

Project 505.0990

Normalisation/harmonisation of method of analysis for pesticides and organic contaminants

Management: ir L.G.M.Th. Tuinstra

Report 91.15

March 1991

Stability of aflatoxin B₁ in animal
feed candidate reference materials

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The investigation was carried out on behalf of and for account of the
Community Bureau of Reference (BCR), Brussels, Belgium (Contract
2880/1/5/310/8712-BCR-NL(10))

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ABSTRACT

Stability of aflatoxin B₁ in animal feed candidate reference materials

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3 tables, 6 figures, 4 references

Two candidate reference materials animal feed were stored at a temperature of -18°C, 4°C, 20°C and 37°C. The stability of aflatoxin B₁ was studied during a period of two years.

A significant decrease in the aflatoxin B₁ content ($\alpha = 0.05$) was measured in the samples stored at 20°C and 37°C. In the samples stored at 4°C and -18°C no decrease in the aflatoxine B₁ content was found during a period of two years.

Keywords: aflatoxin B₁, stability, animal feed, reference material.

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(2)

(1)

SAMENVATTING

Een stabiliteitstest voor het gehalte aan aflatoxine B₁ in twee kandidaat referentiematerialen veevoeder is uitgevoerd bij verschillende temperaturen (-18°C, 4°C, 20°C en 37°C) ten behoeve van het Bureau Community of Reference (BCR) te Brussel.

Een significante afname ($\alpha = 0.05$) werd geconstateerd in de monsters bewaard bij 20°C en 37°C. In de monsters bewaard bij 4°C en -18°C werd gedurende twee jaar geen afname in het aflatoxine B₁ gehalte vastgesteld.

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1 INTRODUCTION

The role of certified materials for verification of the accuracy of methods and for development of new analytical methods is today well established.

In analytical chemistry the role of the Community Bureau of Reference (BCR) of the European Communities (EC) is to assist in improving the quality and accuracy of the analysis to avoid trade barriers between EC member states.

In EC regulation 87/519/87 dated 19 October 1987 limits for aflatoxin B₁ in raw materials and animal feed were established and the BCR specialist group on mycotoxins decided to develop candidate reference materials.

The homogeneity in the animal feed materials was investigated by the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands (Van Egmond, 1990). Our laboratory was contracted to carry out the stability study and results are now reported.

2 MATERIALS AND METHOD

Samples of the batches of a blank animal feed (target level of aflatoxin B₁ lower than 2 µg/kg) and a contaminated animal feed (target level of aflatoxin B₁ about 10 µg/kg) respectively coded RM 375 and RM 376 were provided by RHM Research and Engineering Ltd., High Wycombe, United Kingdom (Howell).

The samples were stored directly after receipt on the 27th of October 1988 at -18°C, 4°C, 20°C and 37°C for a study over two years. The sampling program is given in table I.

Table I Sampling program for the study on stability of aflatoxin B₁ and code of the samples

Period of analysis	RM 375		RM 376			
	storage temperature 4°C	-18°C	storage temperature 4°C	20°C	37°C	
November 1988	00900	00800	-	00825	-	
December	00100	00058	-	00372	00675	
January 1989	00200	00072	-	00433	00725	
March	00350	00100	00875	00474	00750	
May	00400	00150	00901	00512	00776	
June	-	00175	00928	00525	-	
July	-	00200	00960	00560	-	
August	00500	00251	01050	00600	-	
November	-	00275	01100	00651	-	
December	-	00632	00051	-	-	
March 1990	00600	00674	00105	-	-	
June	00700	00737	00194	-	-	
September	00800	00934	00248	-	-	
November	-	01061	00541	-	-	

The method of analysis for aflatoxin B₁ used is based on an extraction of the animal feed with chloroform, filtration and purification of an aliquot portion over a Florisil cartridge, subsequently followed by a C18 cartridge. Final separation and determination is carried out on a C18 reverse phase HPLC column, followed by a post-column derivatization with electrochemically generated bromine (Van Egmond (1989), Kok). To control the procedure blank chemicals, recovery experiments at 20 µg/kg level and analysis of internal control samples stored at 4°C were carried out.

3 RESULTS AND DISCUSSION

The results of all individual analysis of aflatoxin B₁ in the animal feed are given in table II. The results are not corrected for recovery.

Samples were analysed in duplo. The results are visualised in figure 1-4 for the different temperatures. A chromatogram of the animal feed samples is shown in figure 5 and 6. In total 14 recovery experiments at 20 µg/kg level were carried out. The mean was 90.8% (st.dev. 6.0%, range 82-100%).

The results of the reference material 375 are in general below 1.0 $\mu\text{g/kg}$ aflatoxin B₁ being the limit of determination of the method of analysis. The reported results should therefore be considered only as an indication of the content of aflatoxin B₁ in the "blank" animal feed.

Table II Aflatoxin B₁ content in the reference materials animal feed ($\mu\text{g/kg}$ product)

Aflatoxin B ₁ ----- Period of analysis	RM 375 storage temperature 4°C	RM 376 storage temperature			
		-18°C	4°C	20°C	37°C
November 1988	1.1-1.0	10.4-16.4*)	-	10.8-10.8	-
December	0.9-1.1	8.4-10.7	-	8.7- 8.5	6.4-6.9
January 1989	0.8-0.8	8.5- 7.9	-	8.9- 8.5	6.0-8.2
March	0.4-0.3	8.9- 8.3	9.5- 9.5	8.6- 8.3	4.3-4.7
May	0.3-0.3	7.7- 9.1	8.2- 8.2	6.9- 7.6	3.3-3.4
June	-	8.6- 8.4	8.0- 8.1	9.5- 7.6	-
July	-	9.0- 9.3	9.5- 8.7	7.1- 9.3	-
August	0.3-0.4	9.0- 9.4	8.6- 8.7	7.0- 7.7	-
November	-	7.7- 7.7	8.0- 9.0	7.5- 8.1	-
December	-	8.7- 8.5	8.7- 8.8	-	-
March 1990	1.2-0.3	7.7- 7.7	7.7- 8.1	-	-
June	0.5-0.4	8.9- 9.8	9.3- 9.1	-	-
September	0.3-0.3	8.4- 8.2	8.1-10.6	-	-
November	-	7.5- 9.1	7.9- 7.8	-	-
n	18	27	22	18	8
St.dev.(r) ($\mu\text{g/kg}$)		0.67	0.61	0.75	0.81
St.dev.(R) ($\mu\text{g/kg}$)		0.77	0.75	0.95	0.88
mean (mg/kg)	0.6	8.65	8.64	8.41	5.40

*) Dixon outlier, eliminated for statistical evaluation

Using variance analysis, assuming a linear behaviour of the correction for the data in time, a significant decrease ($\alpha=0.05$) was found for the aflatoxin B₁ content at 20°C and 37°C. The slope of the line through all results at -18°C was -0.04 $\mu\text{g/kg/month}$, at 4°C -0.01 $\mu\text{g/kg/month}$, at 20°C -0.18 $\mu\text{g/kg/month}$ and at 37°C -0.76 $\mu\text{g/kg/month}$.

The results obtained in the samples stored at -18°C and 4°C are equal. The mean of all selected data was respectively 8.65 and 8.64 μg aflatoxin B_1 /kg product.

During the stability study also internal control samples were analysed in simplo. The results are given in table III.

Table III Aflatoxin B_1 content in control samples

Sample code	Mean ($\mu\text{g}/\text{kg}$)	St. dev. ($\mu\text{g}/\text{kg}$)	CV (R) (%)	n
89-1	7.4	0.547	7.4	7
89-2	12.5	1.036	8.3	7

The CVs in the control samples do not differ from the results obtained at the temperatures -18°C (8,9%) and 4°C (8,7%).

4 CONCLUSION

Based on the results in the stability study it should be recommended to store the reference materials animal feed in a refrigerator at a temperature of 4°C or lower.

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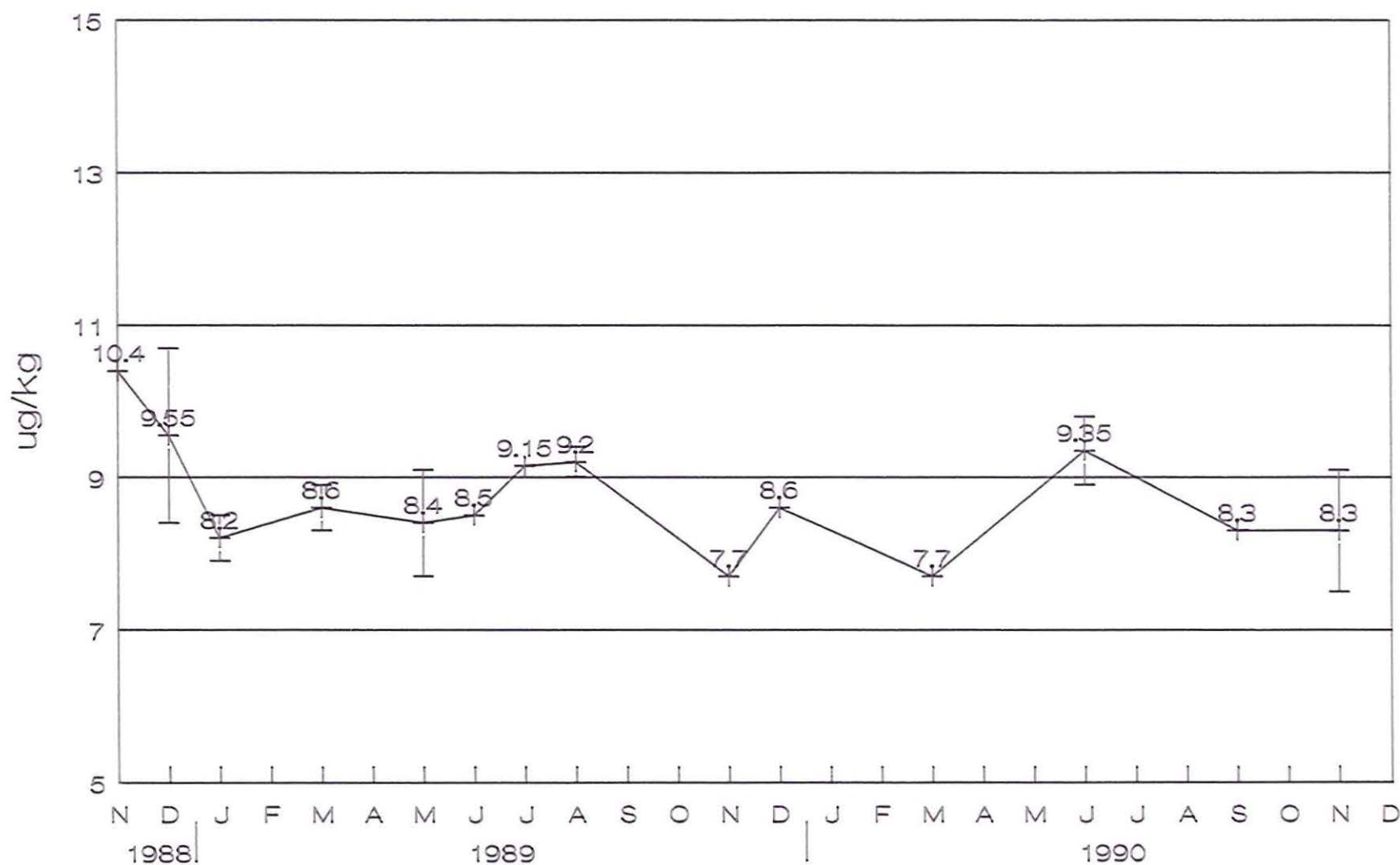
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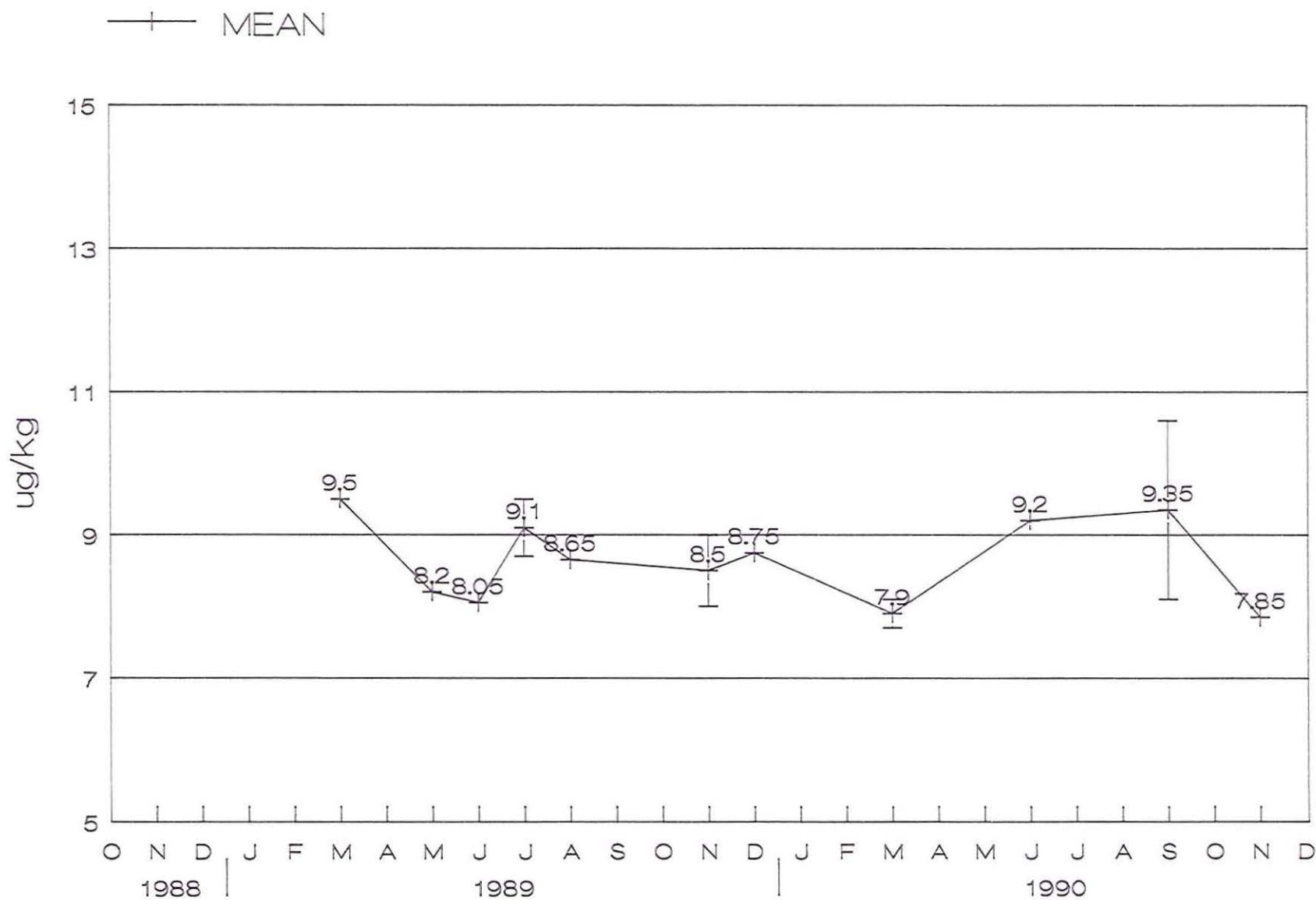
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**Fig.1 Aflatoxin B1 content in RM 376
stored at -18°C**

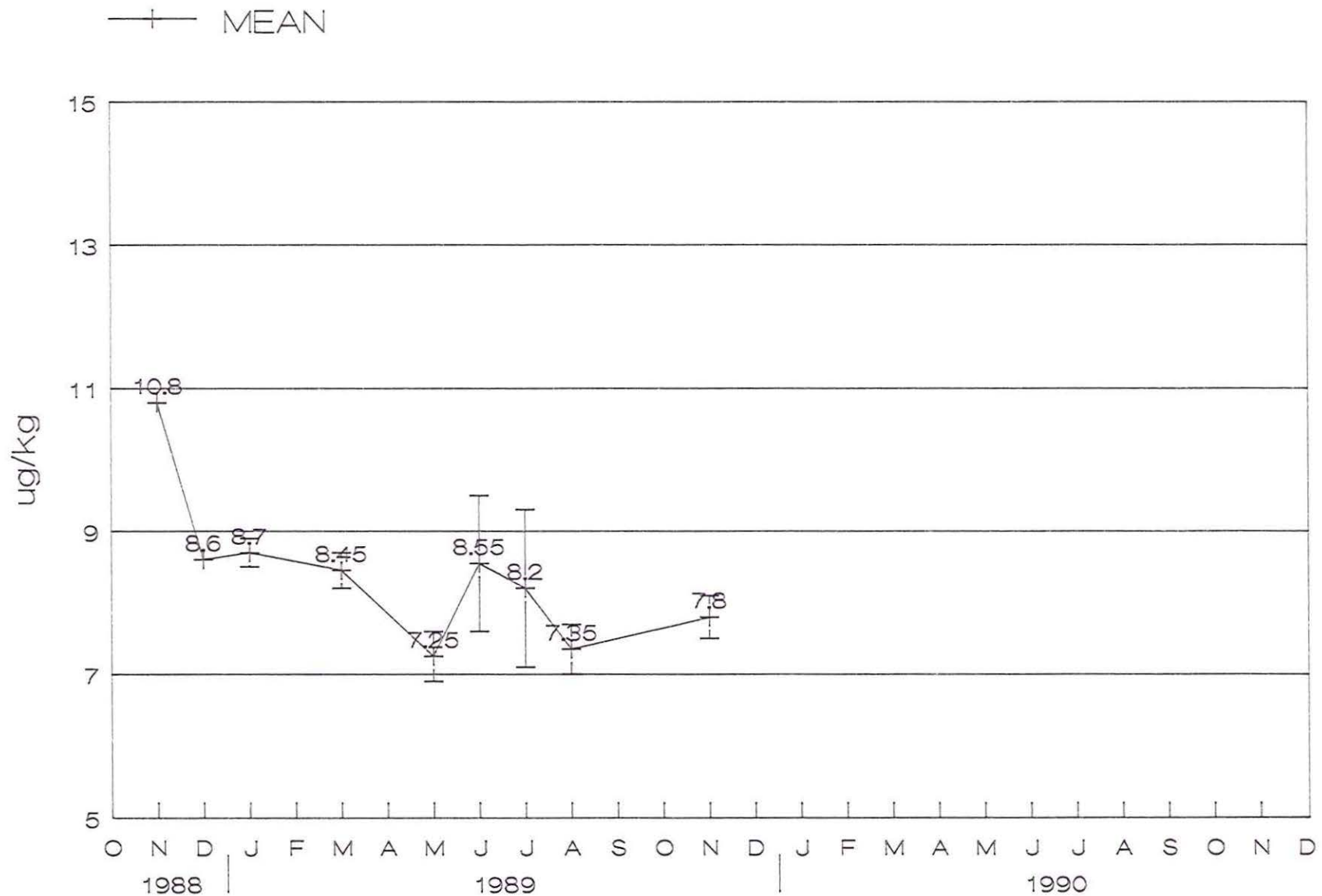
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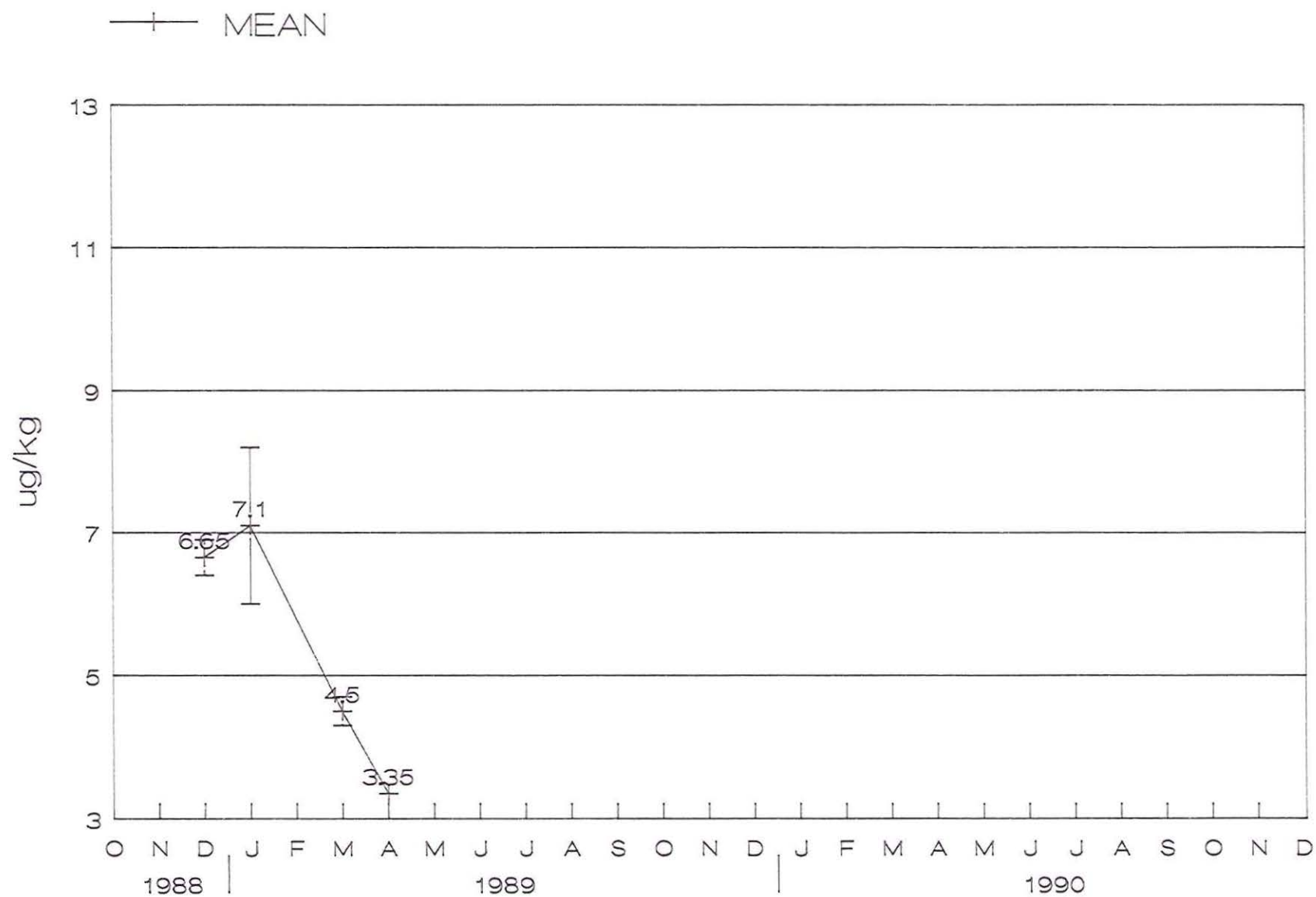
**Fig.2 Aflatoxin B1 content in RM 376
stored at 4 °C.**



**Fig.3 Aflatoxin B1 content in RM 376
stored at 20 °C**



**Fig.4 Aflatoxin B1 content in RM 376
stored at 37 °C**



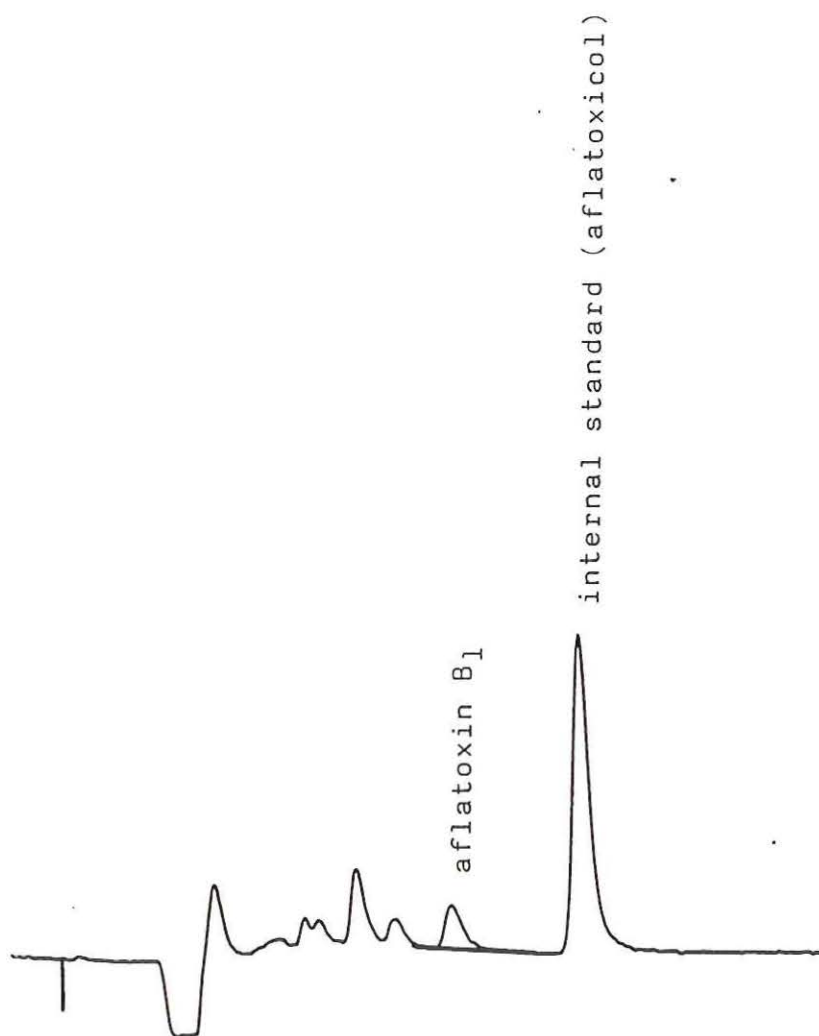


Figure 5. HPLC chromatogram of 0.05 g animal feed (RM 375)

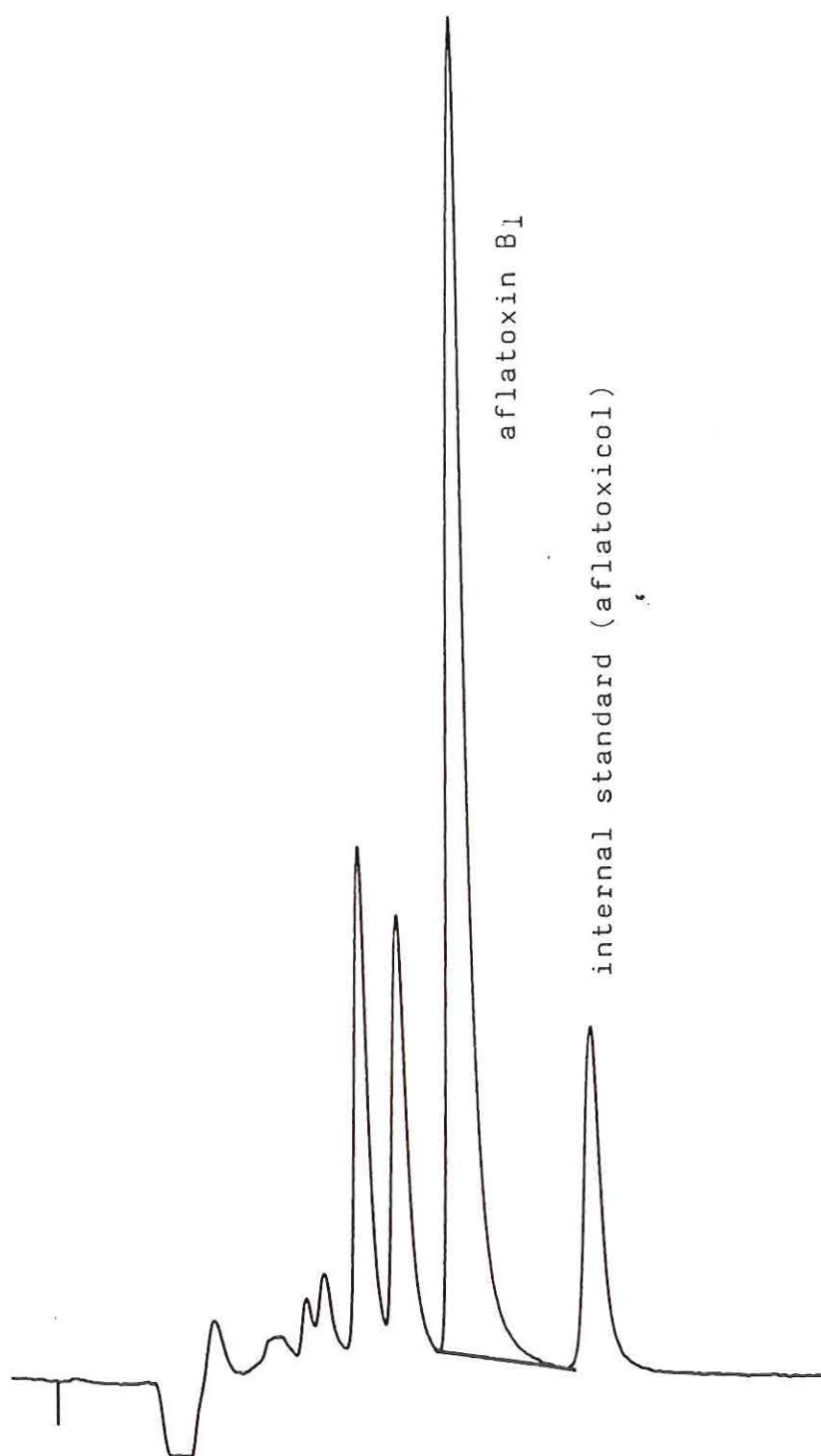


Figure 6. HPLC chromatogram of 0.05 g animal feed (RM 376)