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Research Article

Determination of Rifampicin in Pure form and Pharmaceutical Preparations by Using Merging Zone-Continuous Flow Injection Analysis

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ABSTRACT

A simple, rapid, sensitive and accurate spectrophotometric method has been established for determination of rifampicin in pure form and pharmaceutical preparations by using merging zone-continuous flow injection analysis technique. The proposed method based on formation of a violet blackish colored complex between rifampicin and copper ion (II) at absorptivity maximum (565nm). Chemical and physical parameters of this system were investigated. Beers law was obeyed in the concentration range of (5-100µg.mL^{-1}) with detection limit (3µg.mL^{-1}) and Dispersion coefficient (1.28). The analytical parameters were optimized as the fallowing: flow rate is (2ml.min), reaction coil is (75cm), volume of rifampicin is (150µL), volume of copper ion is (125 µL), and concentration of copper ion is (100µg.mL^{-1}). The method was successfully applied to the analysis of the rifampicin in its pharmaceutical preparations.

Keywords: Drugs, Rifampicin, Flow injection analysis, continuous flow injection analysis, Pharmaceutical preparation.

INTRODUCTION

Rifampicin is a semisynthetic antibiotic, the chemical name of it 3-(4-Methylpiperazinyliminomethyl)rifamycin ($C_{43}H_{58}N_4O_{12}$) figure(1) and the molecular weight is (822.94 g.mol^{-1}), rifampicin is brownish-red crystalline powder, easy soluble in methanol but slightly soluble in acetone, ethanol and water, the melting point of it (183to188°C)[1,2]. Rifampicin is an antibiotic that used to treat various types of bacterial infections such as Tuberculosis, Leprosy and Legionnaires disease, it is used together with some other antibiotics such as isoniazid, ethambutol and pyrazinamide to treat tuberculosis[3,4]. The side effect of this drug is hepatotoxicity, allergic rashes, appetite loss and nausea or immunological disturbances[5]. Several methods have been used for determination of rifampicin in pharmaceutical dosage forms and biological fluids (serum, plasma) such as spectrophotometric[6-12], Liquid Chromatography-Mass Spectrometry[13], UPLC-MS-MS[14,15], Solid-Phase Extraction[15].Flow injection analysis is potential automated technique that used to determine chemical and biological samples because of its simplicity, convenience, rapidity, sensitive, cheap and Consumes small amounts of reagent [16,17]. One of most automated methods in flow injection analysis is continuous flow injection analysis that involved injected of sample and reagent solutions into current stream of carrier[18] then sample solution is mixed with reagent solution at any point to react and this process is controlled by peristaltic pump and reaction coil after that the reaction product passing through detector[19]. Continuous flow injection has some properties such as simplicity, sensitivity and selectivity therefore it is used in many application such as determination of chromate, Iron (II), copper (II) aniline blue dye[20-24]. The present method is based on formation of colored complex between copper (II) ion and rifampicin by using continuous flow injection technique. This method is successfully applied to determine rifampicin in pure form and pharmaceutical preparations.

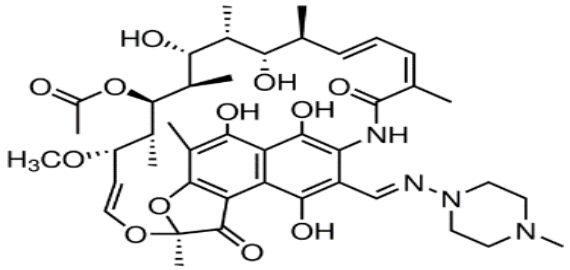

Figure 1: Chemical structure of rifampicin.

EXPERIMENTAL

Apparatus

UV-Visible Spectrophotometer, double –beam, Shimadzu model UV-1800 PC (Japan) with quartz cell of (1cm) path length was used for all spectral and absorbance measurements. Flow Injection system was consist of peristaltic pump (Ismatic, Germany), injection valve (Rheodyne, Altex 210, Supelco-USA), poly vinyl chloride flow tubes of (0.5) mm (Homemade) are used for reaction coils and to transport (reagent,sample) solutions, UV-Visible spectrophotometer, single-beam PD-303UV

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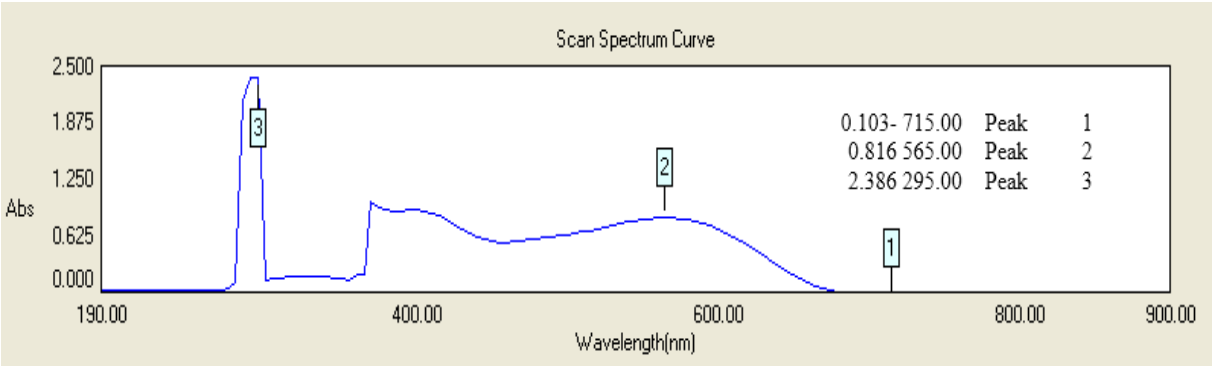


Figure 2: The UV-Visible spectrum of rifampicin –copper ion (II) complex.

Table 1: The repeatability of responses.

Conc. of rifampicin(µg.mL^{-1})	Peak Height (cm) n = 10						Mean	S.D	RSD%
40	3.63	3.61	3.60	3.62	3.62	3.63	3.615	0.0069	0.19
	3.63	3.61	3.62	3.60					

Table 2: The Effect of interferences on rifampicin drug.

Foreign compound	Concentration of Foreign compound (µg.mL^{-1})	Conc. of rifampicin (µg.mL^{-1})		Mean	Error%	Recovery%	R.S.D %
		taken	found				
Ascorbic acid	600	60	60.3	5.58	0.50	100.5	0.53
	60	60	60.1	5.56	0.16	100.16	0.47
	6	60	60.1	5.56	0.16	100.16	0.81
lactose	600	60	60.3	5.58	0.50	100.5	0.45
	60	60	60.2	5.57	0.33	100.33	0.31
	6	60	60.1	5.56	0.16	100.16	0.72

APEL, (Japan) with(flow cell, (450 µL) Helmma) as detector and recorder Kompensograph, (C 1032 Siemens, Germany).

Reagents

All reagents and chemicals used without futher purification and freshly prepared.

Standard solution of rifampicin solution (Semara Drugs Iraq SDI) (250) µg.mL^{-1}

Standard stock solution was prepared by accurately dissolving (0.025) gram of rifampicin in (1mL) of methanol with carefully stir and made up the volume to (100mL) volumetric flask with distilled water. The other standard solution of pure rifampicin drugs were prepared daily by suitable dilution of stock standard solution in water.

Standard solution of copper ion (II) solution (Sigma Alderg) (500) µg.mL^{-1}

Standard stock solution was prepared by accurately dissolving (0.3353) gram of copper ion (II) in (250 mL) calibrated volumetric flask and made up the volume with distilled water. The other standard solution of copper ion solutions were prepared daily by suitable dilution of stock standard solution in water.

Interferences solution (1000) mol.L^{-1}

A solution was prepared by dissolving accurate weighing of (0.1) gram from (Ascorbic acid, lactose) in (100 mL) calibrated volumetric flask and made up to the volume with distilled water.

Pharmaceutical preparations of rifampicin

Rifasynt capsules (Medochemie Ltd, Limassol-Cyprus): 300 mg rifampicin for each capsules.

Rifampicin capsules BP (Ajanta Pharma Limited-India): 300 mg rifampicin for each capsules.

Recommended procedure of Continuous merging zones

125 µL aliquots solution of pure rifampicin prepared at different concentration (1-100 µg. mL^{-1}) were injected into first loop while the second loop was injected by (150) µL of (100) µg mL^{-1} of copper ion (II), the excess of these solutions were drained to waste vent. After that, water was pump by peristaltic pump as flowing carrier. A new colored complex was formed as soon as the solutions of the two loops were mixed in the reaction coil. A sharp peak was depicted in recorder at λmax (565nm) as a result of entrance the complex solution to the cell.

Procedure of Pharmaceutical preparations

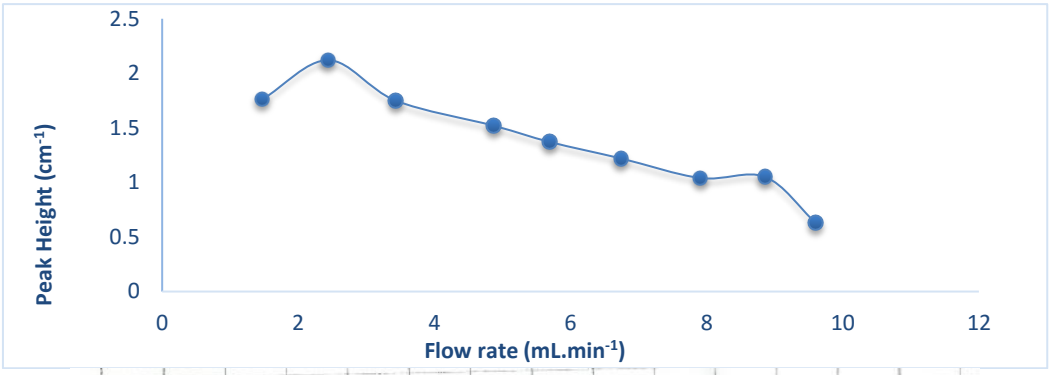
Capsules

A five capsules (300) mg/caps of (rifampicin) were weighted, a portion gram of these capsule which equivalent to (0.03) gram of rifampicin was weighed and dissolved in distilled water and transferred into volumetric flask capacity (100) ml, the volume was completed with water to the mark.

RESULTS AND DISCUSSION

Preliminary studies

In this study, rifampicin drug was mixed with copper ion (II) reagent, a violet blackish colored complex was



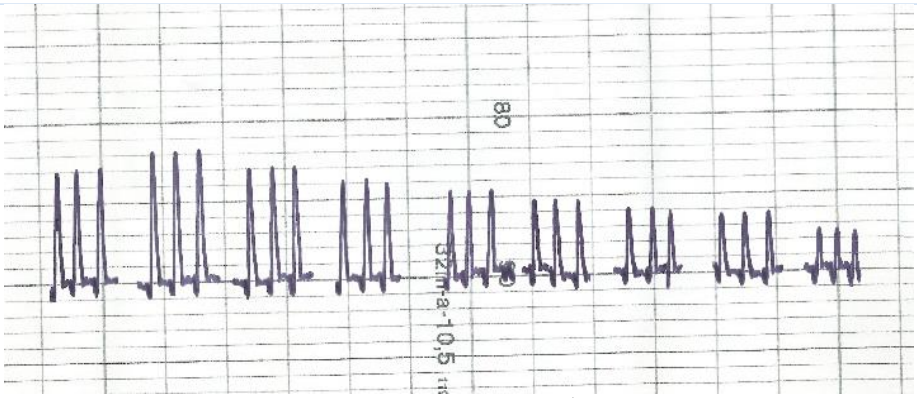
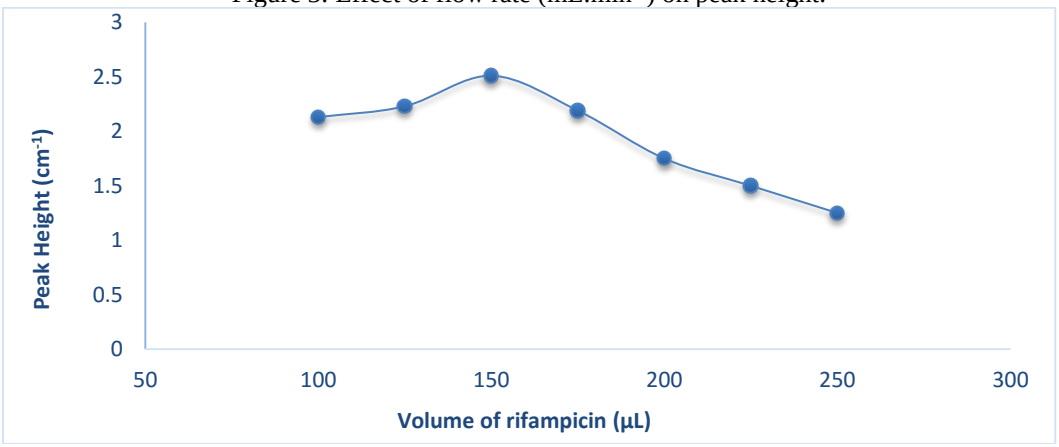


Figure 3: Effect of flow rate (mL.min^{-1}) on peak height.



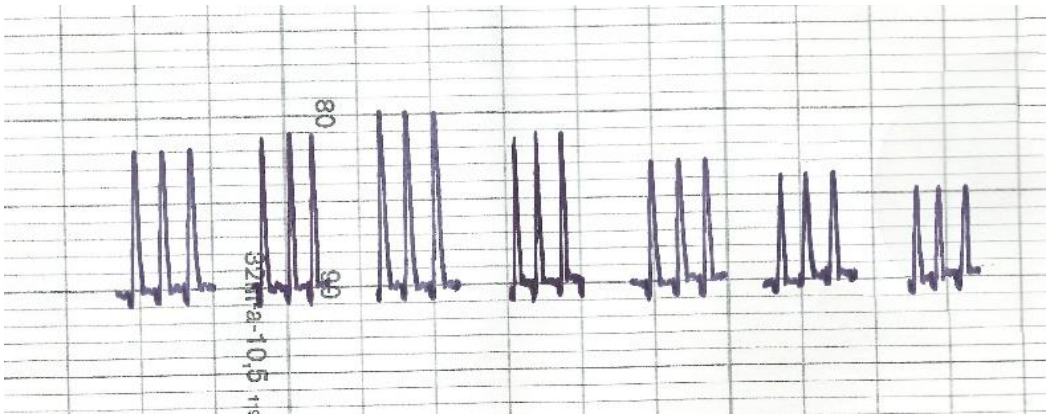
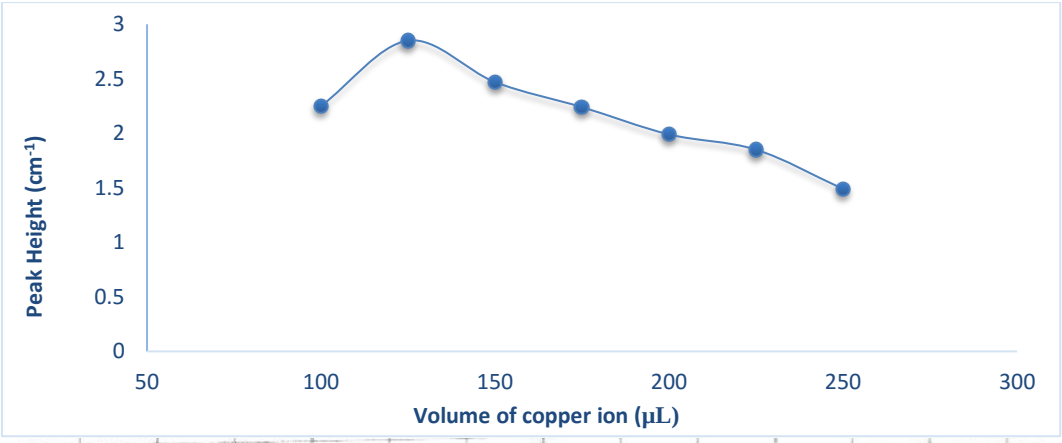


Figure 4: Effect of sample volume (µL) on peak height.



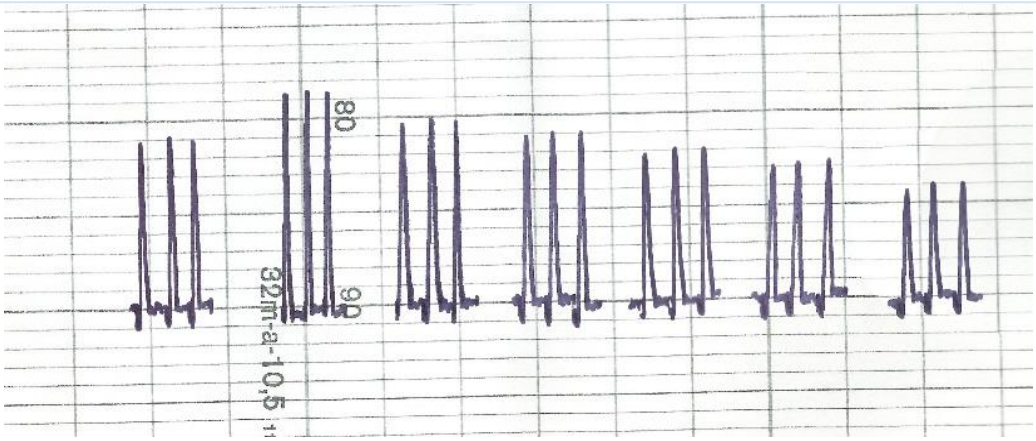
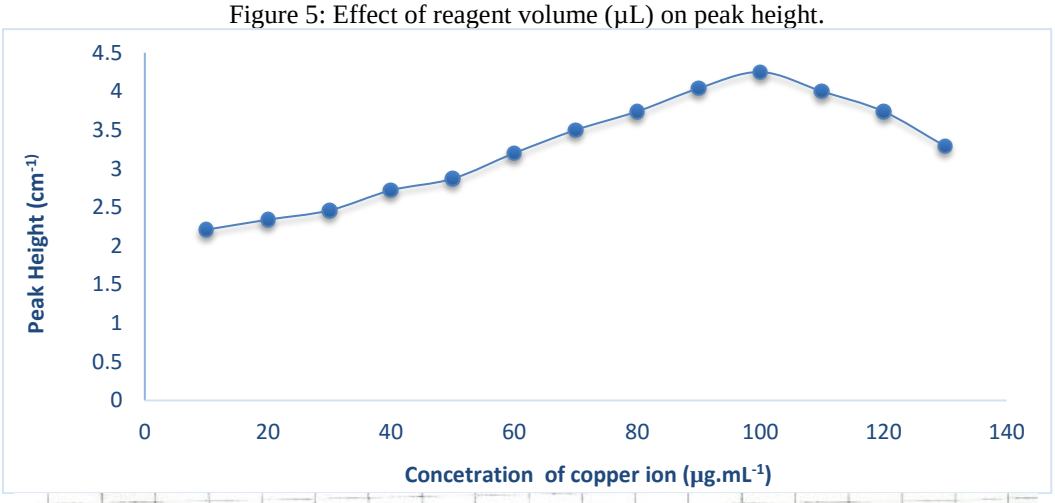


Figure 5: Effect of reagent volume (µL) on peak height.



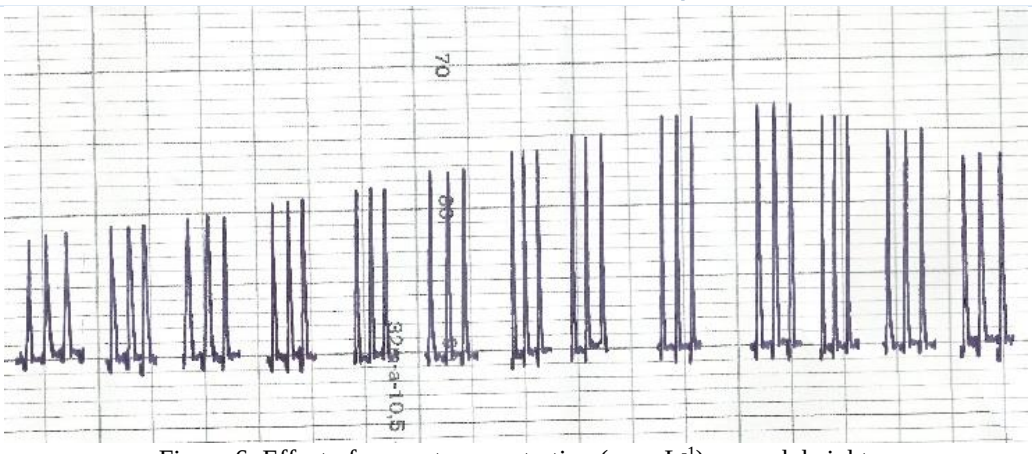


Figure 6: Effect of reagent concentration (µg.mL^{-1}) on peak height.

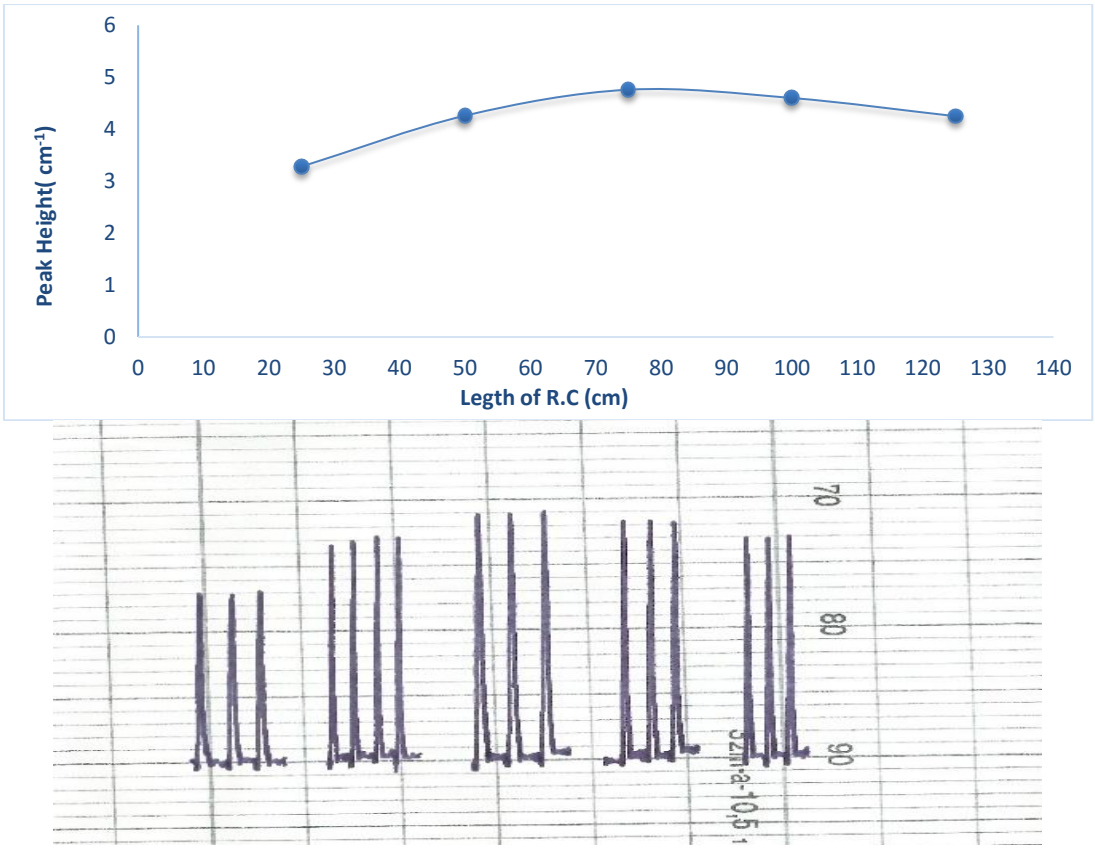


Figure 7: Effect of reaction coil length (cm) on peak height.

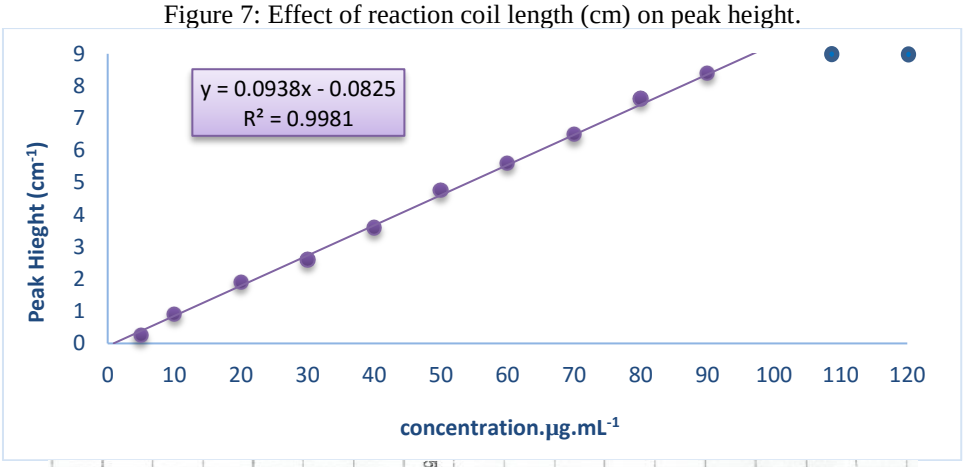


Figure 8: Calibration graph for rifampicin.

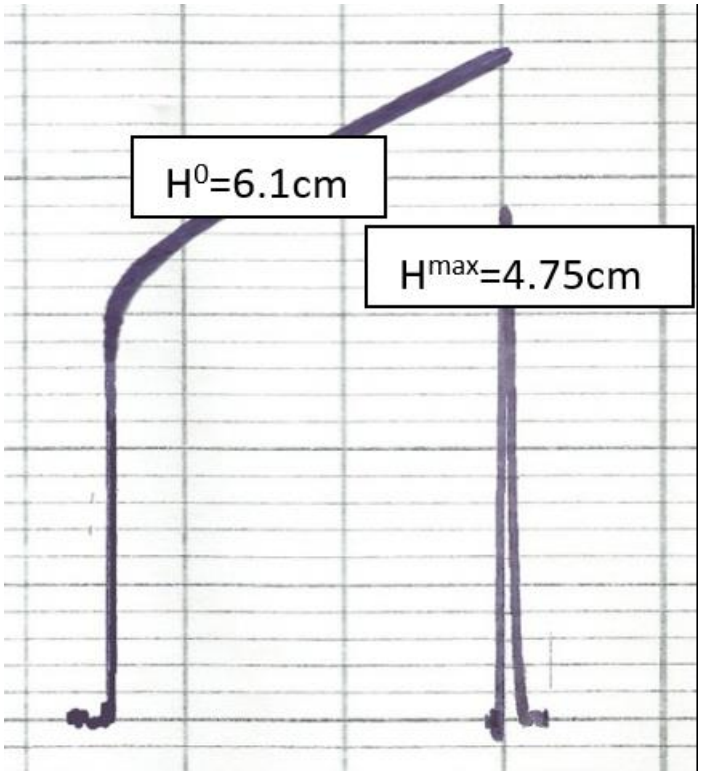


Figure 9: Dispersion Coefficient D.

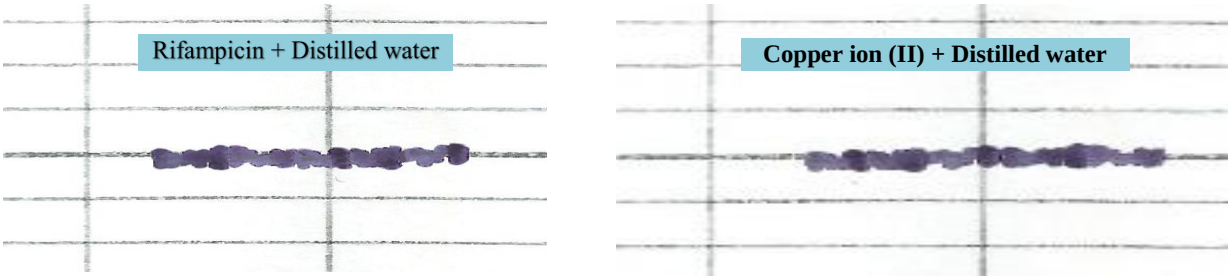


Figure 10: study of Dead Volume.

formed. This product has a maximum absorption at (565nm) figure (2) against blank solution.

Continuous merging zones flow injection analysis

Optimization of the experimental conditions

The effects of various (chemical and physical) parameters on the absorption intensity (Peak height) of the formed product were optimized.

Effect of flow rate

The effect of the flow rate on the peak height was studied in the range of (1.47-9.60ml min^{-1}) that shown in figure (3). This parameter showed that the lower flow rate was gave high response than the higher flow rate because the lower flow rate was provided more time to form the colored product. Thus flow rate (2ml.min^{-1}) gave high response (peak height).

Effect of rifampicin volume

The influence of the sample volume on the peak height was investigated by injected of different volumes (100-250µl) of rifampicin drug. The peak height was increased to maximum at (150 µl) of rifampicin drug after that the peak height was decreased thus this volume at (150 µl) of rifampicin drug was optimized in figure (4).

Effect of copper ion volume

The influence of the reagent volume on the peak height was inspected by injection different volumes (100-250 µl) of copper (II) ion solutions. (125 µl) of it was gave high response. After this volume of copper (II) ion, the peak height was decreased thus (125 µl) of it was chosen as typically volume. The figure (5) was showed this parameter.

Effect of copper ion concentration

A series of concentration of copper (II) ion were prepared from stock solution in range (10-100µg.mL^{-1}) then these solution were injected into the loop of copper (II) ion sequentially. The significant peak height was obtained at (100 µg.mL^{-1}) as can be seen in figure (6). Therefore (100 µg.mL^{-1}) was optimized.

Effect of reaction coil length

The effect of reaction coil length on peak height was studies in the range of (25-125cm) as shown in figure (7), when the reaction coil length transcend (100 cm) the

Table 3: The Pharmaceutical application of rifampicin.

Rifampicin Drug	Concentration of Drug (µg.m:$^{-1}$	Proposed Method				
	Taken	Found	Mean	S.D	RSD%	Error%	Rec%
Rifasynt capsules	25	24.86	2.25	0.036	1.60	-0.56	99.44
(Medochemie Ltd,	75	75.18	6.97	0.047	0.67	0.24	100.24
Limassol-Cyprus)							
Rifampicin capsules BP	25	25.08	2.27	0.026	1.16	0.32	100.32
(Ajanta Pharma Limited-	75	74.97	6.95	0.036	0.51	-0.04	99.96
India)							

absorbance decreased due to the dispersion thus (75cm) was optimized because it was provide greatest mixing for solutions without dispersion.

Calibration curve in Continuous FIA method

A series of rifampicin drug solution were prepared from stock solution in range (1-100µg.mL^{-1}) under (chemical and physical) optimum conditions, a result was showed in figure (8). The calibration curve is linear in the range of (5 -100 µg.m L^{-1}), the detection limit is (3 µg. mL^{-1}).

Determination of Desperation Coefficient (D)

Dispersion coefficient was calculated by conducting two experiments, in the first experiment the component of reaction (rifampicin and copper ion (II)) were mixed in suitable backer outside flow injection system taking into consideration the ratio (rifampicin : copper (II) ion). This mixed solution was passed through FI system to give continuous response that represents H^0 .While the second experiment involve injecting of component of reaction (rifampicin and copper ion (II)) into their loops and the carrier stream was water, in this case the response represents H^{max} that means peak height, the other optimum conditions were fixed in both experiments. The obtained values (H^0, Hmax) from these experiment were showed in figure (9), the equation used to calculate dispersion (D) was:

$$\mathbf{D = H^0 / H^{max}}$$

Where: $\mathbf{H^0}$: peak height without dilution outside the FIA system

$\mathbf{H^{max}}$: peak height with dilution inside the FIA system

The dispersion coefficient D value was (1.28) for concentration (50 µg.mL^{-1}) of rifampicin and (100µg.mL^{-1}) of copper (II) ion, a result was showed the dispersion coefficient (D) value was occured into the limited value of disperation.

Determination of repeatability

Repeatability can be calculated by using large number of repeated injections of sample. (40µgmL^{-1}) of rifampicin drug was used to study repeatability at (chemical and physical) optimum conditions. The result was shown in table (1) that a new FIA system has high precision and efficiency.

Determination of dead volume

Under the optimum parameters, dead volume can be determined by two experiment, in the first one the sample loop was injected with water instead of sample (rifampicin drug) .The same manner was applied on the reagent(copper (II) ion) loop in the second experiment. No signal appear in both experiment, however a sharp peak appear as soon as the injection of sample and reagent in their loops, hence there is no dead volume and new FIA system was operated in high efficiency as in figure (10).

Interferences

In this study, different concentration of certain interference were added to (60 µg.mL^{-1}) of rifampicin individually under optimum parameters. It was noticed there are no inferences where effect on estimation of drug in pharmaceutical preparation. A results were showed in table (2).

Application

The continuous merging zone method was successfully applied for determination of rifampicin in pharmaceutical preparation by using two concentration of each one and treated it in same manner in calibration curve. A results were showed in table (3) the possibility of applying the proposed method successfully in the Pharmaceutical preparation that containing rifampicin.

CONCLUSION

A simple, sensitive, rapid and accurate spectrophotometric method has been developed for determination of rifampicin in pure form and pharmaceutical preparations by using merging zone-continuous flow injection analysis technique. It is based on formation of colored complex between rifampicin and copper ion (II) that exhibits a maximum absorption at (565) nm, the proposed method was applied successfully to determination of rifampicin in pharmaceutical preparations.

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