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STUDY PROTOCOL

APMP.QM-S8 Supplementary Comparison and APMP.QM-P28 Pilot Study

Determination of Mass Fraction of Benzoic Acid, Methyl Paraben and n-Butyl Paraben in Soy Sauce

Background

Preservatives such as benzoic acid and parabens are being tested on a regular basis by regulatory authorities and food testing laboratories as they are widely found in condiments and beverages such as fruit juices, sports drinks and soft drinks. The consumption of excessive amount of preservatives can lead to adverse health effects such as allergy. Also, the levels of these preservatives in food are strictly regulated and the maximum permitted concentrations in each type of food have been established in most countries.

Despite the frequency in which these preservatives are being tested, there is a lack of certified reference materials (CRMs) for use by routine testing laboratories in method validation and as quality controls. In addition, commercial proficiency testing (PT) programmes commonly participated by routine testing laboratories make use of consensus results instead of metrologically traceable assigned values to evaluate the performance of the participating laboratories.

Pilot studies on the determination of preservatives in food matrices have been organised previously by the APMP TCQM. These included APMP.QM-P14 Benzoic acid and sorbic acid in curry paste and APMP.QM-P23 Determination of mass fraction of benzoic acid in orange juice, which were completed in 2010 and 2012, respectively. Till date, there is no

key or supplementary comparison organised on the determination of preservatives in condiments and beverages. In addition, only two NMIs have CMC claims on preservative in beverage/water. Hence, the study would enable participating NMIs/DIs which currently provide or are intending to provide metrological services (e.g. PT programmes or CRMs) for the determination of preservatives in beverages and condiments to underpin their CMC claims.

The study was first proposed as a key comparison and was presented at the APMP TCQM meeting in Taipei in 2013. During the meeting, several NMIs/DIs expressed interest to participate in the study. The study was subsequently presented at the CCQM OAWG meeting in April 2014. The meeting recognised the need for the key comparison to underpin new and existing CMCs and it also noted that the topic involved a condiment (see Objectives) which is commonly used in the Asia Pacific region. Hence, the meeting recommended that the study should proceed as a subsequent study under the APMP. CCQM OAWG members from other RMOs would also be invited to participate in the study. An approval was subsequently obtained from the APMP TCQM Chair to organise this study as a supplementary comparison, along with a parallel pilot study.

Objectives

The comparison aims to enable participating NMIs/DIs to demonstrate their competence in the determination of three common preservatives; namely: benzoic acid, methyl paraben and n-butyl paraben. The chosen matrix material is soy sauce, a traditional condiment commonly used in the preparation of Asian cuisines. Its widespread usage in Asia and increasing popularity in Western countries makes monitoring and testing of levels of preservatives in soy sauce imperative.

Preparation of the Study Material

The soy sauce was sourced from local supermarkets and screened using liquid chromatography-diode array detection (LC-DAD). A brand of soy sauce with no preservatives found was then used to prepare the study material. The soy sauce was

poured into a clean, dry drum and spiked with appropriate amounts of benzoic acid, methyl paraben and n-butyl paraben in methanolic solution. It was then stirred under an atmosphere of nitrogen for 2 hours at room temperature (about 22 °C). Thereafter, the soy sauce was aliquoted into amber glass bottles. A total of 89 bottles of study material were prepared.

The homogeneity of the preservatives in the study material was established by employing gas chromatography-isotope dilution mass spectrometry (GC-IDMS) and LC-DAD. 10 Bottles were randomly and stratifically selected. Homogeneity testing was performed on the 10 bottles, with two subsamples taken from each bottle. Using ANOVA at 95 % level of confidence, the preservatives were found to be sufficiently homogeneous in the study material. The relative uncertainties of the between-bottle homogeneity for benzoic acid, methyl paraben and n-butyl paraben were found to be 0.41 %, 0.26 % and 0.39 %, respectively, using LC-DAD. Similarly, the relative uncertainties of the between-bottle homogeneity for benzoic acid, methyl paraben and n-butyl paraben were found to be 0.37 %, 0.45 % and 0.32 %, respectively, using GC-IDMS.

The stability of the preservatives at 40 °C and 25 °C in the study material was established by LC-DAD and GC-IDMS, respectively. A short-term stability study using isochronous design was carried out over a period of 28 days at a simulated transport temperature of 40 °C. Two randomly selected bottles were transferred from 40 °C to the reference temperature of 4 °C on three occasions over the study period. One subsample was then taken from each bottle and analysed at least five times. Using Student's *t*-test at 95 % level of confidence, no significant instability of the study material was observed. The same approach was used to determine the stability of the preservatives in the study material under the storage temperature of 25 °C using classical design. The study was carried out on five occasions over a period of 243 days. No significant instability of the preservatives in the study material was also observed at 95 % level of confidence. The relative uncertainties of the slope of the linear regression curves plotted from the three results each for benzoic acid, methyl paraben and n-butyl paraben were found to be 0.86 %, 0.94 % and 0.77 %, respectively. The stability of the preservatives in the study material at the storage temperature of 25 °C will be continuously monitored until its distribution.

The Measurands

The mass fraction of benzoic acid, methyl paraben and n-butyl paraben in the study material is in the range of 50 to 200 mg/kg. The study material was intentionally prepared to contain a lower concentration of the preservatives than the regulatory limits of most countries in order to provide a higher level of analytical challenge for the participating NMIs/DIs.

Methods/Procedures

The participating NMIs/DIs should use their own methods for the determination of benzoic acid, methyl paraben and n-butyl paraben.

Each participating NMI/DI will receive one bottle containing about 50 ml of study material. The bottle will be transported at a temperature not exceeding 40 °C. Upon receipt, the bottle should be kept at 25 °C or below before analysis.

The accumulation of precipitating particles is expected to occur in the study material after prolonged storage. It is a normal phenomenon and will not cause any deviation of measurement results as long as the sample bottles are shaken and inverted several times before sampling. The recommended minimum subsample size is no less than 1 g.

CRMs of benzoic acid, methyl paraben and n-butyl paraben which have been certified for use as calibrants are available (See Table 1[1]). The participating NMIs/DIs may use other sources of reference materials, provided that they are purity assessed adequately.

[1] Note: The Table may not contain an exhaustive list of CRMs of benzoic acid, methyl paraben and n-butyl paraben certified for use as calibrants.

Table 1: Sources of CRMs of benzoic acid, methyl paraben and n-butyl paraben.
The product number of the respective CRM is given in the parenthesis.

Benzoic acid	Methyl paraben	n-Butyl paraben
HSA (HRM-1002A)	HSA (HRM-1003A)	HSA (HRM-1004A)
NIM China [GBW(E)100006]*	NIM China [GBW(E)100074]	NIM China [GBW(E)100077]

*Benzoic acid in water.

Internal Standards

Isotopic labelled benzoic acid, methyl paraben and n-butyl paraben are available from Sigma Aldrich and Cambridge Isotopes Laboratories.

Reporting of Results

A Report of Results Form will be provided to the participating NMIs/DIs for completion. The participating NMIs/DIs are expected to report their results based on at least three subsamples. The results should include standard and expanded uncertainties (95 % level of confidence) for the mean of the replicate determinations. The results should be reported in the unit of mg/kg. A complete description of the analytical procedure and the uncertainty estimation should also be provided by the participating NMIs/DIs. NMIs/DIs can choose to determine one or more of the preservative(s).

Evaluation of Results

Results of all participating NMIs/DIs will be evaluated against the supplementary comparison reference value (SCRV). The SCRV and associated uncertainty will be determined from results of NMIs/DIs that participate in the supplementary comparison and employ metrologically traceable calibrants to determine their results.

Core Competency and How far Does the Light Shine?

This study covers a subject which is of wide interest and importance. It enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of common preservatives in soy sauce, using procedure(s) that require simple sample preparation and selective detection in the mass fraction range from 50 to 1000 mg/kg. The study can be extended to include other polar food preservatives (e.g. sorbic acid, propionic acid and other alkyl benzoates) in water, aqueous-based beverages (e.g. fruit juices, tea extracts, sodas, sports drinks, etc) and aqueous-based condiments (e.g. vinegar, fish sauce, etc).

Schedule

Official call for participation:	29 Sep 2014
Deadline for registration:	28 Nov 2014
Distribution of study materials:	26 Jan 2015
Deadline for submission of results:	24 Apr 2015

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Note: A potential DI may also register to participate in the APMP pilot study upon a written agreement from the NMI.