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Determination of tetracycline hydrochloride in some pharmaceutical products using flow injection spectrophotometric technique

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Abstract

A simple, rapid, and sensitive flow injection spectrophotometric system for determination of tetracycline hydrochloride (TCH) in pharmaceutical products. The method is based on diazotization of 2-aminopyridine and subsequent coupling with tetracycline hydrochloride in alkaline medium. The formed azo-dye showed maximum absorbance at 388 nm and the calibration curve in the linear range of 25.0-150.0 µg/ml of tetracycline hydrochloride with correlation coefficient 0.9988, while the detection limit was 10 µg/ml and sample frequency 123 samples/hour. The accuracy and precision data for determination of TCH by flow injection analysis method for triplicate injection at three concentration levels(25, 80 and 150 µg/ml) the relative standard deviation (RSD %) are 1.04, 0.247 and 1.75%, and relative error percent (Erel) are -3.92, +1.06 and -1.9%. The proposed method was successfully applied to the determination of tetracycline hydrochloride in pharmaceutical products. The results obtained were in good agreement with those obtained by reference method.

Introduction

The tetracycline is similar in pharmacologenic properties and antimicrobial activity; they are effective against with wide range of gram positive and gram negative organisms [1]. Tetracycline is a major member of a group of antibiotics with a broad spectrum of activity, which is widely used in medicine and veterinary science to treat bacterial indications [2] . When tetracycline combined with fluid and electrolyte replacement in the treatment of cholera, effectively reduces the volume and duration of diarrhoea as well as the intravenous fluid requirement, tetracycline also rapidly eliminates the cholera vibrio from the stool of cholera patients [3, 4]. The tetracyclines are a well-known and widely used as family of antibiotics. The bacteriostatic activity of tetracycline's lies in their capacity to inhibit protein synthesis by preventing the binding of the aminoacyl t-RNA to the ribosome, because of the similarity between the prokaryotic protein synthesis machinery and that of eukaryotic mitochondria; tetracyclines are also able to interfere with mitochondrial protein synthesis in mammalian cells. The selective permeability of different mammalian cells to tetracyclines led to the hypothesis that these agents could be used to achieve cell proliferation arrest and

applied to the treatment of malignancies. These compounds are particularly useful in several types of both common and rare infections including atypical pneumonias, rickettsial infections, chlamydial infections, Lyme disease and ehrlichiosis [1,5,6,7,8]. A number of spectrophotometric and flow injection analysis methods have already been used successfully for the determination of tetracycline hydrochloride [9, 10, 11, 12,13,14]. Several liquid chromatographic methods have been described [15,16,17], a polarographic technique [18], chemiluminescence [19] and fluorimetric [20] have been used for determination tetracycline hydrochloride.

I. MExperimental

All chemicals used were of as analytical reagent grade, otherwise it is mentioned. Distilled water was used for preparation of solution. Tetracycline hydrochloride (TCH)(Schorlau) stock solution (1000µg/ml): was prepared by dissolving 0.10 g of TCH in 100ml of distilled water; a working standard solution was prepared by a suitable dilution of the standard solution and The stock solution of tetracycline hydrochloride was kept in a refrigerator before and after use (21, 22). 2-Aminopyridine solution (2-AP) (Aldrich, 99 %) (1000µg/ml): was prepared by dissolving 0.10 g of 2-AP in 100 ml distilled water. Sodium nitrite solution (Riedel-De Haen AG, 99.5%) (2%): was prepared by dissolving 2.0 g of NaNO2 in 100 ml of distilled water. Hydrochloric acid solution (E. U., 36.5-39%) (0.5M): was prepared by dilution of concentrated hydrochloric acid solution (Sp. gr=1.18, 36% and M. wt=36g/mol) with distilled water. Sodium hydroxide solution (SCP, 96%) (2%): was prepared by dissolving 2.0 g of NaOH in 100 ml of distilled water.

Apparatus

The following apparatus and instrumentals were used in this study: an absorbance measurement of the method was carried out on a Bio-Tek model 99-90287spectrophotometer. Flow cell with 30µl and 10mm path length quartz was used in the flow injection system. Multi channel peristaltic pump (Watson-Marlow 5012) used for propelling merged streams. A 6-way loop injection valve with various sample loops and two-Channel recorder used for recording the analytical signal (LKB-BROMMIA 2210) was used. The system shown in Fig. (1): was used in which a multi-channel peristaltic pump was used to propelling four lines, the first for 2-AP (0.036%), the second for hydrochloric acid (1.20 M) , the third for sodium nitrite (0.02%) and the fourth for sodium hydroxide (2.4%) solutions with flow rates of 1.1 , 0.9 , 0.5 , and 0.8 ml/min respectively. Teflon tube with 1.0mm internal diameter (i. d) was used. A 100µl of sample was injected via a disposable syringe (5.0ml) through the injection valve into the carrier stream. Three reaction coils were used with lengths 60-cm (RC1), 60-cm (RC2) and 10-cm (RC3). Using the T- shape for debubbling system before the flow reaches the detector and the merged streams were passed through a quartz flow cell (30 µl, 10 mm path length) in a spectrophotometic connected to recorder. The spectrophotometric set at 388nm.

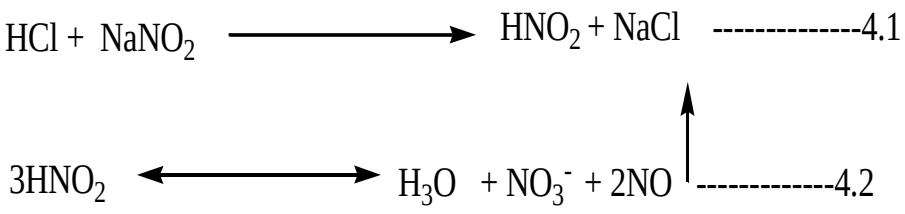
Sample preparation

A- Ten capsules of tetracycline hydrochloride (each one contains 250 or 500 mg tetracycline hydrochloride) (purchased from Erbil market) was weight and mixed, a portion of the powder was weighed accurately, transferred into the 100 ml of volumetric flask and dissolved in distilled water and completed to mark by distilled water (14). The required volume of the sample solution was taken and diluted with distilled water to the range of calibration curve for the determination of TCH.

B- Ten tablets of tetracycline hydrochloride were grounded to fine and mixed thoroughly. A portion of the powder was weighed accurately, transferred into 100 ml of volumetric flask and dissolved in distilled water and completed to mark by distilled water. The required volume of the sample solution was taken and diluted with distilled water to the range of calibration curve for determination of tetracycline hydrochloride [23,24,25].

III. Results and discussion

Physical and chemical characteristics were studied to establish the optimum conditions. When study the optimum conditions the obtained peaks were not stable. The instability of the peak height was attributed to the formation of bubbles of nitrogen mono oxide according to following equations:



Therefore; using the new degassing technique in the flow injection system to remove the bubble of the nitrogen mono oxide in the system, before the flow reaches the detector [26] . The flow split into two stream, one containing the bubbles goes through T-Shape and the other leading to the detector as shown in Fig. (1).

Physical optimization

The influence of different flow rates of 2-AP, hydrochloric acid, sodium nitrite and sodium hydroxide solutions were tested on maximum peak. Different flow rate were obtained by changing the propelling tube diameter. The flow rates 1.1, 0.9, 0.5 and 0.8 ml/min were found to be the optimum values respectively as shown in Fig.(2).

It is well-known that low flow rates can originate a small and enlarge peaks due to a high dispersion of the sample. On the other hand, at very high flow rates the reaction may not progress enough to give an adequate signal [27]. In the FIA system, the height of the response peak highly depends on the residence time of the sample zone, i.e., on tube length, flow rate and sample volume [28]. Fig. (3) show the influence of the lengths of the reaction coils on the peak height. The best response was achieved at lengths of 60-cm, 60-cm, 10-cn and 0.0cm for the system. Therefore, these reaction coil lengths were used for further studied.

Influences of sample volumes injected via an injection valve were tested using different sample volumes (50, 75, 100, 125 and 150µl) of TCH solution. The maximum peak height was obtained with a sample volume of 100µl; therefore, this volume was adopted (Fig. (4)).

Chemical optimization

The influence of concentration of 2-AP solution on the sensitivity was studies and the results are shown in Fig. (5). Increasing of 2-AP from 0.002-0.12%. The results showed that the peak height increase with increasing of the concentration of 2-AP solution was up to 0.036 % as shown in Fig. (5) and then decreases therefore, the 0.036% of 2-AP was selected for subsequent work.

The effects of hydrochloric acid concentrations in range of 0.1-1.5M were estimated on the Peak height. The results are shown in Fig. (6). It was found that 1.2M of HCl was optimums for diazonium ion production; therefore, this concentration was selected for future work.

The influence of the concentration of sodium nitrite solution was examined in the range 0.004-2%. The results showed that the peak height increased with increasing concentration of $NaNO_2$ up to 0.02% as shown in Fig. (7) and then decrease therefore; the 0.02% of sodium nitrite was adopted

The influence of the concentration of sodium hydroxide (as best alkaline medium) on maximum peak height was studied and Fig.(8) shows the results. The 2.4 % of sodium hydroxide gave the maximum peak height; therefore, this concentration was selected for subsequent work.

Under the conditions established, the calibration curve of the determination of TCH was obtained as shown in the Fig. (9). The calibration curve was linearity over the range 25.0-150.0µg/ml with detection limit 8µg/ml and the correlation coefficient (0.9988) with samples frequency 123 samples /hour.

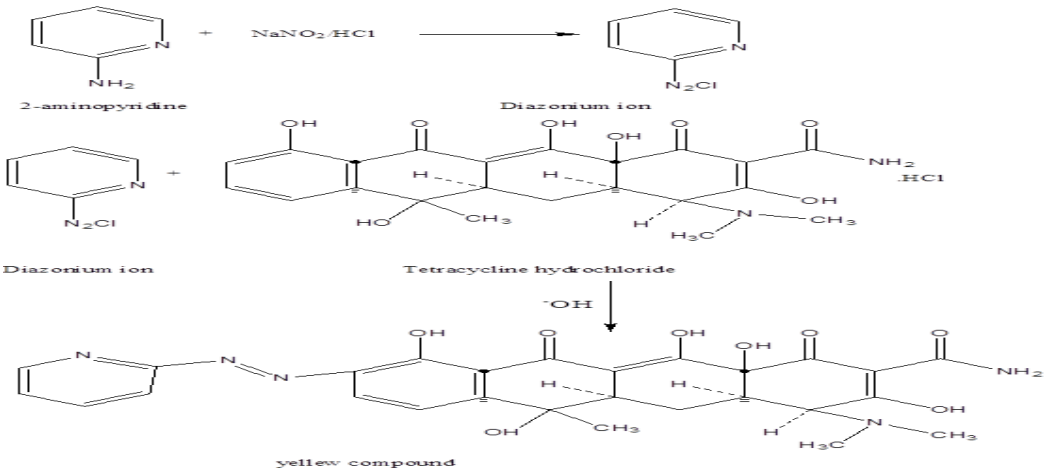
The interfering effects of foreign species which found in pharmaceutical formulation were checked under optimum conditions, through injecting synthetic solution containing pure TCH and varying amount interfering compound. The foreign species must not raise relative error percent ($\geq \pm$ 5 %) of the peak height. Table (1) illustrated the results of interference study.

Application of the methods

The proposed flow injection spectrophotometric method was applied for TCH determination in several commercial pharmaceutical products. A comparison was made between results obtained by the proposed methods and HPLC method as a standard method, when samples were analyzed by Awamedica Company for drugs [29]. The obtained data were in a good agreement with those obtained by standard method and the labeled amount, as show in Table (2), which shows the recoveries of methods and analysis of commercial drugs, respectively.

I. Conclusion:

In the present work new analytical technique has been used for determination of tetracycline hydrochloride in pure or in real samples by using new azo reaction between TCH and 2-AP to form diazonium ion and then forming a new yellow colour coupling compound which represent the concentration of tetracycline in a sample as follows:



This method was new and sensitive flow injection-spectrophotometric technique for determination of tetracycline hydrochloride in pure and pharmaceutical products based on the azo coupling reactions. The main feature of the present method here its low cost and does not require expensive reagents consumption and procedure for tetracycline hydrochloride analysis in pharmaceutical products and they are free from tedious steps like extraction, complex sample treatment and heating. The methods are applicable over the range of concentration with good precision and accuracy. The results obtained by proposed methods are in good agreement when compared with the results obtained by standard method in British pharmacopeias. The systems are suitable the route in the quality control centers for determination of the tetracycline hydrochloride. In addition the methods have acceptable sensitivity, selectivity and reproducibility.

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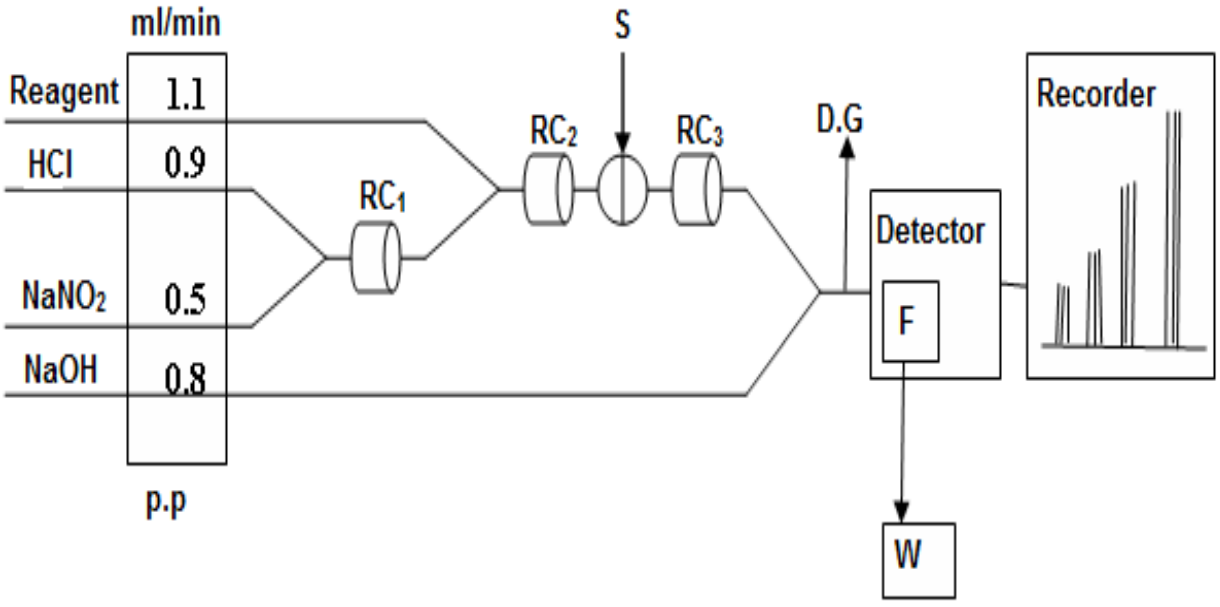

Figure-1 FIA manifold used for determination of TCH by 2-AP reagent: (p. p) peristaltic pump, (S) sample injected, (RC) reaction coils, (D.G) degassing, (F) flow cell and (W) waste.

Table -1: Effect of foreign species on FIA determination of TCH

Foreign species	Maximum Allowable Conc. (µg/ml)	E_r %
Citric acid	65	+1.04
Fructose	1000	+0.81
Glucose	800	+3.99
Lactose	1000	+2.23
Maltose	800	+1.2
Potassium chloride	100	-2.25
Sodium chloride	1000	-0.78
Sodium lauryl suphate	1000	-1.6
Starch	1000	+0.06
Sucrose	800	+2.1

Table - 2: Determination of TCH in commercial drugs by flow injection spectrophotometric methods

Formulation	Composition	Content (mg/Tablet) Declared	found(mg/Tablet) with 2-aminopyridine	Recovery %	found(mg/Tablet) with standard method*	Recovery %
Samacycline	Tetracycline hydrocholirde Hydrochloride	250	256.23	102.49	258.63	103.45
Tetrabact	Tetracycline hydrocholirde Hydrochloride	500	501.16	100.23	497.36	99.47

Tetracycline	Tetracycline hydrocholirde Hydrochloride	250	255.23	102.09	251.29	100.5
Tetracycline hydrocholirdeCapsule	Tetracycline hydrocholirde Hydrochloride	500	495.85	99.17	489.57	97.91
Tetramin	Tetracycline hydrocholirde Hydrochloride	250	255.56	102.22	99.3	105.88
Tetracycline hydrocholirde	Tetracycline hydrocholirde Hydrochloride	100	95.14	95.14	96.64	96.64

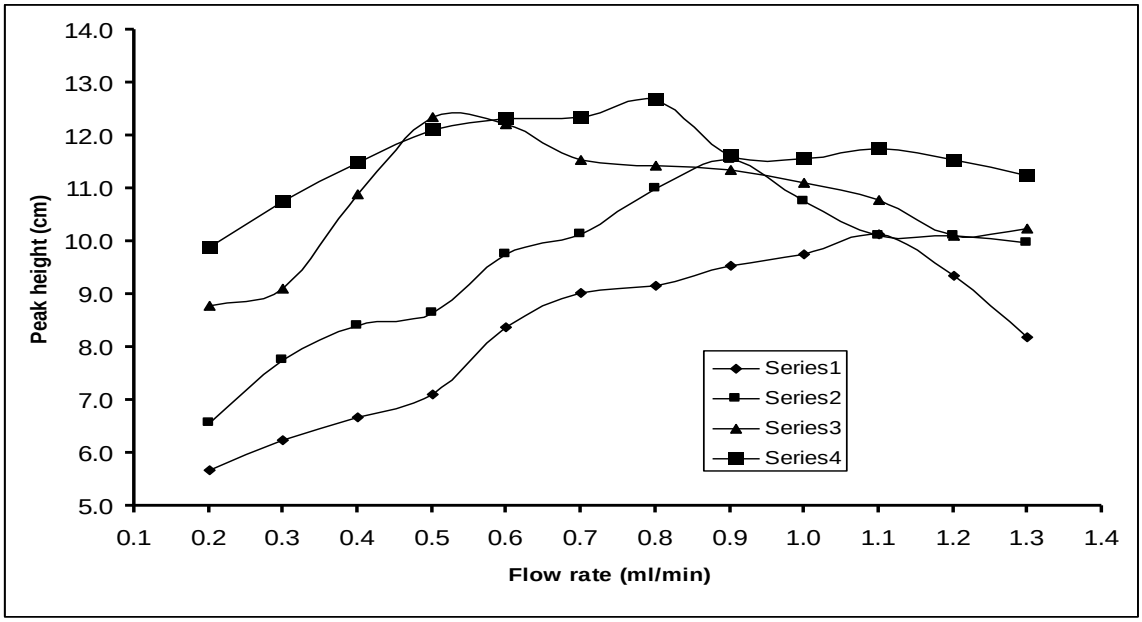

Figure - 2: Effect of flow rates on peak heights: (●) first line reagent, (■) second line hydrochloric acid, (▲) third line sodium nitrite and (■) fourth line sodium hydroxide

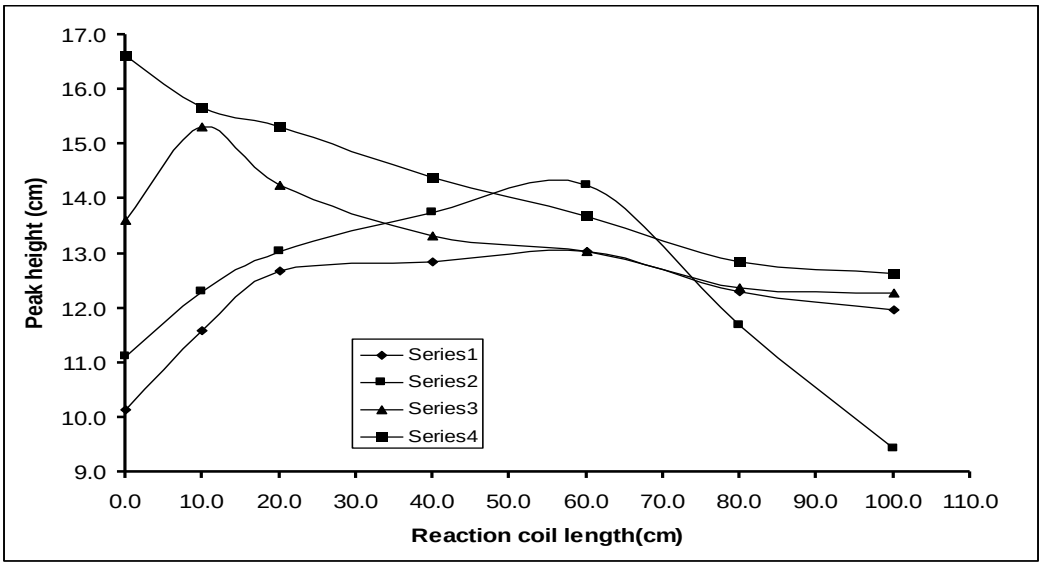

Figure - 3: Effect of reaction coils on peak heights: (●) first reaction coil, (■) second reaction coil, (▲) third reaction coil and (■) fourth reaction coil.

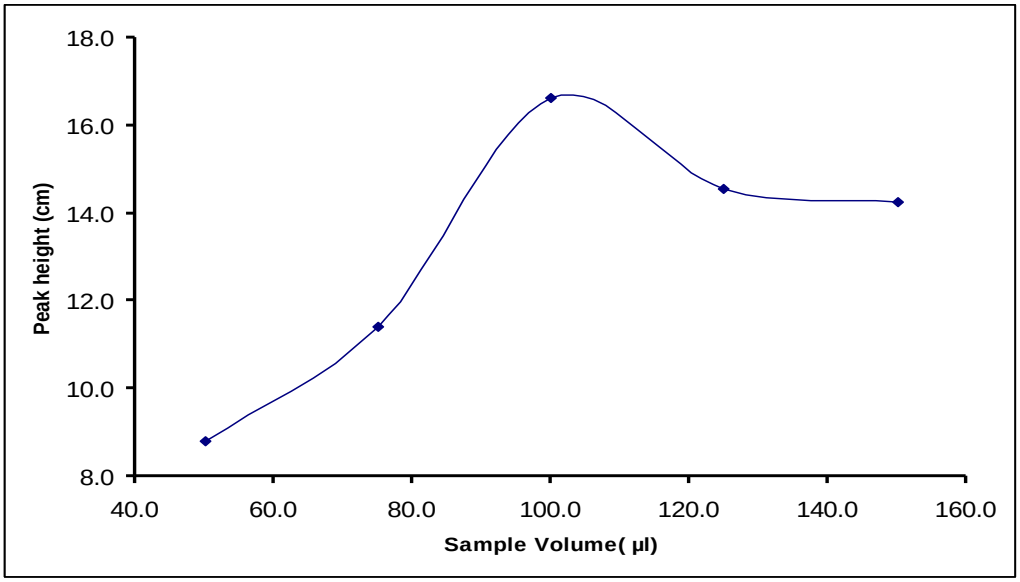

Figure - 4: Effect of sample volume on peak height

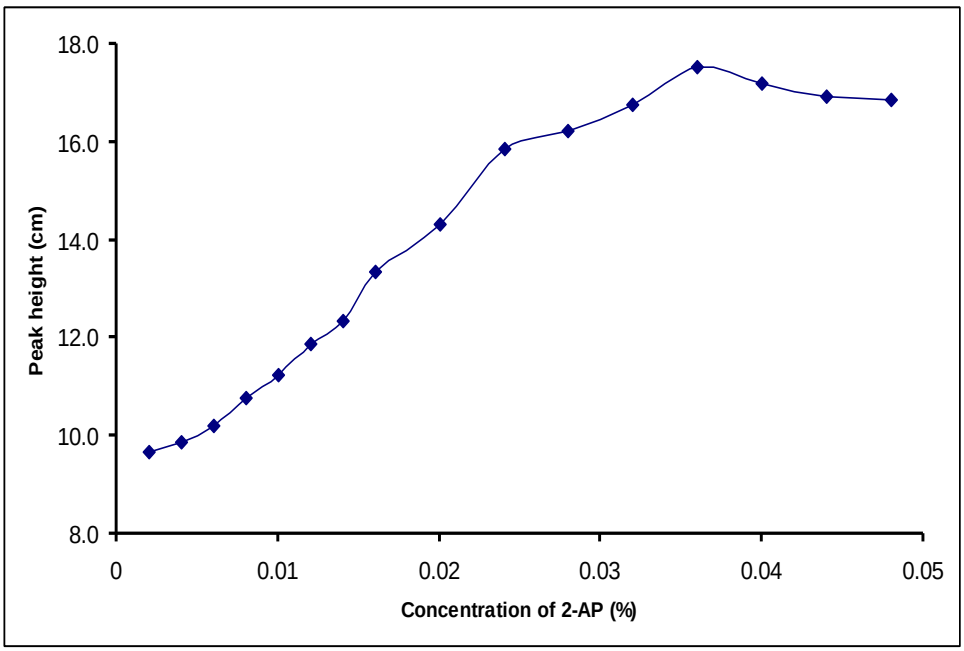

Figure - 5: Effect of the reagent concentration on peak height

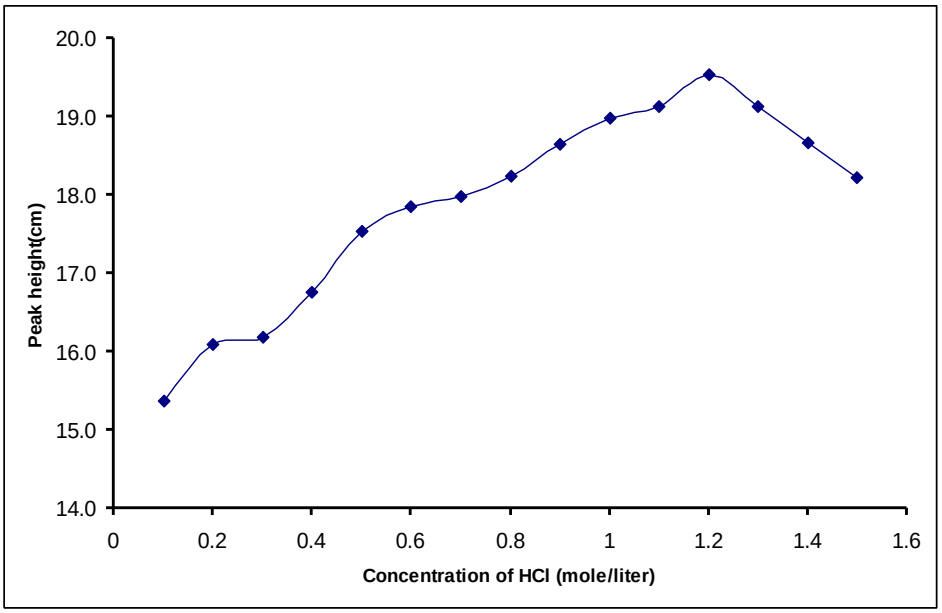

Figure - 6: Effect of the hydrochloric acid concentration on peak height

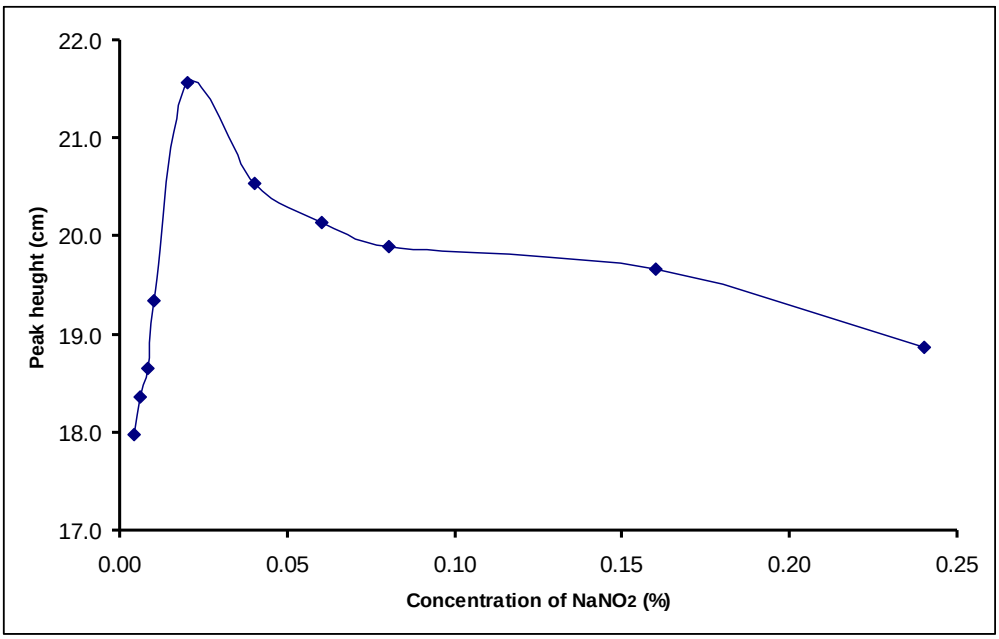

Figure - 7: Effect of the sodium nitrite concentration on peak height

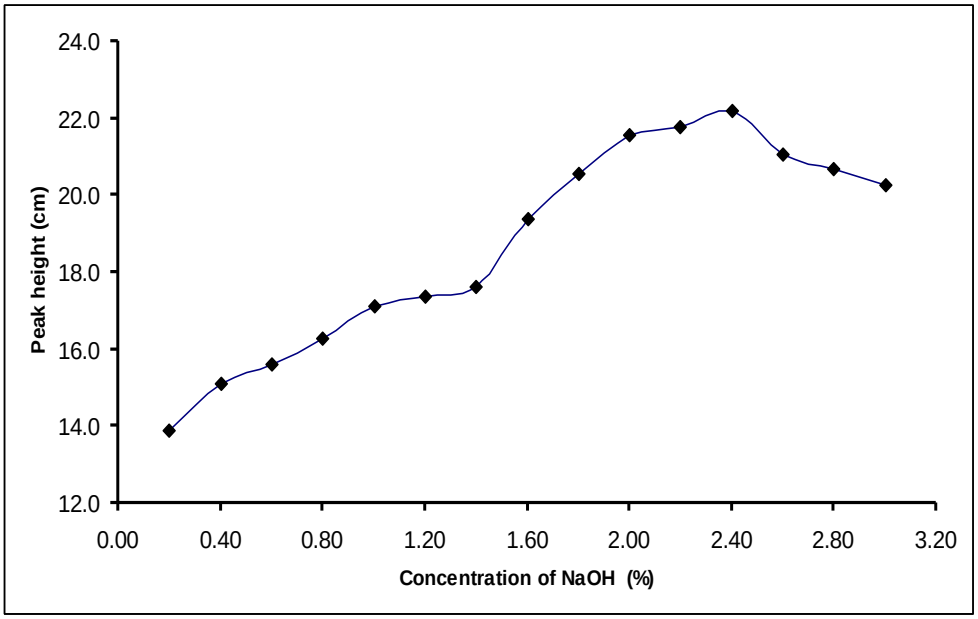

Figure - 8: Effect of the sodium hydroxide concentration on peak height

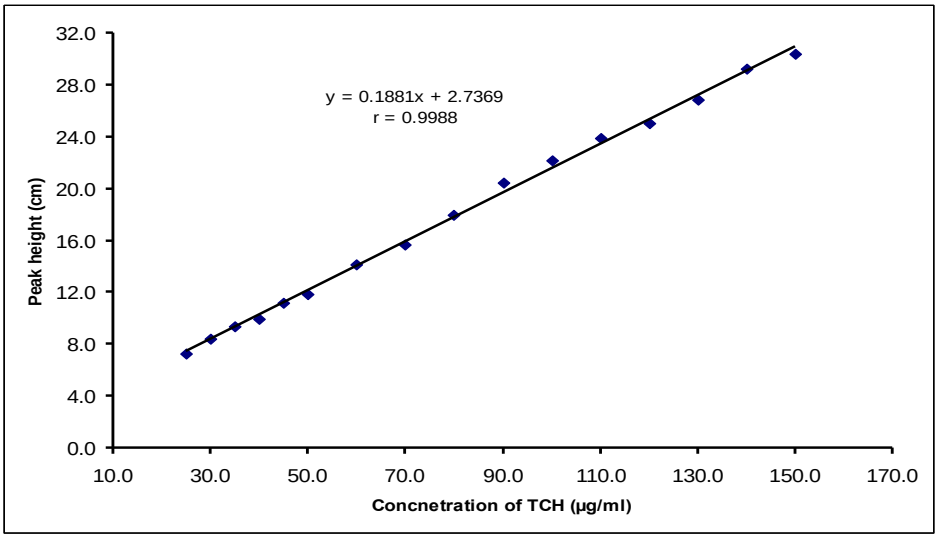

Figure - 9: Calibration curve of flow injection spectrophotometric determination of TCH.

Table - 3: Statistical data of FIS determination of TCH using 2-aminopyridin

Parameter	2-aminopyridin
Beer's law (µg/ml)	25.0-150.0
Sample frequency (s/h)	123
Correlation coefficient (r)	0.9988
Detection limit (µg/ml)	10

Table - 4: Precision and accuracy data of FIS determination of TCH

Reagents	TCH conc.(µg/ml)	SD	RSD%	E_{rel} %
2-aminopyridin	25.0	7.55×10^{-2}	1.04	-3.92
	80.0	4.435×10^{-2}	0.247	+1.06
	150.0	0.53176	1.75	-1.9

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