Spectrophotometric Determination of Isoniazid using 6-Methyl-2-Pyridine Carboxaldehyde as a Derivatizing Reagent

M.Y. KHUHAWAR, F.M.A. RIND AND K.F. ALMANI
Institute of Chemistry, University of Sind, Jamshoro, Pakistan

(Received 12th January, 1998, revised 19th September, 1998)

Summary: 6-Methyl-2-pyridinecarboxyldehyde (MPA) reacts with isoniazid to form hydrazone derivative. The concentration of MPA, pH and warming time for the determination of isoniazid were optimized. The absorbance was measured at 328 nm against reagent blank. Beer's law was obeyed in the range of 1.4-6.8 μg/mL isoniazid. The method was used for the determination of isoniazid contents in pharmaceutical preparations individually and in the presence of ethambutol and rifampicin. The results were obtained with coefficient of variation (C.V) within 0.7-3.8%.

Introduction

The isoniazid is an important and essential drug for the treatment of tuberculosis. It is generally present in pharmaceutical preparations in tablet form individually or in combination with ethambutol, rifampicin and pyrazinamide. A number of analytical methods have been proposed for the determination of isoniazid based on titrimetry [1-3], spectrophotometry [4-6], spectrofluorometry [7,8], thin layer chromatography [9,10], high performance liquid chromatography [11-15] gas chromatography [16-19] and electroanalytical techniques [20-22]. For spectrophotometry a number of derivatizing reagents have been used including chloranil [23], sodium nitroprusside [24], neotetrazolium chloride, tetrazolium violet chloride [25], 2,3,5-triphenyltetrazolium chloride [26], 2-nitroindan-1,3-dione [27], ethyl-8-quinolyloxylacetate [28], tetrazolium blue chloride [29], 2,6-dimethoxy-1,4-benzoquinone [6] 1-fluoro-2,4-dinitrobenzene [30], 4-chloro-5,7-dinitrobenzofuran [31], 6,7-dichloroquinoline-5,8-dione [32], 4-nitrobenzaldehyde [33], pyridoxal [33] and 4-dimethylaminobenzaldehyde [34]. In the present work MPA has been used for the spectrophotometric determination of isoniazid.

Results and Discussion

Isoniazid easily condensed with MPA to form hydrazone derivative (6-methyl-2-pyridincarboxaldehyde-isonicotinoyl-hydrazone) (Fig. 1). It absorbs at 328 nm (λ_{max}) with considerable enhancement in the value of molar absorptivity of isoniazid (20000 $L.mole^{-1}cm^{-1}$) as compared to (ε = 4400 $L.mol^{-1}$ cm^{-1} at 263 nm) for isoniazid without derivatization (Table-1). MPA was examined as a derivatizing reagent for the spectrophotometric determination of isoniazid. The effects of pH, concentration of MPA and heating time on the formation of isoniazid MPA derivative were studied. The pH was varied from 1 to 10. It was observed that derivartization occured in acidic medium and potassium chloride hydrochloric acid buffer pH 1 proved optimal. The reagent MPA concentration was varied between 1-5 mL of 0.025% with an interval of 1 mL and addition of 3 mL

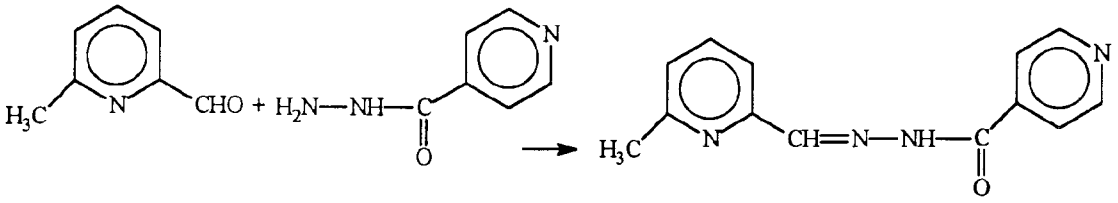

Fig. 1: Structural formula of chemical reaction.

Table-1: Spectrophotometric data

Compound	Max (ε = 1 mole^{-1} cm^{-1}) nm
Isoniazid	263(4400), 214 (5000)
6-methyl-2-Pyridine Carboxaldehyde	271 (10300), 265 (10130), 239 (11200), 208 (9500)
6-Methyl-2-Pyridine-2-Carboxaldehyde isonicotinoylhydrazone	328 (20000)

proved satisfactory. The heating time at 95°C was varied within 0-30 min. with an interval of 5 min. It was observed that a similar absorbance was observed after heating for 5 min. and heating time of 10 min. was considered as optimal. Using the conditions the effect of variation in the concentration of isoniazid was examined. A linear calibration curve was obtained which obeyed the Beer's law within the aqueous concentration range 1.4-6.8 μg/ml of isoniazid with coefficient of correlation (r) of 0.999 (Fig. 2). The validity of the calibration curve was obtained by the analysis of test solution of isoniazid and percent relative error was found within ± 1-7%.

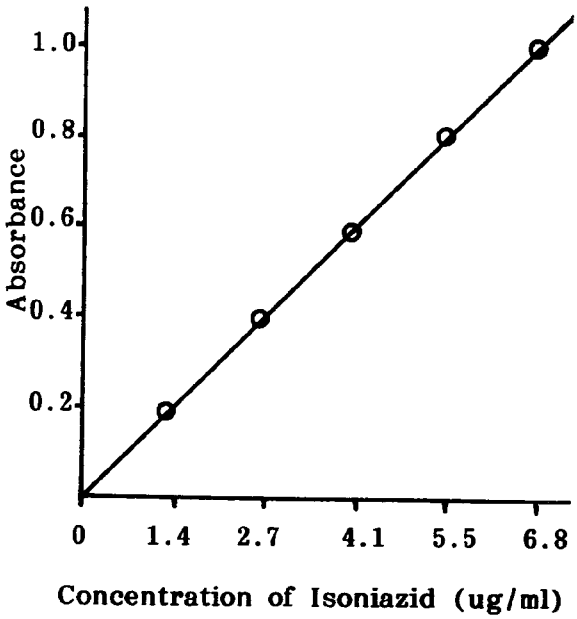

Fig. 2: Spectrophotometric calibration plot of isoniazid.

Isoniazid is present in the pharmaceutical preparations individually and in combination with ethambutol, rifampicin and pyrazinamide. The effect of their presence on the spectrophotometric determination of isoniazid was therefore examined. It was observed that ethambutol did not affect the determination when present twice the concentration of isoniazid. Rifampicin also absorbs at 328 nm and affects the determination. However at pH 1 rifampicin from aqueous ethanolic solution transferred to chloroform to facilitate the determination of isoniazid in aqueous ethanolic phase.Rifampicin in the chloroform could be determined after measuring the absorbance at 475 nm. The effect of pyrazinamide was also examined, but some enhancement in the absorbance of isoniazid was observed when present in twice the concentration of isoniazid. However, it did not affect at the same concentration of isoniazid (1.37 μg/ml).

The tablets isoniazid B.P. and Mayambutol INH were analysed for the contents of isoniazid and tablet Rifinah 450 and Rimactazid 450 were analysed for isoniazid and rifampicin. The results in the table-2 indicate the C.V within 0.2-3.8%. The observed values for the analysis of isoniazid and rifampicin were campared with expected values indicated by the manufacturer and relative deviations were obtained within +2.44-9.1%.

Experimental

A) Spectrophotometric determination of isoniazid

Freshly prepared aqueous solution (1-3 mL) containing (14-68 μg) isoniazid was added MPA (3 mL, 0.025% w/v in ethanol) and potassium chloride hydrochloric acid buffer pH 1 (2 ml). The contents were heated on water bath for 10 min. and volume

Table-2: Analysis of isoniazid and rifampicin in pharmaceutical preparations

1	2	3	4	5	6	7	8
1.	Rimactazid 450	981.15	Isoniazid	300	285.61 (1.39)	–	4.99
2.	Isoniazid B.P.	211.83	Rifampicin	450	95.74 (3.79)	430.12	4.42
			Isonized	100			4.26
3.	Mayambutol-INH	603.01	Isoniazid	100	102.44 (3.88)	–	2.44
			Ethambutol	300	–	–	–
4.	Rifinah 450	1666.12	Isoniazid	300	272.70 (0.19)	–	9.10
			Rifampicin	450	–	429.21 (1.37)	4.62

2. Name of tablets
3. Weight of tablet (mg.)
4. Compounds present
5. Amount of each component reported by the manufactures (mg/tablet)
6. Amount of isoniazid found (mg/table) (C.V %)
7. Amount of Rifampicin found (mg/Tablet) (C.V. %)
8. % relative deviation.

was adjusted to 10 mL with water. The absorbance was measured at 328 nm against a reagent blank.

B. Determination of isoniazid in tablets

Six tablets each isoniazid B.P. (Ferozsons Laboratories, Standard Pharmaceutical Ltd. Nowshera, (Pak)) or Maymabutol 1NH (Leaderle Laboratories, Cynamid (Pak) Ltd. Karachi) were thoroughly ground and the sample (0.1 g) was dissolved in water. The solution was filtered and volume was adjusted to 100 ml. The solution (1 mL) was further diluted to 10 mL and solution (1-3 mL) was transferred to 10 mL volumetric flask and procedure was followed as described in above A.

C. Determination of isoniazid in the presence of Rifampicin

Four tablets each Rifinah 450 (Pacific Pharmaceutical Ltd. Lahore, Pak) or Rimactazid 450 (Ciba Giegy (Pak) Ltd. Karachi) were thoroughly ground and the sample (0.1 g) was dissolved in ethanol with 8-10 mL portion each 5 times on water bath. The solution was filtered and final volume was adjusted to 100 mL. The solution (1 mL) was further diluted to 10 ml with ethanol. The solution (1-2 mL) was transferred to separating funnel and was added water (2 mL), potassium chloride hydrochloric acid buffer pH 1 (2 mL) and chloroform (3-4 mL). The contents were mixed well and the layers were allowed to separate. The organic layer was collected and extraction was repeated with chloroform (2-3 mL). The volume of organic layer was adjusted to 10 mL with chloroform and absorbance was measured at 475 nm for the determination of rifampicin. The aqueous layer was transferred to volumetric flask (10 mL) and was added MPA (3 mL, 0.025% w/v in ethanol). The contents were heated on water bath for 10 min. and remaining procedure was followed as described in A. The amounts of isoniazid and rifampicin in tablets were calculated from the external calibration curve prepared from standard isoniazid and rifampicin solutions (n=5).

MPA (Aldrich), isoniazid (Nabi Qasim Pharmaceuticals, Karachi), rifampicin (Abbott Lab. Pak. Karachi) and chloroform (E. Merck) were used.

Buffer solutions between pH 1-10 at unit interval were prepared from hydrochloric acid (1M), potassium chloride (1M), acetic acid (1M), sodium acetate (1M), sodium bicarbonate (1M), sodium carbonate (saturated), ammonium chloride (1M) and ammonia solution (1M).

Spectrophotometric studies were carried out on Hitachi 220-Spectrophotometer with 1 cm path length silica cells pH measurements were made with Orion 420 A pH meter with glass electrode and combined reference electrode.

Conclusion

A spectrophotometric method has been examined for the determination of isoniazid in pharmaceutical preparations based on derivatization

with MPA at pH 1 in aqeous ethanolic solution. Ethambutol did not affect the determination. Rifampicin was separated by extraction in chloroform before derivatization. Rifampicin in chloroform could also be determined quantitatively.

References

1. U. Muralikrishna, C. Rambabu, K.S. Babu, *Indian J. Pharm. Sci.*, **48**, 138 (1986).
2. K.K. Verma, S. Palod, *Anal. lett.*, **18**, 18 (1985).
3. A.G. Butterfield, E.G. Lovering and R.W. Sears, *J. Pharm.Sci.*, **69**, 222 (1980).
4. S.C. Sharma, S. Das, S.K. Talwar, *J. Assoc. Off. Anal. Chem.*, 70, 679 (1987).
5. B.P. Issopoulos, *J. Pharm.*, 70, 201 (1991).
6. N.M.A. Mahfouz, K.M. Emara, *Talanta*, **40**, 1023 (1993).
7. J. Manes, M.J. Gimeno, J.C. Malto, G. Font *J. Pharm.Biomed. Anal.*, **6**, 1023 (1988).
8. P.C. Ioannou, *Clin. Chim. Acta*, **175**, 175 (1988).
9. K. Kitamura, M. Halta, S. Fukuyama, K. Hozumi, *Anal. Chim. Acta*, **201**, 357 (1987).
10. G.P.R. Carr and B.J. Fish, *Anal. Proc.* **20**, 181 (1983).
11. M. Kim, J.T. Stewart, *Microchim. Acta*, **3**, 221 (1990).
12. A. Walubo, K. Chan, C.L. Wong, *J. Chromatogr. Biomed. Appl.* **105** (*J. Chromatogr.*, 567), 261 (1991).
13. H.I. Seifart, P.B. Kruger, D.P. Parkin, P.P. Van Jaarsveld, P.R. Donald, *J. Chromatogr. Biomed. Appl.* **130** (J. Chromatogr., 619), 285 (1993).
14. A. Walubo, P. Smith, P.I. Folb, *J. Chromatogr., B. Biomed. Appl.*, **658**, 391 (1994).
15. A. Dcfilippi, G. Piancone, R. Costa laia, G.P. Tibaldi, *Chromatographia*, 40, 170 (1995).
16. G. Karlaganis, E. Peretti, B.H. Lauterburg. *J. Chromatogr. Biomed. Appl.*, **64** (J. chromatogr., 420), 171 (1987).
17. Y. Wang, Y. Xin, *Yaowu Fenxi Zazhi*, **8**, 181 (1988).
18. C.P. LoDiico, B.S. Levine, B.A. Golberger, Y.H. Caplan, *J. Anal. taxicol.*, **16**, 57 (1992).
19. M.F. Stewart, A.J. Freemont, T. Richardson, *Clin. Biochem.*, **32**, 229 (1995).
20. G.V. Prokhorova, E.A. Osipova, A.V. Barabanova, *Zh. Anal. Khim.*, **45**, 2246 (1990).
21. J.C. Apostolakis, C.A. Georgiou, M.A. Koupparis, *Analyst*, **116**, 233 (1991).
22. N.H. Li, Z.Zhang, Z.Q. Zhang, *Yaowu Fenxi Zazhi*, **14**, 43 (1994).
23. S.T. Sulaiman, D. Amin, *Microchem. J.*, **28**, 328 (1983).
24. V.P. Kalashnikov, A.F. Mynka, *Farm. Zh. (Kiev)* (4), 45 (1987).
25. P.B. Issopoulos, P.T. Economou, *Analusis*, **20**, 31 (1992).
26. P.B. Issopoulos, *Acta Pharm. Jugosl*, **41**, 123 (1991).
27. V.V. Petrenko, B.P. Zorya, I.V. Pastukhova, *J. Rotbergs, Latv. PSR Zinat. Akad. Vestics. Kim Ser*: (6), 726 (1987).
28. A.H.N. Ahmed, S.M. El-Gizawy, H.I. El-subbagh, *Anal. lett*, **25**, 73 (1992).
29. P.B. Issopoulos, *Acta, Pharm. Hung.*, **61**, 198 (1991).
30. A. Csiba, *Acta, Pharm. Hung.*, **59**, 205 (1989).
31. M.I. Yevegenyev, I.I. Yevgenyeua, N.G. Nikolayeva, N.A. Moskva, F.S. Levinson, B.I. Demchenko, *Kim. Farm. Zh.*, **25**, 80 (1991).
32. M.E. El-Kommos, A.S. Yanni, *Analyst*, **113**, 1091 (1988).
33. P.R. Shah, R.R. Raje, *J. Pharm. Sci.*, **66**, 29 (1977).
34. N.F. Poole, A.E. Mayer, *Proc. Exp. Biol. Med.*, **98**, 375 (1958).