

**HIGH-PERFORMANCE LIQUID  
CHROMATOGRAPHIC DETERMINATION  
OF ISONIAZID, PYRAZINAMIDE,  
AND INDOMETHACIN  
IN PHARMACEUTICAL PREPARATIONS**

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**SUMMARY**

Isoniazid (INH) has been quantitated after precolumn derivatization with 5-methylfuran-2-carboxaldehyde (MFA). Chromatography was performed on a 150 mm × 4.6 mm i.d. YMC-ODS column with water–methanol–tetrahydrofuran, 59:39:2 (v/v) as a mobile phase at a flow rate of 2 mL min<sup>-1</sup>. Detection was performed at 328 nm. By adoption of this procedure pyrazinamide (PZA) and indomethacin (IM) separated completely and could be determined together with INH. Linear calibration plots were obtained between 1.4 and 5.5 µg mL<sup>-1</sup> for INH, between 6.2 and 30.8 µg mL<sup>-1</sup> for PZA, and between 10.7 and 64.08 µg mL<sup>-1</sup> for IM. The method of analysis was used for quantitation of INH, PZA, and IM in pharmaceutical preparations with a coefficient of variation <1%.

**INTRODUCTION**

Isoniazid (INH) is a common drug used alone for treatment of tuberculosis and in combination with pyrazinamide (PZA), rifampicin, and ethambutol. Indomethacin is non-steroidal anti-inflammatory drug. A variety of analytical techniques, for example titrimetry [1,2], spectrophotometry [3–5], spectrofluorimetry [6], electroanalytical techniques [7-9], capillary electrophoresis [10], and gas [11,12] and liquid chromatography [13–20] have been used for the quantitation of INH in pharmaceutical preparations. Liquid chromatography with UV detection is performed either by measuring the natural absorbance of INH at 263 nm or by precolumn derivatization with a suitable reagent. The derivatizing reagents used for HPLC determination of INH are 4-hydroxybenzaldehyde, cinnamaldehyde, salicylaldehyde,

de, and fluorenaldehyde [21–25]. In the current work off line precolumn derivatization was carried out with 5-methylfuran-2-carboxaldehyde (MFA) before HPLC determination of INH individually and in the presence of PZA and IM.

## EXPERIMENTAL

Pure INH, PZA, and IM were obtained from Nabi Qasim Pharmaceutical, Karachi, Pakistan, from Pacific Pharmaceuticals, Lahore, Pakistan, and from Hakimsons Karachi, Pakistan, respectively. 5-Methylfuran-2-carboxaldehyde (MFA), methanol, and tetrahydrofuran (THF) were from E. Merck (Darmstadt Germany). Freshly prepared double-distilled water from an all-glass still was used for HPLC.

HPLC was performed with an Hitachi (Tokyo, Japan) 655A liquid chromatograph with variable wavelength UV monitor, Rheodyne 7125 injector, and Hitachi D-2500 Chromato integrator. Compounds were separated on a 150 mm × 4.6 mm i.d. YMC-ODS column (YMC, Japan) with methanol–water–tetrahydrofuran, 59:39:2 (v/v), as mobile phase at a flow rate of 2 mL min<sup>-1</sup>. UV detection was performed at 328 nm. Spectrophotometric studies were performed with an Hitachi 220 spectrophotometer. IR spectra of KBr discs were recorded with a Perkin–Elmer 1430 IR spectrophotometer.

### Preparation of the 5-Methylfuran-2-carboxaldehyde Derivative of Isoniazid (INH-MFA)

An equimolar solution (0.005 M, 0.068 g) of INH in ethanol–water (2:1; 15 mL) was added to a solution of MFA (0.005 M, 0.55 g) in ethanol–water (2:1, v/v; 10 mL) and hydrochloric acid (0.2 mL, 1 M). The mixture was heated under reflux for 15 min and then cooled. The precipitate obtained was isolated by filtration and recrystallized from ethanol (m.p. = 293°C). Calculated for C<sub>12</sub>H<sub>14</sub>O<sub>2</sub> (%), C = 60.21, H = 5.05, N = 16.20; found (%) C = 60.21, H = 5.02, N = 16.34.

### Spectrophotometric Determination of Isoniazid

MFA (0.5% w/v in ethanol, 2 mL) and potassium chloride–hydrochloric acid buffer (pH 2.5, 2 mL) were added to a solution of INH (0.822–4.11 µg, 1–5 mL). The contents were heated on a water bath at 70–80°C for 10 min and the volume was adjusted to 10 mL with ethanol. The

absorption spectrum was recorded against reagent blank in the range 450–250 nm.

### **HPLC Determination**

MFA (0.5% w/v in ethanol, 2 mL) and potassium chloride–hydrochloric acid buffer (pH 2.5, 2 mL) were added to a solution of INH (1.37–5.48 µg, 1–5 mL) or to a solution containing a mixture of INH (1.37–5.48 µg), PZA (6.2–21 µg), and 1M (10.68–64.08 µg). The contents were heated on a water bath (70–80°C) for 10 min and the volume was adjusted to 10 mL with ethanol. This solution (5 µL) was analysed by HPLC.

### **Analysis of Isoniazid and Pyrazinamide in Pharmaceutical Preparations**

Ten tablets of each of Isoniazid BP (Ferozons Laboratories, Standard Pharmaceutical, Noshera, Pakistan), Myrin or Myrin-P (Lederle Laboratories Division Cynamid, Karachi, Pakistan) were powdered and 21.183 mg from Isoniazid BP tablets, 60.030 mg from Myrin tablets, or 92.229 mg from Myrin-P tablets were dissolved separately by warming with ethanol (3 × 20 mL) at 70°C. The solutions were combined and filtered and the volume was adjusted to 100 mL with ethanol. The solution obtained (5 µL) was analysed by HPLC. For analysis of Myrin or Myrin-P, 10 min after elution the column was washed with methanol for 5 min and the column was then equilibrated with mobile phase for 5 min before the next injection.

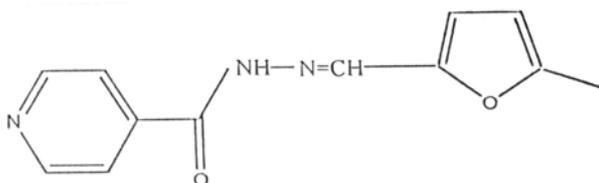
### **Analysis of Indomethacin in Capsules**

Five indomethacin-containing capsules of each of the pharmaceutical preparations Indocin 25 mg (Siza International, Gulburg, Lahore, Pakistan) and Indobid 25 mg (Adamjee Pharmaceuticals, Karachi, Pakistan) were well powdered. The powders (5.324 and 5.235 mg, respectively) were dissolved by warming with ethanol (3 × 20 mL) at 70°C. The solutions were combined and filtered and the volume was adjusted to 100 mL. This solution of IM (5 µL) was analysed by HPLC. The amount of IM in the capsules was evaluated from an external calibration plot.

## **RESULTS AND DISCUSSION**

INH reacted with MFA in 1:1 molar ratio in slightly acidic media to form the derivative INH-MFA. The results of elemental microanalysis

agreed with the expected values. The IR spectrum in KBr indicated bands at 3330 and 3155  $\text{cm}^{-1}$  for  $\nu\text{NH}$ , 1650  $\text{cm}^{-1}$  for  $\nu\text{C=O}$ , 1600  $\text{cm}^{-1}$  for  $\nu\text{C=N}$ , and 1585, 1548, and 1530  $\text{cm}^{-1}$  for  $\text{C=C}$ , as could be expected from the structure (Fig. 1). Spectrophotometric studies revealed the presence of a band

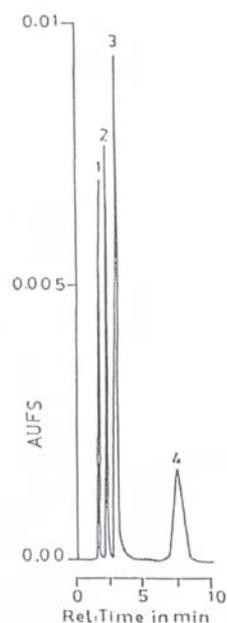


**Fig. 1**

Structural diagram of 1NH-MFA

from INH-MFA at 328 nm with a molar absorptivity of 18500  $\text{L mol}^{-1} \text{cm}^{-1}$  compared with  $\epsilon = 4432$  at 264 nm for INH. Therefore MFA was examined for precolumn derivatization of INH, followed by HPLC determination with UV detection. A YMC-ODS reversed-phase HPLC column was used and it was observed that INH-MFA eluted separately from excess derivatizing reagent MFA. Elution could be achieved isocratically with methanol–water, but better peak shapes were observed when THF was included in the mobile phase. The optimum separation was obtained by use of methanol–water–THF, 39:59:2 ( $v/v$ ), at a flow rate of 2  $\text{mL min}^{-1}$ . UV detection was performed at 328 nm. Under these optimized conditions PZA and IM were also separated completely with resolution factor  $>1.5$  (Fig. 2).

The effect of pH, reagent MFA concentration, and heating time on the formation of INH-MFA were examined. The pH was varied between 1 and 10, the amount of reagent added was varied between 1 and 3 mL (0.5%  $w/v$ ) for each determination, and heating time at 70°C was varied between 5 and 20 min. A constant volume (5  $\mu\text{L}$ ) was injected and average peak height was measured ( $n = 3$ ). These conditions, which resulted in maximum response, were regarded as optimum. It was observed that response to INH-MFA decreased above pH 3 and the response was constant for pH below 2.5. The stability of solutions of PZA and IM at pH 2.5 was examined and no change in the response to PZA or IM was observed after storage of the solutions for 24 h. Use of 1–3 mL reagent MFA resulted in a similar response so 2 mL (0.5%  $v/v$ ) was selected. The same response was also observed after warming for 5 min, but a warming time of 10 min was selected.



**Fig. 2**

HPLC separation of (1) PZ, (2) INH-MFA, (3) MFA, (4) IM. Elution from YMC-ODS column (1.50 mm  $\times$  4.6 mm id) with methanol–water–tetrahydrofuran, 59:39:2 (*v/v*) as mobile phase at a flow rate of 2 mL min<sup>-1</sup> with UV detection at 328 nm

Under these optimized conditions linear calibration plots for simultaneous determination of INH, PZA, and IM were obtained by measuring average peak height ( $n = 3$ ) between 1.36 and 5.48  $\mu\text{g mL}^{-1}$  for INH, between 6.2 and 30.8  $\mu\text{g mL}^{-1}$  for PZA, and between 10.68 and 64.0  $\mu\text{g mL}^{-1}$  for IM. These ranges corresponded to 6.8–27.4 ng per injection (5  $\mu\text{L}$ ) for INH, 31–154 ng for PZA, and 53–320 ng for IM; the correlation coefficient ( $r$ ) for  $n = 5$ -point calibrations were 0.998, 0.9997, and 0.9980 respectively. The detection limits, measured as three times the background noise, were 2.6, 12.4, and 21.4 ng mL<sup>-1</sup> for INH, PZA, and IM, respectively.

The common additives lactose, sorbitol, methylparabin, and propylparabium when added at twice the concentration of INH (2.74  $\mu\text{g mL}^{-1}$ ) did not affect the determination. The method was used for determination of INH in Isoniazid BP and Myrin tablets and for determination of INH and PZA in Myrin-P tablets. The results are summarized in Table I. Myrin contained ethambutol, which did not absorb at the wavelength (328 nm) selected for the determination. Rifampicin is present in the Myrin and My-

rin-P tablets, but it did not elute with the mobile phase used and did not effect the determination. To obtain reproducible results, however, the column was washed with methanol before each injection. Indocin and indobid capsules were analyzed for indomethacin content and the results obtained (Table I) were in reasonable agreement with the expected values with relative deviations of 4% and 4.8%.

**Table I**

HPLC analysis of isoniazid, pyrazinamide, and indomethacin in a pharmaceutical preparation

Name of preparation	Compounds present	Label claim (mg)	Amount found mg (CV%)	Relative deviation (%)
Isoniazid BP	Isoniazid	100	97.0(0.52)	3.5
Myrin	Isoniazid	75	73(0.57)	2.6
	Ethambutol	300	–	–
	Rifampicin	150	–	–
Myrin-P	Isoniazid	60	57.3(0.62)	4.5
	Pyrazinamide	300	292(0.6)	2.7
	Rifampicin	120	–	–
Indocin	Indomethacin	25	24(0.58)	4.0
Indobid	Indomethacin	25	23.8(0.74)	4.8

## CONCLUSION

A simple HPLC method is proposed for determination INH, PZA, and IM in pharmaceutical preparations. Pre-column derivatization with MFA is used for INH. Isocratic elution from a reversed-phase ODS column results in ng per injection detection limits. Analysis of the drugs resulted in CV of 0.54–0.74% with relative deviation up to 4.8%.

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