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Capillary Gas Chromatographic Determination of Isoniazid in Pharmaceutical Preparation by Pre-column Derivatization with Acetylacetone

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Antituberculosis drug isoniazid and toxic substance hydrazine have been determined by capillary column gas chromatography after precolumn derivatization with acetylacetone. Phenyl hydrazine when present together with isoniazid and hydrazine also separated completely from the column HP-5 (30 m × 0.32 mm I.D) connected with flame ionization detection. Phenyl hydrazine was used as an internal standard. The elution was carried out at initial column temperature 120 ºC with heating rate 50 ºC/min up to 280 ºC, with nitrogen flow rate of 1.5 mL/min and split ratio was 20:1. The linear calibration range for isoniazid was observed 6.2-100 µg/mL with detection limit of 3.1 µg/mL corresponding to 150 pg reaching to the detector. Similarly, the linear calibration range for hydrazine was 7.1-57 µg/mL with detection limits of 2.3 µg/mL. The method was applied for the determination of isoniazid and hydrazine from pharmaceutical preparations and relative deviation of isoniazid from labelled values was within 1.0 to 1.4 %. The amount of recovery of isoniazid from pharmaceutical preparations was 97 % with RSD 3 %.

Key Words: Isoniazid, Hydrazine, Acetylacetone, Capillary gas chromatography.

INTRODUCTION

Isoniazid (INH) (isonicotinoyl hydrazine) is the most potent and selective tuberculostatic antibacterial agent in the therapy of tuberculosis[1]. It inhibits the growth of *tubercle bacillus in vitro* in concentration[2] less than 1 µg/mL. It is also employed as a propylactic agent for use in persons constantly exposed to tubercular patients. The INH gains access to all organs and all body fluids including cerebrospinal, renders the drug of special value in treating tuberculosis meningitis and other extra pulmonary forms of the disease. When used alone, it is at least equal to streptomycin in the therapy of tuberculosis. It is believed to affect lipids, nucleic acids, glycolysis or

mycolic acid biosynthesis[3]. The hydrazine (HZ) is a toxic substance and may be present in pharmaceutical preparations of INH as one of the decomposition products[4].

Various analytical techniques such as titrimetry[5,6], spectrophotometry[7-9], spectrofluorimetry[10], atomic absorption[11], chemiluminescence[12], electroanalytical techniques[13-16], kinetic determination[17], flow injection[17,19], thin layer chromatography[20-22], capillary electrophoresis[23,24], liquid chromatography (LC)[25-30] and LC-Mass spectrometry[31,32] have been used for quantification of INH in pharmaceutical preparations and biological samples. The LC with UV detection is either carried out by measuring the natural absorbance of isoniazid at 263 nm or by precolumn derivatization with a suitable derivatizing reagent[33-37]. The determination limits for INH has been reported[38] within 0.5-8.0 μg/mL. The gas chromatography (GC) of INH was carried out after derivatization with trifluoroacetic anhydride or bis(trimethylsilyl)trifluoroacetamide and quantification was by mass spectrometry[39]. The use of acid anhydrides affects the performance of GC column. Acetylacetone (AA) is inexpensive chemical and could react with primary amino group to form stable Schiff base with increase in carbon number of derivative and increase in FID sensitivity. Acetylacetone has been used for HPLC determination of dopamine[40].

Present work examines capillary GC for the determination of isoniazid and hydrazine after derivatization with acetylacetone. The volatile derivatives are easy to elute and separate from capillary GC column with required sensitivity for the analysis of pharmaceutical preparations.

EXPERIMENTAL

All the chemicals used were reagent of pharmaceutical grade. Freshly prepared double distilled water was used throughout the study. Pure isoniazid (INH) was obtained from Nabi Qasim Pharmaceuticals, Karachi, Pakistan. Hydrazine (HZ) (24 %), phenyl hydrazine (PHZ), acetylacetone (AA) and ethanol were purchased from E. Merck, Darmstadt, Germany. The percentage of HZ in HZ solution was determined by the titrimetry. Hydrochloric acid (37 %), potassium chloride, acetic acid, sodium acetate, ammonium acetate, sodium bicarbonate, sodium carbonate, ammonium chloride were also obtained from E. Merck Germany. Buffer solutions in the pH range 1-10 at unit interval were prepared from hydrochloric acid (0.1 M) and potassium chloride (1 M) (pH 1 & 2); acetic acid (1 M) and sodium acetate (1 M) (pH 3 to 6); ammonium acetate (1 M) (pH 7), sodium bicarbonate (1 M) and sodium carbonate (saturated) (pH 8 & 9), ammonium chloride (1 M) and ammonia (1 M) (pH 10).

Fresh solution of INH was prepared by dissolving 10.95 mg INH in ethanol:water (1:1 v/v) and final volume was adjusted to 10 mL. Acetyl-

acetone (1 % v/v) was prepared in 100 mL of ethanol:water (1:1 v/v). The spectrophotometric studies were carried out on double beam Hitachi 220 (Hitachi (Pvt) Ltd. Tokyo, Japan) spectrophotometer with dual 1 cm cells. Gas chromatographic studies were carried out on Agilent model 6890 Network GC system (Agilent Technologies Inc. USA) with split/splitless injector operated in split mode coupled with flame ionization detection (FID), hydrogen generator (Parker Balston, Analytical Gas system H2-90, Parker Hannifin, Havorhill, MA, USA) and pure nitrogen (British Oxygen Company, Karachi). The computer with Chemstation software controlled the gas chromatograph and HP LaserJet 1300 printer was used throughout the study. Capillary column HP-5 (30 m × 0.32 mm I.D) with film thickness of 0.25 μm (J & W scientific GC columns, USA) was used throughout the study. The Orion model 420A pH meter (Orion Research Inc. Boston, USA) with glass electrode with combined reference electrode was used for pH measurements.

Gas chromatographic determination: The solution (1 mL) containing INH (2-40 μg), HZ (2-22 μg) and PHZ (10.9 μg) was added 1 mL of potassium chloride hydrochloric acid buffer pH 2, 1 mL (1 % v/v) AA and heated at 75 ºC for 15 min. The contents were cooled at room temperature and was added 1 mL of chloroform. The contents were mixed well and layers were allowed to separate. Exactly 0.5 mL of chloroform was pipetted out and transferred to screw capped vial. The solvent was evaporated and re-dissolved in 0.2 mL of ethanol. The solution (1 μL) was injected on capillary GC column HP-5 (30 m × 0.32 mm id) with layer thickness 0.25 μm at column temperature 120 ºC with programmed heating rate 50 ºC/min up to 280 ºC with total run time 6.2 min. The nitrogen flow rate was maintained at 1.5 mL/min. The injection port and detector temperatures were fixed at 200 ºC and 300 ºC, respectively. Hydrogen flow rate and nitrogen as make up gas flow rate were fixed at 40 and 45 mL/min, respectively for FID detection.

Determination of isoniazid in pharmaceutical preparations: Ten tablets of each Rematcal & Remister (Novartis Pharma (Pak.) Ltd.) and Myrin P (Leaderle Laboratories Division, Cyanamid Pak. Ltd. Karachi) were powdered and 53.89 mg Rematcal, 55.90 mg Remistar; and 56.12 mg Marin P were dissolved in three portions of each (6 mL) in ethanol:water (1:1). The solution was filtered and final volume was adjusted to 25 mL with ethanol:water (1:1 v/v). The solution (1 mL) was transferred to screw-capped vial and 1 mL of chloroform was added. The content were mixed well and the layers were allowed to separate. The aqueous layer was collected followed by the addition of PHZ (10.09 μg). The GC determination as described earlier was then followed.

Ten tablets of isoniazid (Unexo Lab. (Pvt) Ltd. Lahore) were thoroughly ground and a powder (50 mg) was dissolved in ethanol:water (1:1 v/v)

and solution was filtered. The final volume was adjusted to 100 mL and 1 mL was further diluted to 25 mL. Solution (1 mL) was taken and the GC determination was followed.

Well-mixed Isoniazid syrup (Nabi Qasim Industry Pvt. Ltd. Korangi, Karachi) (5 mL) was dissolved in ethanol:water (1:1 v/v), filtered and volume was adjusted to 100 mL. Solution (1 mL) was again diluted to 25 mL and solution (1 mL) was added PHZ (10.9 μg) and processed as GC determination. The amounts of INH in pharmaceutical preparations were evaluated from external calibration curve and from the ratio of peaks of added internal standard.

Percentage recovery of isoniazid from INH tablets by spiked sample: Isoniazid tablets were processed as determination of isoniazid in pharmaceutical preparations and two portion of solution (1.0 mL each) were taken. A portion was processed as GC determination and the other portion was added INH (25 μg) and again processed as GC determination. Linear calibration was used to measure an increase in the response with added INH and % recovery of INH from isoniazid tablets solution was calculated.

Determination of hydrazine from isoniazid formulations: Well-mixed INH syrup (Nabi Qasim Industry Ltd. Korangi, Karachi) 2 mL containing (10 mg/mL) INH was processed as GC determination. Ten tablets of isoniazid (INH) (Unexo Lab.Ltd, Lahore) were thoroughly ground and a powder (1.2 g, containing 654.6 mg INH) was dissolved in ethanol: water (1:1 v/v) and the solution was filtered and adjusted to 25 mL. The solution 2 mL was taken and PHZ (10.9 μg) was added and the GC determination was followed. The signals corresponding to HZ and PHZ were recorded.

RESULTS AND DISCUSSION

Isoniazid (INH) condenses easily with acetylacetone (AA) to form acetylacetone-isonicotinyl hydrazone. For the selective and sensitive determination of INH and hydrazine (HZ) capillary column gas chromatography was examined. Precolumn derivatization was carried out with AA and elution was examined from the capillary column HP-5 (30 m × 0.32 mm) with layer thickness 0.25 μm at an initial column temperature 120 ºC with heating rate 50 ºC/min up to 280 ºC/ min. The run time was 6.2 min. Nitrogen flow rate was 1.5 mL/min. The detection was performed by FID. The derivatives of HZ and INH gave a single peak with retention times of 2.4 and 4.30 min. The derivatives separated completely from the derivatizing reagent AA. The derivatization conditions were optimized for the quantitative determination of INH and HZ by measuring average peak height/peak area (n = 3). The effects of pH, the concentration of derivatizing reagent and reaction time at 70-80 ºC were examined. A solution 1 μL was injected with split ratio 20:1 and the condition which gave maximum response, was considered optimum.

The pH varied between 1-10 at unit interval and it was observed that derivatization occurred in acidic media (pH 1-3) and a decrease into the response was observed above pH 3. The optimal response was obtained at pH 2 (Fig. 1). The derivatizing reagent concentration varied between 1-3 mL (1 % v/v) at an internal of 0.5 mL. The average peak height/peak area (n = 3) was plotted against the amount of reagent solution added and a similar response was obtained at the amount of 1 mL and above, thus 1 mL (1 % v/v) was used. Heating time varied between 5 to 25 min at 75 ºC and same average peak height (n = 3) was obtained at selected heating time of 10 to 20 min and 15 min.

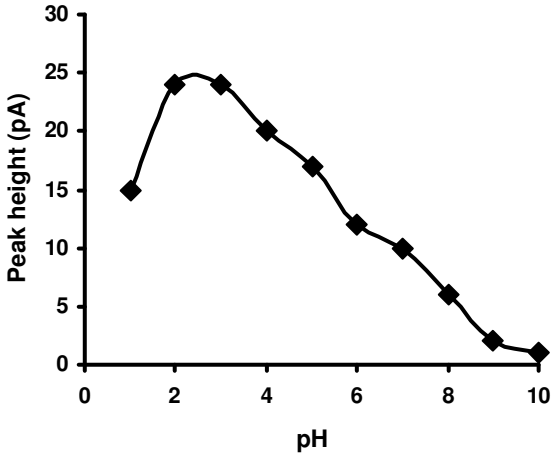

Fig. 1. Effect of pH on the GC elution of AA-INH derivative. GC conditions: The column HP-5 (30 m × 0.32 mm) with film thickness 0.25 μm at an initial column temperature 120 ºC with heating rate 50 ºC/min up to 280 ºC/min. The run time was 7 min. Nitrogen flow rate was 1.5 mL/min and split ratio 20:1. Injection port and detector temperatures were 200 and 300 ºC. Nitrogen make up flow rate was 45 mL/min. FID air and hydrogen flow rate were 450 and 40 mL, respectively

Using the conditions the hydrazino compound PHZ was also examined. The compounds formed derivative with AA, eluted separately (Fig. 2) and did not affect the determination of INH and HZ. PHZ was therefore used as internal standard. The linear calibration curves at the optimized conditions for the determinations of INH and HZ were obtained by measuring average peak height/peak area (n = 3) with 6.2-100 μg/mL and 7.1-57 μg/mL with coefficient of determination (r^2) 0.9904 and 0.9939, respectively. The detection limits measured as three time the background noise were obtained with INH 3.1 μg/mL and HZ 2.3 μg/mL corresponding to 3.1 ng and 2.3 ng/injection (1 μL) and 0.15 and 0.12 ng reaching up to FID detec-

tion. Common additives glucose, magnesium stearate, gum acacia, talcum, methylparabin, lactose and starch when added twice the concentration of INH (109 μg/mL) did not interfere. The method was applied for the determination of INH from pharmaceutical preparations *viz.*, Ramactal, Remister, Myrin P, isoniazid tablets and isoniazid syrup. The results are summarized in Table-1. The isoniazid tablets and isoniazid syrup were analyzed after dissolution of INH in ethanol:water (1:1 v/v). The tablets Remactal, Remister and Myrin P also contained rifampicin, pyrazinamide and ethambutal together with INH. INH after derivatization with AA was extracted in chloroform and pyrazinamide and ethambutol did not elute from GC column and did not interfere the determination of INH. Rifampicin is extracted in chloroform together with INH derivative, but did not elute as symmetrical peak and disturbs the base line due to on column decomposition. However rifampicin separated completely, when extracted in chloroform prior to derivatization of INH in aqueous-methanolic solution and did not affect the determination of INH. The % relative deviations (RD) were obtained 0.66 to 4.0 % from the values labeled by the manufacturer with relative standard deviation (RSD) within 1-3 % (Table-1).

TABLE-1
ANALYSIS OF ISONIAZID IN PHARMACEUTICAL PREPARATIONS USING ACETYLACETONE AS DERIVATIZING AGENT

Name of tablet	Compounds present	Amount of compd. reported (mg/tab)	Amount of isoniazid found in mg/tablet (RSD %)	Relative deviation (%)
Remactal	Isoniazid	150	149.0 (2)	0.6
	Rifampicin	300	–	
Remister	Isoniazid	75	72.0(1.4)	4.0
	Rifampicin	120	–	
	Pyrazinamide	400	–	
	Ethambutanol	275	–	
Myrin P	Isoniazid	75	73.0 (2)	2.0
	Rifampicin	150	–	
	Pyrazinamide	400	–	
	Ethambutanol	275	–	
Isoniazid syrup	Isoniazid	10 mg/mL	9.6 mg/mL (3)	4.0
Isoniazid tablet	Isoniazid	100	99.0 (1)	2.0

The % of INH recovered from pharmaceutical preparations by standard addition was calculated and the amount of recovery was 97 % with RSD 3 %. (Figs. 3 & 4). The presence of HZ was checked in isoniazid syrup and isoniazid tablets. Isoniazid syrup 2 mL containing 10 mg/mL of INH and isoniazid tablets 1.2 g containing 654.6 mg INH were analyzed for HZ.

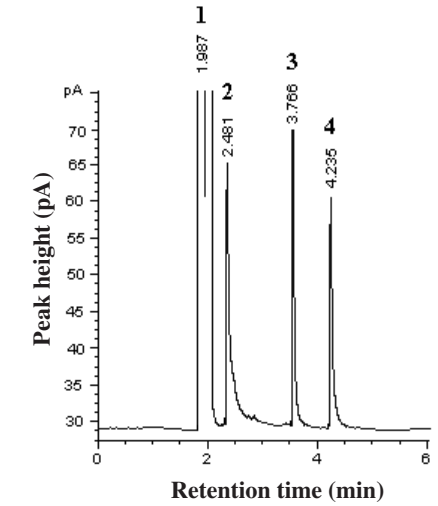

Fig. 3. Capillary GC elution of (1) solvent and AA (2) HZ (3) PHZ and (4) INH as derivative of AA, Condition same as mentioned in Fig. 2

PHZ was added as internal standard. The signals corresponding to HZ and PHZ were recorded. The results in Table-2 indicate the presence of 1.6 μg HZ/10 mg INH in Isoniazid syrup and 4.3 μg HZ/26.18 mg INH in isoniazid tablets. The amounts of HZ found were within the safe limits reported for pharmaceutical formulations[41].

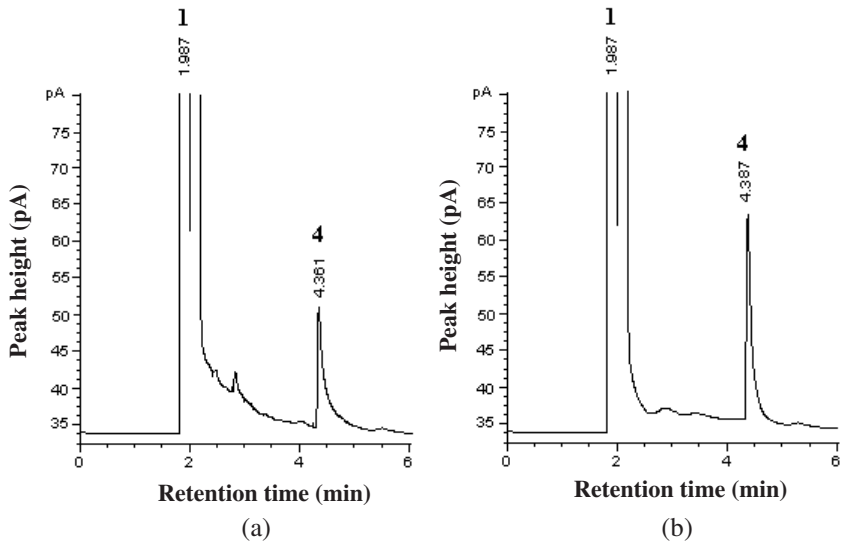

Fig. 3. GC response for measurement of INH as derivative of AA from isoniazid tablet (a) isoniazid tablet (b) after spiking with 25 μg of INH

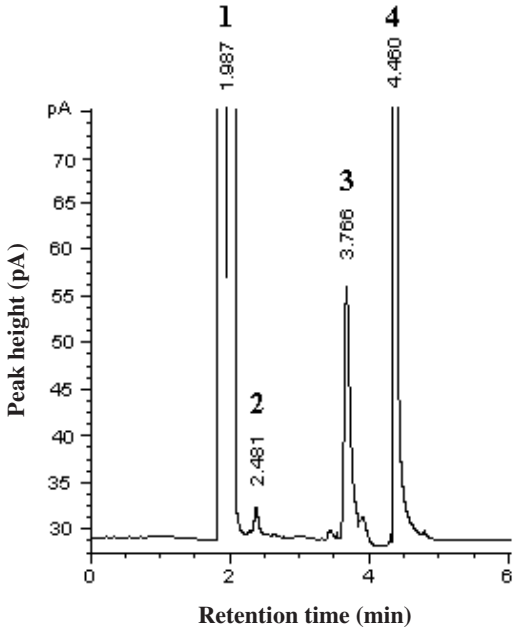

Fig. 4. GC response for measurement of HZ as derivative of AA from isoniazid syrup PHZ used as an internal standard. Conditions as in Fig. 2.

TABLE-2
DETERMINATION OF HYDRAZINE FROM PHARMACEUTICAL PREPARATIONS BY USING AA AS DERIVATIZING REAGENT

Sample	Isoniazid syrup	Isoniazid tablet
Amount of INH labelled in mg	1mL contains10mg of INH	100 mg INH/tablet
Amount of HZ found in µg of INH formulations (RSD %)	1.6 µg HZ/10 mg of INH (2.0)	4.3 µg HZ/26.18 mg of INH in tablets (1.0)

Conclusion

Simple GC procedure has been developed for the determination of INH from pharmaceutical preparation after precolumn derivatization with AA. HZ was also determined from INH formulations and PHZ was used an internal standard. The detection limits were obtained at sub-ng level reaching to the detector. The analysis of pharmaceutical preparation was obtained with RSD 1-3 %.

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