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Analysis of Alkyl Phosphates by Extractive Alkylation

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The development of suitable methods for analysis of the alkyl phosphate metabolites of organophosphorus pesticides in urine has been a rather difficult and persistent problem. Currently available methodology (BRADWAY et al. 1977; LORES & BRADWAY 1977; SHAFIK et al. 1973) suffers from several shortcomings. The extraction of the compounds from urine is often incomplete and inconsistent. The reagent used in derivatization, diazopentane, and its precursor, a substituted nitrosoguanidine, present unacceptable hazards to the analyst, principally because of the demonstrated mutagenicity and/or carcinogenicity of the reagents. In addition, when dimethylphosphorothioate and diethylphosphorothioate are derivatized with diazoalkanes, they to give two isomeric products: a phosphorothionate and a phosphorothiolate (SHAFIK et al. 1970). It is, therefore, uncertain that the ratio of the isomers is constant. The limit of detection is higher than would be the case if only one product were formed.

Several different approaches to the problem are currently being investigated. One particularly promising technique involves extraction of the alkyl phosphate from urine as an ion-pair with a lipophilic quaternary ammonium cation. This approach has been used to determine trace levels of inorganic phosphate in aqueous media (MATTHEWS et al. 1971) and is generally applicable to the analysis of anions of many different types (BROTELL et al. 1973; EHRSSON 1974; GYLLENHAAL & EHRSSON 1975; DE SILVA et al. 1976). In this work, the ion pair is extracted into dichloromethane which contains the alkylating agent, pentafluorobenzyl bromide. Since the non-polar solvent possesses poor solvating ability, it renders the alkyl phosphate highly reactive toward nucleophilic displacement. The resulting pentafluorobenzyl (PFB) ester derivative is thermally stable and highly responsive under conditions of gas chromatographic analysis using a flame photometric detector or nitrogen-phosphorus detector.

EXPERIMENTAL

Apparatus. A Tracor MT-222 gas chromatograph (GC) equipped with either a Tracor Model 702 N-P detector (NPD) or a flame photometric detector (FPD) was used for analysis. A 1.8 m x 4 mm ID glass column packed with 5% OV-210 on 100/120 Supelcoport® was used. Nitrogen carrier flow was 60 mL/min. Column temperature was 160°C.

A Fisher Roto-Rack®, Model 96, operated at 50 rpm was used to mix the samples.

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Glassware. Conical 15-mL graduated centrifuge tubes with Teflon®-lined screw caps were used.

Reagents. The following special reagents were used:
PFBBr: Pentafluorobenzyl bromide (α-bromo-2,3,4,5,6-pentafluorotoluene), Regis Chemical Co. or Aldrich Chemical Co.
TBA: Tetrabutylammonium hydrogen sulfate, Regis Chemical Co.
THA: Tetrahexylammonium hydrogen sulfate, Regis Chemical Co.

Dichloromethane and hexane were pesticide quality.

Sodium sulfate was Soxhlet-extracted with methanol and then dried at 130°C before use.

Alkyl phosphate reference standards were provided by Roger Blinn, American Cyanamid Co.

Methodology

Solutions of THA and TBA were prepared by dissolving 0.01 mole of the appropriate salt in 0.2 M aq. NaOH and diluting to 100 mL with 0.2 M aq. NaOH.

Solutions of standard alkyl phosphates were prepared by weighing accurately 10 mg of the alkyl phosphate salt into a 10 ml volumetric flask and making to volume with deionized water. Dilutions were made as needed.

A 0.1 mL aliquot of standard in water was pipeted into a 15 mL graduated centrifuge tube with a Teflon-lined screw cap. To this was added 1 ml of the THA or TBA reagent, 2 ml of dichloromethane and 25 µL of PFBBr. The tube was tightly capped and placed on a Roto-Rack for 25-30 min at 50 rpm and room temperature. At the end of this period, the layers were allowed to separate and the dichloromethane layer was transferred to a clean tube. The aqueous layer was extracted once with 2 mL of dichloromethane and this was added to the first extract. The combined extracts were concentrated or diluted as required. A little Na_2SO_4 was added to remove traces of water. An aliquot of the solution was injected directly for FPD. For analysis by NPD, an aliquot of the solution was evaporated carefully just to dryness under a gentle stream of nitrogen and the residue was dissolved in hexane.

Urine fortification. A 1.0 ml urine sample was pipeted into a graduated centrifuge tube having a Teflon®-lined screw cap. The urine was fortified with 0.1 ml of standard solutions containing 0.1 µg of the alkyl phosphate. To this was added 1 mL of the THA reagent, 4 mL dichloromethane and 25 µL of PFBBr. The sample was treated as described above.

RESULTS AND DISCUSSION

Each of the six alkyl phosphates (see Table 1) was analyzed

as described. DMTP, DETP, DMDTP and DEDTP were adequately extracted from water as the ion pair with TBA. The extraction of DEP and DMP was incomplete. Using THA as the counter ion, DEP was also extracted and derivatized, although recovery of DMP was still very low.

Table 1. Chemical names and abbreviations of dialkyl phosphate metabolites.

DMP	O,O'-dimethyl phosphate
DEP	O,O'-diethyl phosphate
DMTP	O,O'-dimethyl phosphorothioate
DETP	O,O'-diethyl phosphorothioate
DMDTP	O,O'-dimethyl phosphorodithioate
DEDTP	O,O'-diethyl phosphorodithoiate

Urine samples were fortified with DEP, DMTP, DETP, DMDTP and DEDTP at 0.1 ppm and analyzed using THA as the counter ion. The recovery of these compounds as the PFB derivative was essentially identical to that of similar amounts added to water. Authentic reference standards of the respective PFB derivatives of the five alkyl phosphates were not available. Therefore, the absolute recovery efficiency of the method could not be determined.

Figure 1 shows representative GC chromatograms of the five alkyl phosphate derivatives. The column used was 5% OV-210; the detector was the Tracor Model 702 N-P. Urine extracts gave considerable background with this detector. Therefore, the flame photometric detector was found to be the detector of choice for urinary analyses.

It is characteristic of both the FPD and NPD that there is considerable variation in response from one detector to another. For the particular detectors involved in this study, the response of the alkyl phosphate derivatives was similar on both detectors. A 10% recorder deflection at the lowest practical electrometer attenuation was observed with about 0.2 ng of each derivative. Response was linear on each detector over the range 0.2-10 ng.

Several significant advantages are noted in this method. The first is the simplicity of the method, which minimizes sources of error by minimizing manipulation of the sample. This simplicity also decreases sample analysis time with a subsequent increase in the number of samples which can be analyzed in a given time. Equally important, the reagents used present much less hazard to the analyst. The derivatizing reagent, PFBBr, is a lachrymator and skin irritant. All other reagents appear to be reasonably innocuous. A further advantage is that DMTP and DETP derivatize to a single product each. This permits a lower limit of detection for these two metabolites.

It must be stressed that work on this analytical procedure is by no means complete. There are a number of problems yet to be addressed. A means must be found to increase the recovery and

derivatization of DMP from aqueous solution. Mass spectral and/or nuclear magnetic resonance confirmation of the identities of the derivatives must be obtained. Quantities of each derivative must be prepared and purified to serve as primary reference standards. However, interest in the methodology described in this paper is sufficiently high to warrant publication of these preliminary results.

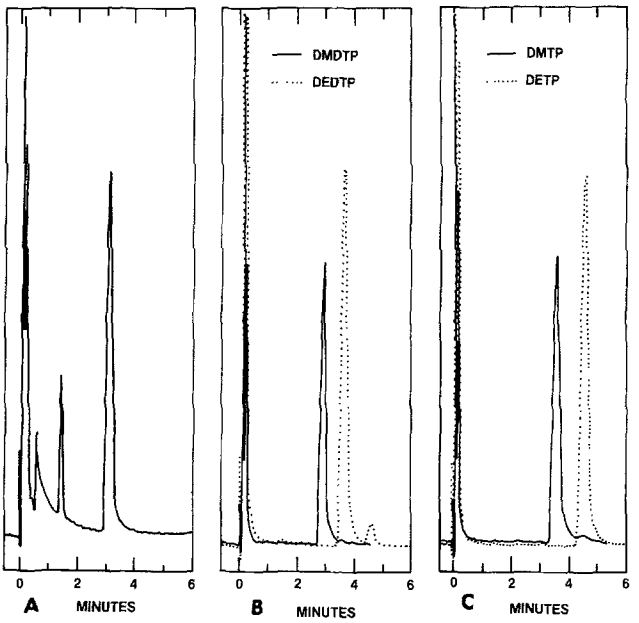

Fig. 1. Gas chromatograms of PFB derivatives of a) DEP (2.5 ng), b) DMTP and DETP (2.5 ng each), and c) DMDTP and DEDTP (2.5 ng each). Detector, Tracor 702 N-P (NPD); column, 5% OV-210; temperature, 160°C; chart, 2 min/inch.

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