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Use of 2-mercaptopyridine for the determination of alkylating agents in complex matrices: application to dimethyl sulfate

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Abstract

A rapid and sensitive method for the determination of alkylating agents in complex reaction mixtures was developed and characterized. Analyses are based on the alkylation of 2-mercaptopyridine by the analyte; the derivative is separated by RP-HPLC and measured by fluorescence detection. When applied to the determination of dimethyl sulfate, the method is linear over four orders of magnitude: 0.01–10 $\mu g\,mL^{-1}$. By using recrystallized 2-mercaptopyridine, quantitation limits of 10 $ng\,mL^{-1}$ can be achieved. Precision of the assay is 2% R.S.D. in the 1–10 $\mu g\,mL^{-1}$ range and about 15% R.S.D. at 10 $ng\,mL^{-1}$. Studies on the pH dependence of the derivatization reaction were key to minimizing interference from the dimethyl sulfate degradation product, monomethyl sulfate, in quenched reaction samples.

Keywords: Dimethyl sulfate; 2-Mercaptopyridine; Alkylating agent; Reversed-phase liquid chromatography; Fluorescence

1. Introduction

Alkylating agents such as dimethyl sulfate and methyl iodide are commonly used in industrial syntheses. The methylating agents, in particular, are extremely hazardous materials. Most efforts to control exposure to alkylating agents have concentrated on the inhalation route. However, since workers can also be exposed to the agent through contact with reaction mixtures, destruction of excess reagent before the reaction mixture is removed from the vessel minimizes the risk of exposure from contact with the solution.

For example, in the recent development of a process involving steroid methylation with dimethyl sulfate, process hazard analysis identified the reaction mixture as a potential exposure risk and set the allowable limit of residual dimethyl sulfate at 10 $\mu g\,mL^{-1}$. To monitor the destruction of dimethyl sulfate in quenched reaction samples and to ensure that its level had been taken below this limit, a new analytical method was developed.

The toxicity of dimethyl sulfate is well documented; a comprehensive review of hazards and exposure limits has been compiled in a World Heath Organization report [1]. Since current occupational exposure control is based on the inhalation route, existing analytical methods have been developed primarily for determining dimethyl sulfate in air samples. The available methods have been reviewed [1] and include gas chromatography with various modes of detection, liquid chromatography of derivatives, with either UV or visible detection, and TLC or UV–vis spectrophotometry of a colored derivative. Although these methods are very sensitive (GC detection limits of 0.03 $mg\,m^{-3}$ are reported for a 1-L air sample [2]; by LC, a limit of 0.05 $mg\,m^{-3}$ was reported [3]), they are not applicable to complex solution samples without the use of extensive sample workup.

In quenched reaction samples, complexity arises not only from the high concentrations of reagents and product but also from the additional reagents added to quench the alkylating agent. In the reaction mixtures addressed by this method, the quenched reaction milieu has high concentrations of steroids, salts, and organic base, and low levels of dimethyl sulfate, in a mixed aqueous/organic solvent. An additional complication

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lies in the fact that this reaction mixture composition is optimized to destroy the analyte.

The HPLC method of Williams [3], previously applied to the evaluation of the destruction of alkylating agents [4], was examined for possible use in measuring low levels of dimethyl sulfate in quenched reaction samples. Based on the methylation of *p*-nitrophenol by dimethyl sulfate to form *p*-nitroanisole, derivatization is done in an acetone solution saturated with sodium-*p*-nitrophenoxide. The anisole derivative was separated from unreacted reagent by extraction with diethyl ether, the ether dried down, and the sample reconstituted in acetonitrile for injection.

Our evaluation showed that the ether extraction could be eliminated, since simple reversed-phase chromatographic systems provided ample separation between the reagent and derivative peaks. Quenched reaction samples, however, presented problems, since the high salt content is incompatible with the acetone-based reagent, and considerable precipitation was observed. To avoid precipitation, approximately equal volumes of sample, reagent, and water were necessary in the derivatization reaction. Under these reaction conditions, derivatization was very slow and seemed to stall after several hours.

In seeking a reagent that would be a better nucleophile than the phenoxide, we examined sulfhydryl compounds and found that 2-mercaptopyridine has very desirable properties for use in such analyses. First, this compound has a UV chromophore, which provides a handle for detection; the (*S*)-methyl derivative also fluoresces, providing additional sensitivity and selectivity. Secondly, this material is soluble both in water and in many organic solvents; therefore, it is ideal for use in mixed aqueous/organic reaction systems. Also, although 2-mercaptopyridine is a thiol compound, it is a nearly odorless solid; thus, it is easy to handle and does not have the usual offensive smell of most sulfhydryl compounds. Lastly, information available in vendors' MSDS documents does not indicate any unusual hazards, suggesting that this reagent can be used with typical laboratory handling practices and personal protection.

This paper describes the development and characterization of a method for determining alkylating agents in complex reaction media by derivatization with 2-mercaptopyridine and quantitation by RP-HPLC.

2. Experimental

2.1. Materials

Dimethyl sulfate, ≥99%, and sodium monomethyl sulfate were purchased from Aldrich (Milwaukee, WI, USA). The derivatizing reagent, 2-mercaptopyridine, 99%, was purchased from Aldrich and used "as is" for most analyses. For high sensitivity analyses, the derivatizing reagent was recrystallized twice from 200-proof ethanol. Acetonitrile, HPLC grade, was from EM Science (Gibbstown, NJ, USA); HPLC-grade water was produced by use of a US Filter (Broadview, IL, USA) purification system.

2.2. Instrumentation

HPLC separations were performed on an Agilent HP1100 system under ChemStation Rev. 7.01 control (Agilent Technologies, San José, CA, USA). Both diode array and fluorescence detection were employed. Data were acquired and processed by a DIAMIR (Varian, Palo Alto, CA, USA) data system. LC-UV-MS was done on an Agilent HP1100 system with MSD and diode array detectors; control was by ChemStation Rev. A.10.01. The mass-selective detector was operated in positive API-ES mode at a fragmentor setting of 80; drying gas was 3.0 L min^{-1} at 100 °C and the capillary was held at 3000 V.

2.3. Chromatographic conditions

Complex samples were separated by gradient HPLC with water:acetonitrile mobile phases in the ratios of 95:5 (A) and 5:95 (B). The gradient profile was as follows: initial, 40% B; gradient from 40% B to 50% B in 5.0 min; step to 100% B at 5.1 min and hold until 8.0 min; step to 40% B at 8.1 min and hold until 11.0 min. Simpler samples, such as those used in kinetics or pH studies, were run isocratically with water:acetonitrile mobile phases.

Flow rate was 1.0 mL min^{-1} throughout. UV detection was at 246 nm with a bandwidth of 8 nm and no reference. Fluorescence detection used an excitation wavelength of 248 nm, emission wavelength of 357 nm, PMT gain of 12, peakwidth of >0.2 min, and attenuation output of 100 LU. Injection volume was 20 μL. Separations were performed at 30 °C on an Agilent Zorbax RX–C18 column, 150 mm × 4.6 mm, with 3.5 μm packing.

Other columns tested included ACE C18, 3 μm (Advanced Chromatography Technologies, Aberdeen, Scotland); Phenomenex Luna C18(2), 5 μm (Phenomenex, Torrance, CA, USA); Waters XTerra RP 18, 5 μm (Waters, Milford, MA, USA); and Supelco Discovery C18, 5 μm (Supelco, Bellefonte, PA, USA).

2.4. Steroid methylation

To about 150 g L^{-1} steroid in 1:1 acetone water containing 50 g L^{-1} potassium bicarbonate, dimethyl sulfate was added to about 75 g L^{-1}. After the reaction was complete, excess dimethyl sulfate was quenched by the addition of triethylamine and additional bicarbonate.

2.5. Reagent and solution preparation

2-Mercaptopyridine was recrystallized by dissolving about 15 g of the reagent in approximately 100 mL hot ethanol, followed by cooling the resulting solution to about 4 °C. The crystals were filtered and washed with ice-cold