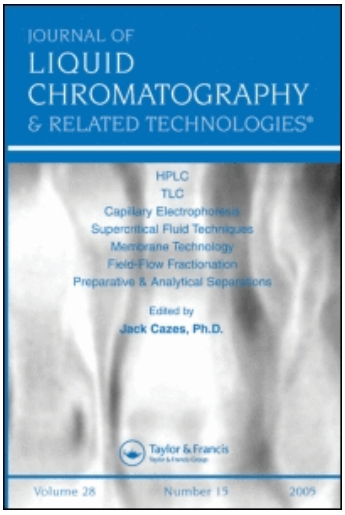

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ANALYSIS OF 2-ACETYLAMINOFLUORENE IN LABORATORY ANIMAL CHOW BY REVERSE PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

A high speed liquid chromatographic procedure for analysis of 2-acetylaminofluorene (2-AAF) in laboratory animal chow is presented. The procedure is rapid and uncomplicated and provides accurate and precise analysis. The reverse phase liquid chromatography allows minimal clean-up of extracts with good column stability and high sample thru-put. Linearity and minimum detectable limit are discussed.

INTRODUCTION

A high speed liquid chromatographic procedure was used in a quality control program to assure dose level and homogeniety of approximately 250 batches of laboratory animal chow per month. The chow meal was treated with a known carcinogen, 2-acetylaminofluorene (2-AAF). The feed was prepared in 20 kilogram batches by spraying 1 liter of an ethanol solution of the agent into a ribbon blendor. Three samples were selected at random from each batch. The concentrations of 2-AAF in the chow were 30, 35, 45, 60, 75, 100 and 150 ug/gm (ppm). In addition, control chow that contained no 2-AAF was processed in a similar manner (ethanol spraying, etc.).

Some physical properties of 2-AAF, and a sensitive fluorometric method for analysis of 2-AAF in animal chow and microbiological media have been described (1).

MATERIAL AND METHODS

Chemicals: 2-Acetylaminofluorene (2-acetamidofluorene) was obtained from Aldrich Chemical Co. (Milwaukee, Wisc.). Basic alumina, Brockman Activity Grade 1, 80-200 mesh was obtained from Fisher (St. Louis,Mo.). The acetonitrile used was glass-distilled by Mallinchrodt Chemical Co. (St. Louis, Mo.). Water was glass-distilled in the laboratory.

Equipment: A Waters (Milford, Mass.) Model 202 liquid chromatograph with sample injector and 280 nm photometer was used. The output signal was quantitated and recorded with a Hewlett-Packard (Avondale, PA.) Model 3370B digital integrator and recorder Model 7127B with variable input module. The liquid chromatographic column was a Varian (Palo Alto, Ca.) bonded octadecylsilane column with 10 micron particle size (2mm I.D. x 25 cm length). An Eberbach (Ann Arbor, Mich.) reciprocal shaker and a Labindustries (Berkeley, Calif.) repetitive dispenser were used, along with a Kontes (Vineland, N.J.) glass column (11.5mm I.D. x 16cm length) for the alumina.

Clean-Up and Liquid Chromatography: Three grams of chow were weighed into a 100ml long-necked round bottom flask with ground glass stopper. Fifteen ml distilled water was added from a repetitive dispenser and the flask was swirled. Fifteen ml acetonitrile was added, and the flask was placed on a reciprocal shaker and run at 100 excursions per minute for 40 minutes. Control samples were treated in the same manner as the 2-AAF coated chow. Extraction efficiency for each dose level was determined by adding a known amount of 2-AAF in solution directly onto the chow and extracting as usual. A clean-up column was prepared by plugging the bottom of an open glass column with glass wool and adding 3 grams of basic alumina. A ten ml portion of the extract was eluted through the column. Ten microliters (ul) of this eluate was injected onto the analytical column. Flow rates ranged from 0.5 to 1 ml of 1:1 acetonitrile-water mixture. The eluted 2-AAF was detected with a 280nm photometer and quantitated with a digital integrator. The concentration of 2-AAF in the unknown sample was

determined by comparison of the peak area with the average area of three standards run at the same nominal concentration as the unknown and corrected for extraction efficiency. Control (zero level 2-AAF) chow samples served as blank values for the procedure.

RESULTS

Table 1 shows the recovery percentage and standard deviation of the analysis for each of the seven different concentrations of 2-AAF. Values listed for each concentration in the table are means from the average of three determinations of extraction efficiency made each time samples were run at a level. Number of runs represented are listed.

Linearity of the procedure between 30 and 150ug 2-AAF per gram of chow is shown in Figure 1. This corresponds to a range of 30 to 150 ng of 2-AAF per 10 ul injection.

The minimum detection limit of the analysis is 10 ug of 2-AAF per gram of chow (10ppm). This level gives 10 ng 2-AAF per injection on the analytical column, and a signal-to-noise ratio of about ten.

DISCUSSION

Passage of the extract through alumina was used to remove co-extracted compounds that would interfere with resolution of the

TABLE 1

2-AAF Analysis - Percent Recovery and Standard Deviation of the Procedure.

Nominal 2-AAF Level	30	35	45	60	75	100	150
Percent Recovery	99.3	99.8	99.8	100.4	99.8	99.4	100.4
Standard Deviation	1.9	1.8	1.7	1.8	2.0	1.5	2.2
Number of Triplicate Recovery Analyses	32	25	23	33	30	22	18

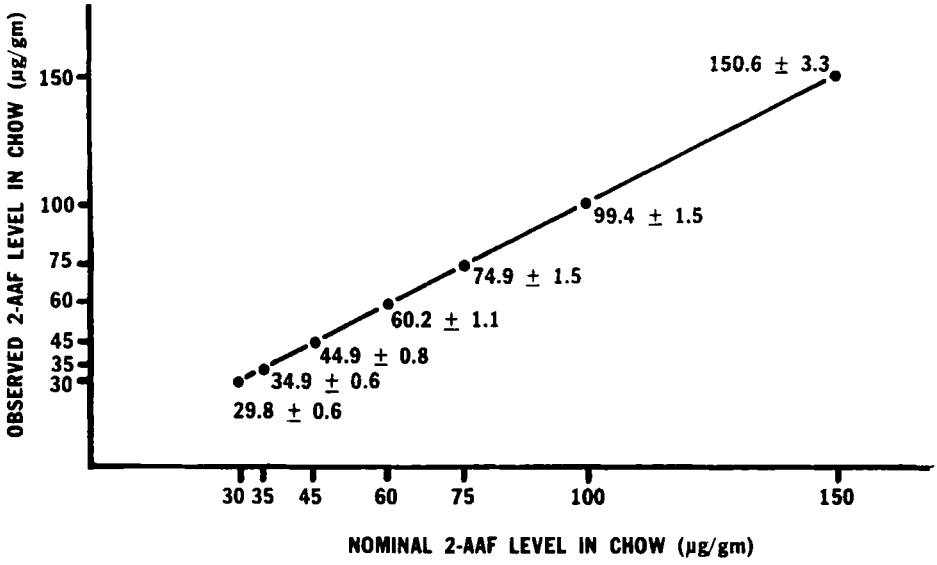

FIGURE 1.

Linearity of the 2-AAF Analytical Procedure.

Mean Recovery and Standard Deviation are Shown at Each Level

2-AAF peak on the analytical column. The background absorbance was reduced, and baseline resolution of 2-AAF was achieved. This was necessary to assure the best accuracy and precision for the digital integrator. The clean-up step was kept brief to maximize the number of samples analyzed per day. The reverse phase analytical column was subjected to a heavy work load, but showed good stability for about 500 injections. When poor chromatographic behavior occured, the column was replaced.

A standard control chart (2) was maintained to follow the recovery values on a daily basis. The confidence limits used were the mean recovery plus or minus two standard deviations. Analyses were repeated on samples where the daily extraction efficiency was outside the confidence limits.

The total time required to conduct the analysis for a single sample was less than three hours. A volume of 50 samples per day (unknowns and extraction efficiency samples) could be handled by two analysts. Considering the good recoveries, the linearity of the procedure, and the high sample volume, a rapid and reproducible method has been presented.

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