneity, long-term stability, characterisation, and calibrant purity

RESULTS AND DISCUSSION (continued)

• All results were reported as sum of OTC and its 4-epimer; all values refer to the reconstituted material (milk)
• ERM-BB492: 12 data sets obtained, 2 (from laboratories 2 and 8) rejected due to technically invalid data
• The 4-epimer was not detected in the material by neither laboratory
• Certified value ± expanded uncertainty (k=2): 101 ± 11 µg/kg

Metrological traceability: The measurand is defined by quantification with LC-UV and LC-MS/MS, different sample preparation procedures were applied. The certified value is the unweighted mean of accepted set of results, using the provided calibrant. The value is traceable to the International System of Units (SI).

• ERM-BB493: 7 data sets obtained, OTC content reported as “below LOD” in all data sets
• Certified value < 5 µg/kg with a 95% level of confidence (corresponds to LOQ of the most sensitive method in the study)

Metrological traceability: The measurand is defined by quantification with LC-MS/MS. The value is traceable to the SI.

REFERENCES