ABSTRACT
Isoniazid (IS) individually or in the presence of pyrazinamide and rifampicin has been determined by high performance liquid chromatography (HPLC) after derivatization with 6-methyl-2-pyridinecarboxaldehyde. Pyrazinamide and rifampicin separate completely from isoniazid derivative and are determined simultaneously. The chromatography is carried out from YMC-ODS column with elution with methanol: water: isopropanol: acetonitrile: sodium acetate (1 mM) 51:42:3:2:2 v/v/v/v/v) with flow rate 1.7 ml/min and UV detection at 333 nm. The method is applied for the analysis of Isoniazid B.P Rambuzid and Myrene-p tablets.

INTRODUCTION
Isoniazide is one of the common drug used for the treatment of tuberculosis individually or in combination with pyrazinamide, rifampicin and ethambutol. Isoniazide in pharmaceutical preparations could be determined by titrimetry (Sarwar et al., 1989; Muralikrishna et al., 1986), spectrophotometry (Issopopoulos, Economou, 1992; Ahmed et al., 1992; Mahfouz, Emara, 1993) spectrofluorimetry (Ioannu, 1988), electroanalytical techniques (Sulaiman & Hameed 1988), gas and liquid chromatography (Matsui et al., 1983; Karlaganis et al., 1987; LoDico et al., 1992; Stewart et al., 1995). The liquid chromatography with UV detection is either carried out by measuring its natural absorbance at 263 nm or by precolumn derivatization with a suitable reagent. The use of precolumn derivatization generally enhances the sensitivity and improves the detection limit of isoniazid. The derivatizing reagents for HPLC determination of isoniazid are 4-hydroxy-benzaldehyde (Gupta and Law, 1988) cinnamaldehyde (Difilippi et al., 1994-95), salicylaldehyde (Walubo et al., 1991 and Kaur & Sangal, 1996) have reported synthesis and antibacterial activity of 6-methylpyridine-2-carboxaldehyde-isonicotinoyl hydrazone complexes. Recently MPA has been examined for the spectrophotometric determination of isoniazid (Khuhawar et. al. in press) in the present work off line precolumn derivatization of isoniazid is carried out with 6-methyl-2-pyridinecarboxaldehyde (MPA) for HPLC determination of isoniazid individually and in the presence of pyrazinamide and rifampicin.

EXPERIMENTAL
Pure isoniazid (Nabi Qasim Pharmaceuticals, Karachi) Pyrazinamide (Pacific Pharmaceuticals Ltd. Lahore, Pak) rifampicin (Abbott Lab. Pak. Karachi) and 6-methyl-2-pyridinecarboxaldehyde (MPA) (Aldrich) were used. Hitachi 655A liquid Chromatography with variable wavelength U.V.
detector, Rheodyne 7125 injection and Hitachi D-2500 chromatointegrator were used. YMC ODS
column 050 x 4.6 mm id) (YMC Co. Ltd., Japan) was used. Elemental microanalysis was carried
out from Elemental micro Analysis Ltd. Devon, U.K.

Isoniazid-6-methyl-2-pyridinecarboxaldehyde was prepared by heating together (15 min)
equimolar (.005M) amounts of isoniazid and (MPA) in ethanol in the presence of few drops of
acetic acid. The precipitate obtained was filtered and recrystallised from ethanol m.p =146°C
Calculated for C13H12N4O expected % C=76.66, H=4.82, N=13.41; found %C=76.44, H=4.46
N=13.15.

A) HPLC Determination

Solution (1-5ml) containing isoniazid (0-680 ug) or a mixture containing isoniazid (0-680
ug), pyrazinamide (0123 ug) and rifampicin (0-822 mg) in ethanol was added MPA (1 ml, 1.2%
w/v in ethanol) and acetic acid (0.2ml). The contents were heated on water bath for 10 min and
volume was adjusted to 10 ml with ethanol. The solution (5 ul) was injected on YMC-ODC
column (150 X 4.6 MM id) and elution was carried out with methanol: water: isopropanol:
acetonitrile: sodium acetate (1 mM) 51:42:3:2:2: v/v/v/v/v/ with a flow rate 1.7 ml/min. The
detection UV was at 333 nm

B) Analysis of isoniazid, Pyrazinamide and Rifampicin in Pharmaceutical Preparation

Tablet Isoniazid B.P (Feroz Sons laboratories, Standard Pharmaceutical Ltd. Noshera, Pak)
(0.212 g), tablet Rambuzid (Abbot Lab. Karachi Pak) (0.7046g) or tablet Myren P (Lederle labo-
ratories Cynamid. Pak Karachi) (0.923 g) was crushed and ground to fine powder. A sample of
21.18 mg from isoniazid B.P. was dissolved in water, 70.46 mg from Rambuzid tablet or 92.29 mg
from Myrene-P was dissolved in ethanol. Each of the solution was warmed on water bath. The so-
lution was filtered before adjusting the final volume to 100 ml. Solution (5 ml) was taken and was
processed as A. The amount of isoniazid, pyrazinamide and rifampicin were evaluated from the
calibration curves constructed from the known amounts of each compounds.

RESULTS AND DISCUSSION

Isoniazid reacts with MPA in acidic solution to form its derivative (Fig. 1) with bathochro-
matic shift from 264 to 333 nm. with considerable increase in the molar absorptivity from 4432 to
25420 1 mole^-1 cm^-1

It was therefore considered to examine MPA for precolumn derivatization, followed by HPLC
determination of isoniazid. Reverse phase YMC-ODS column was examined. It was observed that
MPA and Is-MPA eluted with a binary mixture of methanol and water but an optimal separation
was observed with methanol: water: isopropanol: acetonitrile: sodium acetate (1 mM)
(51:42:3:2:2:v/v/v/v/v/ with a flow rate of 1.7 ml/min with UV detection at 333 nm. At the condi-
tions the elution and the separation of pyrazinamide and rifampicin were examined. Complete
separation was obtained with resolution factor (Rs) between two adjacent peaks > 2.8 (Fig.2).

The effect of pH on the formation of Is-MPA was examined within 1-10. Different buffer so-
Lutions were added to cover the pH range at unit interval and analytical procedure was followed. A constant volume (5ul) was injected and average peak height was measured (n=3). The pH which gave maximum response was considered optimal. The maximum response was obtained at pH=1.

The effect of concentration of isoniazid, pyrazinamide and rifampicin on the average peak height was examined and linear calibration curves were obtained with 6.8-41.1 isoniazid, 6.2-30.8 ug/ml pyrazinamide and 16.4-82.3 ug/ml rifampicin corresponding to 34-205.5 ng 31.0-154.0 ng and 82-411 ng/injection (5 ul) with coefficient of correlation (r) 0.9984, 0.9977 and .09990 respectively. The detection limits measured as three times the background noise were observed 13.6 ng/ml, 12.4 ng/ml and 32.8 ng/ml for isoniazid, pyrazinamide and rifampicin respectively corresponding to 68 pg, 62 pg and 164 pg/injection (5 ul) respectively. The method was applied for the determination of isoniazid, pyrazinamide and rifampicin in tablets Isoniazide B.P, Rambuzid and Myrene P. The results obtained (table 1) indicates coefficient of variation within 0.42-1.7% and indicates close correlation to the expected values reported by the manufacturers.

Table-1
HPLC Analysis of Isoniazid, pyrazinamide and Rifampicin in Pharmaceutical Preparation

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of Tablet</th>
<th>Compounds Present</th>
<th>Amount of each reported by the Mfg.</th>
<th>Amount of each drug found by the HPLC (C.V. %)</th>
<th>% Relative Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Myrene-P</td>
<td>Isoniazid</td>
<td>60</td>
<td>59.33 (1.7)</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pyrazinamide</td>
<td>300</td>
<td>287.91 (0.41)</td>
<td>4.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rifampicin</td>
<td>120</td>
<td>119.70 (0.770)</td>
<td>0.25</td>
</tr>
<tr>
<td>2.</td>
<td>Rambuzid</td>
<td>Isoniazid</td>
<td>75</td>
<td>74.36 (0.99)</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethambutol</td>
<td>300</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rifampicin</td>
<td>150</td>
<td>149.63 (0.87)</td>
<td>0.25</td>
</tr>
<tr>
<td>3.</td>
<td>Isoniazid B.P.</td>
<td>Isoniazid</td>
<td>100</td>
<td>97.24 (1.24)</td>
<td>2.91</td>
</tr>
</tbody>
</table>

Fig.1: Structural diagram of isoniazid derivative.
Fig. 2: HPLC Separation of (1) MPA (2) Pyrazinamide (3) IS-MPA (4) Rifampicin from column YMC ODS (150 x 4.6 mm id) elution with methanol: water: isopropanol: acetonitrile: sodium acetate (1 M) (51:42:2:2 v/v/v/v/v) with a flow rate of 1.7 ml/min. Detection UV at nm.

Fig. 3: Calibration curve of isoniazid as a derivative with MPA conditions as Fig. 2.
REFERENCES


