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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

ethanol. The filtered solids were re-dissolved in hot ethanol and crystallized again. The isolated material was dried overnight at about 40 °C under vacuum. The derivatizing reagent was prepared by dissolving 2-mercaptopyridine in acetonitrile at a concentration of 2 mg mL^{-1}.

Dimethyl sulfate standard stock solutions at about 1500 μg g^{-1} were prepared in dry acetonitrile. The stock solution was diluted to 10 μg mL^{-1} with HPLC grade acetonitrile.

Samples were diluted to about 10 μg mL^{-1} dimethyl sulfate, if necessary, with acetonitrile:water (1:1).

2.6. Derivatization

Concentration of reagent was investigated over the range of 0.1–5 mg mL^{-1} in acetonitrile:water (1:1). Final derivatization conditions involved combining 300 μL reagent with 300 μL buffer (50 mM carbonate, pH 10.0) and adding 200 μL sample, standard, or blank (acetonitrile:water 1:1). Reactions were allowed to stand at room temperature for at least 10 min before injection onto the HPLC.

The reagent, 2-mercaptopyridine, was not combined with the aqueous buffer until immediately before derivatization, to minimize oxidation of the reagent to the disulfide in the presence of base. Similarly, sample was not combined with buffer alone, to minimize degradation of dimethyl sulfate into monomethyl sulfate under these basic conditions.

2.7. Kinetics determinations

Reaction rates were assessed by repeated injections of derivatized samples. For fast reactions, separations were done under isocratic conditions with reduced run times. For these fast reactions, rate constants were calculated by using the Microsoft Excel Solver function to optimize the sum of squares of differences between observed and calculated results. Calculations were done by assuming pseudo-first-order kinetics, and refining the values for the rate constant, k, and the infinite time concentration, C_∞, according to the following equation:

$$C = C_\infty(1 - e^{-kt}) \quad (1)$$

For slow reactions, the rate constant was estimated from the slope of the linear fit (to concentration versus time data) times the initial analyte concentration. The apparent pseudo-first-order rate constants were not corrected for the constant concentration of derivatizing reagent.

2.8. Determination of linearity, precision, and accuracy

Linearity studies were executed over the concentration range of 10 ng mL^{-1} to 10 μg mL^{-1} dimethyl sulfate before derivatization. Reagent blank values were subtracted from each data point. Limit of detection and limit of quantitation were calculated from low-level linearity studies (10 ng mL^{-1} to 100 ng mL^{-1}), using the ICH calculations of 10σ/slope for limit of quantitation and 3.3σ/slope for limit of detection.

Precision studies were carried out from 10 ng mL^{-1} to 10 μg mL^{-1} and evaluated both injection and derivatization variability. At each concentration, six aliquots of sample were derivatized, and one of the derivatized solutions was injected six times.

Accuracy of the analysis was estimated by determining spike recovery from a quenched methylation reaction sample. Concentrations of dimethyl sulfate ranging from 1 ppm to 20 ppm were added to sample; the same concentrations were added to solvent (acetonitrile:water, 1:1) as controls. Since the sample matrix is destructive to analyte, reagent and sample were pre-mixed before adding the dimethyl sulfate spikes. Recovery was calculated relative to the controls after subtracting the response of the unspiked sample.

3. Results and discussion

3.1. Derivatization studies

The reaction used in the determination of dimethyl sulfate is methylation of the 2-mercaptopyridine reagent to form 2-S-methylmercaptopyridine. Identity of the derivative was demonstrated by LC-MS, as well as by coelution of the derivative with a sample of the authentic compound. The reaction is shown in Fig. 1. A second mole of derivative can, in principle, be derived from the monomethyl sulfate that results from the initial reaction.

3.1.1. Reagent concentration

Reagent concentrations over the range of 0.5–5 mg mL^{-1} gave equivalent responses; lower reagent concentrations gave reduced responses. At high reagent concentrations, impurities in the reagent become more of a problem in the HPLC separation. A reagent concentration of 2 mg mL^{-1} ensures complete reaction while avoiding excessive impurity lev-

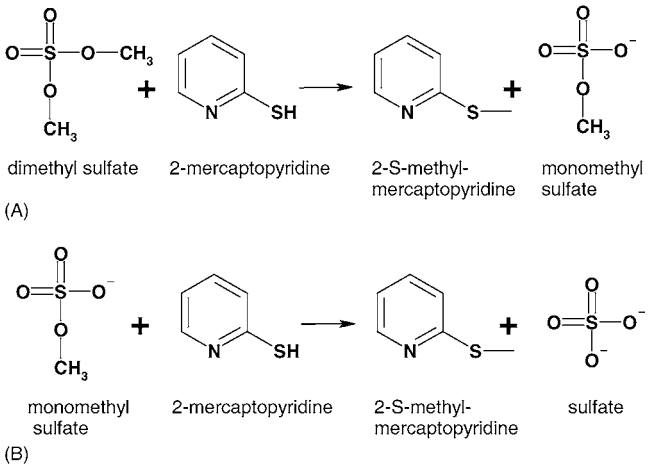

Fig. 1. Reactions of dimethyl sulfate and methyl sulfate with 2-mercaptopyridine.

Table 1
Pseudo-first-order reaction rates for the methylation of 2-mercaptopyridine at room temperature

pH	Buffer	k' (min^{-1})	
		Dimethyl sulfate	Monomethyl sulfate
6.0	Phosphate	0.004	7.4E-09
7.0	Phosphate	0.006	8.7E-09
8.25	Phosphate	0.024	9.8E-09
8.3	Carbonate	0.055	1.3E-08
9.0	Carbonate	0.12	2.6E-08
10.0	Carbonate	0.35	1.3E-07
11.2	Carbonate	0.49	2.1E-07
12	Carbonate	0.54	2.4E-07

All buffers were prepared from 50 mM phosphoric acid or sodium bicarbonate and the pH was adjusted with NaOH.

els. In the proposed derivatization, the amount of reagent is 5.4 μmol, and a 10 μg mL^{-1} sample contains 0.02 μmol analyte, for a mole ratio of about 300.

3.1.2. pH Dependence of reaction rate

The methylation reaction rate is markedly pH-sensitive. Reaction rates were estimated over the range of pH 6–12 by running reactions in the presence of aqueous buffers of varying pH. Due to the large excess of reagent, data were treated as pseudo-first-order.

As shown in Table 1, the reaction rate for dimethyl sulfate with the reagent is on the order of 10^6 faster at a given pH than for the corresponding reaction of monomethyl sulfate. For both reactions, the rate is negligible below pH 8, but increases by about an order of magnitude from pH 8 to 12.

The reaction rate pH profile is paralleled by changes in the UV spectrum of the reagent. Fig. 2 shows spectra of 2-mercaptopyridine over the pH range of 8–12. These spectra show a pronounced shift in absorbance maximum from about 352 nm at pH 8 to about 310 nm at pH 12, with a transition pH of about pH 10. The maximum at 280 nm shows a similar shift to about 270 nm over the same pH range. In work on a related compound, 3-hydroxy-2-mercaptopyridine, Hashem and Saleh attributed the 350 nm band to the neutral species and the 310 nm band to the enol form of the anionic species [5]. This observation is consistent with the greater availability of the anionic sulfur as nucleophile. The actual speciation in solution is more complicated, however, and a large body of literature exists not only on the tautomerism of 2-mercaptopyridine molecules but also on the self-association of these tautomers into hydrogen-bonded dimers, as shown in Fig. 3 [6–15]. The use of pH to shift species distribution may well be important, since Beak estimates that 2-mercaptopyridine exists predominantly (66000:1) as the thioamide [7]. On the other hand, Beak suggests elsewhere that the proton transfers between heteroatoms are so fast that the tautomeric equilibrium constant may be unimportant in describing the chemistry [7].

The million-fold difference in reaction rates between dimethyl and monomethyl sulfate suggests that only the first methylation is important and that the contribution from

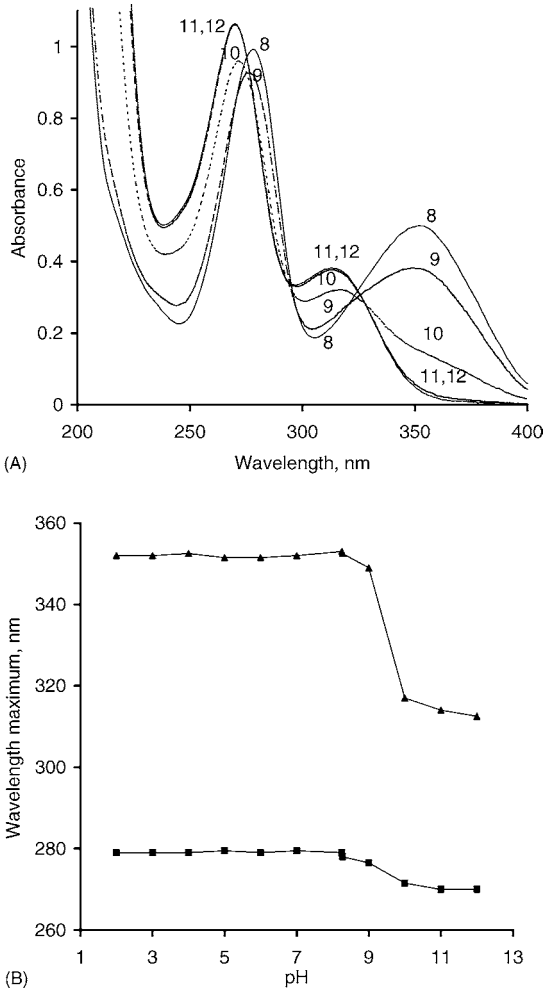

Fig. 2. (A) pH Dependence of the 2-mercaptopyridine UV spectrum in acetonitrile:water (1:1). Labels indicate the pH at which each trace was determined. (B) Shift in peak maxima as a function of pH.

monomethyl sulfate is insignificant. The high concentration of monomethyl sulfate relative to dimethyl sulfate analyte in quenched reaction samples, however, offsets the difference in reaction rates.

3.1.3. Reaction conditions to minimize monomethyl sulfate interference

The contribution from monomethyl sulfate in the derivatization is affected primarily by reaction time. The rapid derivatization of dimethyl sulfate and the slower reaction of monomethyl sulfate are simulated in Fig. 4, in which the levels of 2-S-methylpyridine are calculated from dimethyl sulfate and monomethyl sulfate concentrations of 2 μg mL^{-1} and 70 mg mL^{-1}, respectively, by using rate constants observed at pH 10 and room temperature. While the dimethyl sulfate reacts completely in less than 10 min, the contribution of derivative from the monomethyl salt is linear with time. The interference from the monomethyl salt thus increases with incubation times longer than that required for complete reaction of the dimethyl sulfate. This continuing contribution from the monomethyl salt becomes important if elevated

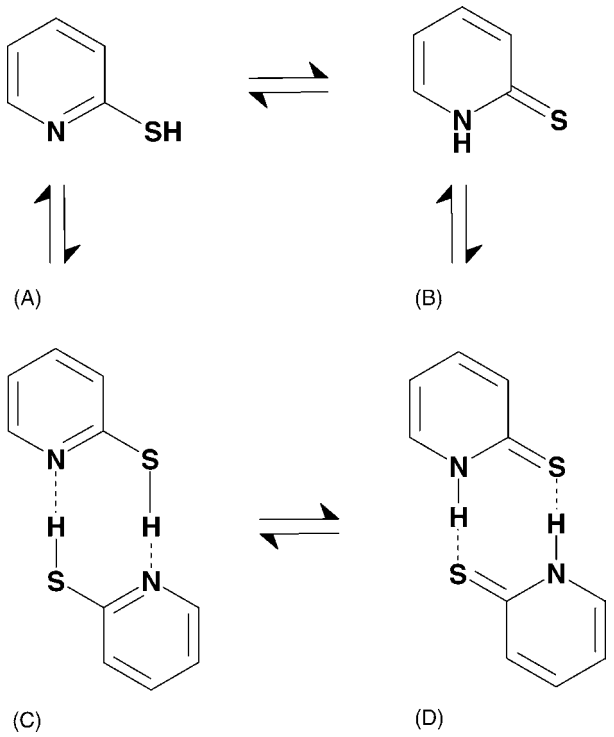

Fig. 3. Proposed solution equilibria for 2-mercaptopyridine tautomers. (A) Thiol, (B) thione, (C) thiol dimmer and (D) thione dimer.

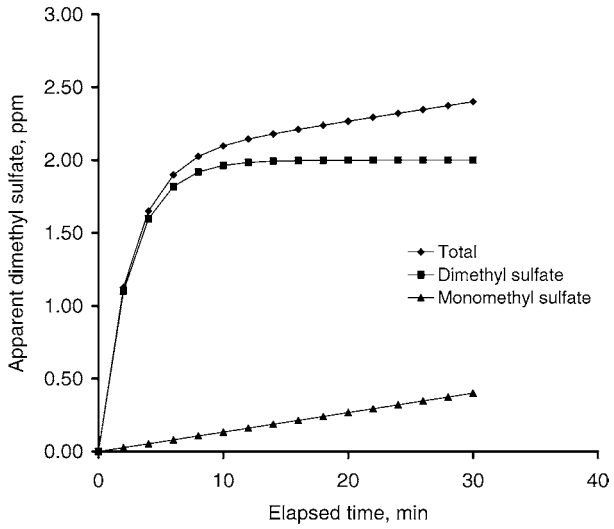

Fig. 4. Calculated responses from dimethyl sulfate at $2\,\mu g\,mL^{-1}$ and monomethyl sulfate at $70\,mg\,mL^{-1}$, based on the rate constants measured at room temperature and pH 10.

temperature or pH are used to accelerate the derivatization; the more rapidly the reaction proceeds, the steeper the slope of the interference/time plot.

3.2. Control of selectivity

3.2.1. Chromatographic separation

Under simple reversed-phase conditions, the derivative peak is well resolved from all other reagent and derivatization

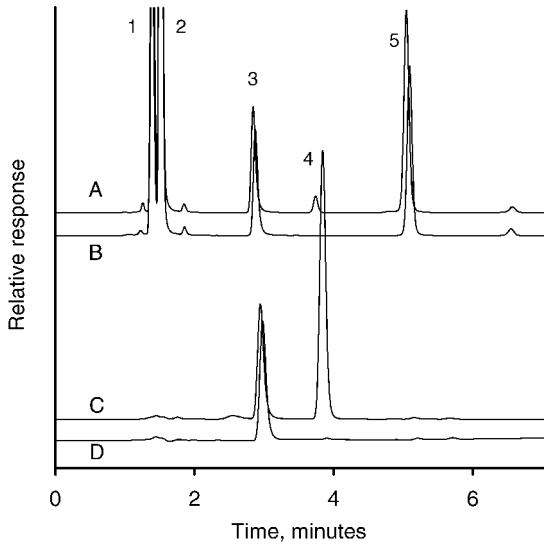

Fig. 5. Chromatograms of $10\,\mu g\,mL^{-1}$ dimethyl sulfate (A and C) and blank (B and D) derivatizations by UV (A and B) and fluorescence (C and D) detection. Peak identifications are in Table 2.

by-product peaks. Fig. 5 shows the separation on a 150 mm × 4.6 mm, 3.5 μm, Zorbax RX–C18 column. Similar separations were obtained on a number of other C18 columns. Although most separations were done with acetonitrile as the organic modifier, satisfactory results could be obtained with methanol as well.

Process reaction mixtures, on the other hand, may contain high concentrations of additional UV-absorbing compounds. Since the reactants and products are at very high concentration, minor components at the 0.1% level can still present interferences with detection of the derivative. Fig. 6A shows the UV trace from a derivatized reaction mixture containing

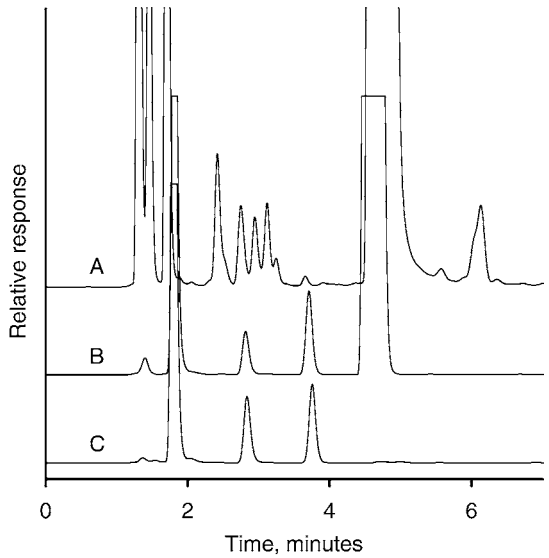

Fig. 6. Separation of a quenched steroid methylation reaction. (A) UV: 246 nm; (B) fluorescence: λ_{ex} = 248 nm, λ_{em} = 357 nm; (C) fluorescence: λ_{ex} = 290 nm, λ_{em} = 357 nm.

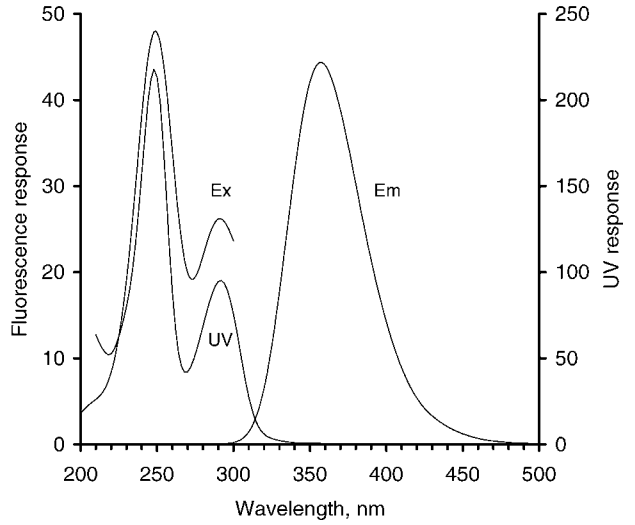

Fig. 7. Absorbance, emission, and excitation spectra for 2-S-methylmercaptopyridine under assay conditions.

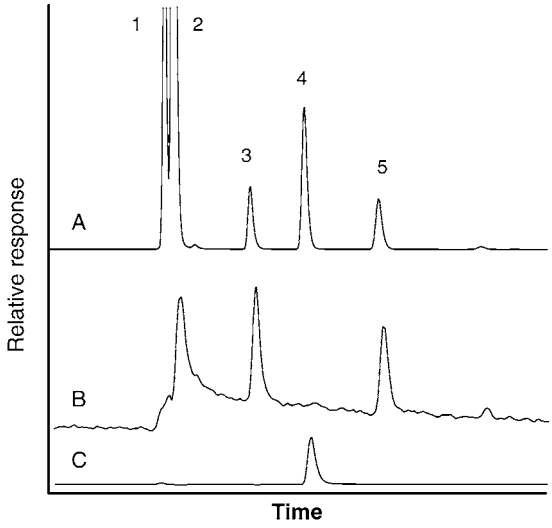

Fig. 8. Chromatograms of derivatized dimethyl sulfate with UV (A), LC/MS (B), and selected ion monitoring at *m/z* 125–127 (C) detection. Peak identifications are in Table 2.

dimethyl sulfate. Although the major reactants are well separated from the derivative, a number of minor components elute in the region of the analyte.

3.2.2. Detection mode

Fig. 7 shows the spectra of the 2-S-methylmercaptopyridine derivative in mobile phase. UV maxima are observed at 248 nm and 292 nm, with the longer wavelength showing about half the absorptivity of the 248 nm band. Sensitivity in UV detection can therefore be maximized by using the lower wavelength.

A major advantage in detection selectivity can be gained by the use of fluorescence detection. As shown in Fig. 7, the 2-S-methylmercaptopyridine derivative shows an emission maximum at about 357 nm when excited at 248 nm. As expected, the excitation spectrum mirrors the UV spectrum. The chromatograms in Fig. 5 demonstrate the selectivity advantage achieved by using fluorescence detection. With fluorescence detection, the derivative gives the major peak in the chromatogram, accompanied by a single reagent peak. Thus, fluorescence detection enhances the response of the analyte relative to the other species in the derivatization reaction.

Fig. 6 gives a direct comparison of UV and fluorescence detection for the determination of dimethyl sulfate in a quenched steroid methylation reaction sample. The selectivity of fluorescence detection eliminates the peak interference observed by UV. Fluorescence detection also results in a signal larger than that generated by UV detection. In these steroid methylation samples, use of λ_{ex} = 290 nm would further increase selectivity, since the steroid is not detected with this excitation wavelength.

3.2.3. Reagent and side-product peaks

Characterization of the other peaks in the UV and fluorescence chromatograms provides information for further optimization of separation and detection conditions, if necessary. The larger peaks were characterized by LC/MS/UV/FL; the UV and fluorescence traces are shown in Fig. 5, and the MS trace in Fig. 8. A summary of results is given in Table 2. The 2-mercaptopyridine reagent elutes very early in the chromatogram and has very little fluorescence. The peaks flanking the derivative have masses consistent with the 2,2′-dipyridyl sulfide and the 2,2′-pyridyl disulfide. The former seems to be an impurity in the reagent as purchased; the latter may be in the reagent but would also be expected to form by oxidation of the reagent during the derivatization. The disulfide excitation and emission maxima are sufficiently different from those of the derivatives that spectral interference is minimal in fluorescence mode. The 2-S-methylmercaptopyridine derivative is fairly volatile and thus is difficult to detect by LC/MS. The presence of this compound was confirmed by selected-ion monitoring, however, as shown in Fig. 8.

3.3. Figures of merit

The method was linear over the range tested, 10 ng mL^{-1} to 10 μg mL^{-1} (slope = 6086 ± 26, intercept = 1.7 ± 95, N = 10, r^2 = 0.9998). Injection and preparation precision are comparable at all concentrations tested, with an assay precision of about 2% R.S.D. at the 10 μg mL^{-1} level and about 13% R.S.D. at the 10 ng mL^{-1} level. The limit of quantitation, derived from low-level linearity data (range 10–100 ng mL^{-1}; slope = 6206 ± 51; intercept = 5.6 ± 2.9; standard error = 3.6; N = 4, r^2 = 0.99985) was 6 ng mL^{-1}, with a corresponding detection limit of 2 ng mL^{-1}. Fig. 9 shows the relative responses of the reagent blank and 10 ng mL^{-1} standard. These limits of detection and quantitation were achievable only with recrystallized reagent; with commercial reagent used “as is” the corresponding limits were 300 ng mL^{-1} and 1000 ng mL^{-1}.

Table 2
Proposed identification of peaks observed by UV detection in derivatized samples

Peak	Retention time (min)	Relative retention time	M + H ion	UV maxima (nm)	Fluorescence emission maxima (nm)	Proposed ID
1	1.3	0.35	105.1	240	392	Unknown
2	1.5	0.40	112.1	280, 352	None	2-Mercaptopyridine
3	2.9	0.77	189.1	242, 289	365	2,2′-Pyridine sulfide
4	3.75	1.00	126.2	248, 291	356	2-S-Methylmercaptopyridine
5	5.1	1.36	221	234, 282	371, 511	2,2′-Pyridine disulfide

Peak identifiers refer to Fig. 5.

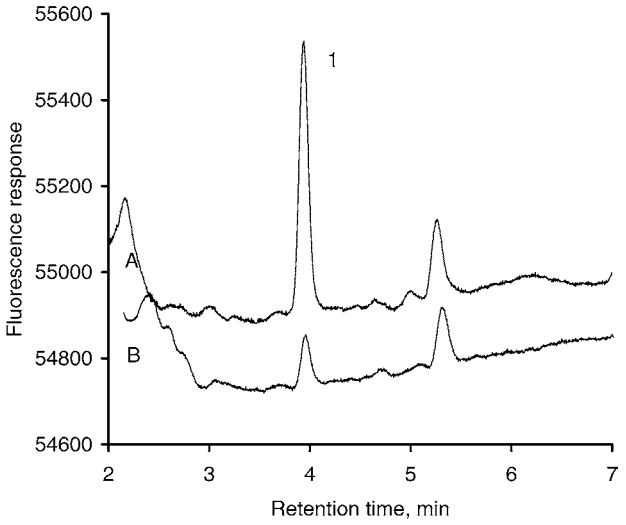

Fig. 9. Signal observed near the quantitation limit. (A) 10 ng mL^{-1} standard; (B) reagent blank prepared with recrystallized 2-mercaptopyridine. Peak 1: 2-S-methylmercaptopyridine.

Table 3
Determination of dimethylsulfate (ppm) in spiked steroid methylation reaction samples

Added	Found	Recovery (%) (n = 3)
0	0.34 ± 0.03	N.A.
1	1.33 ± 0.09	98.9
2.5	3.10 ± 0.04	108.2
5	5.50 ± 0.07	103.2
10	10.23 ± 0.03	98.9
20	20.05 ± 0.06	98.6
Average		101.6
Standard deviation		4.2
R.S.D. (%)		4.1

Over the concentration range of 1–20 ppm, spike recovery from quenched reaction samples averaged 102 ± 4%, with a range of 98.6–108.3% (Table 3).

3.4. Use of recrystallized reagent

Commercial 2-mercaptopyridine tested in this work contained low levels of either *S*-methyl-2-mercaptopyridine or a material eluting at the same retention time. When this material was used in the assay, the levels contributed to the dimethyl sulfate assay were between 20 ng mL^{-1} and 70 ng mL^{-1}. Typical blank contributions with the recrystallized reagent are on the order of 1–3 ng mL^{-1}. Since these levels are insignificant when assaying samples at the 10 μg mL^{-1} level, no work was done to reduce blank levels further. However, the noise level in the baseline suggests that if the reagent peak could be further diminished, there is room for further decrease in the detection and quantitation limits.

3.5. Solution stability

We tested the stability of 1000–2000 μg mL^{-1} stock solutions of dimethyl sulfate in acetonitrile and confirmed the findings of Lunn and Sansone that these stock solutions are stable for weeks [16]. Loss of dimethyl sulfate from these stock solutions was about 0.1% per day when stored at room temperature.

Working standards, on the other hand, are quite unstable and must be used immediately after preparation. Kinetic data show that the 10 μg mL^{-1} standards prepared in acetonitrile have a half-life of about 12 h. This rate of decomposition translates into about a 30 min usable working time in which to retain 98% of the expected dimethyl sulfate.

In contrast to the working standards, derivatized standards and samples are stable for at least 2 days. No appreciable decrease in response was observed over a 55-hour period. Similarly, the derivatizing reagent, 2-mercaptopyridine, is stable in solution at room temperature for at least 2 days.

3.6. Application to alkylating agents other than dimethyl sulfate

Derivatization of 2-mercaptopyridine is well suited to the analysis of alkylating agents other than dimethyl sulfate. All methylating agents result in the same 2-(*S*)-methylpyridine derivative, so a common chromatographic system can be used. Longer-chain alkylating agents such as diethyl sulfate and di-*n*-propyl sulfate give well-resolved peaks, as shown in Fig. 10.

Use of the method for other alkylating agents may require optimization of the reaction conditions. Methyl iodide has a fast reaction rate with 2-mercaptopyridine, and room temperature incubation for 10 min is adequate for complete reaction. Methyl methanesulfonate, on the other hand, has a reaction half-life of about 1 h. Similarly, reactions with diethyl sulfate and di-*n*-propyl sulfate are much slower than with dimethyl sulfate. For use of the assay with the less active alkylating

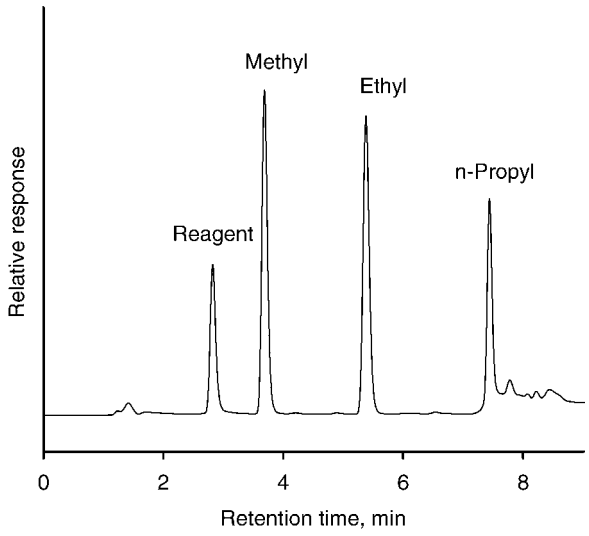

Fig. 10. Separation of 2-(*S*)-alkyl-mercaptopyridine derivatives derived from dimethyl sulfate, diethyl sulfate, and di-*n*-propyl sulfate.

agents, optimization of reaction conditions to include a heating step should be considered.

4. Conclusions

Methylation of 2-mercaptopyridine by dimethyl sulfate provides a rapid and sensitive method for the determination of this alkylating agent. Use of RP-HPLC with fluorescence detection provides detection limits in the ng mL^{-1} range. The selectivity provided by fluorescence detection allows measurements of dimethyl sulfate in complex solution matrices.

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