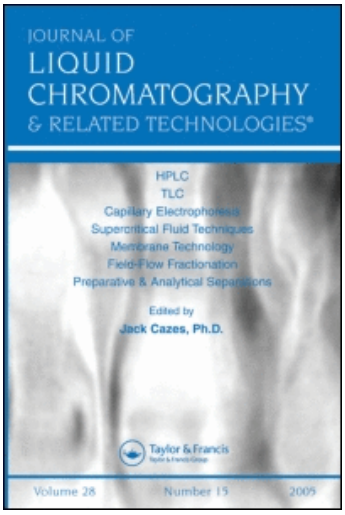

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A Simple and Rapid Method for the Determination of 2-Acetylaminofluorene in Laboratory Diets

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A SIMPLE AND RAPID METHOD FOR THE DETERMINATION OF 2-ACETYLAMINOFLUORENE IN LABORATORY DIETS

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ABSTRACT

A simple and rapid method for the determination of 2-AAF in animal feeds was developed using high performance liquid chromatography. The column employed was an octadecyl bonded silica support with 80% methanol as mobile phase. Fluorene was added as an internal standard. The method is applicable for concentrations of 250-1200 μg AAF/kg diet.

INTRODUCTION

The carcinogen 2-acetylaminofluorene (2-AAF) has been used extensively to induce cancer in laboratory animals. Its physical properties have been discussed (1) and various methodologies

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developed for its analysis by gas liquid chromatography and fluorometry have been reviewed (2). West and Oiler (3) have also published a method for determining 2-AAF in laboratory animal chow by reverse phase liquid chromatography. We are describing here a method for determining 2-AAF in semi-purified diets by high pressure liquid chromatography (HPLC). The method requires no preliminary clean-up and utilizes fluorene as an internal standard.

MATERIALS

Chemicals

2-AAF and fluorene were obtained from Aldrich Chemical Co. (Milwaukee, WI). The acetonitrile and methanol used as solvents were obtained from Burdick and Jackson (Philadelphia, PA) and were glass distilled.

Synthetic Diets

The semipurified diets containing added 2-AAF and control diets were prepared commercially according to the AIN-76 reference standard (4) (Bio Serv Inc., Frenchtown, NJ). They contained 10 or 40% of isolated soybean protein or vitamin-free casein, 5% corn oil, 5% cellulose, 3.5% minerals, 1% vitamins, 0.3% dl-Methionine and 0.2% choline bitartrate. The balance of the mixture contained sucrose and cornstarch in a ratio of 1:3. The protein content was varied at the expense of the carbohydrate. Several batches of diet were also prepared in the laboratory, spiked with 2-AAF in ethanolic solution, dried, thoroughly mixed, and assayed to determine recovery of the chemical.

Analytical Procedure

Five grams of diet were weighed into a 100 ml round bottom flask and 50 ml of acetonitrile containing the internal standard, fluorene, at a concentration of 300 μg/ml, were added via a pipet dispenser. The flask was then stoppered and shaken mechanically

for 30 minutes at about 100 cycles/min. After the solids were allowed to settle, 10.0 ml of extract were transferred to a 3-inch glass funnel lined with a 12.5 cm Whatman filter paper. The filtrates, collected in 22 ml glass scintillation vials, were analyzed as described below.

Instrumentation

The analytical system consisted of a Tracor model 995 isochromatographic pump (Tracir Avi, Austin, TX) and a Rheodyne loop injector equipped with a 20 µl loop (Rheodyne, Berkeley, CA) and U.V. detection at 285 nm. The data were recorded by a Hewlett-Packard model 3380 electronic integrator, which was programmed to record results directly in ppm when compared to an internal standard. A Whatman PXS-10-25, ODS-2 reverse phase octadecyl bonded column with an attached C-18, 37-50 µ bonded pre-column (4" x 1/8") were used for separation. The mobile phase employed was 80% methanol:20% water at 1 ml per min flow rate.

RESULTS AND DISCUSSION

Complex matrices like diets and tissues often contain components which are extracted and interfere with subsequent analyses. The extracts prepared as described here did not appear to contain interfering components in the area of the chromatogram of present interest. The chromatogram of a control diet extract showed no peaks. Subsequent chromatograms of diet extracts with added internal standard and 2-AAF are shown in Figure 1. Fluorene, a degradation product of 2-AAF, and chosen for the internal standard (1), was well-separated from 2-AAF. Its presence in diet extracts prepared after diets were stored as long as 90 days could not be demonstrated. This is to be expected since the formation of fluorene from 2-AAF requires hydrolysis of the amide followed by deamination. Conditions favoring these reactions would not be expected in feed stored at 4°C. Though oxidative and hydroxylated products might be expected, none were found.

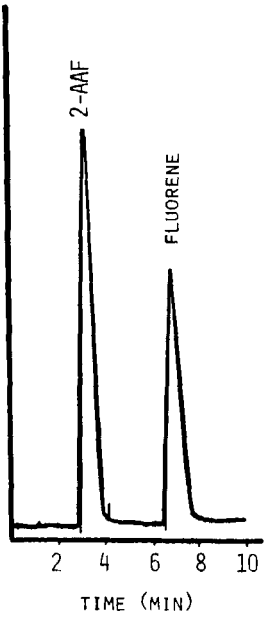

FIGURE 1. Chromatogram of diet extract containing 2-AAF and fluorene, an internal standard. Correlation: 25 cm x 4.2 m O.D.S. column (Whatman, 10 μ, Nutley, NJ). Mobile phase: 80% methanol:H_2O at 1 ml/min. Detection method: 285 nM.

The data shown in Table 1 indicate satisfactory overall recovery of 2-AAF from semi-purified diets to which the carcinogen and internal standard had been added in the laboratory. The difference in carcinogen content from that expected was slightly greater than one percent.

The data presented in Table 2 indicate a considerable difference both in the error between replicate samples and the percentage deviation from the amount presumed to be present in the diet mixture. The 54 samples analyzed to obtain these data were all obtained from diets provided by the same commercial supplier. They demonstrate the importance of analyzing dietary formulations before feeding of toxic or carcinogenic compounds which depend upon the amount consumed for their biological effects. The

TABLE 1

Recovery of Added 2-AAF from Control Diets

% Protein	Concentration of 2-AAF Added µg/g	n*	Concentration of 2-AAF Found µg/g ± σ	% Recovery
10	250	6	257.2 ± 4.5	102.9 ± 1.3
	333	6	344.1 ± 4.5	103.4 ± 1.3
	666	6	669.7 ± 13.7	100.6 ± 2.1
	1000	6	1005.9 ± 6.2	100.6 ± 0.6
40	333	6	348.3 ± 4.4	104.6 ± 1.3
	500	6	524.0 ± 4.9	104.8 ± 1.0
	666	6	695.9 ± 7.3	104.5 ± 1.1
	700	6	710.5 ± 7.5	101.5 ± 1.1
	1000	6	985.4 ± 9.4	98.5 ± 0.9

*Each sample result was the average of duplicate injections into the HPLC.

TABLE 2

Determination of 2-AAF in Commercially Prepared Diets

Diet Number	% Protein	Concentration of 2-AAF Added µg/g	n	Concentration of 2-AAF Found µg/g ± σ	% Recovery
1*†	10	333	6	306.4 ± 17.5	92.0 ± 4.3
		666	6	576.2 ± 20.4	86.5 ± 3.0
		1000	6	973.4 ± 47.6	97.3 ± 4.8
	40	333	6	332.7 ± 58.1	99.9 ± 17.5
		666	6	614.7 ± 11.7	92.3 ± 1.8
		1000	6	935.3 ± 53.0	93.5 ± 5.3
2*†‡	10	333	3	340.6 ± 6.5	102.3 ± 1.8
		666	3	671.1 ± 32.0	100.8 ± 4.8
		1000	3	990.4 ± 57.0	99.0 ± 5.7
	40	333	3	294.4 ± 8.4	88.4 ± 2.6
		666	3	641.0 ± 28.8	96.2 ± 4.3
		1000	3	951.6 ± 8.8	95.2 ± 0.9

*Diets supplied at two different time intervals and from different batches.

†Determined by duplicate injection of diet extract into the HPLC.

‡One injection of diet extract into the HPLC.

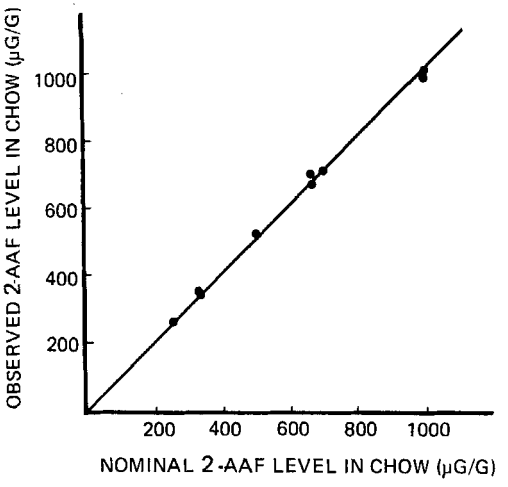

FIGURE 2. Relationship between observed and nominal 2-AAF level in chow.

linearity of the analytical method developed is illustrated in Figure 2. Reliable determinations were made for concentrations ranging from 250 µg to 1200 µg/kg of 2-AAF in the diet. The currently used analytical column has shown no signs of deterioration after analyses of over 500 diet extracts. It is necessary, however, to employ a short precolumn packed with 37-50 µ octadecyl stationary phase, which should be replaced after 200-225 analyses.

ACKNOWLEDGEMENT

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