Simultaneous Quantitative Determination of Melamine and Cyanuric Acid in Cow's Milk by Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry

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Introduction: Nitrogen content of milk may change as a result of natural variation in the milk and/or the collection process or, unusually, as a result of deliberate adulteration of the milk with specific nitrogen-containing compounds Melamine (MEL) is an organic base and a trimer of cyanamide, with a 1,3,5-triazine skeleton, which contains 66% nitrogen by mass. It is commonly combined with formaldehyde to produce a melamine resin, which is used in the manufacture of countertops, fabrics, glues and flame retardants. Cyanuric acid (CA; 1,3,5-triazine-2,4,6-triol) can be produced either as a byproduct during the manufacturing process of MEL. Once absorbed into the blood stream, the melamine and cyanurate concentrate together and interact in the urine-filled renal micro- tubules, subsequently crystallizing to form a large number of round, yellow crystals. These crystals will then damage the renal cells that line the tubes, resulting in progressive tubular blockage and cellular degradation. The Kjeldahl method, the traditional standard technique for measuring protein content by indirectly measuring the nitrogen content in food , is not able to separate endogenous nitrogen content from adulterants nitrogen content. This way, is necessary analytical methodology with sensibility and specificity for the detection of economically motivated adulteration like as LC-MS/MS.

Objective: The goal is to catch the economic adulteration in cow's milk by quantification of compounds used as adulterants (MEL) or presents as impurities (CA) using a fast method for extraction and mass spectrometry.

Material and Methods: For extraction, acetonitrile with 2% formic acid was added to each tube containing sample milk and the tubes were vigorously shaken for 60 s. Gain, the tubes were shaken for 30 min in a horizontal table (180rpm, Nova Ética®) folowed by centrifugation at 4500 rpm (~1840 × g) for 20 min. So, an aliquot of 1 mL of the extract was transfered into eppendor and again centrifugated (10,000rpm, 0°C) for 20min. An aliquot of 800 µL was transfered to vial and analyzed was carried out using an AB Sciex 5500 QTRAP mass spectrometer (AB Sciex, Framingham, MA, USA) in multiple reaction monitoring (MRM) mode, using electrospray ionisation (ESI) at 600 ◦C in negative mode for CA and positive mode for MEL.

Results and Discussion: Validation parameters (linearity, matrix effect, specificity, precision, robustness and trueness) were assessed and demonstraed in accordancy with Commission Decision 2002/657/EC). LOQ obtained was 37.50 $\mu g.L^{-1}$ (below the LMR 125 $\mu g.L^{-1}$ for both compounds).

Conclusion: The method presented here is fast, easy, and accurate for analysis of the two potential markers in milk adulteration with minimal sample preparation allowing maximized throughput for the analysis of many samples in a short time period.

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