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A COMPARATIVE ANALYTICAL DETERMINATION OF ISONIAZID IN PURE AND PHARMACEUTICAL PREPARATIONS

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ABSTRACT : This investigation involves development of a new spectrophotometric and a reverse phase-high performance liquid chromatography (RP-HPLC) methods for the determination of isoniazid (INH) in pure and pharmaceutical preparation. Spectrophotometric method was based on the charge-transfer complex form coloured blue of the drug (INH) with $K_3Fe(CN)_6$ and $FeCl_3$ in normal medium with a maximum absorbance at 710 nm. A Linear calibration graph was in the range of 0.5-6 mg/L, with a correlation coefficient of 0.9991, detection limit of 0.033mg/L, the molar absorption coefficient is 12.534 x10^3 L/mol.cm, sandel sensitivity (S) 10.94 x10^{-3}µg/cm^2, relative standard deviation RSD% of 1.89% and relative error 1.3%. RP-HPLC method with a column C_{18} (150mm × 4.5mm, 5µm) and a mobile phase containing (75% ammonium acetate + 12.5% acetonitril + 12.5% methanol), PH = 3, at a flow rate of 1ml/min., 10 µL sample loop and UV detector was set at ë$_{max}$ 270 nm was applied. Calibration graph was in the range of 0.5-15 mg/l with a correlation coefficient of 0.9996 detection limit of 0.012 mg/land a relative standard deviation of 0.05% and the relative error 0.8%. The two methods were applied successfully to determine the content of (INH) in pharmaceutical preparation with recovery of98-110%.

Key words : Isoniazid, spectrophotometry, charge transfer complex, HPLC, dosage form.

INTRODUCTION

Isonizaid (INH) is a first-line antituberculosis drug. It has been widely used since 1952 alone in the prophylaxis, and also in combination with other antituberculars), Isoniiazid (pridine-4-carboxilic acid hydrazide or isonicotinic acidhydrazide) (Fig. 1) is a white, odourless, crystalline powder that is soluble in water (14%) and has a melting point of 171.4 c (Shabani *et al*, 2018).

The analysis of INH spectrophotometry (Swamy *et al*, 2014; Li and Yang, 2006), a number of derivating reagents have been used including Istatinl (Abbas and Homoda, 2003), Vanillin (Oga, 2010), Tiron-KIO_4 (Gowda *et al*, 2002), uranyl acetate (Kalia *et al*, 2006), Tiron-$NaIO_4$ (Gowda *et al*, 2005), 2-Hydroxy-1,4-naphoqunone (Rind *et al*, 2005), Ethyl vanilin (Kashyap *et al*, 2012), performance Liquid chromatography (Ayyappan *et al*, 2011; Gupta and Sood, 2005), gas chromchromatography (GC) (Khuhawar and Zardari, 2006, 2008), ion selective electrode-potentiometry (Pasakova *et al*, 2011), titrimetry (Nagendra *et al*, 2002), LC/LC-MS (Bhutani *et al*, 2007), Voltametry (Azad and Ganesan, 2012; Wahdan, 2005), amperometry (Chen *et al*, 2012), chemical uminescence spectrometry (Bowan *et al*, 2012) and capillary electrophoresis (Liu *et al*, 2011; Driouich *et al*, 2003). The present paper describe a spectrophotometric method for the determination of isoniazid in pharmaceuthcal proposed method was applied successfully for the determination of the analytical in pharmaceutical preparation and RP-HPLC methods were compared successfully with spectrophotometry and were applied for the analysis of pharmaceutical samples containing isoniazid.

Experimental

Instrumentation

Spectroscopic analysis was carried out using Jasco v-650 Japan double beam UV- Visible spectrophotometer with 10 mm path length quartz cells was used for the analytical purpose. The separation was achieved by using HPLC Shimadzu LC- 20A, Japan, Sensitive balance ± 0.0001 g, ultrasonic water bathe and pH meter.

Chemical

All chemicals used were of analytical –reagent grade.

Isoniazid as pure standard powders were supplied by Samara Drug Industries (SDI), Iraq. Accurately weighed 0.01 g pure samples of Isoniazid were transferred to 100 ml calibrated volumetric flask, dissolved and made up to the mark with water.

A. UV-Vis spectrophotometry method

Aliquots of 0.0005 M $K_3Fe(CN)_6$ solution (3.0ml) and 0.005M $FeCl_3$ solution (3ml) were transferred in to 25 ml volumetric flask. Accurate volume of the working solution of Isoniazid 0.5-2.0mL were add to the flasks respectively and the volumes were diluted with distilled water. The contents of transferred to the spectrophotometric cell at room temperature. The absorbances were recorded at 710 nm as function of time against reagent blank. In the second procedure, the absorbance was measured at affixed time of 50 min and was plotted againt the finil concentration (INH) and the cotent of the drug was calculated from the eitherthe calibration graph or regression equation.

A (1-1) Procedure for drug assay in pharmaceuticals tablets

Ten tablets each Isoniazid (manufactured by Svizera Labsmbia, India or kick Farma turkey was thoroughly grounded into a fine powder and the amount 0.0176 gm and 0.0109 gm corresponding to 100 mg INH were transferred to separate beakers and dissolved in water.The solutions were filtered and the volume was adjusted to 100 ml (Table 1).

RESULTS AND DISCUSSION

A (1-1) absorption spectra

It was found preliminary that the reaction of isoniazid with $K_3Fe(CN)_6$ and $FeCl_3$ in neutral media forming blue colored at ë$_{max}$(710 nm) (Fig. 2).

Table 1 : Optimum condition for the determination of isoniazid using the spectrophotometric

Parameters	**Value**
$K_3Fe(CN)_6$ (0.0005M)	3mL
$FeCl_3$(0.005M)	0.5mL
Order of addition	Drug+$FeCl_3$+$K_3Fe(CN)_6$
pH	Natural
Temperature	Room temp.
Fixed time	50 min.
λ_{max}	710nm

Table 2 : shows a summary of analytical data for the determination of isoniazid using spectrophotometric method.

Analytical data	**Value**
Linear range	0.5-6.0 mg/l
Correlation coefficient	0.9991
LOQ	0.1130 mg/l
Regression equation	Abs=0.0914x[C]+0.0441
RSD%	1.89%
Detection limit(LOD)	0.033 mg/L
Intercept	0.0441
Sandals sensitivity(s)	10.94x10^{-3} μg/cm^2
Molar absorptivity(£)	12.534x10^3 L/mol.cm

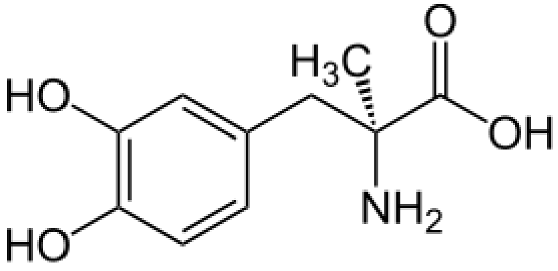

Fig. 1 : Chemical structure of isoniazid.

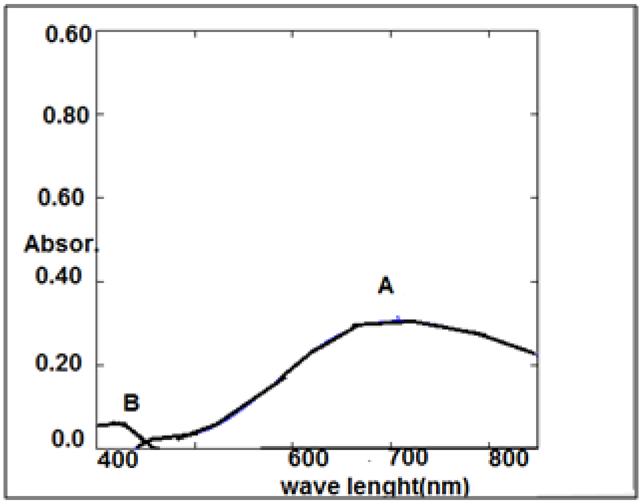

Fig. 2 A : The absorbance spectrum for Isoniazid after being complex with blank solution. **B:** The absorbance spectrum for Isoniazid before being the complex with $K_3Fe(CN)_6$ and $FeCL_3$.

A (1-2) Optimization of the experimental conditions

The influence of various reaction variables such as concentration of reactants,volume $K_3Fe(CN)_{6,}$volume $FeCl_{3,}$ media reaction, order of addition, time and temperature were investigated (Table 1).

A(1-3) -UV-Vis spectrophotometry calibration graph

To increasing volume (0.05-3.5) ml of 100 mg/l of standard INH solution, the following reagents have been added in the following order $FeCl_3$ 0.5 ml, $K_3Fe(CN)_6$ 3 ml has been finally added, the volume completed to 25 ml in a volumetric flask with distilled water. The absorbance has been measured at 710 nm against the blank. Table(2) shows a summary of analytical data for the determination of isoniazid using spectrophotometric method, while Fig. 3 shows the calibration curve.

A(1-4) Accuracyand precision of proposed methods

This study was carried out to assure the closeness of thetestresultsobtained by the analytical method to the true value. For study method (INH).were determined at three different selected concentrations within the Beer's law limited 1, 2, 4 mg/l for isoniazid.

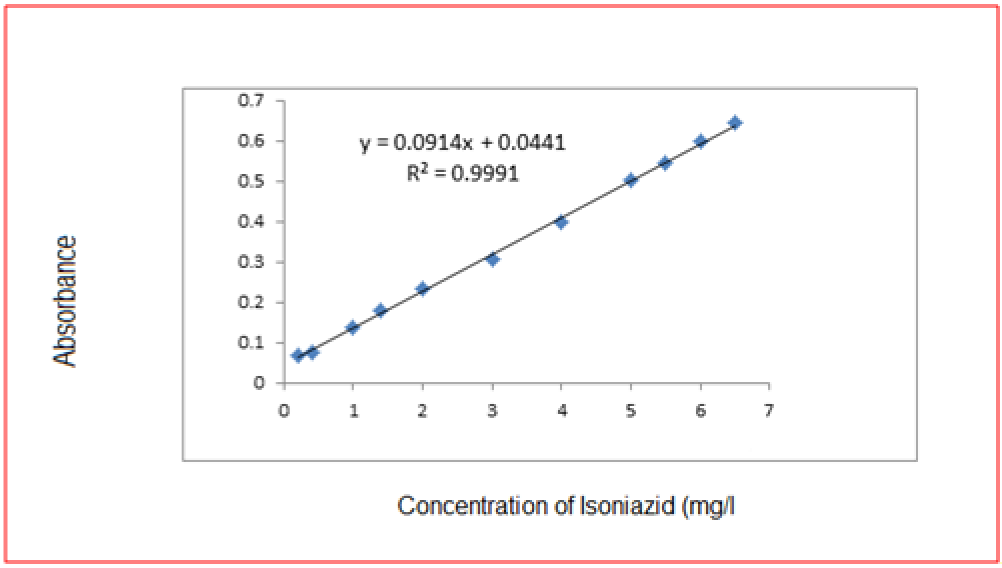

Fig. 3 : Calibration graph for the determination of isoniazid by the spectrophotometric method.

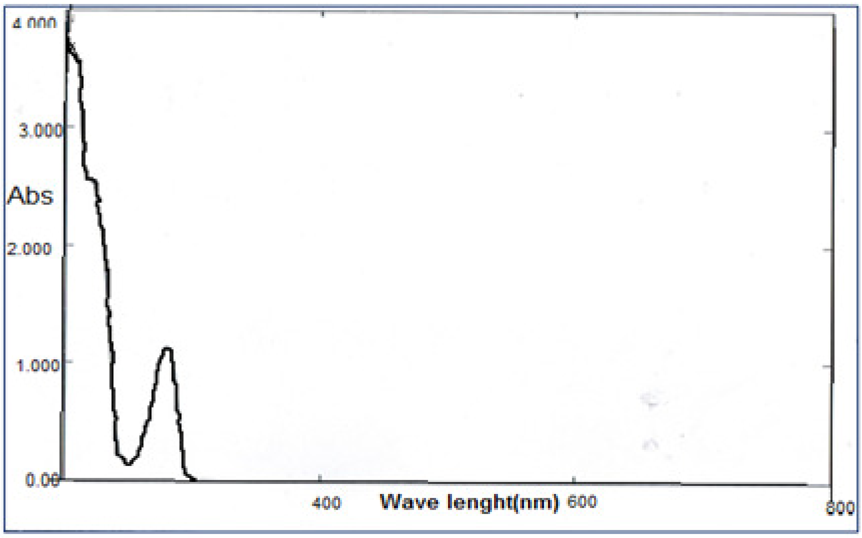

Fig. 4 : Selection of wavelength.

A (1-5) Procedure for drug assay in pharmaceuticals tablets

Ten tablets each Isoniazid (manufactured by Svizera Labs India or Farma Turkey) was thoroughly grounded to a fine powder and the amount 0.0176 gm and 0.0109 gm corresponding to 100 mg INH were transferred to separate beakers and dissolved in water.The solution were filtered and the volume were adjusted to 100 ml (Table 3).

B -HPLCmethod

B(1-1) Absorption spectra

The absorption spectrum of 100 mg/l of isoniazid prepared shown that the maximum absorbance of isoniazid at 270 nm, therefore, 270 nm has been used for UV-detection (Fig. 4).

B(1-2) Chromatographic condition

Separation had been completed the usage of a Phenomenex C18 column (150 × 4.6 mm, 5ìm) and a cellular segment composed of ammonium acetate: methanol: acetonitril (75:12.5: 12.5 v/v%) at a glide charge of 1 ml/min with $ë_{max}$ at 270 nm. Injection extent became 10 ìl and elution time became near 8 min.Under the defined HPLC parameters standard chromatogram, which indicates a clean the peak at affordable t_R 1.717 min.

Table 3 : The obtained results of Isoniazid in pharmaceutical tablets.

Drug sample	Rec%	Err%	SD	RSD%
Isoniazid pure(SDI)	98	-1.2	5.5	**0.05**
Isoniazid (Indian)	98	-2	1	1.02
Isoniazid(turkey)	110	+10	3.5	3.18

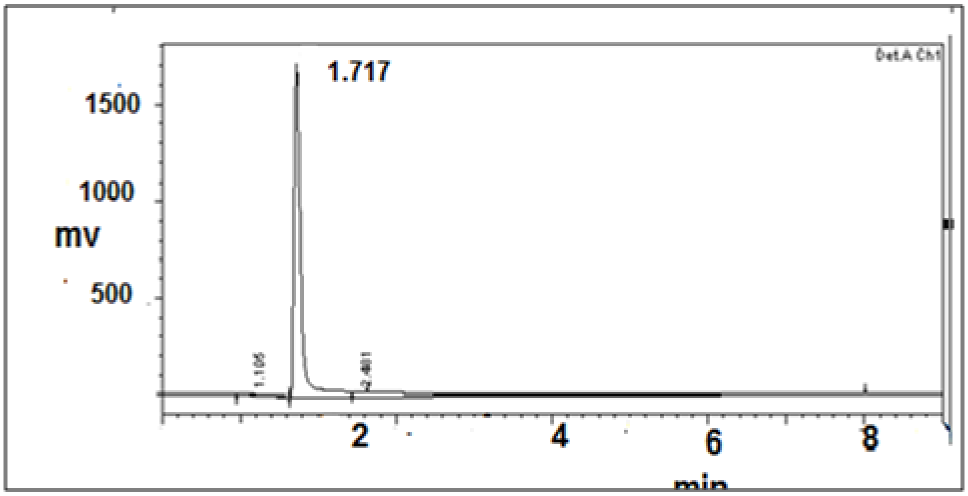

Fig. 5 : Chromatogram of isoniazid using optimum condition.

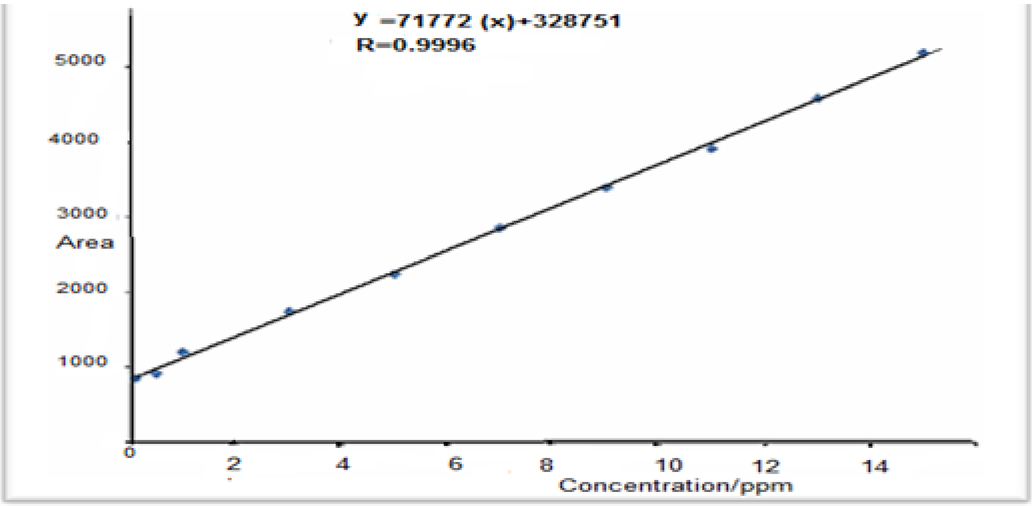

Fig. 6 : Calibration graph of isoiazid using HPLC method.

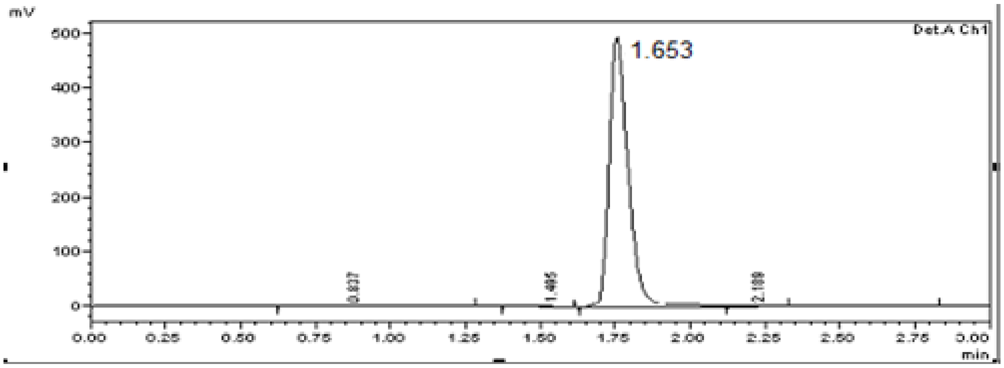

Fig. 7 : Chromatogram of pharmaceutical preparation (Indian company).

Summary of analytical data for the determination of isoniazid using HPLC method (Table 4).

(1-3) HPLC calibration graphs

A 10ìl of the same old answers include Isoniazidhave been injected intriplicate run from which the linear regression equation changed into calculated. A calibration graphs have been built through the plotting vicinity below top as opposed to concentrations and a regression equations have beencalculatedareprovided in Table 2, which proven that the linear dynamic variants for isoniazid turned into inside the variety 05-15 mg/L proven in Fig. 6. The statistical analytical values the correlation coefficient (R^2), restrict of detection (LOD) anddifferent parameters for HPLC strategies had been tabulated in Table 5. Good linearity became located with an R^2 of (0.9996). The linear regression statistics for the calibration

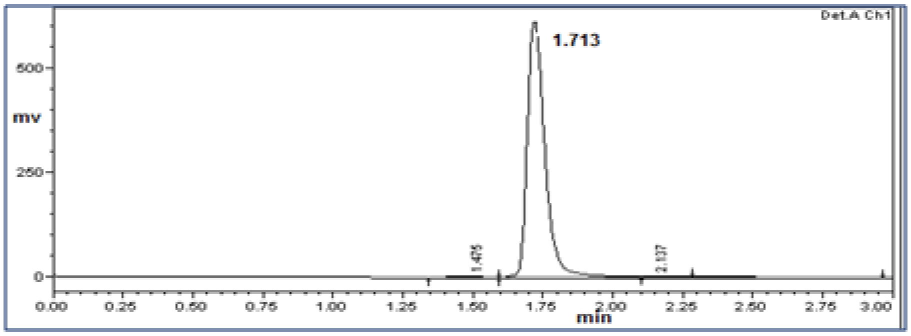

Fig. 8 : Chromatogram of pharmaceutical preparation (Turkish) company).

Table 4 : shows a summary of analytical data for the determination of isoniazid using HPLC method.

Parameter	Recommended value
Column	C18 (150mm x 4.5mm : 5µm)
Organic modifir	12.5% acetonitril+12.5% methanol
Buffer	75% amonium acetate
pH	3
Flow rate	1ml.min^{-1}
Column temperature	30C¾
Detecter	U.V detector at 270 nm
Injected sample	10 µm

Table 5 : Analytical data for the determination of isoniazid using RP-HPLC.

Analytical data	Value
Linear range	0.5-15mg/L
Correlation coefficient	0.9996
Regression equation	Auc=71772[C]+328751
RSD%	0.05
Slope	71772
Detection limit(LO.D)	010.0 mg/L
Calculated t-value	2506>3.16

Table 6 :

UV-Vis method					HPLC method				
Isoniazid (mg/L)		Rec%	Error%	R.S.D% n=3	Isoniazid(mg/L)		REC%	Error%	R.S.D% n=3
Taken	Found				Taken	Found			
1	9.93	98.3	-1.7	1.9	4	3.906	98	-2	0.1
2	1.99	99.5	-1.1	2.4	6	5.906	99.5	-0.5	0.04
4	3.95	98.75	-1.2	1.37	8	7.902	99	-0.1	0.02

Table 7 : The statistical comparison of results for the spectrophotometric and RP-HPLC.

The method	Regressionequation	Linearity(mg/l)	Correlation Coefficient	Rec%	RSD%
Spectrophotometric	$y=0.0914_{x}+0.0441$	0.2-6.0mg/l	0.9991	98.85	1.89
RP-HPLC	$Y=71772_{X}+328751$	0.5-15mg/L	0.9996	99	0.05

plot are indicative of an amazing linear dating among awareness version and region .

Accuracy and precision and recovery of proposed methods

This study was carried out to assure the closeness of the test result sobtained by the analytical method. For this study three different selected concentrations 4, 6, 8 ìg/ml of Isoniazid were chosen for the HPLC method. In table 6, which revealed that the suggested method for detection of Isoniazid was interesting and quiteconvenient with respect to the methods and parameters calculated. While, the data accuracy and precision methods of proposed methods were summarized in Table 6.

Accuracy and precision methods of proposed methods summarized in Table 6.

Comparison the two methods

The two proposed methods were compared as shown in Table 7.

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