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Analytical Methods

Determination of formaldehyde in food and feed by an in-house validated HPLC method

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ABSTRACT

Formalin is carcinogenic and is detrimental to public health. The illegal addition of formalin (37% formaldehyde and 14% methanol) to foods to extend their shelf-life is considered to be a common practice in Bangladesh. The lack of accurate methods and the ubiquitous presence of formaldehyde in foods make the detection of illegally added formalin challenging. With the aim of helping regulatory authorities, a sensitive high performance liquid chromatography method was validated for the quantitative determination of formaldehyde in mango, fish and milk. The method was *fit-for-purpose* and showed good analytical performance in terms of specificity, linearity, precision, recovery and robustness. The expanded uncertainty was <35%.

The validated method was applied to screen samples of fruits, vegetables, fresh fish, milk and fish feed collected from different local markets in Dhaka, Bangladesh. Levels of formaldehyde in food samples were compared with published data. The applicability of the method in different food matrices might mean it has potential as a reference standard method.

1. Introduction

Food contamination and food adulteration are significant problems in Bangladesh (Ali, 2013; Noman & Atahar, 2013). A lack of strong regulatory controls, weak infrastructure for transport, storage and refrigeration and increasing consumer demand for fresh produce have led to an increase in fraudulent practices to increase shelf-life of food products. Food adulteration can have a detrimental impact on the health of a population, as adulterants can lead to developmental defects, chronic diseases, or death. Children, in particular, are more vulnerable to unsafe food, and is a major cause of child mortality (United Nations (UN), 2012).

Formaldehyde (HCHO) is a common air pollutant and a gas at ambient temperature. In its liquid form as formalin (35–40% aqueous solution stabilized with methanol), it is widely used in the manufacture of household products such as paint, furniture laminates and cleaning fluids. It is a proven carcinogen and, therefore, detrimental to public health (International Agency for Research on Cancer (IARC), 2004). In Bangladesh and South-East Asian countries, formalin has been reported to be added fraudulently to foods to extend shelf-life (Riaz, Moin, Tasbira, Naz, & Kumar, 2011). On occasions, tonnes of fruits and vegetables allegedly adulterated with formalin have been destroyed by authorities to protect consumers. There is no scientific evidence in the country corroborating the actual presence of this adulterant in foods and, generally, colorimetric qualitative tests are used during inspections. However, as formaldehyde is naturally present at varied concentrations in foods, its qualitative detection is not conclusive evidence of adulteration. To date, formalin adulteration in Bangladesh has only been evidenced by media reports.

The lack of accurate *fit-for-purpose* methods to determine formaldehyde in food and the pervasiveness of formaldehyde in nature make the detection of illegally added formalin challenging. Moreover, formaldehyde content in fresh food products varies with development stages and environmental factors. Formaldehyde occurs naturally in free and bound forms. Formaldehyde can bind reversible arginine, tyrosine and lysine protein residues yielding methylol groups, Schiff bases, methylene bridges and imidazolidinone adducts. Primarily, free formaldehyde is of toxicological interest and it is the compound measured as a potential adulterant (Metz et al., 2006; Rehbein, 1987).

The presence of formaldehyde as a breakdown product of hexamethylenetetramine is permitted in cheeses in Europe to a maximum residue limit (MRL) of 25 mg/kg (Directive 95/2/EC). Formaldehyde has also been permitted as preservative in gelling additives up to 50 mg/kg (Directive 2009/10/EC). Given the great

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variability of formaldehyde in foods, a more general MRL has not been set. The use of formaldehyde as preservative for feed is still under discussion at the European level (European Commission (EC), 2002, European Food Safety Authority (EFSA), 2014b) although a concentration of 2.5 g/kg is already permitted in the USA (United States Environmental Protection Agency (USEPA), 2010). According to the European Food Safety Authority (EFSA) (2014a), daily exposure to formaldehyde from food of animal and plant origin should not exceed 100 mg/kg food per day. Average dietary exposure is estimated to be about 11 mg/kg food per person per day (Agence Francaise de Securite Sanitaire des Aliments (AFSSA), 2004).

The official method for the determination of formaldehyde in foodstuffs is based on a colorimetric reaction where sample distillates are mixed with sulfuric acid yielding a purple color if formaldehyde is present. The intensity of the color is proportional to formaldehyde concentration and can be measured by UV spectrophotometer (AOAC 931-08, 1931). Titrations and acetylcholine have also been used to detect and quantify relatively the presence of formaldehyde in foods (European Pharmacopoeia 6.0 method., 2008; Lee, Su, & Chang, 1984). Currently, a colorimetric-based kit is being used during inspections in Bangladesh to detect adulteration with formaldehyde (Noordiana, Fatimah, & Farhn, 2011; Riaz et al., 2011). Drawbacks of this and other colorimetric methods are their poor specificity, selectivity, prolonged analysis times and highly acidic conditions, which together lead to over-reporting and/or false positives (Bicking, Cooke, Kawahara, & Longbottom, 1998).

Other techniques, such as LC and GC, have been proven to be more selective and accurate in determining formaldehyde in water (Tomkins, McMahon, & Caldwell, 1989), mushrooms (Claeys et al., 2009), milk (Kaminski, Atwal, & Mahadevan, 1993), fish (Jianrong, Junli, & Lifang, 2007; Tai-Sheng, Tzu-Chun, Ching-Chuan, & Hwui-Mei, 2013) and shrimps (Radford & Dalsis, 1982). There are a number of methods and extraction procedures available in the literature, which emphasizes the need of a harmonized reference method with broad applicability. To support regulatory authorities, the present study aimed to optimize and validate an HPLC method for the accurate determination of formaldehyde in food products. The applicability of the method was demonstrated in three different matrices: milk, mango and fish. A range of food products collected from local Dhaka markets were further screened for formaldehyde content.

2. Materials and methods

2.1. Chemicals

Solvents were of analytical grade (SIGMA–Aldrich, Buchs SG, Switzerland). 2,4 dinitrophenylhydrazine (2,4 DNPH) was purchased from Merck (Darmstadt, Germany). Formaldehyde in water certified reference material (CRM) (4815 mg/L) was from SIGMA–Aldrich (Buchs SG, Switzerland).

2.2. Formaldehyde solution

The certified value for formaldehyde in water CRM was 47.5 mg/L ± 8.91 (mean ± st. dev.) with an expanded uncertainty of 1.82, (k = 2.23). A stock solution of formaldehyde in water (500 mg/L) was prepared using deionized water. A matrix free calibration curve was prepared at six concentrations: 1, 2, 5, 25, 50 and 100 mg/L. For matrix matched calibrations, matrix samples (mango, fish and milk) were spiked before extraction at 1, 2, 5, 25, 50 and 100 mg/L. To calculate the bias of the method, a stock solution of formaldehyde CRM at 47.5 mg/L concentration was prepared following the instructions provided.

2.3. 2,4 dinitrophenylhydrazine working solution

2,4 DNPH was recrystallized prior to use. Recrystallization was performed by dissolving 10 g of 2,4 DNPH in 100 mL in hot analytical grade acetonitrile to form a saturated solution. After complete dissolution, the solution was cooled to room temperature, capped in a brown bottle and stored overnight at 4 °C for crystallization. The crystals were collected by vacuum filtration. 150 mg of 2,4 DNPH crystals were accurately weighed, dissolved in 49.5 mL of acetonitrile and mixed with 0.5 mL of phosphoric acid (85%).

2.4. Derivatization kinetics and sample preparation

Derivatization kinetics followed the procedure described by Claeys et al. (2009) but was slightly modified. Edible parts of the food; fruit flesh and fish fillets were used for the analysis. For derivatization kinetics, mango samples were ground, homogenized and spiked with 10 mg/L of formaldehyde standard. To sample aliquots of 5 g, 5 mL of acetonitrile were added, and the sample vortexed and then sonicated for 30 min. The samples were centrifuged at 5000 rpm for 5 min and the supernatant was passed through a 90 mm diameter Whatman® 541 (Hardened Ashless) filter paper (SIGMA–Aldrich, Buchs SG, Switzerland). Two and half milliliter of 2,4 DNPH was added to the extract and mixed well. Samples were incubated at 40 °C for 30, 60, 90 and 120 min in a shaking water bath (model BS-11, Oxon, UK). Formaldehyde was quantitatively converted to its Schiff base in 60 min. In all experiments, derivatization time was set to 60 min. After incubation, the acetonitrile layer was collected, membrane filtered (0.45 μm) and injected into the HPLC.

2.5. High performance liquid chromatography conditions

Analyses were performed on a C_{18} Luna column (25 cm × 4.6 mm id., 5 μm particle size), (Phenomenex, Utrecht, The Netherlands) using a HPLC (model SPD-M20A) coupled to a photodiode array detector (both manufactured by Shimadzu, Kyoto, Japan). The wavelength was set to 355 nm and the oven temperature at 30 °C. Separation was achieved using isocratic elution with a mixture of water/methanol (35:65, v/v). The flow rate was 1.0 mL/min and the injection volume 20 μL. The total run time was 12 min.

2.6. Method validation

The method was validated in terms of specificity, linearity, range, limit of detection (LOD), limit of quantification (LOQ), repeatability, intermediate precision and robustness. The specificity of the method was tested by injecting reagent blank (2,4 DNPH and phosphoric acid), sample blank and formaldehyde solution individually. For linearity the determination coefficient (R^2) was calculated from the responses of 0.1, 1, 2, 5, 25, 50 and 100 mg/L standards. The limit of detection was calculated by the expression 3.3 $s_{y/x}$/slope, based on the assumption that, the standard deviation of the signal of a solution with a concentration near to the blank is roughly the standard deviation of y-residuals ($s_{y/x}$). General, there is a normal distribution of 5% of occurring error type a or b and the curve intercepts at zero. The quantification limit was estimated by the expression 10 $s_{y/x}$/slope (Miller & Miller, 1993). For repeatability and recovery studies, 5 samples of each of the matrices were spiked at nominal concentrations at the LOQ, 2xLOQ and 5xLOQ levels and extracted by the method described in Section 2.3. Recoveries were expressed in % and repeatability as