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Lab Highlights

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DERIVATIZATION OF PRIMARY AND SECONDARY AMINES WITH A POLYMERIC ANHYDRIDE FOR IMPROVED DETECTION IN HPLC

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The identification and quantitative determination of aliphatic and related amines is a commonly encountered problem in organic analysis. The gas chromatographic determination of these amines at low concentrations is limited by adsorption and decomposition in the column, tailed elution peaks, and the low sensitivity of the compounds in the gas chromatographic detectors. A common method to overcome these limitations is to convert the original analyte into a derivative that has a selective sensitivity improvement in the GC detector, e.g., an electron capture or flame ionization detector. Several derivatization reagents, such as flophemesyl chloride, pentafluorobenzoyl chloride, dimethylthiophosphinic chloride, and 1-fluoro-2,4-dinitrobenzene, have been reported for this purpose.

Amines have also been difficult to analyze in HPLC for several reasons, including: low detection sensitivities, variable ionization during separation, and strong interaction with many HPLC supports. However, these compounds are easily derivatized to give products that are strongly UV absorbing, fluorescent, or electrochemically active. The resulting derivatives are usually separable with minimal difficulty and have better detectabilities. Today, most of the derivatizations performed for HPLC involve the use of homogeneous reactions, in which the reagent is present in solution, and is mixed with a solution of the substrate of interest. Recently, there has been some work on the use of solid phase reagents for derivatizations to be used in conjunction with HPLC. Several advantages in using solid phase reagents for derivatization have been discussed (1).

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Some years ago, Digenis reported in several papers on the preparation and reactivity of various polymeric activated anhydrides containing various incorporated labels (2). All of this work dealt with the possible use of such polymeric reagents for synthetic organic purposes, such as a multi-step preparation of penicillin derivatives and related compounds. However, none of this work involved any HPLC approaches, and there was no suggestion at that time that such polymeric reagents might prove useful for either off-line or on-line derivatizations related to HPLC analyses or applications. We have pursued these reactions, now off-line, using a polymeric anhydride containing o-acetylsalicyl as the labelling moiety. We have investigated the reactivities of various primary amines (propylamine, etc.) and secondary amines (diethylamine, etc.) toward this polymeric anhydride, and have been able to show that with mild, off-line conditions (60°C, 20 mins) it is possible to obtain up to 96% of the expected, known derivatives. Standards were prepared, characterized by m.p., UV, IR, NMR, MS, and elementary analysis, and these were then used as external standards to determine the percent reaction of amines with the polymeric anhydride. The derivatives can be monitored by a UV detector at 196nm, an electrochemical detector (EC) in the oxidative mode, with or without post-column photolysis (HPLC-hv-EC). The minimum detection limits of underivatized and derivatized amines were determined. There is a 3-4 orders of magnitude lowering of detection limits as direct result of these off-line derivatizations. The minimum amount of amine that can be detected by such derivatization has also been determined.

1. I. S. Krull, H.-H. Xie, S. Colgan, U. Neue, T. Izod, R. King, and B. Bidlingmeyer, J. Liquid Chrom., 6, 605 (1983); ibid/. 6(S-2), 125 (1983).
2. G. E. Martin, M. B. Shambhu, S. R. Shakshir, and G. A. Digenis, J. Org. Chem., 43, 4571 (1978).