Improved Extraction Procedure for 2,3,5-Triiodobenzoic Acid from Milk and Milk Products

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While studying 2,3,5-triiodobenzoic acid (TIBA) residues in milk and milk products using gas chromatography, it became necessary to develop an extraction and clean-up procedure which would concentrate TIBA and/or its metabolites, separate the compound from interfering milk constituents, and give a low background signal. This was necessitated since most extracting solvents were found to form emulsions with milk or milk products (ether, methanol, ethanol, isopropyl alcohol, and aqueous formic acid) or were expensive (acetonitrile). The earlier extraction method as used by Lisk (1) does not include a clean-up procedure.

Procedures

Clean-up and Extraction Procedure. Milk or milk product samples weighing 100 g. were placed in a tared 500 ml. erlenmeyer flask on a magnetic stirrer and titrated dropwise with H_3PO_4 (85%-N.F.) until the mixture was at a pH of 2.5-3.0 (about 4 ml. per 100 g. sample). A minimum of 250 ml. of technical grade acetone was added and the mixture allowed to stir for 60 minutes. The resulting slurry was settled in an ice bath for about 1 hour (speeds filtration) and then filtered with vacuum on a 150 C scintered glass funnel. Acetone was evaporated from the filtered

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solution, 10 ml. of a saturated aqueous solution of NaCl was added to the aqueous residue, and the solution titrated to a pH of 9.5 with an aqueous solution of NaOH.

Depending upon the fat content of the milk products the clean-up step was varied. For whole milk, skim milk and buttermilk, the basic solution was extracted in a 250 ml. separatory funnel once with 50 ml. of solvent grade ether, the aqueous phase saved and the ether phase backwashed with 25 ml. of a 5% aqueous solution of NaCl at a pH of 10. The aqueous phases were combined and the ether clean-up phase discarded. For butter and cream the basic solutions were extracted as above and the combined aqueous phases re-extracted two more times in a like manner.

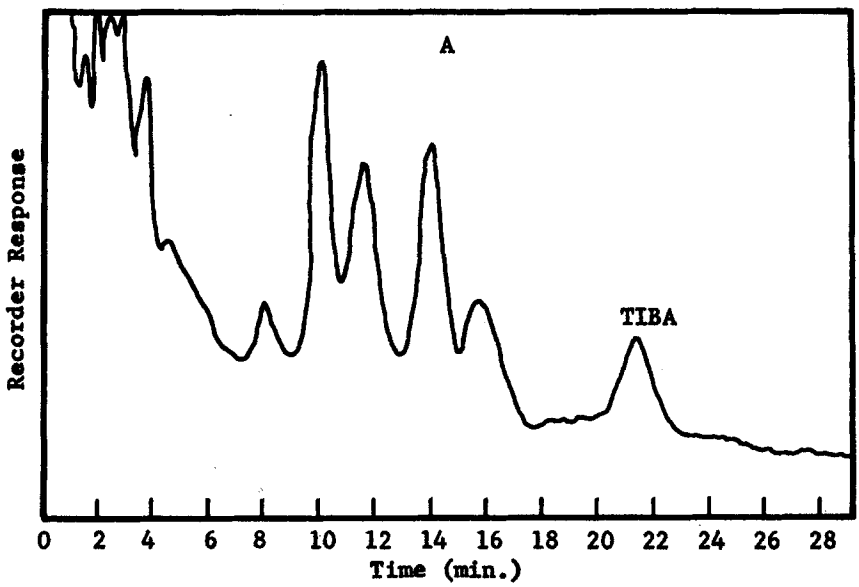
The cleaned up aqueous phase was then titrated to a pH of 2.5-3.0 with H_3PO_4 (85%-N.F.) and extracted with three 50 ml. portions of reagent grade diethyl ether (Mallenckrodt Chemical Works, St. Louis, Missouri). The three ether phases were combined and evaporated to dryness. Etheral diazomethane prepared from "Diozald," N-methyl-W-nitroso-p-toluenesulfonamide, (Aldrich Chemical Co., Inc., Milwaukee, Wisconsin) was used to esterify the residue which had been reconstituted in 5 ml. of reagent grade diethyl ether. The diazomethane was added dropwise until a yellow color persisted, then a 2-5 ml. excess of the diazomethane added, and the reaction allowed to proceed to completion (60 min.). The esterfied solution was then carefully evaporated to dryness (35°-40°C) and the residues were quantitatively transfered to a 10 ml. volumetric flask with Spectrophotometric Grade benzene

(J. T. Baker Chemical Co., Phillipsburg, N. J.) washes. For gas chromatographic analysis, 0.1-1.0 microliters of the extract were used. Since Jarboe (2) has shown that TIBA undergoes photodecomposition, all samples were protected from light at all times.

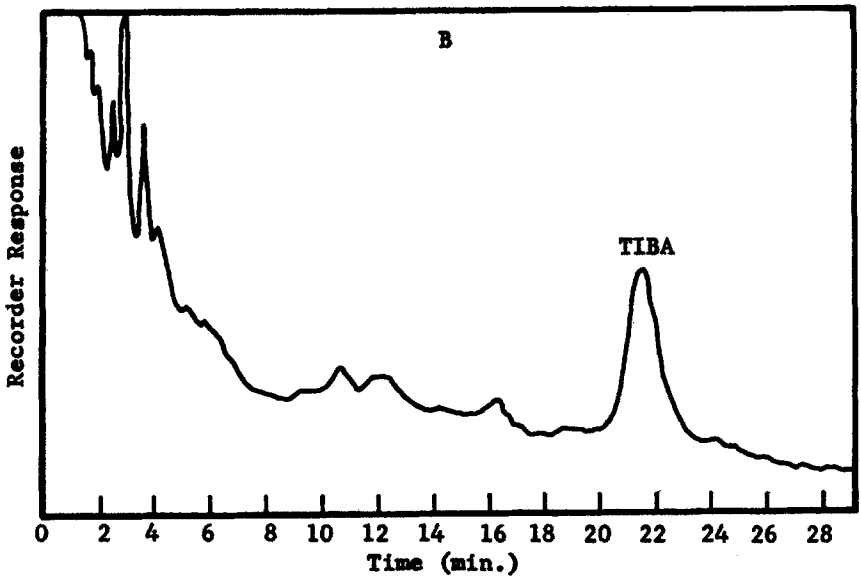
Gas Chromatography. For analysis of the esterified residues a dual column gas chromatograph (Varian Aerograph, Walnut Creek, California - Model 204-1B) equipped with 250 millicurie tritium foil electron capture detectors was used. The gas chromatographic operating conditions were: injector temperature - 270°C, column temperature - 190°C, detector temperature - 210°C, carrier gas flow - 35-40 ml./min. (high purity nitrogen), high voltage - E.C.-1, and attenuator-variable depending upon sample size. Because of the high operational temperatures, it was impractical to use glass columns. For this reason, a 10 ft. stainless steel column which had been treated with trimethylchlorosilana (TMS - Applied Science, Labs., State College, Pennsylvania) for 1 hour, rinsed with acetone, and dried was used. The packing for the column was 10% QF-1 on 60-80 mesh Gas Chrom Q (Applied Science, Labs., State College, Pennsylvania).

Results and Discussion

With a 100 g. sample, TIBA was easily quantitated at the 0.05 ppm level with the above method. To evaluate the efficiency of the extraction procedure, ^{14}C-labeled TIBA was added to raw milk and the sample was carried through the extraction procedure. Aliquots of the ether extraction phase were counted in a liquid scintillation counter. The average recovery of the added activity



A. Whole Milk without Clean-up Steps



B. Whole Milk with Clean-up Steps

Figure 1. Typical Gas Chromatographs of Whole Milk Spiked with TIBA

was 95.1% with a standard deviation of 1.9%, indicating that essentially all of the labeled TIBA or its degraded products were remaining in the acid form for extraction from the acidic aqueous solution. Next raw milk samples to which TIBA at the 0.1 ppm level and at the 1.0 ppm level had been added were used to help evaluate recoveries. These samples were carried through the extraction procedure (without the clean-up step) and analyzed by gas chromatography. An average of 91.6% and 89.2% was recovered from the 0.1 ppm and 1.0 ppm level, respectively. Duplicate samples of cream and butter with 2 ppm TIBA added were subjected to the extraction and clean-up steps and analyzed on the G. C. An overall recovery of 96.0% and 94.4% was obtained for the cream and butter, respectively.

Typical gas chromatographs of raw milk are shown in Figure 1. Graph A represents a sample of raw milk with 1 ppm TIBA added which was not subjected to the clean-up steps and Graph B represents raw milk with 2 ppm TIBA added which was subjected to the clean-up procedure.

References

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2. R. H. Jarboe, Jr., J. B. Data, and J. E. Christian, J. Pharm. Sci., 57 (no. 2), 323 (1968).