Short Method for the Detection of Chick Edema Factor in Fats, Oils and Fatty Acids by Electron Capture Gas Chromatography

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ABSTRACT

The electron capture gas liquid chromatography test method for chick edema factor has been modified by replacing the alumina column step with an additional sulfuric acid cleanup and a caustic wash which permits a reduction in sample cleanup time. Samples are subjected to double preliminary sulfuric acid cleanup and caustic wash and extracted each time with trimethyl pentane. The final extract is washed with sulfuric acid and examined by electron capture gas chromatography. Gas chromatographic peaks with retention times vs. Aldrin (Ra values) between 8 and 45 are indicative of the presence of chick edema factor.

INTRODUCTION

The chick edema factor (CEF) has been characterized as a group of toxic polychlorinated chemicals found randomly in trade processed fats and oils and fatty acids. The detectable concentration of the CEF in fat by electron capture gas chromatography is in the low nanogram range.

According to x-ray crystallographers Cantrell et al. (1) the chemical structure of the CEF is 1,2,3,7,8,9 hexachlorodibenzo-p-dioxin. This anthracene base skeleton was accepted instead of the earlier phenanthrene based model.

The possible sources of the CEF in fats and oils, its potential precursors, the reaction mechanism of formation, its isomers and the related toxicity were treated in detail by Higginbotham et al. (2) and Verrett (3).

Extensive reviews of methods of CEF detection in fats and oils were presented by Firestone (4) and Ress et al. (5).

Our critical evaluation of the official method (6) based on several hundred analyses points at several weaknesses associated with the alumina column fractionation in the cleanup process. Many elements in the alumina column fractionation can alter severely the efficiency of the cleanup process in terms of CEF signal recovery. These constants are difficult to regulate to keep them uniform: (a) activation time and temperature of alumina; (b) density and uniformity of column packing; (c) elution rate; (d) humidity prevailing in the laboratory.

In our short method we omitted the alumina column fractionation altogether, thus eliminating all the above mentioned potential causes affecting CEF signal recovery. Instead we added a second preliminary sulfuric acid treatment and a caustic wash was applied after the trimethylpentane extraction, just before the final sulfuric acid wash.

EXPERIMENTAL PROCEDURE

Reagents and Apparatus

Only glass containers were used for the test and storage of reagents; all glassware was rinsed with trimethyl pentane before use. Reagents were 50% KOH solution in water (w/w), reagent grade concentrated H_2SO_4, reagent grade CCl_4, and chromato quality 2,2,4 trimethyl pentane (TMP) (Matheson, Coleman and Bell). Standard Aldrin solution was made by dissolving Aldrin in TMP to make 0.1 μg/ml solution. The CEF low positive reference sample was 1.5% reference toxic fat in USP cottonseed oil or other suitable vegetable oil. (The reference toxic fat is available from the Division of Pesticides, Bureau of Science, Food and Drug Administration, Washington, D.C.)

The gas chromatographic column was of glass, 6 ft long x 1/5 in. ID, packed with 3% SE 52 silicone gum rubber on 60-80 mesh Gas Chrom Q or 60-80 mesh acid washed Chromosorb W (Applied Science Laboratories, State College, Pa.). The column was conditioned for 2-5 days at 250 C using nitrogen as a carrier gas. A tritium source, concentric type electron capture detector is recommended. Stable baseline for carrying out analysis should be obtained. Optimum electrometer settings and detector voltage neces-

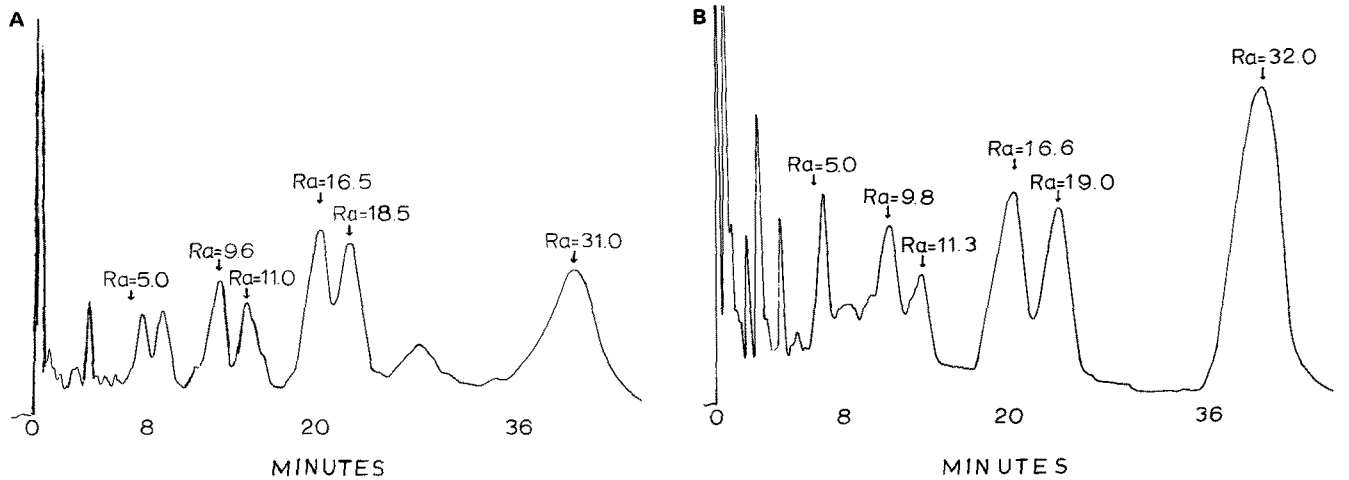

FIG. 1. A: Gas chromatogram of strong CEF positive fat, Official AOAC Method (injected sample size, 0.1 μliter). B: Gas Chromatogram of strong CEF positive fat, Short Method (injected sample size, 0.1 μliter).

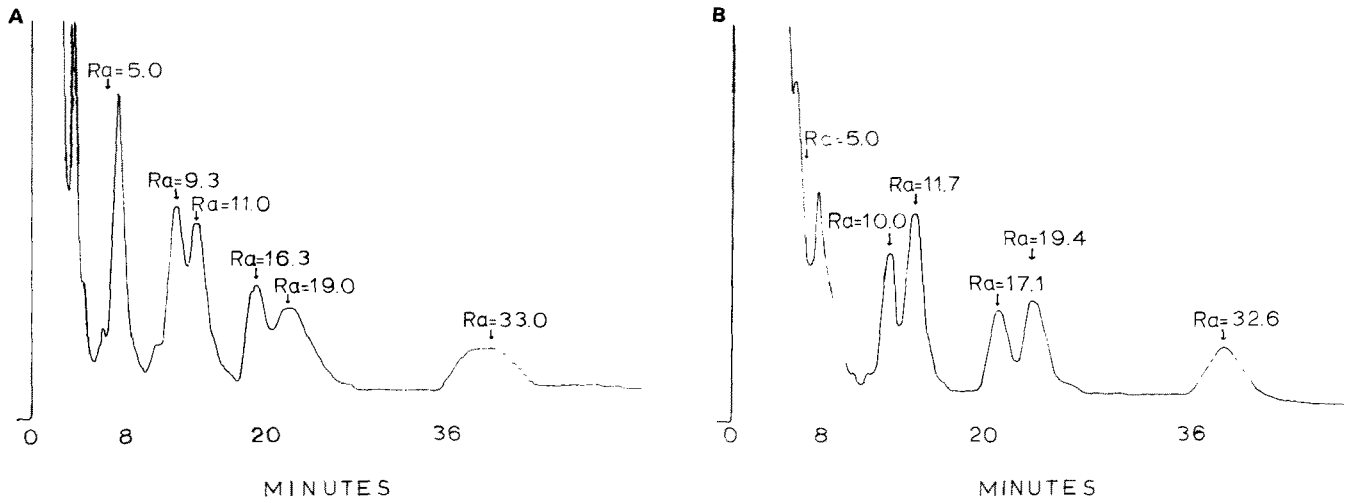

FIG. 2. A: Gas chromatogram of low CEF positive toxic reference standard fat, Official AOAC Method (injected sample size, 1.0 μliter). B: Gas chromatogram of low CEF positive toxic reference standard fat, Short Method (injected sample size, 1.0 μliter).

sary to achieve the required sensitivity for detection of CEF using the low positive reference sample should be determined. Nitrogen flow rate should be high enough to elute Aldrin in 1-1.5 min (Ra). Chart speed was 1 in./5 min. To establish Ra factor, standard Aldrin solution must be injected before each reference or test sample.

Determination

Analysis of 1.5% reference toxic fat should be performed with each batch of samples. About 3.0 g of sample of 1.5% low positive reference fat must be weighed into a 125 ml Erlenmeyer flask, and 10 ml of CCl_4 added, the flask shaken for 30 sec, 10 ml of H_2SO_4 added, the solution shaken again for 30 sec and let stand for 1 min. 25 ml TMP are then added, and the solution is shaken for 1 min. Layers are then allowed to separate and the upper layer is carefully decanted into a 250 ml beaker. Extraction is again performed with 2 x 25 ml TMP, shaking each time for 30 sec, and separating the layers. TMP extracts are combined and evaporated just to dryness using compressed air and low heat.

The residue is taken up in 10 ml TMP, shaking to dissolve the residue. 10 ml concentrated H_2SO_4 is added and shaken for 30 sec; 10 ml TMP is added, and the solution is shaken again for 30 sec, and the layers separated. The upper layer is decanted into a 250 ml Erlenmeyer flask and extraction is repeated with 2 x 10 ml TMP.

TMP extracts are combined into a separatory funnel and washed with 30 ml 30% solution KOH, shaking for 1 min. The mixture must be allowed to stand 10 min, and the layers separated. The lower KOH layer is then washed with 10 ml of TMP. TMP layers are combined and rinsed with 10 ml concentrated H_2SO_4, shaking for 30 sec; separated TMP layer is decanted into a 250 ml beaker. H_2SO_4 layer is washed with 2.x10 ml TMP, and upper layers are combined.

Combined TMP extracts are evaporated to 2-5 ml using compressed air and low heat. The residue is transferred into a small vial and evaporated almost to dryness using compressed air and low heat. The evaporation is completed with compressed air and no heat. The residue is taken up in 0.1 ml TMP, the vial is lightly stoppered and rotated so that solvent wets sides of vial.

One μliter of sample solution is injected into a calibrated gas chromatograph. Calibration is performed with standard Aldrin solution and 1.5% reference toxic fat. One μliter of the reference toxic fat solution, prepared in the same manner as the sample should be used for the calibration. Peaks at Ra 8-13 are due to a number of hexachloro-dibenzo-p-dioxin isomers; two peaks at Ra 16-22 are due to the two heptachloro-dibenzo-p-dioxin isomers, and a peak at Ra 30-45 is due to octachloro-dibenzo-p-dioxin (5). Ra values of sample peaks are compared with Ra values of peaks from reference toxic fat. Reagents are checked for possible interferences by running a blank with each batch of reagents. The same procedure is followed for the blank

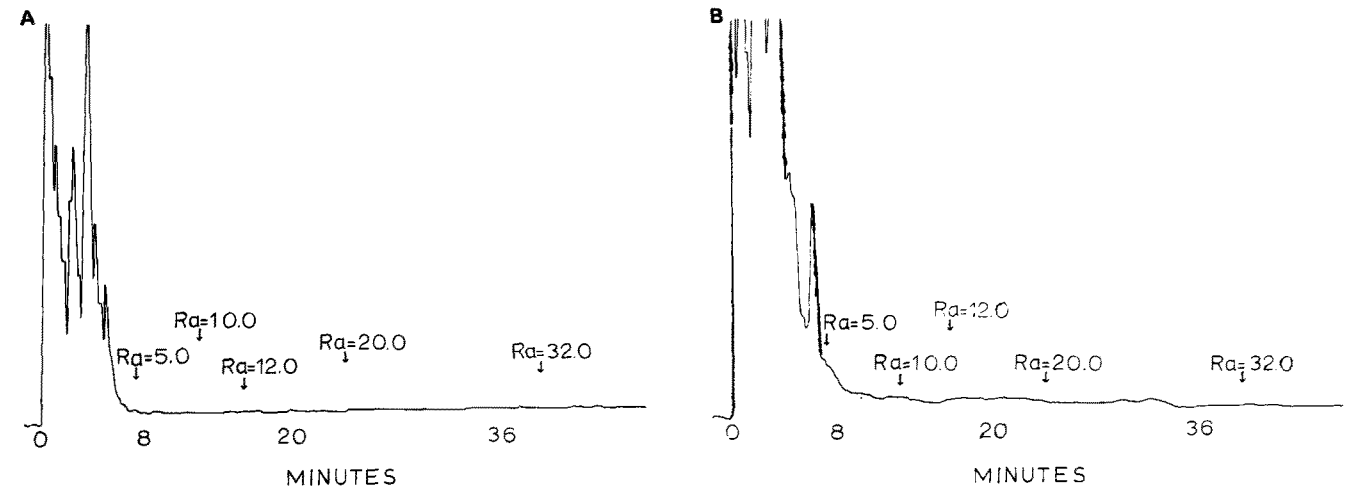

FIG. 3. A: Gas chromatogram of CEF negative fat, Official AOAC Method (injected sample size, 1.0 μliter). B:Gas chromatogram of CEF negative fat, Short Method (injected sample size, 1.0 μliter).

as for a sample, using the same solvents but no oil. The chromatogram from the blank should show a smooth low baseline in the interval Ra 8-Ra 45.

RESULTS AND DISCUSSION

Advantages of this short method are:

First, by omitting the alumina column fractionation the method eliminates all potential causes affecting CEF signal recovery as mentioned earlier.

Second, the method is much shorter and faster on an individual assay basis—that is, within 2-3 hr one can obtain the result. The official method treats six to eight samples simultaneously but it takes one and a half days to obtain the results.

Disadvantages of the short method are:

The demand for constant and attentive work during the analysis, making work overlap impossible for the operator.

A larger amount of impurities is present—as indicated on the GC chart—in the Ra 1-5 region. Also, in the Ra 5-20 region a slight base-line elevation (5-8%) may be encountered.

For qualitative comparison between the official and short methods the following charts illustrate the CEF signal presentation with three different conditions:

Chromatograms 1/a and 1/b present strong CEF positive fat.

Chromatograms 2/a and 2/b present low CEF positive (FDA-reference) fat.

Chromatograms 3/a and 3/b present negative CEF fat.

In some cases the short method exhibits impurities of unknown character, present randomly in the Ra 8-25 region, but not interfering in the qualitative and quantitative recognition of the CEF signal domain. Any relatively high molecular weight, heat stable compound having electron withdrawal properties in the nanogram concentration level could generate peaks in the 8-25 Ra region.

In order to clean up these impurities, further research is necessary.

This new method of CEF detection in fats and oils can be used as a fast screening test but not as a substitute for the official test method.

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