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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF ISONIAZID, PYRAZINAMIDE AND RIFAMPICIN IN PHARMACEUTICAL PREPARATIONS

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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

Isoniazed is one of the common ding used for the treatment of tuberculosis individually or in combination with pyrazinamide, rifampicin and ethambutol. Isoniazid 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) spectroflourimetry (loannu, 1988), electroanalaytical techniques (Sulaiman & Hameed 1988), gas and liquid chromatography (Matsui et al., 1983; Karlaganis et al., 1987; LoDico et al., 1992; Stewarte et al., 1995). The liquid chromatography with UV detection is either canned 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 (Abbot Lab. Pak. Karachi) and 6-methyl-2-pyridinecarboxaldehyde (MPA) (Aldrich) were used. Hitachi 655A liquid Chromatography with variable wavelength U.V.

detector, Rheodyne 7125 injector 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 healing 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 $C_{13}H_{12}N_4O$ expected % C=76.66, H=4.82, N=13.41; found %C=76.44, H=4.46 N=13.15.

A) HPLC Determination

Solution (1-5m1) 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 m./min. The detection UV was at 333 nm

B) Analysis of isoniazid, Pyrazinamide and Rif ampicin 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 Myrene P (Lederle laboratories 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 solution 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 bathochromatic 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 conditions the elution and the separation of pyrazinamide and rifampicin were examined. Complete separation was obtained with resolution factor (Rs) between two adjecant 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

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

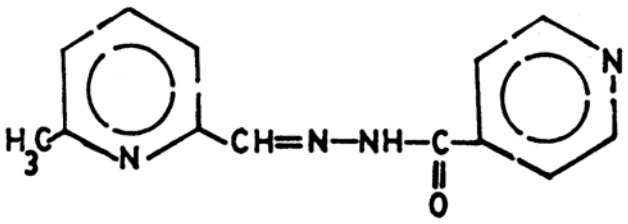

Fig.1: Structural diagram of isoniazid derivative.

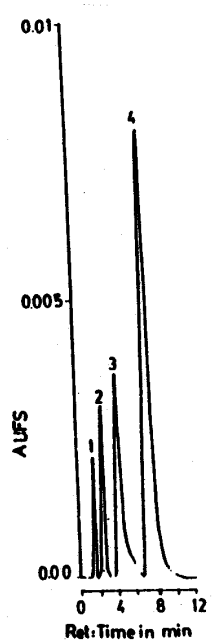

Fig.2: HPLC Separation of (1)MPA (2) Pyrazinamide (3) IS-MPA (4) rifampicin from column YMC ODS (150 x 4.6 mm id) elution with methanol: water: isopropanol: acetonitrile: sodium acetate (1m M) (51:42:3:2:2 v/v/v/v/v/ with a flow rate of 1.7 ml/min. Detection UV at nm

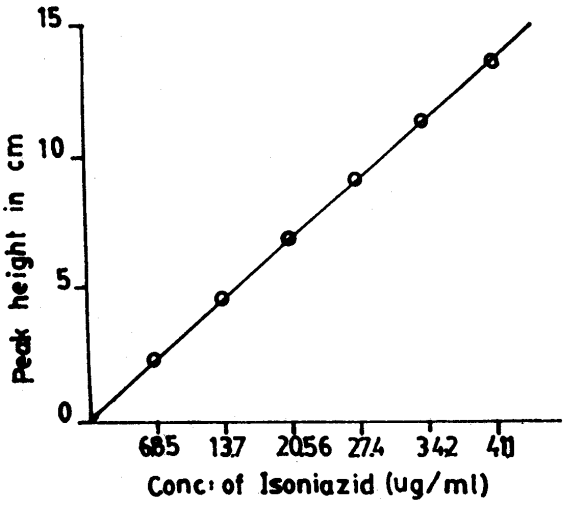

Fig.3: Calibration curve of isoniazid as a derivative with MPA conditions as Fig.2

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