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Determination of Aflatoxins G_1 and G_2 Using Ion Mobility Spectrometry

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ABSTRACT

This work describes a rapid and sensitive ion mobility spectrometry method for the determination of aflatoxins G_1 and G_2 (AFG_1 and AFG_2). The effective instrumental parameters were investigated and optimized. After optimizing, the calibration curves for AFG_1 and AFG_2 were linear in the range of 1 to 300 ng. Relative standard deviation was 8% and limit of detection was 0.5 ng. The capability of the proposed method was evaluated for the determination of AFG in spiked pistachio nut as a real sample that satisfactory results were obtained.

Key words: Aflatoxin G (AFG), Aflatoxin B (AFB), Ion mobility spectrometry (IMS), Pistachio nut.

INTRODUCTION

Aflatoxins (AF) belong to the group of mycotoxins, toxic fungal metabolites found as contaminants in various agricultural commodities under favourable temperature and humidity. AFs are difuranocumarine derivatives produced by *Aspergillus Flavus* and *Aspergillus Parasiticus* through a polyketid path way[1–3]. These compounds are carcinogenic, mutagenic and teratogenic to the most animals and humans. Among 20 identified AFs, the four major AFs are B_1, B_2, G_1 and G_2 (Fig. 1) named based on their fluorescence under UV light (blue or green) and relative chromatographic mobility during TLC[5]. Those AFs occur naturally in wide variety of agricultural products including cereals, nuts and dried fruits. Pistachio nuts are especially sensitive samples to AFs contamination. Some reports indicate natural contamination with AFB and AFG mainly in countries with warm and humid climate[6-8].

In order to control AFs levels in agricultural products, several analytical methods have been developed TLC [9], HPLC with different detectors[10-13] and enzyme linked immunosorbent assay[14] are the most commonly used. These methods are usually time consuming and require harmful solvents and well equipped laboratories. Therefore, it is still necessary to develop a sensitive, rapid and low cost method for the determination of AFs.

Here we report a method for determination of AFs ion mobility spectrometry (IMS). IMS is a gas phase ion separation technique that allows analytes to be identified on the basis of ion mobility. The

principles of IMS are well described in book and literature[15,16]. In brief IMS characterizes chemical compounds using gas phase motilities of ions in weak eclectic fields at ambient pressure. The mobility of an ion is determined by the ion velocity that is measured in the drift tube of the spectrometer where an electric filed is applied. During their drift, ions are separated based on their size, shape, and charge. Therefore, different ions reach the detector at different drift times, which are their characteristic. The number of ions reach to the detector is considered as a measure of the analytes concentration. IMS has applied for the rapid and sensitive detection of trace amounts of a broad range of compounds[17-20].

We previously, reported the capability of IMS equipped with positive corona discharge ionization source in determination of AFB_1 and AFB_2 in pistachio nut as a real sample[18]. Here, the method was extended for the determination of AFG_1 and AFG_2 and also the simultaneous determination of AFB and AFG in pistachio sample investigated.

EXPERIMENTAL

Materials and Chemicals

Pistachio samples were supplied by Pistachio Co. (Rafsanjan, Iran). The materials and solvents used for this research were purchased from Merck. AFs (B and G) were obtained from sigma Co. Stock standard solutions were prepared by dissolving analytes in methanol. The concentration of the solutions was determined using a UV-vis instrument as explained in Ref.[21] and stored below 5 °C until use. Working standard solutions were prepared by diluting stock standard in methanol. Immunoaffinity columns (Afla Test) were supplied by Vicam of USA.

Instrumentation

The ion mobility spectrometer used in this work was constructed in Isfahan University of Technology. The spectrometer, equipped with corona discharge ionization source was operating in the positive mode. The instrument was described in details in Ref.[17]. The newly designed injection port[18] for direct introducing liquid samples into the IMS was used. The injection port was filled with steel wool to enhance evaporation rate of the solvent and analyte. A UV-vis spectrophotometer (Shimadzu, 160-A, Japan) with 1 cm matched quartz cells was also used to verify the concentration of AF standard solutions.

Sample Preparation

The extraction and purification of AFs were performed based on the AOAC method[22]. 25 g grounded pistachio sample was used for each experiment. 5 g of NaCl and 125 mL of methanol solution (70%) were added to the grounded pistachio sample. The mixture was then blended at high speed for 2 min and filtered. 15 mL of the filtered solution was then mixed with 30 mL of water and filtration repeated. 15 mL of filtered solution was passed through Afla test affinity column at a flow rate of 1–2 drops per second. The column was twice washed with 10 mL water and dried with air. Finally, it was eluated by passing 1 mL methanol. This solution was then used for determination of AFs using IMS method.

RESULTS AND DISCUSSION

Optimization of the IMS Operation Parameters

Preliminary studies indicated the possibility of using IMS to determine AFG. AFG_1 and AFG_2 were separately injected into the IMS cell. Fig. 2 shows the ion mobility spectrum of AFG1 which contains only one peak. The spectrum of AFG_2 was similar to AFG_1, i.e, a single peak appeared at a drift time identical to that of AFG_1. Based on their structures (Fig. 1) and molecular weight (328 and 330), this behavior was predictable. IMS is not able to resolve two ions with similar structure that differ by only 2 amu. This was the case in our previous

Table 1. The optimized experimental conditions of IMS for determination of AFG

Parameter	Setting
Length of drift tube	11 (cm)
Drift field	600 (V cm^{-1})
Corona voltage	2200 (V)
Drift gas flow (N_2)	600 (mL min^{-1})
Carrier gas flow (N_2)	300 (mL min^{-1})
Injection port temperature	220 (°C)
IMS cell temperature	190 (°C)
Typical shutter grid pulse width	100 (μs)

work, where it was shown that AFB_1 and AFB_2 have identical spectra[18].

To obtain the best sensitivity for determination of AFG the parameters such as the corona and drift voltage, the injection and cell temperature, the flow rates of drift and carrier gas, and the shutter grid pulse width were investigated and optimized. The optimized experimental conditions for determining of AFG are given in Table 1 which are the same as those previously obtained for determination of AFB[18]. Among all parameters, temperature is the most important one. The spectrum was recorded at different temperature for injection port as well as for the IMS cell. Figs 3 and 4 show the effect of temperature (injector and cell, respectively) on the sensitivity. As shown, increasing the injection port temperature enhances the peak height. Similarly the cell temperature improves the sensitivity.

Table 2. The analytical parameters for the determination of AFG_1 and AFG_2

R^2 (G_1, G_2)	(0.9887, 0.9986)
LDR (ng)	1–300
LOD (ng)	0.5
RSD%	8

Table 3. Determination of AFG_1 or AFG_2 for spiked pistachio samples

Sample	Recovery%	Spiking levels (ng)
1	4.0	97
2	7.0	102
3	3.0	104
4	6.0	97.8
5	5.0	95.8

Analytical Parameters

The calibration curves for AFG were prepared with spiking different volumes of the analyte standard solutions into uncontaminated pistachio sample as blank. The blank was analyzed by IMS in which no AFG peak was observed. The analytical parameters for the determination of AFG were given in Table 2. According to this Table and Ref.[18], the results show the wide linear range in comparison with the determination of AFB. Furthermore, the precision is also improved for determination of AFG.

Determination of AFG_1 and AFG_2

To evaluate the analytical applicability of the proposed method, it was applied to the determination of AFG_1 and AFG_2 in spiked pistachio nuts. The results are given in Table 3. The recoveries are close to 100 % and indicate that the developed method can be used to the determination of AFG in pistachio samples.

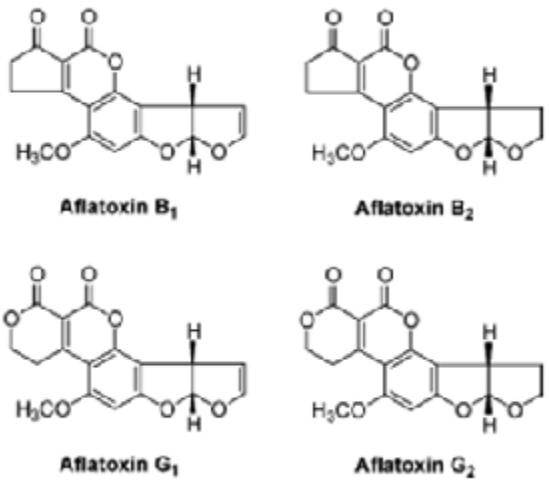

Fig. 1: Chemical structures of AFs B_1, B_2, G_1 and G_2 [4]

The possibility of determination of the total AFG_1 and AFG_2 was investigated. As mentioned before, the product ions of AFG_1 and AFG_2 completely overlap and appear at same drift time (13.7 ms). This means that IMS cannot separate and distinguish between AFG_1 and AFG_2. This problem was also observed in determination of AFB_1 and AFB_2[18]. To achieve to the determination of total AFG_1 and AFG_2, the response factors of them were evaluated and compared based on the slopes on their calibration curves. Statistical calculations show that the response factors slightly differ. Therefore, the determination of total AFG can be performed by IMS method.

Analysis of Mixtures of AFB and AFG

The other objective of our work was the development of IMS method for the simultaneous determination of mixtures of AFB and AFG without pre-separation. Binary mixtures of AFB_1 and AFG_1 were injected into the instrument at conditions given at Table 1. The ion mobility spectrum of this mixture is shown in Fig. 5. The product ions were appeared in the range of 15 to 16.8 ms so that the corresponding peaks did not overlap. The peak appeared at lower drift time is originated from AFG_1 whereas the second peak originates from AFB_1. The obtained results were unacceptable due to influence AFB_1 and AFG_1 signal on another.

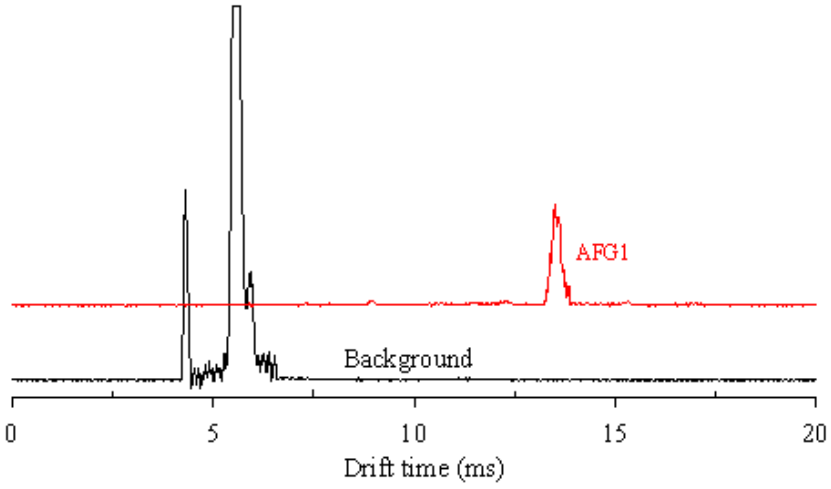

Fig. 2: Ion mobility spectra of AFG_1 or AFG_2 and background

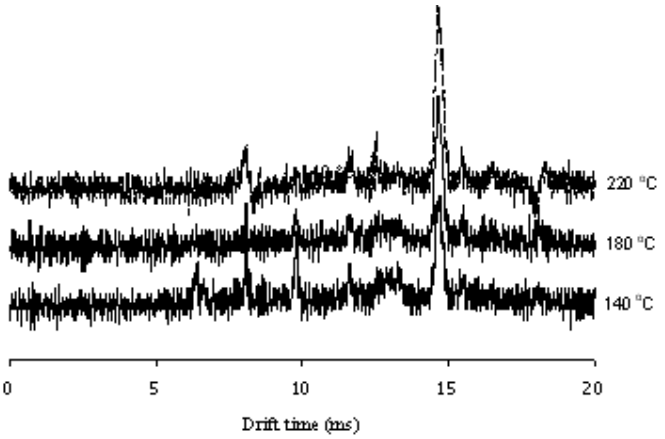

Fig. 3: The effect of injection temperature on the peak height for AFG_1

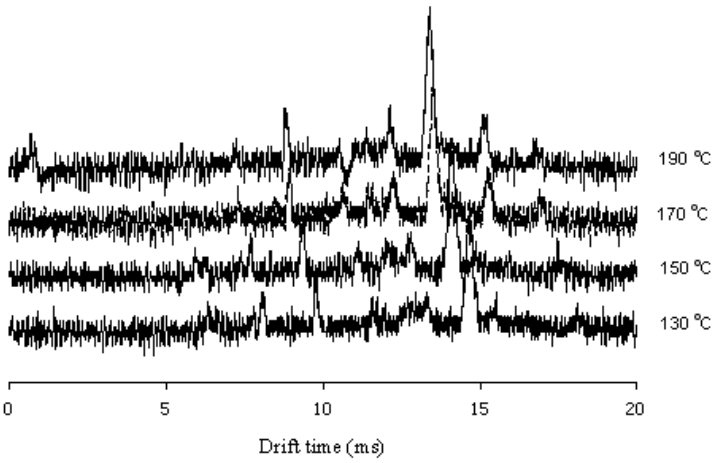

Fig. 4: The effect of cell temperature on the peak height for AFG_1

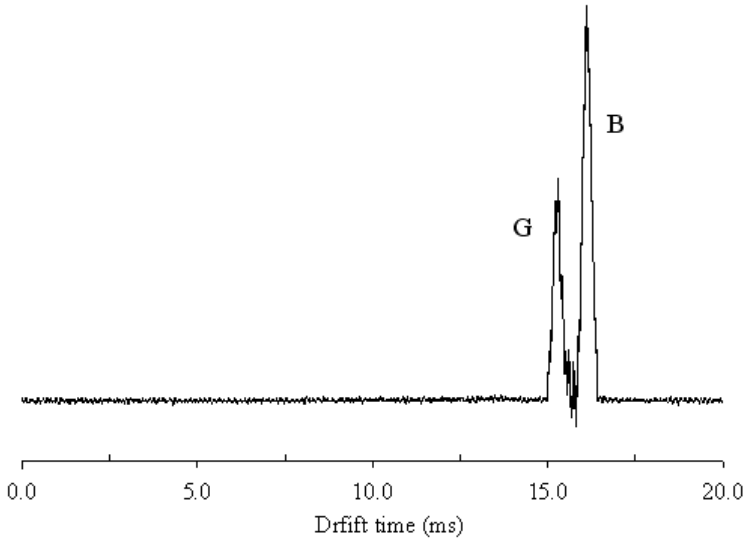

Fig. 5: Ion mobility spectrum of AFB_1 and AFG_1 in mixture

Therefore, a separation technique such as HPLC is required in the simultaneous determination purposes.

CONCLUSION

IMS using corona discharge ionization source permits rapid, sensitive and reproducible determination of AFG_1 and AFG_2. The reasonable results were achieved that showed the developed method can be applied as a simple and low cost method comparison with to other methods for the determination of AFs.

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