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# Determination of 2-methylpyrazine and pyrazinamide in reaction mixtures by gas and high-performance liquid chromatography[a]

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ABSTRACT

Pyrazinamide is an antitubercular drug, commonly prepared by ammoxidation of 2-methylpyrazine (2-MP), which also may be prepared by several routes. Rapid, sensitive and selective analytical methods are essential for monitoring the reactions during process development. Methods based on gas chromatography were developed for monitoring the reactions during the preparation of 2-MP and a high-performance liquid chromatographic procedure was used to separate and determine the ammoxidation products of 2-MP. The methods were utilized successfully in analysing the reaction streams.

## INTRODUCTION

Pyrazinamide (PZA) is a drug used in the treatment of pulmonary tuberculosis[1,2] and is manufactured by several ways[3], including the hydrolysis of 2-cyanopyrazine (2-CP), obtained by ammoxidation of 2-methylpyrazine (2-MP). Pyrazine may be obtained as a byproduct during this ammoxidation process. The raw material, 2-MP, necessary for this ammoxidation is prepared by several routes. Pyrazine derivatives are useful not only in the preparation of pyrazinamide, but also in the manufacture of many other industrial products, *e.g.*, flavouring agents, pharmaceuticals and agricultural chemicals[4]. The reactions chosen for the preparation of 2-MP and PZA in the present studies are shown in Fig. 1.

PZA levels in biological fluids have previously been determined by spectrophotometric[5,6], gas chromatographic–mass spectrometric (GC–MS)[7] and high-performance liquid chromatographic (HPLC)[8–12] techniques. GC analysis of reaction mixtures obtained in the synthesis of 2-MP from EDA and PG (route I) and ammoxidation products of 2-MP (route IV) was reported by Forni *et al.*[13,14].

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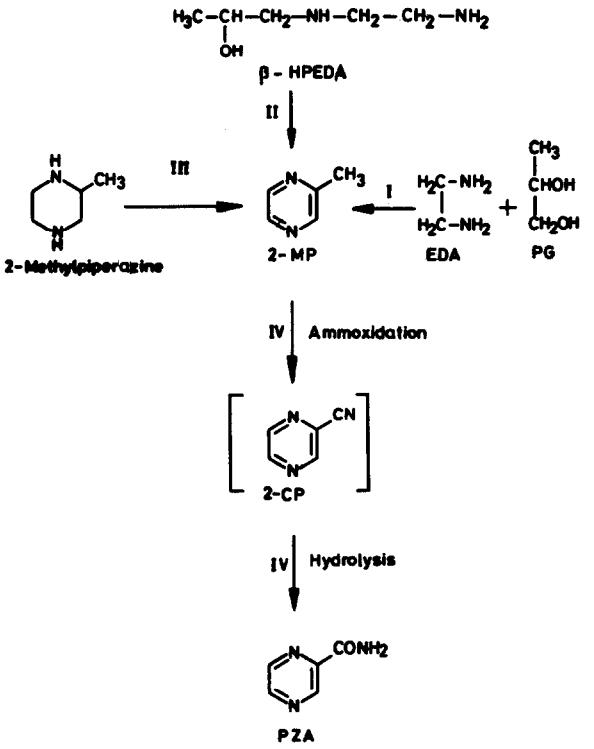


Fig. 1. Reactions for the preparation of 2-MP and PZA. β-HPEDA = β-hydroxypropylethylenediamine; PG = propylene glycol; EDA = ethylenediamine.

Attempts to standardize these methods in our laboratory are not successful. It was found that the stationary phase deteriorated quickly and that EDA was not eluted from the column.

In addition, no GC or HPLC methods are available for the analysis of reaction mixtures obtained in the preparation of 2-MP from β-hydroxypropylethylenediamine (β-HPEDA) (route II) and 2-methylpiperazine (route III). Therefore, there is an increasing need for rapid and selective methods for the determination of raw materials, intermediates and finished products in reaction streams during process development of 2-MP and PZA.

In this study, methods based on GC and HPLC were developed for the determination of 2-MP and PZA in reaction mixtures and their application to monitoring the reactions during process development is demonstrated.

## EXPERIMENTAL

### *Reagents*

2-MP and pyrazine were purchased from Aldrich (Milwaukee, WI, U.S.A.), PZA from IDPL (Hyderabad, India), 2-CP by Armour Chemicals (Bombay, India), EDA from Qualigens Fine Chemicals (Bombay, India) and PG from S.D. Fine

Chemicals (Bombay, India). All solvents used in HPLC except ethanol were obtained from Spectrochem (Bombay, India). Ethanol, 2-chloropyridine and 2,3-dichloropyridine (internal standards) were purchased from Fluka (Buchs, Switzerland). Chromosorb 101 was supplied by Sigma (St. Louis, MO, U.S.A.), other stationary phases employed in GC were purchased from Analabs (Norwalk, CT, U.S.A.) and stationary-phases used in HPLC from Waters Assoc. (Milford, MA, U.S.A.). All other reagents were of analytical-reagent grade.

Doubly distilled water from a glass apparatus was used throughout. All glassware was cleaned, rinsed with acetone and silanized before use.

*Instrumentation*

The GC system consisted of Perkin-Elmer Model 910 chromatograph equipped with a flame ionization detector in conjunction with a Perkin-Elmer 1-mV potentiometric strip-chart recorder. The following GC conditions were used for the analysis of reaction mixtures of 2-MP obtained from route I: injection temperature, 300°C; detector temperature, 300°C; column, 10% Carbowax 20M (12 ft. × 1/8 in.) coated on Chromosorb W AW (80–100 mesh); carrier gas, nitrogen at a flow-rate of 45 ml/min; column temperature, programmed from 80 to 120°C at 5°C/min. The column was saturated with aliquots of ammonia before the sample was analysed. The following GC conditions were used for the analysis of reaction mixtures of 2-MP from routes II and III: injection temperature, 230°C; detector temperature, 250°C; stationary phase, Chromosorb 101 (6 ft. × 1/8 in.); oven temperature, 250°C; carrier gas, nitrogen at a flow-rate of 40 ml/min.

The HPLC system consisted of a Waters Assoc. Model ALC/GPC/244 chromatograph with an isocratic solvent delivery system (Model 6000A) equipped with a U6K injector, a Model 440 absorbance fixed-wavelength detector (254 nm at 0.1 a.u.f.s.), combined with a Chromatopak EIA integrator (Shimadzu, Kyoto, Japan). Analyses of process streams of PZA (route IV) were performed on a normal-phase μPorasil (10 μm particle size) column (30 mm × 3.9 mm I.D.) with isooctane–ethanol–acetic acid (75:24:1, v/v/v) as mobile phase at a flow-rate of 1.5 ml/min. The mobile phase was freshly prepared and filtered through Millipore HF 0.5-μm filters and the solvent was degassed before use.

*Chromatographic analyses*

Standard mixtures with different concentrations of 2-MP, EDA and PG together with 2-chloropyridine (internal standard) were prepared and analysed by GC. Standard solutions with different concentrations of 2-MP were prepared in order to construct a calibration graph for the determination of 2-MP reaction products by routes II and III. Sample volumes of 1 μl were injected in each instance.

Standard mixtures with different concentrations of 2-MP, pyrazine, 2-CP and PZA together with 2,3-dichloropyridine (internal standard) were prepared and analysed by HPLC; 5 μl of the sample were injected.

## RESULTS AND DISCUSSION

*GC analysis of reaction mixtures of 2-MP (route I)*

The relative retention times of EDA, PG and 2-MP on Carbowax 20M

TABLE I

RELATIVE RETENTION TIMES (RRT) AND RESPONSE FACTORS OF EDA, PG AND 2-MP BY GC

| *Compound* | *RRT* | *Response factor* |
|---|---|---|
| EDA | 0.26 | 3.65 |
| PG | 0.41 | 2.17 |
| 2-MP | 0.57 | 1.26 |
| 2-Chloropyridine (internal standard) | 1.00 | 1.00 |

stationary phase are given in Table I. Stationary phases with different polarities such as Porapak Q, OV-17 and QF-1, and also Chromosorb 101[14], were tried for the separation of these compounds, but EDA was not quantitatively eluted and response factors calculated for PG and 2-MP were not reproducible. This problem was overcome by saturating the stationary phase with ammonia as the compounds under investigation are basic. It was observed that 5-$\mu$l aliquots of ammonia solution, injected six times, was sufficient for complete elution of all the compounds.

A typical chromatogram illustrating the separation of EDA, PG and 2-MP is shown in Fig. 2. Standard mixtures were prepared and analysed by GC. Response factors were calculated and presented in Table I. Results obtained in the analysis of standard mixtures are given in Table II, and are in close agreement with the true values. Reaction mixtures were analysed utilizing the developed method and the results are given in Table III.

*GC procedure for the analysis of reaction mixtures of 2-MP (route II)*

A procedure for monitoring the conversion of $\beta$-HPEDA to 2-MP by GC using

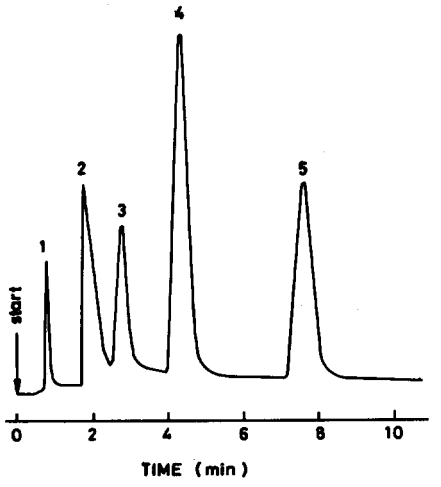


Fig. 2. Typical gas chromatogram showing the separation of the reaction mixture of 2-MP (route I). Peaks: 1 = solvent; 2 = EDA; 3 = PG; 4 = 2-MP; 5 = 2-chloropyridine (internal standard).

TABLE II

RESULTS OF ANALYSIS OF STANDARD MIXTURES OF EDA, PG AND 2-MP BY GC

Average of triplicate determinations.

| *Mixture No.* | *EDA* | | *PG* | | *2-MP* | |
|---|---|---|---|---|---|---|
| | *Taken (%)* | *Found (%)* | *Taken (%)* | *Found (%)* | *Taken (%)* | *Found (%)* |
| 1 | 25.31 | 25.46 | 24.89 | 24.70 | 49.80 | 49.33 |
| 2 | 21.43 | 21.27 | 22.86 | 22.72 | 55.71 | 55.89 |
| 3 | 16.58 | 16.40 | 20.08 | 20.19 | 63.34 | 63.12 |
| 4 | 34.92 | 35.61 | 32.23 | 32.05 | 32.85 | 32.64 |
| 5 | 10.47 | 10.41 | 13.74 | 13.91 | 75.79 | 76.50 |
| 6 | 5.58 | 5.65 | 8.28 | 8.38 | 86.14 | 86.09 |

TABLE III

RESULTS OF ANALYSIS OF REACTION MIXTURES OF 2-MP BY GC (ROUTE I)

Average values obtained from duplicate GC runs.

| *Mixture No.* | *EDA (%)* | *PG (%)* | *2-MP (%)* |
|---|---|---|---|
| 1 | 30.62 | 16.53 | 57.75 |
| 2 | 36.73 | 20.09 | 43.18 |
| 3 | 27.95 | 14.81 | 60.30 |
| 4 | 40.58 | 22.33 | 37.87 |
| 5 | 50.34 | 26.71 | 23.59 |

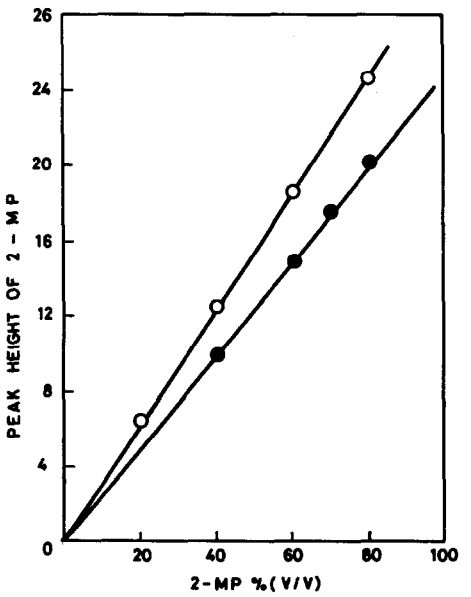


Fig. 3. Calibration graph for the determination of 2-MP in reaction mixtures obtained by routes II (○) and III (●).

TABLE IV

RESULTS OF ANALYSIS OF REACTION MIXTURES OF 2-MP BY GC (ROUTE II)

| *Mixture No.* | *2-MP formed (%)* | *Unconverted β-HPEDA (%)* | *Pyrazine (%)* |
|---|---|---|---|
| 1 | 53.50 | 35.00 | 9.50 |
| 2 | 57.00 | 40.00 | 2.50 |
| 3 | 73.50 | 20.00 | 6.50 |
| 4 | 72.00 | 22.00 | 4.00 |

the external standard method was devèloped. Mixtures with different concentrations of 2-MP and β-HPEDA were prepared and a calibration graph was constructed of peak height (ordinate) *versus* concentration of 2-MP (abscissa) (Fig. 3). Results of the analysis of reaction mixtures are given in Table IV.

*GC procedure for the analysis of reaction mixtures of 2-MP (route III)*

A similar method was developed for the analysis of reaction mixtures of 2-MP from route III. The calibration graph is shown in Fig. 3. Results of the analysis of reaction mixtures are given in Table V. The results obtained are in close agreement with the yields obtained after isolating the products.

*HPLC analysis of 2-MP reaction mixtures of pyrazinamide (route IV)*

Normal-phase HPLC with μPorasil as stationary phase and isooctane–ethanol–acetic acid (75:24:1, v/v/v) as mobile phase separated all the components, *i.e.*, 2-MP, 2-CP, PZA and pyrazine. A typical chromatogram is shown in Fig. 4. Relative retention times along with capacity factors are given in Table VI. Quantitative analysis was carried out by the internal standard method. Many compounds were tried as internal standards and 2,3-dichloropyridine was found to be most suitable. Response factors determined by well established procedures[15] are given in Table VI. It was found that the response factors for all the components are much less than 1, which indicates that the detector response is good.

The validity of the method was checked by analysing synthetic mixtures and the results are presented in Table VII. The results obtained are in good agreement with the true values.

TABLE V

RESULTS OF ANALYSIS OF REACTION MIXTURES OF 2-MP BY GC (ROUTE III)

| *Mixture No.* | *2-MP formed (%)* | *Unconverted 2-methylpiperazine (%)* | *Pyrazine (%)* |
|---|---|---|---|
| 1 | 64.00 | 35.00 | 2.00 |
| 2 | 57.50 | 40.50 | 2.50 |
| 3 | 80.50 | 17.00 | 2.50 |
| 4 | 85.50 | 12.50 | 2.00 |

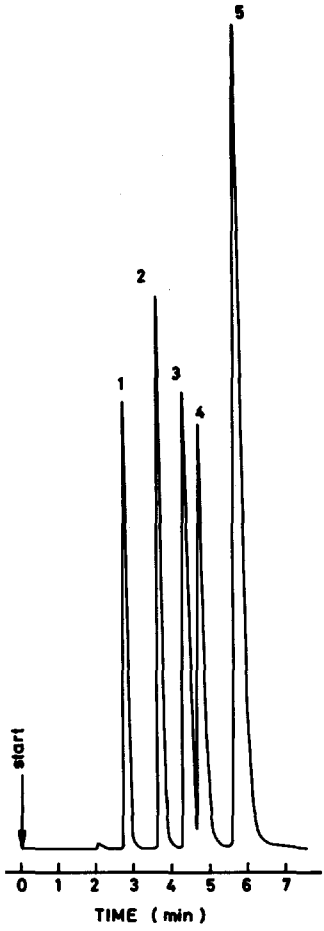


Fig. 4. Typical high-performance liquid chromatogram showing the separation of reaction mixtures of pyrazinamide (route IV). Peaks: 1 = 2,3-dichloropyridine (internal standard); 2 = 2-CP; 3 = 2-MP; 4 = pyrazine; 5 = PZA.

TABLE VI

RELATIVE RETENTION TIMES (RRT), RESPONSE FACTORS AND CAPACITY FACTORS OF THE CONSTITUENTS OF REACTION MIXTURES OF PYRAZINAMIDE BY HPLC

| *Compound* | *RRT* | *Response factor* | *Capacity factor (k')* |
|---|---|---|---|
| 2,3-Dichloropyridine (internal standard) | 1.00 | 1.00 | 0.37 |
| 2-CP | 1.88 | 0.25 | 0.82 |
| 2-MP | 2.22 | 0.26 | 1.15 |
| Pyrazine | 2.43 | 0.13 | 1.36 |
| PZA | 2.93 | 0.23 | 1.84 |

TABLE VII

RESULTS OF ANALYSIS OF SYNTHETIC MIXTURES OF PYRAZINAMIDE BY HPLC

Average of triplicate determinations.

| *Mixture No.* | *2-MP* | | | *2-CP* | | | *Pyrazine* | | | *PZA* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Taken (%)* | *Found (%)* | *Error (%)* | *Taken (%)* | *Found (%)* | *Error (%)* | *Taken (%)* | *Found (%)* | *Error (%)* | *Taken (%)* | *Found (%)* | *Error (%)* |
| 1 | 55.99 | 56.55 | 1.00 | 27.87 | 27.47 | 1.44 | 13.83 | 13.72 | 0.80 | 2.26 | 2.22 | 1.77 |
| 2 | 18.29 | 18.56 | 1.48 | 42.11 | 42.36 | 0.59 | 5.60 | 5.57 | 0.54 | 34.00 | 33.51 | 1.44 |
| 3 | 19.89 | 20.09 | 1.01 | 19.84 | 19.82 | 0.10 | 10.65 | 10.53 | 1.23 | 49.61 | 49.56 | 0.10 |
| 4 | 30.46 | 30.10 | 1.18 | 28.98 | 29.04 | 0.21 | 26.51 | 26.89 | 0.30 | 14.05 | 13.96 | 0.47 |
| 5 | 5.64 | 5.54 | 1.77 | 4.30 | 4.36 | 1.39 | 1.72 | 1.69 | 1.74 | 88.34 | 88.41 | 0.08 |

TABLE VIII

RESULTS OF ANALYSIS OF REACTION MIXTURES OF PYRAZINAMIDE BY HPLC

Average values obtained from duplicate GC runs.

| *Mixture No.* | *2-MP (%)* | *2-CP (%)* | *Pyrazine (%)* | *PZA (%)* |
|---|---|---|---|---|
| 1 | 97.23 | 1.96 | – | 0.82 |
| 2 | 85.85 | 0.70 | 11.90 | 0.01 |
| 3 | 47.78 | 28.73 | 22.43 | 1.08 |
| 4 | 42.51 | – | 24.16 | 33.32 |
| 5 | 44.45 | 52.11 | 0.96 | 2.46 |
| 6 | 41.87 | 54.67 | 0.77 | 2.66 |
| 7 | 47.71 | 47.69 | 3.15 | 1.43 |
| 8 | 37.26 | 2.70 | 8.51 | 51.54 |
| 9 | 34.78 | – | 10.47 | 54.75 |
| 10 | 40.54 | – | 4.23 | 55.22 |

Experiments on the ammoxidation of 2-MP were carried out under different conditions and with various catalysts. Typical samples of the reaction mixtures collected were analysed by the developed procedures and the results obtained are given in Table VIII. It can be seen that the amounts of PZA and 2-CP formed are different in each instance, depending on the experimental conditions.

## CONCLUSIONS

A rapid and accurate GC method was developed for the determination of 2-MP, EDA and PG in reaction mixtures, in which presaturation of the column with ammonia before sample analysis enhanced the accuracy. A GC method was established for determining 2-MP in the conversion of $\beta$-HPEDA to 2-MP. Reaction mixtures obtained during the dehydrogenation of methylpiperazine were also monitored by GC under similar conditions.

In addition, an HPLC procedure for the determination of ammoxidation

products of 2-MP was developed. The time required for complete elution of the compounds is 10 min whereas the reported GC method requires nearly 20 min. Further, in the reported GC procedure, the stationary phase material deteriorates after a few months of use, whereas such a drawback is not observed in the developed HPLC method.

The methods developed are applicable to the assay of pyrazinamide and for monitoring the reactions during its process development. Further, the procedures are useful in the assay of the components of reaction mixtures in the process development of 2-methylpyrazine, which is the starting material for the manufacture of pyrazinamide.

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