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Determination of Sodium Fluoroacetate (Compound 1080) in Poison Baits by HPLC

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Sodium fluoroacetate, also known as compound 1080, has been widely used as a poison for controlling various vertebrate pests. The field use of such a highly toxic compound necessitates the analysis of baits and tissues to monitor the exposure of operators, agricultural stock and protected wildlife species (RAMMELL and FLEMING 1978). Two general analytical methods are currently used, one based on a final fluorine estimation and the other on gas-liquid chromatography (GLC). In the fluorine methods the sample extract is ashed and the fluorine determined either colorimetrically (AOAC 1980) or with a fluoride-selective electrode (PETERS and BAXTER 1974). The fluorine methods, although sufficiently sensitive for most purposes, are necessarily non-specific. The GLC methods which have been described are cumbersome and generally semi-quantitative (STAHR et al. 1974, PETERSON 1975, STEVENS et al. 1976). An improved GLC method using pentafluorobenzyl derivatisation and electron-capture detection was proposed recently (OKUNO and MEEKER 1980) but the presence of interfering peaks and low, variable recoveries were a problem.

The preparation of fluorescent derivatives of monocarboxylic acids using 4-bromomethyl-7-methoxycoumarin (Br-Mmc) and their separation by high-performance liquid chromatography (HPLC) has recently been reported (LAM and GRUSHKA 1978). We have adapted this method to the analysis of sodium fluoroacetate. A simple but efficient method for recovering this compound from baits is also described.

MATERIALS AND METHODS

Chemicals. Br-Mmc was prepared as described by SECRIST et al. (1971). Fluoroacetic acid (Pfaltz and Bauer) was used as an external standard after standardisation against sodium hydroxide. Sodium fluoroacetate (98% pure) obtained from Rentokil (N.Z.) Ltd., was used for recovery experiments. 18-Crown-6 was obtained from Sigma. Other chemicals used were of analytical grade and water was glass-distilled.

Sample preparation. Poison baits containing 100-1000 mg sodium fluoroacetate/kg were minced and a 20 g sample homogenised in 200 ml water using an MSE Atomix homogeniser. The homogenate was made up to 1000 ml with water, shaken for one minute and left overnight at room temperature. After reshaking and a five minute settling period,

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1.00 ml of the suspension was mixed with 100 µl 5 M orthophosphoric acid in a 50-ml round-bottom flask. The flask was attached to a manifold connected to an Edwards ES 100 vacuum pump, the mixture frozen in dry ice/isopropanol and the system evacuated to a pressure of 0.05 mbar. The 50-ml flask was then placed in a water bath at 25-30 °C and the distillate collected in a 25-ml pear-shaped flask cooled in dry ice/isopropanol. When most of the solution had distilled over, the 50-ml flask was heated to a final temperature of 60 °C for five minutes. The vacuum was then released and the distillate transferred to a 5-ml pear-shaped flask containing one drop 1% phenolphthalein solution. Potassium hydroxide (0.1%) was added dropwise until the solution just turned pink and then 60 µl 0.05% propionic acid in methanol was added to adjust the pH of solution to the range 6.5-7.5. The mixture was evaporated to dryness at 40-45 °C on a rotary evaporator and then derivatised.

Derivatisation. Samples were derivatised at 55 °C in a stoppered flask with 0.65 ml Br-Mmc (2 mg/ml in acetone) and 0.20 ml 18-crown-6 (0.8 mg/ml in acetonitrile) while stirring the solution with a magnetic follower.

HPLC analysis. Extracts were chromatographed on a 25 cm RP-8 reverse phase column (Brownlee Laboratories). The HPLC system comprised a Tracor 995 pump, a Rheodyne 7120 injector with 100-µl loop, and a Varian Fluorichrom fluorescence detector fitted with a deuterium lamp. A 360 nm band filter was used for excitation and a 400 nm cut-off filter together with a 410 nm band filter were used for emission. Detector response was monitored on a Perkin Elmer Sigma 10 data station. Before injection, 2 ml acetonitrile: water (2:3) was added to the derivative solution to increase its polarity. If a 20-µl loop was used for injection this addition was unnecessary.

RESULTS

Derivatisation. Derivatisation of a 50 µg fluoroacetate standard was complete after 15 minutes at 55 °C with no change in fluorescence peak area when heating was continued for 60 minutes. A derivatisation time of 30 minutes was therefore used routinely. Refluxing, as advocated by LAM and GRUSHKA (1978) for other carboxylic acids, was found to be unnecessary under the conditions used here. The derivative, once formed, was stable for at least a week at room temperature in the solvent mixture acetone:acetonitrile: water (65:100:120).

HPLC analysis. The best binary solvent found for separation of the fluoroacetate derivative from the derivatives of acetate and formate was acetonitrile:water (1:2). However, complete separation was obtained only with a ternary solvent containing ethyl acetate. The solvent mixture used routinely was acetonitrile:ethyl acetate: water (9:2:22) at a flow rate of 1.5 ml/min. A typical separation of Br-Mmc derivatives prepared from a poison bait containing 500 mg sodium fluoroacetate/kg is shown in Figure 1. The relative

retention times of the formate, acetate, fluoroacetate and propionate derivatives were 0.68, 0.81, 1.00 and 1.42 respectively.

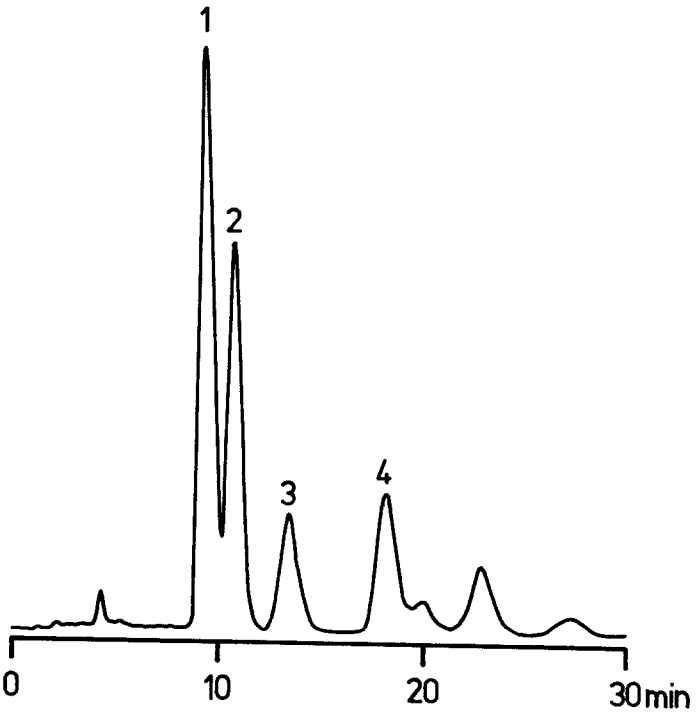

Figure 1. HPLC trace of Br-Mmc derivatives prepared from a poison bait distillate: 1 formate, 2 acetate, 3 fluoroacetate, 4 propionate. The 100 µl injection contained the equivalent of 0.35 µg sodium fluoroacetate.

Figure 2 shows the linearity of response and consistency of derivatisation for eight fluoroacetic acid standards containing the equivalent of 1-50 µg sodium fluoroacetate (0.035-1.75 µg/100 µl injection). The detection limit for sodium fluoroacetate under the conditions used was 0.2 ng/100 µl injection.

Recovery. The analysis in duplicate of bait samples spiked with 100 mg and 1000 mg sodium fluoroacetate/kg gave mean recoveries of 83% and 99% respectively. The mean and standard deviation for six replicate analyses of a single bait extract were 682±33 mg/kg.

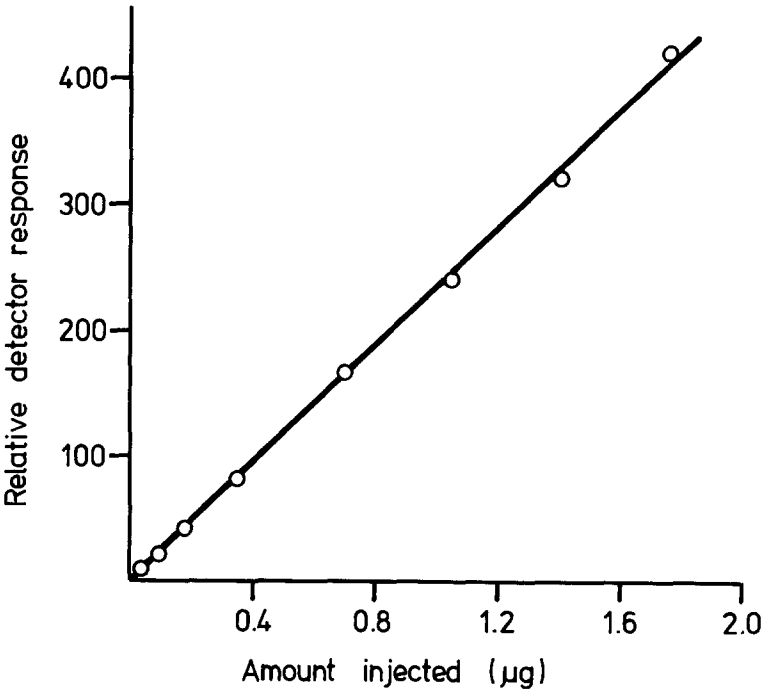

Figure 2. Detector response to fluoroacetate derivatives with amounts expressed as sodium fluoroacetate.

DISCUSSION

The determination of low levels of sodium fluoroacetate in biological samples has been hindered by its high solubility in water and correspondingly low solubility in organic solvents, its instability when heated in alkaline solution, and the lack of any specific group in the molecule that would facilitate identification. The vacuum distillation and HPLC method developed in this study largely overcomes these difficulties. The distillation technique described is similar to that successfully used to recover acetate from biological samples (TOLLINGER et al. 1979). The technique is much simpler than that used for fluoroacetate in the AOAC and other methods and reduces the analysis time considerably. The recoveries of 83-99% compare favourably with the 40% recoveries reported for the most recent GLC method (OKUNO and MEEKER 1980).

The distillate obtained is relatively clean and could be analysed by a variety of methods for its fluoroacetate content. HPLC offered considerable promise in view of the recent published work on its usefulness for the analysis of other carboxylic acids. The simplest HPLC method for volatile carboxylic acids separates the underivatised acids with phosphate buffer on a reverse phase

column and uses their absorption at 210 nm for detection (BUSH et al. 1979). This method was used here for the initial development of the vacuum distillation technique but was found to be too insensitive and non-specific. Early HPLC methods for carboxylic acids were based on UV-absorbing derivatives such as the p-bromophenacyl esters (DURST et al. 1975). The bromophenacyl ester of fluoroacetic acid was successfully prepared (unpublished work), but the fluorescent Br-Mmc derivative showed more promise for biological extracts and was developed in preference. Br-Mmc itself is not difficult to prepare and appears to have a long shelf life. Br-Mmc derivatives are also simple to prepare and can be stored for some days if necessary before HPLC analysis. Since the derivatisation only occurs in non-aqueous solutions the distillate must first be evaporated to dryness. It is crucial that during this evaporation the solution remains close to neutrality as fluoroacetic acid is lost by evaporation from solutions of low pH and is degraded to glycolic acid in solutions of high pH. The addition of propionic acid was found to be a convenient way to maintain the required pH. The broad linear range of detector response to the fluorescent derivative facilitates accurate analysis of widely varying fluoroacetate levels.

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