**ABSTRACT**

This procedure describes the extraction of Aflatoxin M₁ from milk samples using an immunoaffinity solid phase extraction procedure followed by liquid chromatography tandem mass spectrometry analysis. The method allows for superior clean up and selective detection allowing for a detection limit of 10.0 ng/l. The method was evaluated by participation in a proficiency testing scheme hosted by FAPAS.

**INTRODUCTION**

Aflatoxins are secondary Aspergillus fungal metabolites which pose a serious health risk for humans due to their carcinogenic properties. Aflatoxins occur naturally and are produced by many species, most notably Aspergillus flavus and Aspergillus parasiticus. The four major aflatoxins include: Aflatoxin B₁, Aflatoxin B₂, Aflatoxin G₁, and Aflatoxin G₂. Among them Aflatoxin B₁ is the most carcinogenic and is classified by the International Agency for Research on Cancer (IARC) as group 1 of human carcinogens. Aflatoxin M₁ is a metabolite of Aflatoxin B₁ and is found in the milk of cows that ingested feed contaminated with Aflatoxin B₁. Aflatoxin M₁ is relatively stable to thermal processes like pasteurization and sterilization. Different analytical techniques can be used to determine Aflatoxin M₁ in milk. The need for derivatization is a disadvantage of some methods such as HPLC-FD and gas chromatography. Cross-reactivity may occur in ELISA trials and the need for a confirmatory quantitative technique such as LC-MS/MS was applied in the laboratory to ensure that Aflatoxin M₁ can be determined.

**MATERIALS AND METHOD**

**FLUID MILK:**

Measure 30mℓ fluid milk into a 50mℓ polypropylene centrifuge tube. Centrifuge the sample at 1540 g for 15 minutes. Separate the fat (top) layer from the defatted (skim) milk. Use 20mℓ of the defatted milk for further analysis. Filter the 20mℓ defatted milk through a 0.45mμ filter prior to loading onto the Aflatoxin M₁ immunoaffinity columns.

**MILK POWDER:**

- Weigh 5g milk powder.
- Heat 50mℓ purified water to 50 – 60 °C.
- Add 30mℓ preheated water to the milk powder. Stir for 10 minutes.
- Transfer to a measuring cylinder and bring the volume to 50mℓ with the remaining preheated water.
- Centrifuge the sample at 5000 g for 15 minutes.
- Centrifuge the sample at 1540 g for 19 minutes. Separa-