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LIQUID CHROMATOGRAPHIC DETERMINATION OF MORPHOLINE AND ITS THERMAL BREAKDOWN PRODUCTS IN STEAM–WATER CYCLES AT NUCLEAR POWER PLANTS

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SUMMARY

Morpholine and its amine breakdown products in aqueous samples were derivatized with dabsyl chloride in the presence of sodium bicarbonate and the resulting precolumn derivatives determined by high-performance liquid chromatography with visible detection at 456 nm. The analytical column was a μBondapak C_{18} reversed-phase device. The breakdown products were determined in the concentration range of 0.25–10 μg/ml in water, with a relative standard deviation of 0.38–7.08%. The amine detection limits were 0.01–0.03 μg/ml for 20-μl injections. Chromatographic analysis of 100-ml grab samples after acidification and concentration demonstrates the success of this technique for determining the quantity (ng/ml) of ammonia, methylamine, ethylamine, ethanolamine and 2-(2-aminoethoxy)ethanol in the thermal cycle at Gentilly 2 nuclear power plant. The recovery for the complete assay procedure varied between 92.0 and 106.0% depending on the product studied.

INTRODUCTION

Morpholine (6–14 μg/ml) is added to the thermal cycle of Hydro-Québec's Gentilly 2 nuclear generating station (CANDU-PHW 600 design) to keep the pH between 9 and 10 in order to counteract the corrosive action of any carbon dioxide present in the system. Recent laboratory investigations have shown that morpholine decomposes at temperatures and pressures close to the operating conditions at Gentilly 2 (260°C and 4.55 MPa) to give ammonia, methylamine, ethylamine, ethanolamine, 2-(2-aminoethoxy)ethanol and some organic acids[1]. These substances may themselves corrode the system components or thermally break down into corrosive organic or inorganic by-products, which may lead to premature equipment failure. Sensitive methods are needed to analyse morpholine breakdown products from operating steam–water systems in order to determine their contribution to the organic contaminants and investigate their corrosiveness for construction materials.

The methods generally used for determining ammonia and aliphatic amines in water samples include gas chromatography[2], continuous-flow fluorometric tech-

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niques[3], ion-exchange separation techniques[4,5] and high-performance liquid chromatography (HPLC). The HPLC techniques described in the literature involve precolumn derivatization with *m*-toluoyl chloride[6], 4-dimethylaminoazobenzene-4'-sulphonyl choride (dabsyl chloride)[7,8], 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole[9], 5-dimethylaminonaphthalene-1-sulphonyl chloride (Dns chloride)[10] or acetylacetone[11] in conjection with either fluorescence, UV–VIS or Raman spectroscopic detection. These techniques were developed specifically to solve analytical problems in the field of biomedical and environmental studies, and none was designed to determine simultaneously ammonia, methylamine, ethylamine, ethanolamine, 2-(2-aminoethoxy)ethanol and morpholine as needed for this particular application.

This work presents a method based on reversed-phase HPLC for separating and quantifying the amines present in the thermal cycle of a nuclear plant via the formation of dabsylated derivatives. It allows the amount (ng/ml or ppb) of amines in water samples to be determined following concentration by evaporation. Amine levels in samples collected at Gentilly 2 are reported.

EXPERIMENTAL

Apparatus

The HPLC studies were performed with a Series 5500 chromatographic system (Varian, Walnut Creek, CA, U.S.A.) equipped with a 20-μl loop injection valve (Model 7126; Rheodyne, Cotati, CA, U.S.A.) and an ultraviolet–visible detector (Model UV-200, Varian) with a cell capacity of 4.5 μl. The detector was set at 456 nm. A μBondapak C_{18} column (30 cm × 3.9 mm I.D., 10 μm; Waters Assoc., Milford, MA, U.S.A.) was used and held at a steady temperature of 30°C. The mobile phase, consisting of a water–ethanol mixture, was pumped at 1.0 ml/min by using gradients. The chromatograms were recorded and integrated on a Varian Model DS 651, Vista Series.

Reagents and solvents

The dabsyl chloride of HPLC grade was obtained from Regis Chemical (Morton Grove, IL, U.S.A.). The sodium bicarbonate, hydrochloric acid and amine used as standards were ACS grade, while the acetone and ethanol were HPLC grade from Anachemia (Montréal, Canada); the ethanol contained 5% (v/v) isopropanol. The water used to prepare the standard solutions was purified by means of a Milli-Q filter system (Millipore, Bedford, MA, U.S.A.) and filtered over a 0.45-μm Millipore membrane for preparation of the chromatographic eluents. The Gentilly 2 samples were collected in polyethylene bottles and stored at 4 to 10°C for a maximum of 2 days before analysis.

Dabsylation procedure for standard solutions

The dabsylation procedure was a modification of that of Lin and Lin Shiau[12]. A 1.0-ml volume of an amine standard solution (0.25–300 μg/ml water) was mixed with 4.0 ml of dabsyl chloride (1.25 mg/ml acetone) and 0.6 ml of an aqueous solution of sodium bicarbonate (25 mg/ml). This mixture was allowed to stand in the dark at ambient temperature for 1 h prior to injecting 20-μl aliquots on the HPLC column (final pH of 9.4).

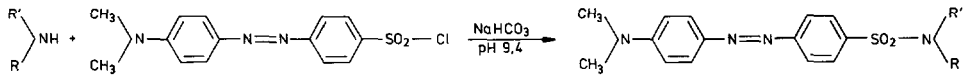
Dabsylation procedure for steam–water cycle samples

The pH of 100-ml samples was adjusted to 4.0 by a 0.01 *M* hydrochloric acid solution. The acidified samples were concentrated by gently boiling them in a beaker covered with a ribbed watch-glass, and stirring with a magnetic stirrer until the volume was reduced to 2.5 ml, giving a concentration factor in the range of 40. The amines contained in this concentrated solution were subsequently dabsylated as described above.

RESULTS AND DISCUSSION

Spectrophotometric properties

The poor ultraviolet absorptivity of morpholine and its decomposition products calls for precolumn derivatization for trace determination by HPLC. Dabsyl chloride has proven to be a good practical derivatizing agent for amines[7]. The reaction consists in condensing the amine with dabsyl chloride at pH 9.4 and analysing the chromophoric dabsylamides by detection in the visible region. The reaction is described by the following equation:



Amine Dabsyl chloride Dabsylamide

The UV–VIS spectra of the dabsylated morpholine and its decomposition products in the range of 300–600 nm were recorded on a Philips PU 8820 UV–VIS spectrophotometer. Table I lists the absorption maxima, λ_{max}, and molar extinction coefficients of these derivatives. Since the absorption maxima occurred at 453.0–458.5 nm, the derivatives were detected at 456 nm in the HPLC analysis. The extinction coefficients of the derivatives were between 6580 and 6930 l mol^{-1} cm^{-1}, showing that a reasonable sensitivity was attained.

Chromatographic separation

Optimum separations of the dabsylamides were obtained on a μBondapak C_{18} column when a gradient elution of the mobile phase was used (this gradient is defined in Table II). These experimental conditions produced the chromatogram of Fig. 1 when a mixture of 10 μg/ml of each dabsylated amine was injected. The elution peaks at 2.8 and 5.3 min are due to the dabsyl chloride and its acid form respectively, whereas the peak at 12.3 min was not identified in the present study. The dabsylamides were eluted between 22 and 29 min; their retention times are reported in Table III. The same-day relative standard deviation of the retention times was less than 0.54%, which indicates an excellent reproducibility in the separation. The chromatogram shows an excellent resolution for the dabsylated ammonia and methylamine, whereas partial separation is noted for dabsylated ethanolamine and 2-(2-aminoethoxy)ethanol as well as for dabsylated ethylamine and morpholine. The stability of the dabsylamides was determined by measuring the signal at 456 nm for different reaction times. Table IV shows that the absorbance for each derivative reached a maximum value in 1 h and

TABLE I

ABSORPTION MAXIMA AND MOLAR EXTINCTION COEFFICIENTS FOR DABSYLATED AMINES

Amine	λ_{max} *(nm)*	ε_{456}[a] *(l mol^{-1} cm^{-1})*	*Ethanol–water*[b] *(%, v/v)*
Ammonia	455.5	6580	60–40
Ethanolamine	456.0	6700	60–40
2-(2-Aminoethoxy)ethanol	455.5	6760	60–40
Methylamine	458.5	6600	63–37
Ethylamine	453.0	6880	70–30
Morpholine	458.5	6930	73–27

[a] Calculated from the chromatographic peaks of dabsylated amines according to the equation proposed by Nishikawa[11]

$$\varepsilon_{456} = (A/L)(WR/M)$$

where A is the absorption at peak height, L the path length of the detector cell (0.4 cm), M the amount of amine injected (mol), W the peak width at half-height (min) and R the flow-rate of the mobile phase (0.001 l/min). Based on standard solutions of 10 μg/ml of amines.

[b] Solvent composition used to study the UV–VIS spectra, corresponding to the gradient at the moment each peak is eluted.

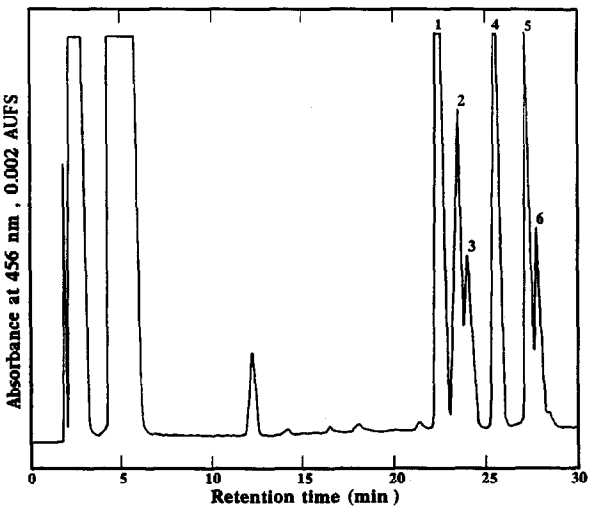


Fig. 1. Chromatogram of an HPLC separation of the dabsyl derivatives of (1) ammonia, (2) ethanolamine, (3) 2-(2-aminoethoxy)ethanol, (4) methylamine, (5) ethylamine and (6) morpholine. A 20-μl amount of an amine mixture (10 μg/ml solution of each amine) was injected. Chromatographic conditions as described in the Experimental section.

TABLE II
GRADIENT ELUTION OF THE MOBILE PHASE
Flow-rate 1.0 ml/min.

Operation	*Time (min)*	*Water–ethanol (%, v/v)*	*Pressure (atm)*	*Elution conditions*
Initial	0	75:25	160	–
Run	0–21	40:60	210	Linear
Hold	21–25	40:60	210	Isocratic
Run	25–35	0:100	180	Linear
Reverse	35–38	75:25	160	Linear
Conditioning	38–50	75:25	160	Isocratic

TABLE III
RETENTION TIMES OF DABSYLATED AMINES

Amine	*Retention time (min), mean[a] ± S.D.*	*R.S.D. (%)*
Ammonia	22.31 ± 0.12	0.54
Ethanolamine	23.23 ± 0.08	0.34
2(2-Aminoethoxy)ethanol	23.89 ± 0.11	0.46
Methylamine	25.61 ± 0.09	0.35
Ethylamine	27.71 ± 0.12	0.43
Morpholine	28.28 ± 0.13	0.46

[a] Average of 30 replicates.

TABLE IV
STABILITY OF DABSYLATED AMINES

Amine[a]	*UV–VIS[b] detector response (456 nm) versus time (h)*							
	0.5	*1*	*3*	*6*	*24*	*48*	*72*	*96*
Ammonia	6.88	8.12	8.05	8.17	8.13	8.08	8.10	8.10
Ethanolamine	2.54	2.33	2.53	2.40	2.48	2.48	2.49	2.48
2-(2-Aminoethoxy)ethanol	1.67	1.91	1.98	2.00	1.98	2.09	1.89	1.89
Methylamine	4.75	4.92	4.75	4.58	4.58	4.62	4.76	4.78
Ethylamine	3.30	3.14	3.24	3.78	3.24	3.39	3.24	3.24
Morpholine	1.53	1.54	1.51	1.50	1.49	1.60	1.53	1.52

[a] Solutions of 10 μg/ml of amine used.
[b] Peak area for a 20-μl injection expressed in $\mu V\ s \cdot 10^5$.

remained constant at least for 96 h when the reaction mixture was stored in the dark at ambient temperature. Consequently, the reaction time of the dabsylation was set at 1 h.

Quantitative response of the method

Table V reports the detection limits, analytical precision and calibration data for the dabsylated amines. The sensitivity achieved is 0.01 μg/ml for ethanolamine, 0.02

TABLE V
DETECTION LIMITS, ANALYTICAL PRECISION AND CALIBRATION DATA FOR DABSYLATED AMINES

Amine	*DL*[a] *(μg/ml)*	*QL*[b] *(μg/ml)*	*Analytical precision*			*Regression line and correlation coefficients*[e]
			Amount of amine[c] *(μg/ml)*	*Response at 456 nm*[d] *mean ± S.D.*	*R.S.D. (%)*	
Ammonia	0.03	0.15	0.25	4.64 ± 0.27	5.82	$y = 80680x + 25456$
			0.50	6.62 ± 0.16	2.41	$r = 0.9999$
			1.00	10.34 ± 0.11	1.06	
			2.50	22.92 ± 0.28	1.22	
			5.00	42.84 ± 0.75	1.75	
			10.00	83.22 ± 0.47	0.56	
Ethanolamine	0.01	0.05	0.25	0.64 ± 0.016	2.50	$y = 23790x - 515$
			0.50	1.13 ± 0.016	1.42	$r = 0.9999$
			1.00	2.38 ± 0.017	0.72	
			2.50	5.70 ± 0.22	3.86	
			5.00	11.86 ± 0.18	1.51	
			10.00	23.77 ± 0.30	1.26	
2-(2-Aminoethoxy)ethanol	0.03	0.15	0.25	0.59 ± 0.04	6.77	$y = 19848x + 1643$
			0.50	1.18 ± 0.07	5.93	$r = 0.9996$
			1.00	2.27 ± 0.12	5.28	
			2.50	4.85 ± 0.29	5.97	
			5.00	10.39 ± 0.30	2.88	
			10.00	19.92 ± 0.52	2.61	

Methylamine	0.02	0.10	0.25	3.14 ± 0.08	2.54	$y = 44807x + 18484$
			0.50	4.09 ± 0.17	4.15	$r = 0.9996$
			1.00	6.32 ± 0.22	3.48	
			2.50	12.34 ± 0.45	3.65	
			5.00	24.99 ± 1.15	4.60	
			10.00	46.46 ± 0.54	1.16	
Ethylamine	0.03	0.15	0.25	1.13 ± 0.08	7.08	$y = 31528x + 2793$
			0.50	2.00 ± 0.10	5.00	$r = 0.9999$
			1.00	3.26 ± 0.08	2.45	
			2.50	8.23 ± 0.14	1.70	
			5.00	15.86 ± 0.42	2.65	
			10.00	31.90 ± 0.12	0.38	
Morpholine	0.03	0.15	5	7.66 ± 0.51	6.65	$y = 6.62x^2 + 15345x - 26040$
			10	15.30 ± 0.72	4.70	$r = 0.999$
			25	33.74 ± 1.31	3.88	
			50	71.30 ± 2.83	3.96	
			100	150.46 ± 5.38	3.57	
			125	211.15 ± 3.46	1.64	
			150	241.50 ± 2.58	1.07	
			175	284.90 ± 2.35	2.58	
			300	516.95 ± 9.83	1.90	

[a] Detection limit.
[b] Quantification limit (QL = 5 DL).
[c] Amine concentration in aqueous sample prior to dabsylation.
[d] Peak area in $\mu V\ s \cdot 10^4$ (average of five replicates).
[e] Number of points: 5.

μg/ml for methylamine and 0.03 μg/ml for ammonia, 2-(2-aminoethoxy)ethanol, ethylamine and morpholine in water samples. These detection limits were estimated with a 95% confidence interval using the statistical approach proposed by McAinsh *et al.* [13]. The dabsylated amines were determined with 0.38–7.1% relative standard deviation in the range of 0.25–10 μg/l, except for morpholine whose concentration varied from 5 to 300 μg/l. An high degree of precision is achieved whatever the amount and nature of the amine involved. The linearity in the response of the UV–VIS detector (peak area) as a function of the amine concentration, expressed by the correlation coefficients, is reported in Table V. Coefficients of over 0.9996 were obtained for the breakdown products, indicating an excellent linearity in calibration. However, dabsylated morpholine shows a second-degree polynomial regression whose characteristics are given in Table V.

HPLC analysis of dabsylated samples of purified water with no amines added revealed the presence of two interference peaks whose elution times correspond to those of dabsylated ammonia and methylamine. The peak areas are constant (ten different preparations) and equivalent to the values caculated for the *y*-intercept of the calibration graphs for dabsylated ammonia and methylamine. The sodium bicarbonate used in the preparation of the derivatives was identified as the source of contamination but in no way does it affect the analytical precision of the method.

Analysis of real samples

The HPLC method was applied to samples collected at Gentilly 2 which revealed the presence of morpholine in the range of 6–14 μg/ml and very small amounts of decomposition products which could not be quantified with the required precision. The samples were then acidified and concentrated by evaporation following the second

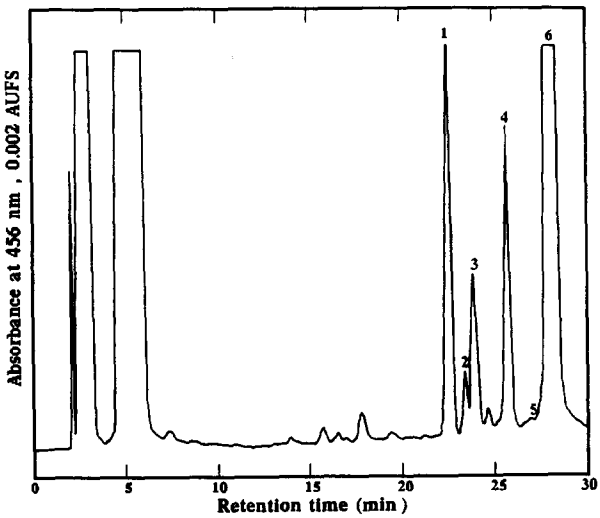


Fig. 2. Typical chromatogram of an HPLC separation of the dabsyl derivatives of morpholine and its amine breakdown products in a sample collected at Gentilly 2 (main steam sample). For peak identification and corresponding concentration, see Fig. 1 and Table VI, respectively. Chromatographic conditions as described in the Experimental section.

TABLE VI
ANALYSIS OF MORPHOLINE AND ITS AMINE BREAKDOWN PRODUCTS IN A GRAB SAMPLE COLLECTED AT GENTILLY 2 AFTER CONCENTRATION AND DABSYLATION

Amine	*Amount (ng/ml)*
Ammonia	28.9
Ethanolamine	20.6
2-(2-Aminoethoxy)ethanol	85.2
Methylamine	54.0
Ethylamine	5.2
Morpholine	6260

TABLE VII
ANALYSIS OF A STANDARD SOLUTION OF AMINES FOLLOWING CONCENTRATION OF THE AQUEOUS SOLUTION BY EVAPORATION

Amine	*Amount (ng/ml)*		*Recovery (%)*	*R.S.D. (%)*
	Added	*Found*[a]		
Ammonia	30	29.8	99.3	1.9
Ethanolamine	20	21.2	106.0	4.3
2-(2-Aminoethoxy)ethanol	85	82.6	96.0	2.6
Methylamine	54	51.1	94.6	3.6
Ethylamine	5	5.4	92.0	8.0
Morpholine	8300	8000	96.4	0.7

[a] Average of five replicates.

procedure described in the Experimental section. The representative chromatographic results illustrated in Fig. 2 and reported in Table VI show that morpholine decomposition products are present in the thermal cycle at Gentilly 2 in quantities of several ng/ml. Elution of low amounts of dabsylated ethylamine beside high amounts of dabsylated morpholine limits the ethylamine determination accuracy to 70%.

The risk of losing amines during the evaporation step, despite the acidification of the sample, was assessed. The results in Table VII reveal that the amounts of amines found after concentration are very similar to those in the initial sample. The recovery percentages were over 92%, with relative standard deviations of 0.7 to 8.0%. For this evaluation, the amines investigated were added to the water samples in proportions close to those determined in real samples (Table VI).

CONCLUSION

The analytical method developed during this research has proven to be a viable procedure for determining trace amounts of amine breakdown products of morpholine and is being used to monitor these compounds in steam–water cycles of nuclear and thermal power plants.

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