Waters Lab Highlights

An Internal Communication of Applications and Techniques

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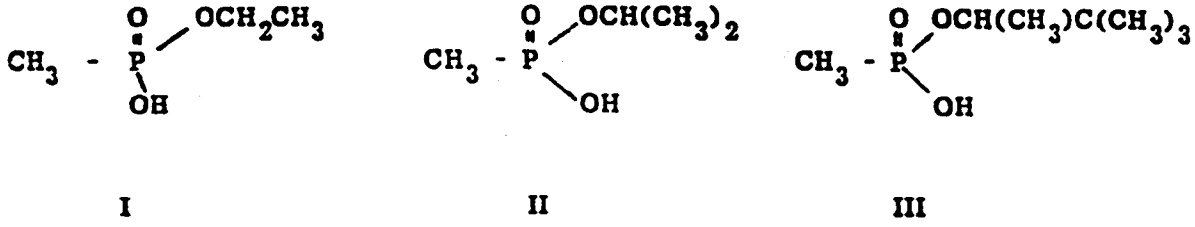
High Performance Liquid Chromatographic Analysis of Alkyl Methylphosphonic Acids by Derivatization

The ability to easily detect, separate, and quantitate trace amounts of phosphonic acids (degradation by-product of organophosphorus nerve agents) in aqueous solutions is of significant importance to the Army. This capability is needed in combat situations for the detection of nerve agent use as well as in the laboratory for the analysis of intelligence samples. Also, valuable kinetic and other chemical data on the organophosphorus nerve agents could be generated by such analytical methods.

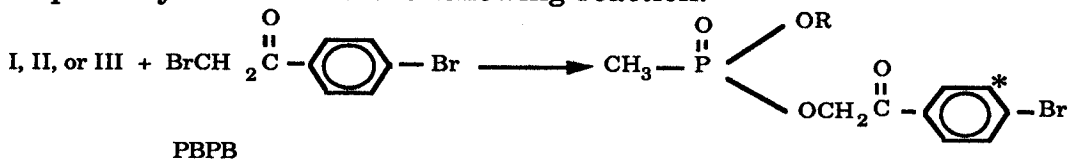
Described here is the development of an reaction technique for analysis for phosphonic acids by reverse-phase HPLC.[1] The technique involves the esterification of compounds I, II, and III (Figure 1) separately and in a combined mixture with p-bromophenacyl bromide (PBPB), an ultraviolet chromophore, on a microscale to attain an ultraviolet absorbing phosphonate species. The esters are then separated by reverse-phase HPLC and quantitated by UV detector response (Figure 2).



I II III

Figure 1: Structures of alkyl methylphosphonic acids.

The first step in the enhancement reaction technique for the analysis of I, II, and III by reverse-phase HPLC is the conversion of the acids to their corresponding p-bromophenacyl esters* via the following reaction:



PBPB

Where R = - CH_2CH_3 = Compound IV (from Compound I)

R = - $CH(CH_3)_2$ = Compound V (from Compound II)

R = - $CH(CH_3)C(CH_3)_3$ = Compound VI (from Compound III)

* Since these derivatives are of nerve gas degradation products, the derivatives may be toxic. Please contact the authors in reference 1 for toxcicity information and safety precautions.

Waters
Division of MILLIPORE 34 Maple Street Milford, MA 01757 508-478-2000

Brian A. Bidlingmeyer

The PBPB is a highly reactive esterifying agent as well as a strong ultraviolet absorbing chromophore. The 18-Crown-6 solubilizes and chemically activates potassium bicarbonate in the reaction medium. This permits the ready synthesis of the novel chromophore-bearing p-bromophenacyl ester derivatives in acetonitrile-water. Esterification of the acids decreases the polarity of the analytes permitting ready separation by reverse-phase HPLC.

The qualitative capability of this technique is illustrated in Figure 2 in which the complete separation of IV, V, VI, and PBPB is achieved in 25 minutes. The PBPB peak in the chromatogram is well separated from that of V and VI, eliminating any interference in the analysis from excess PBPB. Detection limits for the three acids were 43, 59, and 62 nanograms respectively.

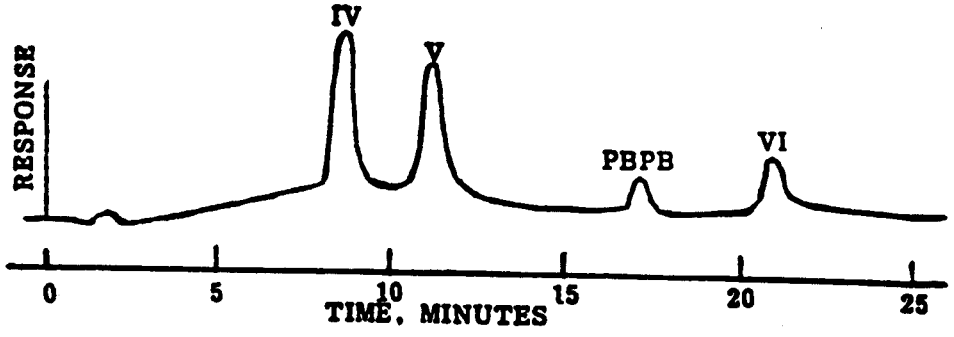


Figure 2: Separation of Alkyl Methylphosphonic p-Bromophenacyl Esters (from reference 1). Analytical separations were performed under the following conditions: sample size, 200µl; flow rate, 2.0ml/min; column temperature, ambient; mobile phase, water-acetonitrile; elution mode, linear gradient (no. 6), 20-55% acetonitrile, 25 min; UV detector, 254nm. Column: µ-Bondapak™ C_{18} (3.9mm I.D. x 30cm).

* * *

For Investigational use only. The performance characteristic for this procedure has not been established.The following trademarks are the property of Millipore Corporation, Bedford, MA 01730 USA: µ-Bondapak

* * *

Reference

1 P. C. Bossle, J.J. Martin, E.W. Sarver, US Army Armament Research and Development Command, Aberdeen Proving Ground, Maryland (21010). Technical Report ARCSL-TR-83002.

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