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## RAPID ESTIMATION OF TRACE AMOUNTS OF ETHYLENIMINE BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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### SUMMARY

A rapid procedure utilizing high-pressure liquid chromatography is described for determining trace quantities of ethylenimine down to 0.01 ppm. The basis for the method is the quantitative reaction of ethylenimine with the 1,2-naphthoquinone-4-sulphonate ion (Folin's reagent) to give 4-(1-aziridiny)-1,2-naphthoquinone. Compared with the spectrophotometric procedure, this method provides a high degree of specificity and sensitivity.

The method has been employed to determine ethylenimine in the pyrolysis products of polyethylenimine.

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### INTRODUCTION

Many analytical methods are known for ethylenimine (EI), but only a few are applicable to the determination of the trace amounts necessary for toxicological studies<sup>1,2</sup>. Epstein *et al.*<sup>3</sup> have used a spectrophotometric method utilizing the reagent 4-(4-nitrobenzyl)pyridine. However, other alkylating agents interfere in this procedure, and, in our experience, its sensitivity is limited. Rosenblatt *et al.*<sup>4</sup> have reported an alternative spectrophotometric procedure employing Folin's reagent for amino acids<sup>5</sup> and this has subsequently been used for estimating trace quantities of EI in air down to 1 ppm<sup>6</sup>. This method is based on the rapid reaction between EI and the 1,2-naphthoquinone-4-sulphonate ion. The product, 4-(1-aziridiny)-1,2-naphthoquinone, is readily extracted into chloroform and determined spectrophotometrically at 420 m.

Our interest in the toxicity of the pyrolysis products of polyethylenimine (PEI), which is used extensively in the paper and textile industries, necessitated a sensitive and selective method for determining EI. The usual procedure with Folin's reagent was precluded because many of the amines produced on pyrolysis of PEI react with Folin's reagent to give coloured products that interfere<sup>4,7</sup>. This paper reports an improved technique, utilizing high-pressure liquid chromatography (HPLC), for estimating the concentration of the 1,2-naphthoquinone derivative formed by the

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reaction of EI with Folin's reagent. This procedure eliminates interferences due to amines, thereby providing greater accuracy, and extends the sensitivity for quantitative analysis down to 0.01 ppm of EI in aqueous solution.

## EXPERIMENTAL

### *Materials*

Folin's reagent was prepared fresh daily by dissolving 0.104 g of the sodium salt of 1,2-naphthoquinone-4-sulphonic acid (BDH, Poole, Great Britain) in distilled water (100 ml). Ethylenimine (Koch-Light, Colnbrook, Great Britain) was redistilled from potassium hydroxide prior to use. The buffer employed was 0.05 M sodium phosphate (pH 11.7). 4-(1-Aziridinyl)-1,2-naphthoquinone was synthesised as described by Gauss and Petersen<sup>8</sup> and recrystallized twice from ethanol. Methylamine was prepared from the hydrochloride; other amines were reagent grade. Isopropanol and dichloromethane were spectroscopic grade; chloroform was analytical grade.

### *Apparatus*

A Varian 4100 Series liquid chromatograph, equipped with a 10- $\mu$ m Micro Pak column (50 cm  $\times$  2.2 mm I.D., Varian) at ambient temperature and 254-nm UV detector, was employed for chromatographic analyses. Dichloromethane containing 1% (v/v) isopropanol was used as the mobile phase with a flow-rate of 80 ml/h and a column pressure of 2500 p.s.i. Sample injections were made on-column, using a stop-flow technique, through a high-pressure septumless injector (Varian Aerograph) with a 25  $\mu$ l or 100- $\mu$ l syringe (S.G.E.).

### *Calibration*

Aqueous standard solutions of EI with concentrations in the range 0.01–1.0  $\mu$ g/ml were freshly prepared. Aliquots (50 ml) of the standards were treated with phosphate buffer (1 ml) and Folin's reagent (10 ml) in separatory funnels for 3 min. The solutions were shaken with two 5-ml aliquots of chloroform, and the extracts were combined and set aside for HPLC. A calibration graph was prepared by plotting EI concentration against peak height. Chloroform solutions containing known amounts of 4-(1-aziridinyl)-1,2-naphthoquinone were similarly subjected to HPLC in order to prepare a calibration graph.

### *Determination of EI in the pyrolysis products of PEI*

A dry weighed sample of PEI (MW ca. 60,000) was pyrolysed at 500° in a Pyrex tube under a stream of air or nitrogen (100 ml/min). The pyrolysis products were swept into a stainless-steel loop (1/8 in. O.D.) suspended in liquid nitrogen. One end of the loop was attached to a gas-scrubbing bottle containing Folin's reagent (10 ml), buffer (1 ml) and distilled water (50 ml) for the collection of EI<sup>6</sup>. A stream of nitrogen was passed in the other end and the loop slowly warmed to 70° to release the trapped EI. The scrubbing solution was extracted with chloroform and an aliquot of the extract subjected to HPLC.

### *Chromatographic procedure*

A 25- $\mu$ l aliquot of the chloroform extract was taken for standards or samples

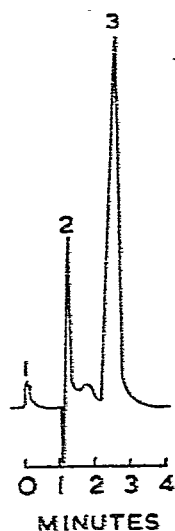


Fig. 1. Chromatogram of the chloroform extract of an aqueous EI solution treated with Folin's reagent. 1 = Injection peak; 2 = solvent peak; 3 = 4-(1-aziridiny)-1,2-naphthoquinone. Operating conditions: 10- $\mu$ m Micro Pak column at ambient temperature; flow-rate, 80 ml/h; pressure, 2500 p.s.i.; elution solvent, 1% isopropanol in dichloromethane.

that contained 0.1 to 1.0  $\mu$ g/ml of EI and injected on to the column. For samples containing 0.01–0.1  $\mu$ g/ml of EI a 100- $\mu$ l aliquot was injected. The column effluent was monitored with UV detector sensitivity between 0.02 and 0.16 absorbance units f.s.d. as necessary.

#### Thin-layer chromatography (TLC)

The chloroform extracts obtained from the reaction of Folin's reagent with various amines (Table II) were subjected to TLC on plastic sheets covered with a 0.25-mm layer of silica gel F<sub>254</sub> (E. Merck, Darmstadt, G.F.R.) using chloroform-methanol (10:1) as the mobile phase.

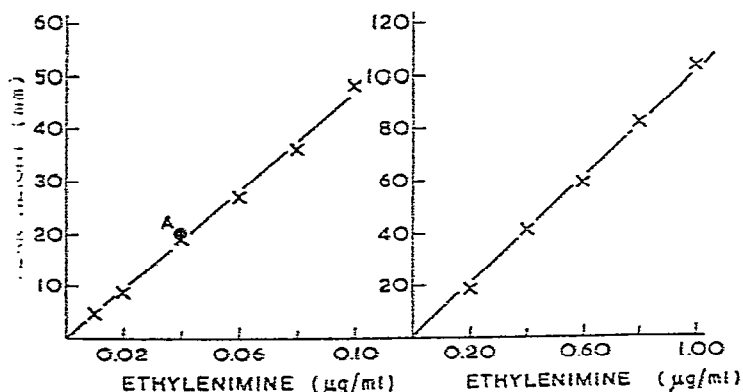


Fig. 2. Calibration graphs for aqueous solutions containing 0.01–0.1  $\mu$ g/ml (left) and 0.2–1.0  $\mu$ g/ml (right) of EI. Point A (left graph) was obtained with 0.04  $\mu$ g/ml EI solutions containing 0.4  $\mu$ g/ml of the following amines: (1) aniline; (2) diethylamine; (3) *n*-butylamine.

TABLE I

## EFFICIENCY OF CONVERSION AND RECOVERY OF EI AS 4-(1-AZIRIDINYL)-1,2-NAPHTHOQUINONE

Figures in brackets refer to the theoretical quantity of EI required to produce the given amount of 4-(1-aziridiny)-1,2-naphthoquinone.

4-(1-Aziridiny)- -1,2-naphthoquinone ( $\mu\text{g/ml}$ )	Peak height (mm)	EI ( $\mu\text{g/ml}$ )	Peak height (mm)	Recovery of EI (%)
0.23 (0.05)	31	0.05	31	100
0.46 (0.10)	61	0.10	60	98
0.69 (0.15)	101	0.16	100	93
0.93 (0.20)	134	0.21	126	90
1.16 (0.25)	173	0.26	164	91
1.40 (0.30)	188	0.31	188	97

## RESULTS AND DISCUSSION

HPLC of a chloroform extract of an aqueous solution of EI treated with Folin's reagent afforded the chromatogram illustrated in Fig. 1; an authentic sample of 4-(1-aziridiny)-1,2-naphthoquinone in chloroform produced an identical chromatogram. Fig. 2 shows the linear calibration graphs obtained for standard aqueous solutions containing 0.01–0.1 and 0.2–1.0  $\mu\text{g/ml}$  of EI. It is apparent that EI can be readily determined down to the 0.01  $\mu\text{g/ml}$  level and detected below the 0.005  $\mu\text{g/ml}$  level by this method. The recovery efficiency for EI was evaluated by subjecting equimolar solutions of 4-(1-aziridiny)-1,2-naphthoquinone to HPLC under identical conditions and comparing peak heights with those obtained for EI after derivatization with Folin's reagent. Table I shows that the recoveries of EI exceeded 90% in the concentration range 0.05–0.3  $\mu\text{g/ml}$ .

TABLE II

TLC  $R_f$  VALUES OF 4-SUBSTITUTED 1,2-NAPHTHOQUINONE DERIVATIVES OF VARIOUS AMINES

Thin layer: Silica gel F<sub>254</sub> (Merck) on plastic sheet. Solvent: Chloroform–methanol (10:1).

Amine	$R_f$
Ethylenimine	0.82
<i>n</i> -Butylamine	0.77
Diethylamine	0.65
Aniline	0.65
Dimethylamine	0.55
Isopropylamine	0.55
<i>n</i> -Propylamine	0.50
Ethylamine	0.43
Allylamine	0.43
Methylamine	0.36
Ethanolamine*	—
Ammonia*	—

\* Derivative not extracted into chloroform.

TABLE III

ESTIMATION OF EI RELEASED ON PYROLYSIS OF PEI: A COMPARISON OF METHODS FOR DETERMINING TRACE AMOUNTS OF EI

<i>Analytical procedure</i>	<i>Dry weight of PEI (g)</i>	<i>EI detected (<math>\mu</math>g)</i>	<i>EI released per 100 g of PEI (mg)</i>
4-(4-Nitrobenzyl)pyridine <sup>3</sup>	0.20*	0	—
Folin's reagent spectrophotometric <sup>4</sup>	0.20*	70	35
Folin's reagent HPLC	0.20**	3.3	1.65
	0.14*	2.8	2.0

\* Pyrolysis in atmosphere of air.

\*\* Pyrolysis in nitrogen atmosphere.

Several primary and secondary amines that react with Folin's reagent similarly to EI, and interfere in the spectrophotometric determination, were examined as sources of interference in this method. TLC of the 1,2-naphthoquinone derivatives of these amines provided the  $R_F$  values given in Table II. These data show that the aniline, diethylamine and *n*-butylamine derivatives are those most likely to interfere. Solutions containing 0.04  $\mu$ g/ml of EI and a 10-fold excess of the foregoing amines were subjected to the normal analytical procedure. The peak heights obtained for the EI derivative in these mixtures were equal (point A, Fig. 2), and remained essentially unchanged from that given by a solution containing only EI (0.04  $\mu$ g/ml). The amine derivatives were not visibly eluted from the column unless the polarity of the mobile phase was increased.

In Table III three methods are compared for determining the quantity of EI formed on pyrolysis of PEI. Clearly, the method utilizing 4-(4-nitrobenzyl)pyridine is insensitive to such low concentrations of EI and the spectrophotometric method with Folin's reagent gives high results due to interference from the various amines formed on pyrolysis of PEI. In contrast, the use of Folin's reagent with subsequent chromatographic analysis of the derivatized EI provides a high degree of specificity and sensitivity. With certain adjustments to the chromatographic procedure, this method could probably be extended to the specific determination of C-substituted aziridines and certain primary or secondary amines that react quantitatively with Folin's reagent.

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