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Note

Detection of benzidines on thin-layer chromatograms with fluorescamine

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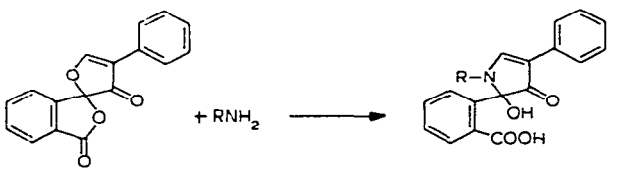
In general, the qualitative and quantitative gas chromatographic (GC) analysis of amines as free bases at low concentrations is limited by adsorption and decomposition in the column and tailed peaks. In order to overcome these limitations, the amino groups and other functional groups if present in the molecule have been masked by different types of derivative formation reactions prior to GC analysis. The derivatives used include acetyl[1–3], trimethylsilyl[4], enamines[3,5], trimethylsilylenamines[6], trimethylsilylheptafluorobutyryl[7], trifluoroacetyl[8], pentafluoropropionyl[9], heptafluorobutyryl[10], *p*-tosylamides[11], and isothiocyanates[12,13]. Amino acids have been determined by GC as alkyl chloroformates with derivatizing agent[14,15]. Alkyl chloroformate reacts readily with amino, imino, phenolic, hydroxyl, sulphydryl, and imidazolic NH groups in aqueous alkaline media at room temperature to provide corresponding compounds with N-, O-, and S-substituted alkyloxycarbonyl groups. A recent report in this journal based upon this chemistry for the determination of specific phenolic amines has been reported[16].

Another approach to the measurement of primary amines is the formation of a fluorescent derivative. The reaction of fluorescamine with a primary amine is shown in Fig. 1. Fluorescamine (I) reacts with primary amines (II) to form intensely fluorescent substances (III), providing the basis for a rapid and highly sensitive assay for compounds containing a primary amine group, such as amino acids, primary amines, peptides, and proteins[17]. Application of fluorescamine as a rapid spot test for solid dosage exhibits in forensic toxicological analysis has been reported[18]. The fluorescamine test only yields a bright aquamarine (blue-green) fluorescent product with primary amines; thus, this test makes a clear cut distinction between amphetamine



I II III

Fig. 1. The reaction between fluorescamine (I) and a primary amine (II) yielding a highly fluorescent derivative (III).

and methamphetamine. Previous common spot tests yielded the same results with these two amines. Fluorescamine is 100 times more sensitive in detecting amphetamine extracted from urine on thin-layer chromatograms than ninhydrin[18,19].

Reported here is a sensitive thin-layer chromatographic (TLC) spray development based on fluorescamine for benzidine (4,4′-diaminobiphenyl) and 3,3′-dichlorobenzidine. These compounds are members of the Environmental Protection Agency (EPA) priority pollutant list. These compounds present variability and difficulty in performing their measurement with direct analysis by GC–mass spectrometry (MS).

EXPERIMENTAL

Reagents

Fluorescamine TLC spray, prepared commercially in 1,2-dichloroethylene, was purchased from Whatman, Clifton, NJ, U.S.A., Cat. No. 4911-109. Precoated TLC plates (LKGF Linear-K), 5 × 20 cm glass plates coated with a 250-μm thickness of silica gel, were also purchased from Whatman.

Known amounts of benzidine and 3,3′-dichlorobenzidine were spotted on TLC chromatograms using a Drummond (Broomall, PA, U.S.A.) 0–10-μl Microdispenser, Cat. No. 210.

Standard solutions were purchased from Supelco, Bellefonte, PA, U.S.A., "Standards for EPA Consent Decree Water Protocol", Cat. No. 4-8808.

All mobile phase solvents were purchased from Burdick & Jackson Labs., Muskegon, MI, U.S.A.

Fluorescence examinations were performed in a Chromato-Vue® box (manufactured by Ultra-Violet Products, San Gabriel, CA, U.S.A.) using the long wavelength (366 nm) for excitation.

Procedure

Appropriate amounts of authentic compounds were spotted on precoated silica gel plates 2 cm from the bottom of the plate and developed in hexane–methyl *tert.*-butyl ether (50:50) until the solvent front migrated 18–19 cm up the plate. Chromatogram development time was approximately 40 min. After being air dried, the plate was placed in a fume hood and sprayed with the fluorescamine solution (note the recommended safety precautions of avoiding skin contact and breathing) and viewed for fluorescence. The compounds appeared as yellowish spots within minutes after spraying.

RESULTS AND DISCUSSION

The mobile phase hexane–methyl *tert.*-butyl ether (50:50) resolved 3,3′-dichlorobenzidine from benzidine. R_F values were 0.57 for 3,3′-dichlorobenzidine and 0.30 for the slower migrating benzidine. Recently, methyl *tert.*-butyl ether has been reported to provide more resolving power than diethyl ether in TLC and high-performance liquid chromatographic systems[20]. Also, this higher boiling ether (boiling point 55–56°C compared to 34–35°C for diethyl ether) has been reported to have a relatively small tendency to form dangerous peroxides[21].

By analyzing different amounts of benzidine and 3,3′-dichlorobenzidine, it was

observed that 50 ng can be easily detected using this technique. The colors of the fluorescent products were different for the two condensation products. 3,3′-Dichlorobenzidine was the usual aquamarine fluorescence, but benzidine yielded a butter yellow fluorescent product. Possibly, the reaction product with benzidine contains two molecules of fluorescamine. Previous experience has shown that aquamarine fluorescent products were formed with a wide variety of low-molecular-weight primary amines[18].

Spiking 50 ng/μl of each into complex industrial effluent extracts revealed no interference in their detection by TLC. Base-neutral methylene chloride extracts were prepared according to recommended EPA protocol[23].

Application of this screening technique to industrial effluents requires fluorescent examinations before spraying the developed chromatogram with fluorescamine to avoid misinterpreting results. Many organic compounds contain endogenous fluorescence. Accordingly, it is important to examine the developed plate for both short-wavelength (254 nm) and long-wavelength (366 nm) spots in order to detect any interferences with the fluorescamine spray development for benzidines. It is convenient to circle the short-wavelength spots and draw a dashed line around long-wavelength fluorescent spots with a sharp pencil. When screening an industrial effluent, it is important to analyze a standard of the benzidines on the same TLC plate as a control. Also, it is recommended that the industrial extract be analyzed in duplicate. To one of the duplicate spots, a known amount of benzidines, same as control, should be overlayed as a standard addition before development to aid the analyst in the interpretation of results obtained. The control functions to document the performance of the overall procedure. The standard addition functions to demonstrate detection of the target compounds in a complex matrix and monitor any chromatographic development abnormalities due to the industrial extract.

Recently, fluorescamine has been described as an aid to detect primary polycyclic aromatic amines (PPAAs) in synthetic crudes such as shale oil and coal liquids[24]. The health and environmental effects of these petroleum substitutes are being extensively investigated to determine whether the use of these supplemental energy sources presents significant hazards. At least three classes of organic compounds have been isolated from synthetic crude oils which possess mutagenic activity: polycyclic aromatic hydrocarbons (PAHs), aza-arenes, and PPAAs. PPAAs appear to be present at relatively low concentrations, but the potency of this class of mutagenic compounds is much greater than that of PAHs. These authors pointed out that a successful analytical procedure must be capable of handling: (a) the trace concentrations of PPAA; (b) the complexity of the sample matrix; and (c) the potentially high molecular weights and polar nature of the individual components.

The procedure described in this paper provides a sensitive, quick, and inexpensive method to detect benzidine and 3,3′-dichlorobenzidine. Experience in our laboratory analyzing standard commercial solutions of these two compounds by GC–MS, without derivatization, as recommended by the EPA[23] has demonstrated highly variable detection limits for these two primary amines. It is the opinion of this author that the organic environmental chemist has not used TLC and spot tests as frequently as other scientific areas such as clinical, toxicological, or forensic science. Fluorescamine is a reagent which should be utilized for TLC and spot tests in environmental measurements of primary amines.

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