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Ligand substitution kinetic assay of antitubercular drug isoniazid in pure and pharmaceutical formulations

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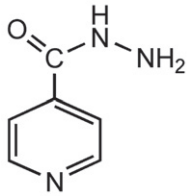
ABSTRACT

The interaction of isoniazid (INH) with $[Fe(CN)_5(H_2O)]^{3-}$ in aqueous solution at pH 3.8 was found to give intensely golden yellow color product, $[Fe(CN)_5(INH)]^{3-}$, which strongly absorbs at 435 nm without any interference with the reactants. The effect of several reaction variables on the rate of the complex $[Fe(CN)_5(INH)]^{3-}$ formation was studied and optimized. Thus a simple, rapid, selective and economical analytical method based on the ligand substitution (LS) kinetic assay of INH in pure as well as in dosage forms involving the reaction between $[Fe(CN)_5(H_2O)]^{3-}$ and INH has been reported. The isoniazid can be determined in the range 1.37–13.71 μg mL^{-1} by measuring the initial rate of the complex formed during the course of the reaction at 435 nm. The detection limit has been established to be 0.15 μg mL^{-1} of INH. Recovery experiment was carried out to ensure the accuracy and the precision in the quantification of INH. The proposed method has successfully been applied for the analysis of INH in pure samples and a number of its pharmaceutical formulations with excellent accuracy and precision while the results were compared with those obtained by the official analytical method, and were in well agreement. The common excipients used as additives in pharmaceuticals do not interfere in the proposed method. It was possible to do 20 analyses per hour.

1. Introduction

Tuberculosis, also known as M. tuberculosis due to its causative agent pathogen *Mycobacterium tuberculosis*, is an important public health concern due to world's main cause of death from a single infectious agent having global prevalence of millions of people [1,2]. In 1990, 2.5 million persons were estimated to have died of tuberculosis [2]. In fact, this dreaded disease is responsible for more than two million deaths worldwide annually [3]. Pyridine-4-carboxylic acid hydrazide *i.e.* 4-pyridine formohydrazide (**I**) is commercially known as isoniazid (INH), isonicotinyl hydrazide or hydrazide of nicotinic acid. INH is the primary anti-tubercular drug widely used for the chemotherapy of all kinds of tuberculosis, and especially tubercular meningitis [4]. It is also used as a prophylactic agent for persons constantly exposed to tubercular patients [5,6]. In the therapy of tuberculosis it has been used alone or widely used incorporation with streptomycin, rifampin, p-aminosalicylic acid, nicotinamide, ethambutol, *etc.* [7–9]. INH is a pro-drug that is activated by mycobacterial catalase-peroxidase enzyme (CPE) in the presence of manganese ions, nicotinamide adenine dinucleotide (NAD) and oxygen [10–13]. INH inhibits the growth of *Tubercle bacillus in vitro* in concentration less than 1 μg mL^{-1} [5,6].



I

INH is also known to hydrolyze with the passage of time and inappropriate storage conditions, and gives hydrazine which is a known carcinogen and considerably toxic. Thus, probably due to the release of free hydrazine upon its hydrolysis, INH has been reported to be carcinogenic in mice [14]. Therefore, because of its medicinal application, the determination of INH in the pharmaceutical preparations and biological fluids is of great importance as part of public health programs. As a consequence of the increasing control demanded in industrial and pharmaceutical processes, it led to greater interest in

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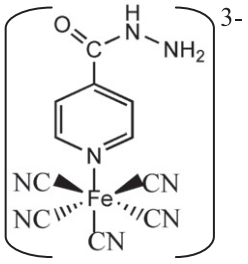
the development methods for its determination in various samples [3,4,7–9,14]. Therefore, a number of analytical methods have been reported in the literature to determine INH, each of which has been developed in a different way involving different techniques. These different analytical techniques that have been employed for the quantitation of INH in pure, pharmaceutical and biological samples are: titrimetric [15–18], colorimetric [19–23], spectrophotometric [3,4,7–9,24–37], fluorescence spectrometric [38–42], atomic absorption spectrometric [43,44], chemiluminescence [45–49], flow injection analysis [50–55], capillary electrophoresis [56–60], kinetic determination [14,61], electroanalytical techniques (voltammetry, screen-printed carbon electrode) [62–66] and various chromatographic techniques including capillary gas chromatography [6], thin layer chromatography [67,68], electrokinetic chromatography [59,69], liquid chromatography or high-performance liquid chromatography (HPLC) [70–84]. In addition Safavi and Bagheri have designed an optical sensor for indirect determination of INH [85].

A wide linear dynamic range and simple instrumentation using continuous flow chemiluminescence (CL) method for the determination of INH by oxidation with *N*-bromosuccinimide (NBS) has been reported [45] but it suffers from instability of the oxidant NBS for the detection of the CL signal and results in poor sensitivity. Chromatographic methods including capillary electrophoresis have been the most frequently used method for the determination of INH in various samples, including human urine and blood (*vide supra*) [6,59,67–84]. However, the direct application of many of these methods is limited due to insufficient sensitivity. HPLC is useful for the determination of low concentrations of INH in simple materials but its main disadvantage is the complicated procedure; INH must be separated or has to be extracted before the determination, which in addition to making it laborious increases the risk of error [86]. The majority of liquid chromatographic techniques involved ultra violet (UV) detection by measuring the absorbance of INH at 263 nm or after pre-column derivatization with a suitable derivatizing reagent [73,74,78] but procedures to separate INH before its UV or colorimetric detection make them laborious and time consuming [86]. Also the high instrumental and operational costs prohibit the use of these methods by many laboratories. Some sensitive flow injection analysis (FIA) methods have also been developed for the determination of INH but FIA always requires additional detection system [46,48–55]. Zhao et al. proposed a FIA method for INH determination based on luminol–Mn(II)–KIO_4 CL system with INH as a sensitizer [46]. However, the method has low sensitivity. The micellar electrokinetic capillary chromatography (MEKC) [59] and cation-selective exhaustive injection-sweeping-micellar electrokinetic chromatography [69] have also been used for the quantitation of INH in pharmaceutical preparations and biological samples but they become quite expensive.

In one of the early reports Peters et al. reviewed the methods previously employed for the determination of INH and reported their often inadequate specificity [38,86]. The conversion of an aldehyde or ketone to the corresponding hydrazone, by reaction with INH containing the hydrazine moiety, is generally quantitative and gives intensely colored product. This procedure has mostly been exploited as a colorimetric method for determining INH [19–23]. Most of the spectrophotometric procedures involve condensation reaction to form hydrazine derivatives which are absorbed maximally in UV–visible regions, and formed the basis of spectrophotometric determination of INH in various samples [3,4,7–9,24–37]. Spectrophotometric methods are simple, but their main disadvantage is poor selectivity, which necessitates preliminary separation before derivatization through condensation reaction and makes them time consuming. Ellard et al. have carried out the comparison between colorimetric and fluorimetric methods, and reported that the most sensitive and specific method for the determination of INH in serum is the fluorimetric method but this method cannot be applied directly to the determination of INH in real sample [38]. After Peters et al. there has not been any review of the methods reported for the determination of INH [86].

Zhang et al. established a spectrophotometric method to determine INH with high sensitivity and good selectivity using potassium ferricyanide, $[Fe(CN)_6]^{3-}$, as spectroscopic probe reagent where iron(III) is reduced to iron(II) by INH [3]. In this method, the *in situ* formed iron(II) reacts with $[Fe(CN)_6]^{3-}$ to give soluble prussian blue complex, the absorbance of which is measured at 735 nm to calculate the amount of INH. The parameters with regard to determination were optimized, and the reaction mechanism discussed but it was not a kinetic method. In available literature, only two kinetic methods for the quantitative determination of INH have been found which offer very good sensitivity [14,61]. Routine laboratory testing of drugs in the market is crucial to protect public health especially in developing countries where counterfeit and substandard drugs have become a major challenge to health care services. Thus there is further need for the development of simple, inexpensive, sensitive and specific methods for assessing the quality of drugs in the market. As many of the reported methods involve significant cost, a LS-kinetic assay for INH, as being reported here, was developed by us as an in-house method as a part of our continued interest in developing analytical methods based on catalytic oxidation [87–91] and ligand substitution reactions [92–98]. Thus the goal of the present reported work was to carry out extensive literature survey on determination of INH in various samples (*vide supra*) [3,4,6–9,14–86] and to develop a sensitive, selective, rapid, accurate and economical method for the determination of INH pure and pharmaceutical preparations and is reported herein. The procedure is based on the uncatalyzed LS reaction of $[Fe(CN)_5(H_2O)]^{3-}$ with INH which leads to the quantitative formation of a substituted, highly stable and pharmaceutically important product $[Fe(CN)_5(INH)]^{3-}$ (**II**) that absorbs strongly at 435 nm. The kinetics and mechanistic studies of the formation of $[Fe(CN)_5(INH)]^{3-}$ have very recently been reported by us [13]. The method is simple, and requires only one reagent, $[Fe(CN)_5(H_2O)]^{3-}$, which can easily be obtained and is stable.



II

2. Experimental

2.1. Reagents

All chemicals used were of analytical reagent grade and the solutions were prepared with deionized distilled water. The sodium amminopentacyanoferrate(II) trihydrate, $Na_3[Fe(CN)_5(NH_3)]\cdot 3H_2O$, was synthesized by a reported method [99] and characterized by UV–visible absorption spectra and CHN microanalysis. Analytical calculation for $Na_3[Fe(CN)_5(NH_3)]\cdot 3H_2O$ was %C 18.42, %H 2.76, %N 25.77 while found was %C 18.36, %H 2.75, %N 25.64. The stock solutions of $Na_3[Fe(CN)_5H_2O]$ were obtained by quantitative hydrolysis of $Na_3[Fe(CN)_5NH_3]\cdot 3H_2O$. The isoniazid (BDH, UK) was used for preparing the solutions of required concentrations. The stock solution of 1.0×10^{-2} mol L^{-1} INH was prepared by the accurate weighing of

its calculated amounts and dissolution in the de-ionized distilled water. The solution of $Na_3[Fe(CN)_5H_2O]$ was stored in a dark amber colored bottle to avoid any photodecomposition and probable oxidation. All other common laboratory chemicals used were of the best grade available and used without further purification. To maintain the pH of reaction mixture during different kinetic runs, potassium hydrogen phthalate–NaOH buffer solution was prepared by the reported procedure [100]. The standard BDH buffers of pH 4, 7 and 10 were used to standardize the pH-meter before its use. The potassium nitrate (Merck) was used to maintain the ionic strength, μ, of the reaction mixture.

2.2. Apparatus

The acquisition of kinetic data *i.e.* absorbance at fixed wavelength, as a measure of initial rate, and spectra were recorded on a double beam Shimadzu UV-240 spectrophotometer attached to a thermostatic cell compartment having 10 mm matched quartz cuvettes. Measurements of pH of the working solutions were made with a Toshniwal digital pH-meter, model CL-46, using a combined glass electrode. All the glassware used were of certified 'A' grade, which were scrupulously cleaned and steamed before their use.

2.3. General procedure

The experimental conditions were established after a detailed kinetic study of the indicator reaction. The concentration of the reactants and other conditions were selected at which the initial rate of the reaction as well as the sensitivity showed maximum. The experiments were performed at ambient temperature, 25.0 ± 0.1 °C. Thus the temperature of the reactants in each kinetic run was maintained at 25.0 ± 0.1 °C by immersing them in a thermostat in advance for 30 min. The indicator reaction under study was carried out in acidic medium where pH of all the reactants was adjusted to 3.80 ± 0.02 as optimum value.

The 2.0 mL reactants of desired concentrations were mixed in a 10.0 mL Borosil volumetric flask in the sequence: INH and buffer while the substrate $[Fe(CN)_5(H_2O)]^{3-}$ solution was added last, immediately before every kinetic run. The reaction mixture was diluted to 10.0 mL with the buffer. The properly shaken reaction mixture was transferred to a 10 mm path length spectrophotometric cuvette kept in a temperature controlled cell compartment. The progress of reaction was monitored where the initial rate of the reaction was determined by measuring the increase in absorbance of the complex $[Fe(CN)_5(INH)]^{3-}$, formed during the course of the reaction at 435 nm at which the product absorbs strongly without any interference from the reactants. The fixed time absorbance as a measure of the initial rate was used to optimize the dependence of the reaction variables. Three calibration models involving relationship between the initial rate *i.e.* absorbance at fixed time (t_n = 2, 5 and 7 min) and known INH concentrations were established (*vide infra*) under optimum conditions. A set of sample solutions with different known concentrations of INH was prepared and recovery was carried out under the optimum conditions to quantify the [INH] and also in unknown samples.

3. Results and discussion

3.1. Investigations of the indicator reaction

Very recently we have reported the kinetics and mechanism of the formation of an anti-tubercular complex $[Fe(CN)_5(INH)]^{3-}$ based on substitution reaction between $K_4[Fe(CN)_6]$ and isoniazid *i.e.* isonicotinohydrazide (INH) catalyzed by Hg^{2+} in aqueous medium where the uncatalyzed reaction with $K_4[Fe(CN)_6]$ is quite slow [13]. The catalyzed reaction was studied spectrophotometrically at 435 nm (the λ_{max} of the golden yellow colored complex $[Fe(CN)_5(INH)]^{3-}$) as a function of pH, ionic strength, temperature, and concentration of the reactants and the catalyst Hg^{2+}. However, the LS reaction between aquapentacyanoferrate(II) *i.e.* $[Fe(CN)_5(H_2O)]^{3-}$, and INH in aqueous acidic medium also leads to the formation of the same substituted highly stable and pharmaceutically important product, $[Fe(CN)_5(INH)]^{3-}$ (**II**) and this uncatalyzed reaction is quite fast to apply for the development of kinetic method for INH.

The hydration of $Na_3[Fe(CN)_5NH_3]\cdot 3H_2O$ quantitatively generates $[Fe(CN)_5(H_2O)]^{3-}$ which further reacts with INH to give the desired product. The overall reaction occurring between $[Fe(CN)_5(H_2O)]^{3-}$ and INH yielding substituted anti-tubercular complex $[Fe(CN)_5(INH)]^{3-}$ is represented by Eqs. (1) and (2).

$$[Fe(CN)_5(NH_3)]^{3-} + H_2O \rightarrow [Fe(CN)_5(H_2O)]^{3-} + NH_3 \quad (1)$$

$$[Fe(CN)_5(H_2O)]^{3-} + INH \rightarrow [Fe(CN)_5(INH)]^{3-} + H_2O. \quad (2)$$

The complex, $[Fe(CN)_5(INH)]^{3-}$, formed in the above reaction was fully characterized by elemental analysis (calculated: %C 28.4, %H 3.2, %N 24.0 and found: %C 28.1, %H 3.4, %N 23.7) and ^1H NMR(D_2O), 500 MHz: δ 9.11 (d, 2H, H_2 and H_6), δ 7.41 (d, 2H, H_3 and H_5), indicating that the nitrogen atom of the pyridine ring is coordinated with Fe^{2+}. The anionic complex $[Fe(CN)_5(INH)]^{3-}$ which absorbs strongly at 435 nm, which is attributed to the metal to ligand charge transfer (MLCT) transitions, at which all subsequent measurements were made. The absorption spectrum of the complex $[Fe(CN)_5(INH)]^{3-}$ at optimum reaction conditions is presented in Fig. 1.

3.2. Optimum conditions of reaction variables

The experimental parameters affecting the reaction rate between $[Fe(CN)_5H_2O]^{3-}$ and INH yielding substituted anti-tubercular complex, $[Fe(CN)_5(INH)]^{3-}$ formation were carefully studied and optimized.

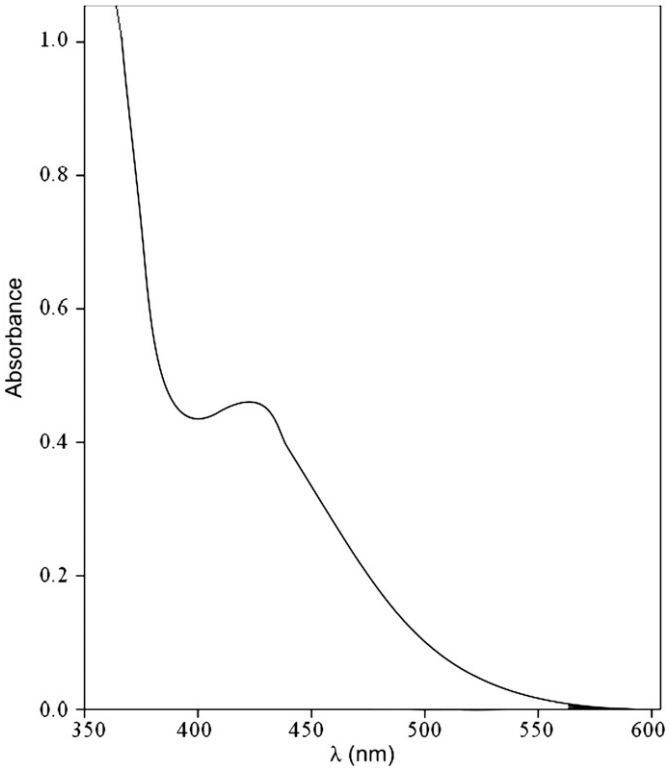


Fig. 1. Absorption spectrum of the product, $[Fe(CN)_5(INH)]^{3-}$ under the conditions: $[Fe(CN)_5(H_2O)^{3-}] = 6.0 \times 10^{-5}$ mol L^{-1}, $[INH] = 6.0 \times 10^{-4}$ mol L^{-1}, pH = 3.80 ± 0.02, ionic strength, μ = 0.05 mol L^{-1} (KNO_3) and temperature = 25.0 ± 0.1 °C.

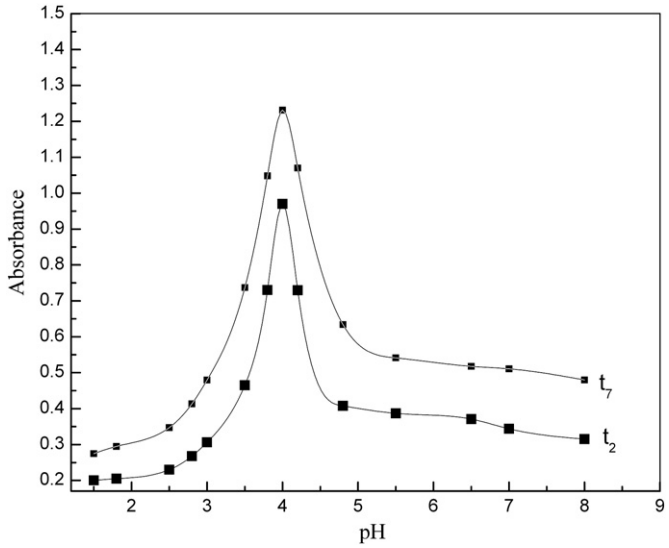


Fig. 2. Effect of pH on the absorbance under the conditions: $[Fe(CN)_5(H_2O)^{3-}] = 6.0 \times 10^{-5}$ mol L^{-1}, [INH] = 6.0×10^{-4} mol L^{-1}, ionic strength, μ = 0.05 mol L^{-1} (KNO_3) and temperature = 25.0 ± 0.1 °C.

3.2.1. Effect of pH

The reaction is pH dependent. Thus the optimum maintenance of pH is essential. The effect of pH on the rate of complex formation was studied using fixed time procedure in the pH range 1–8. The pH up to 6 was varied using potassium hydrogen phthalate–NaOH or HCl buffer. However, in the higher pH region, the pH of the solutions was maintained using 5.0 mol L^{-1} solution of NaOH. The results in terms of the variation in the initial rate *i.e.* fixed time absorbance, at 435 nm after 2 and 7 min of mixing the reagents, with the pH of the medium are shown in Fig. 2. It was known from our previous study that a pH 3.5 gives the highest rate of formation of $[Fe(CN)_5(INH)]^{3-}$ [13] but the substrate used in the present case was $[Fe(CN)_5H_2O]^{3-}$ *i.e.* $[Fe(CN)_5(NH_3)]^{3-}$ as precursor, and thus the optimum pH of 3.80 ± 0.02 was observed. Therefore pH 3.80 was selected for further studies.

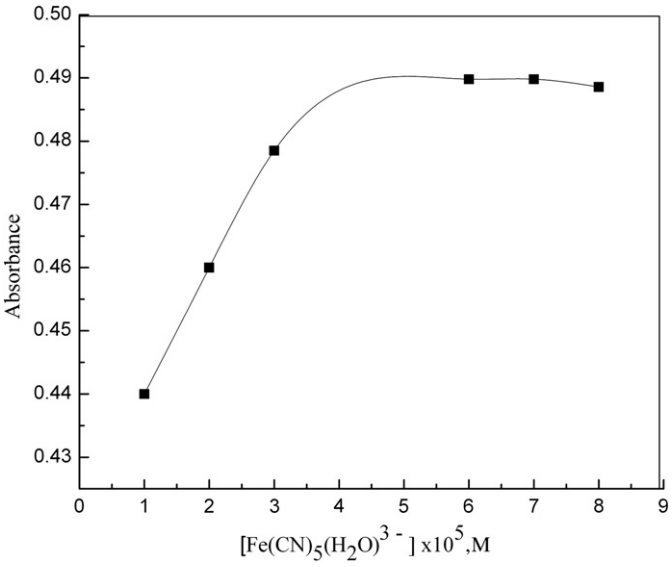


Fig. 3. Effect of $[Fe(CN)_5(H_2O)^{3-}]$ under the conditions: [INH] = 6.0×10^{-4} mol L^{-1}, ionic strength, μ = 0.05 mol L^{-1} (KNO_3), temperature = 25.0 ± 0.1 °C.

3.2.2. Influence of $[Fe(CN)_5(H_2O)^{3-}]$

The effect of $[Fe(CN)_5(H_2O)]^{3-}$ concentration on producing the maximum initial rate *i.e.* absorption intensity in formation of $[Fe(CN)_5(INH)]^{3-}$ was investigated at optimum pH 3.80 ± 0.02 in the concentration range 1.0–12.0 × 10^{-5} mol L^{-1}. Low concentration range of the complex was used to prevent any dimerization [101]. The plot between absorbance *versus* $[Fe(CN)_5(H_2O)^{3-}]$ is shown in Fig. 3. As shown in Fig. 3, the rate of reaction was increased by increasing $[Fe(CN)_5(H_2O)]^{3-}$ concentration. It was observed that the rate of the reaction leveled off after 4.0×10^{-5} mol L^{-1}. Thus 6.0×10^{-5} mol L^{-1} was accepted for further study since it provided the maximum initial rate value. However, when [INH] was higher at 10.0×10^{-5} mol L^{-1} then $[Fe(CN)_5(H_2O)^{3-}]$ was taken at 1.1×10^{-4} mol L^{-1} *i.e.* 10% excess over the ligand INH to have complete coordination by INH.

3.2.3. Influence of temperature and ionic strength (μ)

The effect of temperature on the sensitivity was examined in the range of 15–40 °C. The reaction was also studied at higher temperatures but it was found that the complex, $[Fe(CN)_5(INH)]^{3-}$, undergoes degradation at higher temperatures. As expected the reaction followed the Arrhenius equation *i.e.* the reaction rate is increased with increasing temperature. The reaction proceeds with reasonable rate at 25 °C. Therefore, room temperature (25.0 ± 0.1 °C) was recommended as the optimum temperature, for indicator reaction system, for further study. The effect of ionic strength (μ) on the reaction was also studied, in 0.01–0.3 mol L^{-1} range, using KNO_3. Then ionic strength of the reaction medium 0.05 mol L^{-1} was selected for further study as it provided suitable change in absorbance value.

3.2.4. Influence of INH concentration and analytical characteristics

In order to establish the calibration model for the analytical system, INH concentration was varied in the concentration range 1.0×10^{-6}–6.0×10^{-5} mol L^{-1} under the optimized conditions: 6.0×10^{-5} mol L^{-1} $[Fe(CN)_5(H_2O)^{3-}]$, pH 3.80 ± 0.02, μ 0.05 mol L^{-1} (KNO_3) and temperature 25.0 ± 0.1 °C. The plot of the fixed time absorbance (t_n) after 2, 5 and 7 min *versus* [INH] was found to be linear in the concentration range 1.0–10.0 × 10^{-5} mol L^{-1}. Thus the calibration graphs were obtained by plotting fixed time absorbance after 2, 5 and 7 min *versus* INH concentration using the general procedure (*vide supra*) under the optimal conditions and are shown in Fig. 4. From the results of the experiments, 2, 5 and 7 min were chosen as the optimal

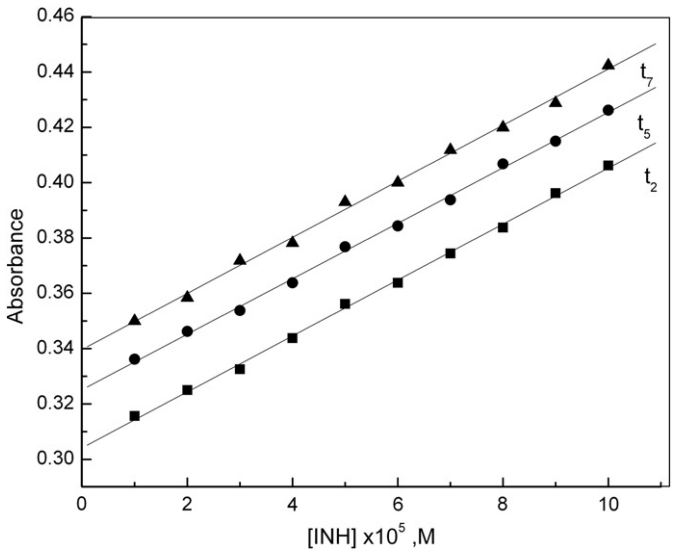


Fig. 4. Calibration curve for the determination of [INH] under the conditions: $[Fe(CN)_5(H_2O)^{3-}] = 1.1 \times 10^{-4}$ mol L^{-1}, ionic strength, μ = 0.05 mol L^{-1} (KNO_3) and temperature = 25.0 ± 0.1 °C.

Table 1
Characteristic features of [INH] *versus* absorbance calibration graphs at different fixed times under the optimum reaction conditions where $[Fe(CN)_5(H_2O)^{3-}] = 1.1 \times 10^{-4}$ mol L^{-1}, was taken 10% excess over the ligand INH, pH = 3.80 ± 0.02, ionic strength, μ = 0.05 mol L^{-1} (KNO_3) and temperature = 25.0 ± 0.1 °C.

[INH] (μg mL^{-1})	Time (min)	Calibration/regression equation	Correlation coefficient (r)	Sensitivity (M^{-1})
1.37–13.71	2	$A_2 = 1.012 \times 10^3$ [INH] + 0.1817	0.9919	1.012×10^3
1.37–13.71	5	$A_5 = 1.003 \times 10^3$ [INH] + 0.2276	0.9991	1.003×10^3
1.37–13.71	7	$A_7 = 1.014 \times 10^3$ [INH] + 0.2879	0.9985	1.014×10^3

Table 2
Accuracy and precision of INH determination by proposed method under the optimum reaction conditions: $[Fe(CN)_5(H_2O)^{3-}] = 1.1 \times 10^{-4}$ mol L^{-1}, pH = 3.80 ± 0.02, ionic strength, μ = 0.05 mol L^{-1} (KNO_3) and temperature = 25.0 ± 0.1 °C.

Concentration INH added (μg mL^{-1})	A_2			A_5			A_7		
	[INH][a] found ± s (μg mL^{-1})	Recovery (%)	Error (%)	[INH][a] found ± s (μg mL^{-1})	Recovery (%)	Error (%)	[INH][a] found ± s (μg mL^{-1})	Recovery (%)	Error (%)
1.526	1.532 ± 0.02	100.39	+0.32	1.546 ± 0.05	101.31	+1.31	1.499 ± 0.16	98.23	−1.77
2.040	2.034 ± 0.03	99.70	−0.29	2.074 ± 0.04	101.67	+1.67	2.050 ± 0.03	100.49	+0.49
2.568	2.560 ± 0.03	99.67	−0.31	2.524 ± 0.07	98.28	−1.71	2.592 ± 0.05	100.93	+0.93
3.056	3.068 ± 0.03	100.39	+0.39	3.072 ± 0.05	100.52	+0.52	3.040 ± 0.08	99.54	−0.46
3.426	3.478 ± 0.03	101.52	+1.51	3.412 ± 0.07	99.59	−0.41	3.442 ± 0.03	100.47	+0.47
3.818	3.799 ± 0.03	99.50	−0.49	3.898 ± 0.08	101.30	+1.30	3.836 ± 0.04	99.69	−0.31
5.048	4.998 ± 0.05	99.00	−0.99	5.020 ± 0.16	99.45	−0.55	5.016 ± 0.04	99.37	−0.63
7.098	7.130 ± 0.06	100.45	+0.45	7.026 ± 0.08	98.98	−1.01	7.072 ± 0.06	99.63	−0.37
9.264	9.186 ± 0.13	99.16	+0.84	9.282 ± 0.04	100.19	+0.19	9.222 ± 0.08	99.55	−0.45
		Average = 0.16			Average = 0.14			Average = 0.23	

The ± s values represent relative standard deviation of the mean for five determinations (%).
[a] Mean of five determinations.

times, because they provided the best correlation coefficient and sensitivity (Table 1).

The linear regression equations correlating the fixed time absorbance (A_t) values to the INH concentration range 1.0 to 10.0 × 10^{-5} mol L^{-1} (1.37 to 13.71 μg mL^{-1}) are given in Table 1. The study of the accuracy of the proposed method was confirmed with five independent determinations for the recovery of INH from the solutions of various concentrations under the optimum conditions and the results are given in Table 2. The calculated recoveries for the measured concentrations compared to the spiked concentrations in the calibrated range were 98 to 101%. The limit of detection was determined to be 0.15 μg mL^{-1} of INH.

3.3. Interference of excipients

The extent of interferences by some excipients that generally present in the pharmaceutical preparation was determined by the recovery experiments from the solutions containing 2 μg mL^{-1} INH and large amounts of diverse species. It was found that the excipients studied do not interfere in the present method, even when present in large excess to 1000 times. The maximum error ±3% obtained was considered quite satisfactory and the interesting results are given in Table 3.

3.4. Application to assay of INH in pharmaceutical preparations

The developed kinetic spectrophotometric method for determining INH was successfully tested in pharmaceutical samples. The average mass per tablet was determined by weighing twenty tablets and finely grounded. The amounts equivalent to 100 mg of INH from the finely ground material were accurately weighed and transferred into different 100 mL calibrated flasks containing 70 mL de-ionized distilled water. The flasks with INH content were sonicated for 15 min and the dissolved sample diluted to 100 mL with de-ionized distilled water. The solutions were filtered through a 0.45 μm Millipore Whatman filter paper. To bring the [INH] within the calibration range, the solutions were diluted with de-ionized distilled water. The diluted solutions were directly analyzed, using the calibration models established (Table 1) for the determination of INH (*vide supra*).

Six types of pharmaceutical samples were analyzed for INH content and the results are presented in Table 4. The results were statistically compared with the standard method in respect to the accuracy and the precision. The results on INH determination from the developed method were in good agreement with the results of the standard method [102]. The mean recoveries were found in the range of 98–102% (five determinations) which clearly demonstrated that the developed method may be suitable for the quick determination of INH in pharmaceutical samples.

4. Conclusion

An accurate, sensitive, rapid, economical and viable LS-kinetic spectrophotometric assay for INH was developed based on LS reaction between $[Fe(CN)_5(H_2O)]^{3-}$ and INH. The method was applied for the determination of INH in pharmaceutical samples and the results

Table 3
Determination of INH at 2.0 μg mL^{-1} level in presence of excipients under the optimum reaction conditions: $[Fe(CN)_5(H_2O)^{3-}] = 1.1 \times 10^{-4}$ mol L^{-1}, pH = 3.80 ± 0.02, ionic strength, μ = 0.05 mol L^{-1} (KNO_3) and temperature = 25.0 ± 0.1 °C.

Additives	[Excipient]/[INH]	Recovery (%) ±RSD (n = 5)
Galactose	1000	99.6 ± 0.8
Sorbitol	1000	100.4 ± 0.4
Sugar	500	100.2 ± 0.6
Sodium lauryl sulfate	1000	100.8 ± 0.2
Calcium sulfate	1000	99.2 ± 0.5
Iodide	1000	100.5 ± 0.5
Citrate	500	100.4 ± 0.8
Oxalate	1000	99.6 ± 0.6
Talc	500	100.8 ± 0.2
Gum acacia	500	100.6 ± 0.2
Sodium chloride	500	100.7 ± 0.4
Glucose	1000	99.2 ± 0.5
Lactose	500	100.4 ± 0.6
Sodium alginate	500	100.5 ± 0.3
Carboxy methylcellulose	1000	99.8 ± 0.4
Magnesium stearate	500	99.3 ± 0.8
Starch	500	100.4 ± 0.3

RSD: Relative standard deviation (%).

Table 4
Determination of INH in pharmaceutical preparations under the optimum reaction conditions: $[Fe(CN)_5(H_2O)^{3-}] = 1.1 \times 10^{-4}$ mol L^{-1}, pH = 3.80 ± 0.02, ionic strength μ = 0.05 mol L^{-1} (KNO_3) and temperature = 25.0 ± 0.1 °C.

Type of drug used	Label claim per tablet or per mL (mg)	Amount of drug found in mg ± RSD ($n = 5$); proposed method	Amount of drug found in mg ± RSD ($n = 5$); official method [102]
Isokin tablet (Warner)	100	99.6 ± 0.6	99.4 ± 0.2
Isokin liquid (Warner)	20	19.8 ± 0.4	20.2 ± 0.6
Isonex tablet (Pfizer)	100	100.2 ± 0.5	100.4 ± 0.5
Solonex tablet (Macleods)	300	299.6 ± 0.6	299.5 ± 0.6
Ipcazide tablet (IPCA)	100	99.4 ± 0.2	99.5 ± 0.4
Ipcazide liquid (IPCA)	20	19.8 ± 0.4	19.6 ± 0.2

RSD: Relative standard deviation (%).

correlated well with the ones obtained from the standard method. The proposed method is advantageous over many spectrophotometric methods as the heating, extraction, and use of oxidant, organic dye or catalyst are avoided minimizing the source of errors in the determination of INH. The recovery study data has clearly substantiated the reproducibility and accuracy of the method. The recommended LS-kinetic assay is well-suited for the estimation of INH in pharmaceutical preparations and can reduce turnaround time.

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