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Technical note

Sensitive method for the detection of fumonisin B_1 in human urine

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Abstract

Fumonisins are a group of carcinogenic mycotoxins that occur worldwide. This paper reports a sensitive method for the detection and quantitation of fumonisin B_1 in human urine. Amberlite XAD-2 non-ionic polymeric adsorbent resin is used prior to strong anion-exchange (SAX) cartridge clean-up. As much as 100 ml of undiluted human urine can be loaded onto the column. Recoveries obtained using this method were 93.6, 95.1 and 94.4% when samples were spiked with 10, 50 and 500 ng/ml of fumonisin B_1, respectively. This method is highly reproducible (R.S.D.$<$5%) and gives good sample clean-up, which is suitable for HPLC analysis.

Keywords: Fumonisins; Mycotoxins

1. Introduction

Fumonisins are a group of carcinogenic mycotoxins produced by *Fusarium moniliforme* [1]. Fumonisins are found to cause leukoencephalomalacia in horses, pulmonary oedema in pigs, liver cancer in rats and they are epidemiologically correlated with high incidences of human oesophageal cancer in some parts of the world [2]. Fumonisins have been shown to occur naturally worldwide in maize [3] and recently they have been shown to occur in Indian maize and sorghum [4]. Consumption of mouldy sorghum and maize containing high levels of fumonisin B_1 has resulted in a food-borne disease outbreak in humans [5].

Exposure of humans to different levels of fumonisins through staples in different regions of the world is considered to be of great concern [6]. Toxicokinetic studies with labelled and unlabelled fumonisins have shown that a fraction of the ingested fumonisin is excreted in urine [7,8] and, hence, urine can act as a better indicator for monitoring the exposure of populations to fumonisins.

For the detection and quantitation of fumonisin B_1 in rat urine, a strong anion-exchange (SAX)-based single step clean-up method was developed by Shephard et al. [9]. Urine contains high concentrations of solutes that interfere with clean-up using a SAX cartridge [9]. Therefore, to get acceptable recovery and reproducibility, small volumes (250 μl) of rat urine were diluted several fold before being loaded onto the SAX cartridge. This method cannot be used for the detection of fumonisin B_1 in human urine. The present paper reports a sensitive method for the detection of fumonisin in human urine.

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2. Experimental

Treated Amberlite was equilibrated overnight in methanol–water (1:3, v/v). The column was prepared on a sintered glass column (300×29 mm) to a height of 15 cm. A 100-ml sample of human urine was spiked with fumonisin B_1 and the pH was adjusted to 3.5 with 1 *M* HCl [10]. The sample was loaded onto the column at a flow-rate of 1 ml/min. The column was washed successively with 100 ml of methanol–water (1:3, v/v) and with 50 ml of methanol–water (1:1, v/v). Adsorbed fumonisin B_1 was eluted using 100 ml of methanol. The eluted fumonisin B_1 was evaporated to dryness on a rotary evaporator at 60°C.

The eluate was reconstituted in methanol–water (1:3, v/v) and the SAX clean-up was carried out as suggested by Shephard et al. [9], except that 0.5% acetic acid was used in the elution step instead of 5%, as suggested by Shephard et al. [9]. Detection was carried out using the method of Stack and Eppley [11].

3. Results and discussion

After clean-up on Amberlite and SAX cartridges, the sample was easily chromatographed without any interfering or coeluting peaks. A chromatogram of a spiked human urine sample is shown in Fig. 1. Recoveries of fumonisin B_1 in human urine spiked at low (10 ng/ml), medium (50 ng/ml) and high (500 ng/ml) levels are shown in Table 1. Reproducibility for these levels of spiking were found to be 4.35, 2.58 and 3.03 R.S.D., respectively. The detection limit of the method was found to be less than 8 ng/ml.

Inclusion of the pre-SAX Amberlite XAD-2 polymeric adsorbent resin clean-up successfully removes the interfering solutes from the urine without compromising the recovery of fumonisin B_1. Although this method had a detection limit that was only 6.25 times lower than that of the previous method, it had a much lower working range (8 ng/ml) when compared to the earlier method. In addition, the use of 0.5% acetic acid in methanol instead of 5% improved the baseline separation.

The small amounts of fumonisin that are found in the urine of people who consume a fumonisin-contaminated diet can be detected using our method. It is not only useful in biological monitoring of the exposure of humans to fumonisin B_1, but can also be useful for farm animals.

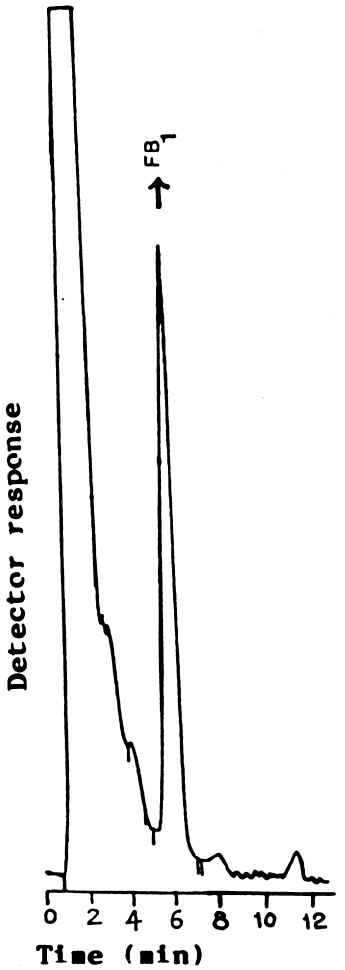

Fig. 1. Liquid chromatogram of OPA derivative of fumonisin B_1 in a spiked human urine sample (fluorescence detector, 335 nm excitation and 440 nm emission).

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Table 1
Recovery of fumonisin B_1 in a spiked human urine sample

Amount of fumonisin B_1 added (μg)	Concentration (ng/ml)	Amount of fumonisin B_1 recovered (μg)	Recovery (%)
1	10	0.94±0.04	93.6±4.35
5	50	4.76±0.13	95.1±2.58
50	500	47.22±1.53	94.4±3.03

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