A Gas Chromatographic Method for Sodium Fluoroacetate (Compound 1080) in Biological Materials

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Since its development in the early 1940's (KALMBACH 1945), sodium fluoroacetate (compound 1080) has found widespread use as a rodenticide and predator control agent. Almost as soon as its use became common, methods were developed to detect 1080 in such materials as poison bait formulations, foodstuffs, soils, and vegetation (RAMSEY and CLIFFORD 1949, RAMSEY and PATTERSON 1951). However, the analytical methods developed thus far lack either the specificity or the sensitivity to detect 1080 at levels below about 1 ppm in such samples as animal stomach tissue or stomach contents. Since 1945, the U.S. Bureau of Sport Fisheries and Wildlife has been called on to analyze many samples for 1080; most have been stomachs and/or stomach contents of dogs, coyotes, and carrion-eating birds. Because such analyses sometimes have legal implications, a method was developed that is highly specific and sensitive enough to detect the toxicant at levels as low as 0.1 to 0.2 ppm.

EXPERIMENTAL

The method takes advantage of the relatively high acid strength of fluoroacetic acid. The nonhalogenated acids normally associated with a sample of biological origin (acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, etc.) have dissociation constants (K_a) equal to approximately 2×10^{-5}, while fluoroacetic acid has a value of almost 2.2×10^{-3}. Basic anion exchange resins can therefore be used to remove it from aqueous solution. Chromatographic columns packed with the resins effectively remove fluoroacetic acid from solution, but release of the fluoroacetate by percolating a dilute strong base through the column is inefficient and results in a large excess of base that interferes with subsequent steps. However, fluoroacetic acid can be removed very efficiently (85-96%) from an aqueous solution containing up to 2,000 times its weight of nonhalogenated carboxy acids by simply stirring with a weakly basic anion exchange resin, filtering off the resin beads, and recovering the absorbed fluoracetic acid as its sodium salt. When the pH is adjusted to 7.3-7.5, the filtrate may be reduced to dryness without volatilization of free fluoroacetic acid or loss of fluoroacetate through defluorination.

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Because fluoroacetic acid is stable in hot, concentrated sulfuric acid, nearly quantitative recovery (98+%) is possible by distillation at 195° to 200° C. A micro distillation apparatus, consisting of a large test tube fitted with an ST joint and cold-finger condenser, permits very efficient recovery of dry fluoroacetic and other aliphatic acids, mostly n-butyric acid. This is advantageous, as the butyric acid serves very well as a "keeper" for the relatively volatile fluoroacetic acid, especially when quantities are very small. The acids are rinsed from the condenser with absolute n-propanol (or ethanol), concentrated sulfuric acid is added, and the mixture is heated for a few minutes to produce n-propyl (or ethyl) esters of the recovered acids for gas chromatography. (The n-propyl esters chromatograph with the best resolution and the least interference from the injection solvent, although the ethyl esters may be satisfactorily chromatographed as well.) Column packings normally suited to the chromatography of free fatty acids proved unsuitable for fluoroacetic acid per se, but esters of fluoroacetic and other acids normally present chromatography very well on Johns-Manville's Chromosorb 102* (porous styrene-divinyl benzene copolymer). Good sensitivity (10 µg of n-propyl or ethyl fluoroacetate, equivalent to approximately 0.1-0.2 ppm of 1080 in the starting sample) is achieved by detection with a flame ionization detector. Most aliphatic esters of fluoroacetic acid are not commercially available except at high, custom-synthesized prices, but a reference standard of n-propyl or ethyl fluoroacetate may be conveniently prepared by heating fluoroacetic acid with absolute n-propanol or ethanol and a small amount of concentrated sulfuric acid. Under these conditions, the conversion of fluoroacetic acid to its n-propyl or ethyl ester is again nearly quantitative (98+%). Quantitation is done in the usual manner, by comparing GC peak area of the sample with that of the standard. The 1080 equivalent is found by multiplying the amount of fluoroacetic acid equivalent recovered by 1.28. Overall recoveries vary somewhat with the type of sample. Recoveries from typical samples of canine stomach and/or stomach contents containing 10 ppm 1080 will average about 70%, and from those containing 1 ppm, about 60%. A typical sample and reference standard mixture are illustrated by Figures Ia and Ib.

METHOD

Grind or chop as finely as possible 50-100 g of sample, add about 50 ml of water, and blend or stir thoroughly. Pour off as much of the water as possible through a large funnel lined with a layer of coarse cheesecloth and repeat with two or more 50-ml volumes of water to collect a total of about 150 ml. Acidify the filtrate with 3 ml of 1:1 (v/v) concentrated sulfuric acid-distilled water. Slowly add 30% (w/v) phosphotungstic acid to

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* Reference to trade names does not imply Government endorsement of commerical products.

ROWE et al. (1971) found that residues of endrin and dieldrin had greatly declined in oysters and in water from the Mississippi River in Louisiana from 1964-1966 to 1968-1969. Whether endrin was related to the extirpation of the brown pelican in Louisiana is unknown because tissues or eggs of these birds were never analyzed for residues. Endrin was not detected in tissues or eggs of brown pelicans collected from Florida, California, and South Carolina from 1969 through 1972 (BLUS et al., 1971).

Relationships between residues in the egg and biological effects -- The relationship of DDE to shell thinning of the Louisiana brown pelican eggs followed that described in an earlier study in which DDE accounted for all or most of the shell thinning of brown pelican eggs from South Carolina, Florida and California (BLUS et al., 1971). The relationship of DDE to eggshell thinning was significant ($P<0.05$; $r = 0.374$; $\hat{Y} = 91.16 - 16.561 \log_{10} X$ where Y is the percent of the pre-1947 eggshell thickness and X is the logarithm of the DDE residue).

The percentage thinning of eggshells of Louisiana pelicans was less than the DDE-induced thinning associated with reduced reproductive success of captive birds of other species (HEATH et al., 1969; LONGCORE et al., 1971). Furthermore, eggshell thinning and residues of the eggs of Louisiana pelicans were similar to that found in eggs of pelicans from Florida (BLUS, 1970; BLUS et al., 1971; BLUS et al., 1974a). The breeding population of brown pelicans in Florida has been stable since 1968 when the population census of the nesting colonies was originated (WILLIAMS and MARTIN, 1968; LOVETT E. WILLIAMS, JR., 1974 Personal Communication); the reproductive success of one large Florida colony is said to be normal (SCHREIBER and RISEBROUGH, 1972).

The residues of DDE in the Louisiana eggs are generally below those associated with subnormal reproductive success of the brown pelican in South Carolina (BLUS et al., 1974b). However, the level of dieldrin tends to be slightly higher in the Louisiana eggs, and 18 of the 36 eggs contain a level of dieldrin considered potentially detrimental to reproductive success (BLUS et al., 1974b). Thus, there is the possibility that dieldrin may have some influence on reproductive success in Louisiana brown pelicans, but the effects of dieldrin were not satisfactorily separated from those of DDE in the South Carolina study (BLUS et al., 1974b). The low rate of reproductive success may be influenced by the unusual conditions existing in establishment of a small breeding colony in an

water to the funnel and make alkaline (light pink) to phenolphthalein by adding drops of 20% (w/v) sodium hydroxide, shaking between additions. Transfer the aqueous phase to a 50-ml beaker. Scavenge residual water from the separatory funnel with an additional 2-3 ml of water and add this to the separated aqueous phase.

To regenerate the acids removed from the ether extract as their sodium salts and remove excess sodium hydroxide, use a strongly acidic cation exchange resin (Abmerlite IR-120, 20-50 mesh). Immerse pH meter electrodes (preferably the combination type, which take less space) in the 50-ml beaker containing the sample solution. Add 0.5 g of the resin beads and stir continuously with a small magnetic stirrer. After 2-3 minutes, note the pH and add about 0.1 g more of the resin beads. Continue to stir for 2-3 minutes and again note the pH. Continue stirring and adding more beads, about 0.1 g at a time, until the pH remains constant (equilibrium normally occurs at about pH 3). Then filter out the resin beads through a small Hirsch funnel (size 01) into a 50-ml filter flask. Transfer the filtrate to a second 50-ml beaker, rinse the filter flask with 4-5 ml distilled water, and add the rinse to the beaker.

To remove fluoroacetic and other carboxy acids, use a weakly basic anion exchange resin (Amberlite IR-45, 20-50 mesh). To the sample solution add 80-100 mg of the resin beads and stir continuously for 40 minutes with a magnetic stirrer (see note).

Note: At this point, good recovery of fluoroacetic acid depends on good recovery of the ion exchange beads from the sample solution. Since a conventional magnetic stirring bar partially grinds the beads, I constructed a simple device to eliminate this problem. A stirring bar is made by inserting a 5/8-inch piece of soft iron wire into a 3/4-inch piece of 1/8-inch pyrex tubing and sealing the ends of the tubing with a gas-air torch. Two or three circular loops are made in one end of about a 3-inch piece of 0.02-inch-diameter stainless steel wire and placed around the bar. The other end of the wire is passed through a pin hole in a disc of stiff paper or thin sheet metal of a size to fit over a 50-ml beaker. The wire is secured on the top side of the disc by inserting through a small stop (I used a piece of teflon sheet stock cut from the liner of a bottle cap with a No. 1 cork borer). This assembly is placed on the 50-ml beaker and the wire length adjusted so that the stirring bar is in the center of the beaker and about 1/8 inch above the bottom.

Recover the beads by filtration through a small funnel and a lightweight, porous filter paper. (Save the filtrate until chromatography has been completed. If more fluoroacetic acid was present than the beads could absorb, more acid will have to be recovered with additional beads.) Lift out the filter paper containing the beads and spread it out on absorbent paper to absorb water and leave the beads just moist. Scrape all of the beads off the paper with a flat, narrow-bladed spatula and transfer to a

50-ml beaker containing 15 ml of distilled water made alkaline with 0.15 ml of 20% (w/v) sodium hydroxide. Again stir continuously for 20 minutes. (During this step, a conventional stirring bar may be used, as some grinding of the beads is unimportant.)

Filter off the beads through the small Hirsch funnel and wash the beaker and the beads with 2-3 ml of water. Transfer the filtrate to a 20-ml beaker, immerse the pH meter electrodes in the solution, and with dilute sulfuric acid, very carefully adjust the pH to 7.3-7.5. (For this, it is convenient to use 10%, 1%, and 0.1% solutions of the acid. If, by accident, the pH goes lower than 7.3, make alkaline with a small amount of 0.1% sodium hydroxide and again adjust to pH 7.3-7.5 with 0.1% acid.) Add 2 ml of pH 7.4 buffer solution (Clark and Lub's $NaOH-KH_2PO_4$) and transfer to a micro distillation vessel. (The vessel consists of a 24/40 ST outer joint, Corning No. 6580, whose length has been adjusted so that when the open end has been drawn down and sealed to form a test tube, a mating 24/40 ST cold-finger condenser, Corning No. 6949-5, will fit with about one-fourth inch of space between the tip of the condenser and the bottom of the tube. A small hole should be drilled or blown about one-fourth inch below the lower end of the ground joint to serve as a vent.) With a stream of air directed into the tube, heat at about 90° C in a water bath until the sample has reached complete dryness. Cool the tube and add 0.2 ml concentrated sulfuric acid. Rotate the tube so that all of the surface that had been exposed to the sample solution is coated with a film of the acid. Install the cold-finger condenser, being careful not to touch the sides of the tube. Immerse the tube about 2 inches into a small mineral oil bath and heat at 195° to 200° C for 40 minutes. Lift out the cold-finger condenser, again being careful not to touch the sides of the tube. Transfer the distillate to a small culture tube (17 x 105 mm) with a 1.0-ml syringe containing 1.0 ml of redistilled absolute n-propanol or ethanol. Hold the cold-finger about 15° above horizontal with the tip of the condenser just above a small funnel. Going from top to bottom, move the syringe along the top side of the condenser, slowly dispensing the alcohol. This will effectively wash off all of the condensate. Add 1 µl of concentrated sulfuric acid to the culture tube, cap tightly, and heat at 95° to 100° C for 20 minutes. The sample is now ready for gas chromatography.

Prepare a reference standard of n-propyl or ethyl fluoroacetate by adding 7.5 µl of fluoroacetic acid (density at 25° C = 1.330 g/ml) and 20 µl concentrated sulfuric acid to 10.0 ml absolute n-propanol or ethanol and heating at 90° to 95° C for 15 minutes. (High purity--99+%--fluoroacetic acid is available at moderate cost from Columbia Organic Chemicals Co., Inc., Columbia, S.C.). One µl of this solution is equivalent to 1 µg of fluoroacetic acid or 1.28 µg of 1080. Operate the chromatograph under the following conditions:

Column	- 3' x 1/8" soft aluminum with Johns-Manville Chromasorb 102 (100/120 mesh)
Oven temperature	- 180° C for propyl esters and 155° C for ethyl esters
Detector	- Hydrogen flame ionization
Carrier flow (N_2)	- 30-35 ml/min
Hydrogen	- 25 ml/min
Air	- 250 ml/min
Electrometer sensitivity	- 1.6 x 10^{-10} A (for FS on a 1-mV recorder)

Under these conditions, 1.0 µg equivalent of the esterfied standard fluoroacetic acid reference will produce a GC peak of about 45% FS. Retention time will be about 4-5 minutes. As most samples are less than fresh, they will contain acetic, propionic, iso-butyric, butyric, and iso-valeric acids, as well as minor amounts of other acids. The n-propyl and ethyl esters emerge from the GC column in the order: acetate, fluoroacetate, propionate, iso-butyrate, n-butyrate, iso-valerate, and n-valerate. To save time during the gas chromatographic step, the esters following the fluoroacetate may be made to emerge much faster if the column temperature is raised to about 200° C just after the fluoroacetate has emerged. (At 180° n-propyl-n-valerate has a retention time of about 21 minutes.) If confirming identification of fluoroacetic acid is required, the analysis can be repeated with detection of the ethyl ester by replacing the n-propanol with ethanol at the esterification step or *vice versa*.

Even greater specificity may be achieved by GC-mass spectrometry. A 100-g sample containing about 1 ppm 1080 will yield enough n-propyl or ethyl fluoroacetate to produce a good mass spectrum. The eight most prominent ions in the n-propyl fluoroacetate spectrum and their relative intensities are: m/e 27, 31%; m/e 33, 37%; m/e 41, 34%; m/e 42, 66%; m/e 43, 89%; m/e 61, 100%; m/e 79, 30%; and m/e 91, 29%. For the ethyl ester they are: m/e 27, 30%; m/e 29, 100%; m/e 33, 28%; m/e 43, 9%; m/e 45, 13%; m/e 61, 61%; m/e 78, 13%; m/e 79, 7%.

CONCLUSIONS

The method described is relatively simple and requires no unusual equipment. For samples such as animal stomachs, the method is considerably more sensitive, more specific, and less time-consuming than previously described methods. A typical sample requires about 8 hours, compared with 3-4 days for the most commonly used older method (A.O.A.C., 1965:399-402). The method should be considered semiquantitative, as recoveries vary somewhat with the type of sample. With minor modifications, it could be adapted to analyzing other sample types such as water, soils, vegetation, etc.

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