

Comments on methods

NOTE: these are compiled comments received by several analytical experts. They are intended to give some guidance but should not be considered as complete evaluations of each method.

HPLC-MS/MS is with GC-MS/MS amongst the most reliable methods for low level quantification of melamine in a variety of different sample matrices. It will deliver high sensitivity and selectivity for a broad range of different products. Samples may be extracted with an acetonitrile/water mixture. The extract may then be cleaned up by liquid/liquid extraction followed when necessary by solid phase extraction SPE. Isotope dilution approach is strongly recommended to provide reliable quantification.

GC-MS/MS is with LC-MS/MS amongst the most reliable methods for low level quantification of melamine in a variety of different sample matrices. It will deliver high sensitivity and selectivity for a broad range of different products. This approach is less sensible to matrix effect compared to LC-MS/MS. Samples are generally extracted with an acetonitrile/water mixture. The extract may then be cleaned up by liquid/liquid extraction followed when necessary by solid phase extraction SPE followed by a derivatization step. Isotope dilution approaches using isotopically labelled internal standards may provide more reliable quantification, but is not mandatory.

HPLC-MS will be characterised by a slightly lower selectivity compared to LC-MS/MS and thus a significantly higher limit of quantification. A SPE purification step should be preferably adopted in this case to compensate for the weaker specificity of MS versus MS/MS. Positive ion exchange SPE should be avoided in order to make possible the simultaneous measurement of cyanuric acid and melamine.

GC-MS will be characterised by a slightly lower selectivity compared to GC-MS/MS and thus a significantly higher limit of quantification. A SPE purification step should be preferably adopted in this case to compensate for the weaker specificity of MS versus MS/MS. Derivatisation is mandatory before injection into the system. Positive ion exchange SPE should be avoided in order to make possible the simultaneous measurement of cyanuric acid and melamine.

HPLC-UV can provide quantitative information (usually using UV at 240 nm). Samples are extracted with acetonitrile/water mix and analysed by ion-pair HPLC. However, analyses of complex samples like candy and cookies will give a lot of interfering compounds that also will absorb UV at 240 nm. Therefore, development of an LC-UV requires intensive validation by a mass spectrometric based technique for each food matrix and may also require a selective sample preparation procedure. Often positive samples are validated by a second analysis. However, this is normally used to eliminate false positives, whereas false negatives will not be discovered. From the point of food safety it is more important to find the false negatives (thus samples that do contain melamine above the limit even though analysis show they are below).

ELISA is an enzyme-linked immunosorbent assay (ELISA) that determines a quantitative level of melamine and is intended for use in milk, milk powder, wheat gluten and pet food. Melamine is extracted from a sample by vortex or sonication, and the extract pipetted into the antibody-coated microwell. The quantity of melamine is determined via a colour reaction, and a microplate (or strip-) reader is required to measure the optical density. Test kits (i.e. coated microplates and reagents) are commercially available; they can prove very useful as screening tool. However care has to be taken with respect to sample preparation in order to avoid interferences. Also currently available test have reported detection limits that may not be sufficiently sensitive. Validated for various food matrices and sample preparation methods adapted to achieve lower detection and quantification limits are currently under development.