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Flow injection Spectrophotometric determination of Penicillamine in Pharmaceutical Formulation Using 1, 2-naphthoquine-4-sulfonate.

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ABSTRACT

Flow injection analysis (FIA) method, using spectrophotometric detection, is proposed for the determination of Penicillamine (PA).The proposed method is based on the reaction between Penicillamine (PA) and 1, 2-naphthoquine-4-sulfonate(NQS) at alkaline medium (pH 11.5) to form deep brown-purple adduct, exhibiting maximum absorption (λmax) at 498 nm. A detailed study of the physico-chemical parameters affecting the systems performances has been carried out. Under optimized reaction condition, the method was linear regression equation of the calibration curve is A=0.0252+0.1203C (µg/mL), with a linear regression correlation coefficient of 0.9991. The detection limit is 3.00 µg/mL. The method has been successfully applied to the determination of Penicillamine in pharmaceutical formulation.

Keywords: Flow injection analysis, Penicillamine, 1, 2-naphthoquine-4-sulfonate, pharmaceutical formulation.

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INTRODUCTION

Penicillamine (PA) (2-Amino-3-mercapto-3-methylbutanoic acid) is naturally occurring sulfur – containing amino acid that belongs to the amino-thiol family. It is the main product of the decomposition of penicillin antibiotics [1]. Penicillamine is a chelating agent, which is used to aid the elimination of copper in the treatment of hepatolenticular degeneration (Wilson's disease) [2]. It has been also used in cystinuria, in heavy metal poisoning and for the treatment of rheumatoid arthritis [3]. Fig.1 shows the chemical structure of Penicillamine.

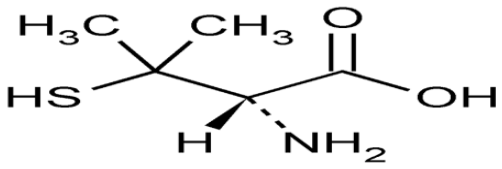

Fig. 1: The Chemical Structure of D- of Penicillamine (PA) (2-Amino-3-mercapto-3-methylbutanoic acid)

Several methods have been reported for the analysis of PA in both pharmaceutical preparations and biological samples. These methods include chemiluminescence [1], spectrophotometry [2-6], spectrofluorometric [7], high performance liquid chromatography (HPLC) [8] and capillary electrophoresis (CE) [9]. Most of the reported colorimetric methods are time consuming or lacking selectivity due to the problem of interference with degradation product of coloring agents. For these reasons, the need for a fast, sensitive, simple and selective method is obvious, especially for quality control analysis of pharmaceutical products containing Penicillamine.

Highly selective, sensitive and rapid flow-injection spectrophotometric analysis (FIA) method is used for the determination of Penicillamine (PA) in Pharmaceutical Formulation. The methods were based on The reaction between Penicillamine and 1, 2-naphthoquine-4-sulfonate [NQS]. Fig. 2 shows the chemical structure of [NQS].

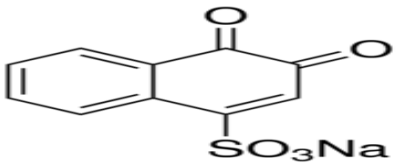

Fig. 2: The chemical structure of1, 2- naphthoquine-4-sulfonate [NQS].

NQS has been used as a color-developing reagent in the spectrophotometric determination of many pharmaceutical amines [10-12]. The applications of NQS for determination of pharmaceutical bearing amine group have recently been reviewed by Elbashir et al. [12].

EXPERIMENTAL

Apparatus

Analytical balance sensitive Denver Instrument, Spectrophotometer Labomed.in G single beam, USA, and a spectrophotometer Shimadzu UV-1700 spectrophotometer, Recorder Pen Siemens C 1032, Hitter thermal Ardeas 51, peristaltic pump Germany, Ismatic, files Interaction with the radius of 0.5 mm, homemade valves, pipes load of Teflon, flow cell volume of 450 µL, pH meter.

Unit

The various parameters affecting the unit have been investigated and selected for a final method evaluation; the following results allow the operator to choose different operation conditions, many new

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designs of valves were developed by the researchers [13-16]. We have been designing four new systems for flow injection analysis, as in Fig.s : 3,4,5 and 6.

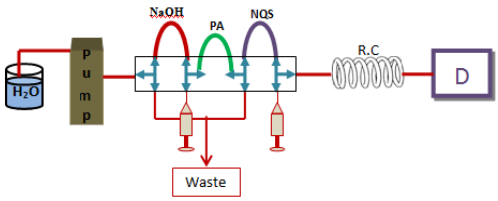

Fig.: 3 Designer 1

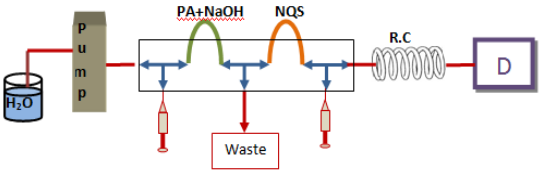

Fig.: 4 Designer 2

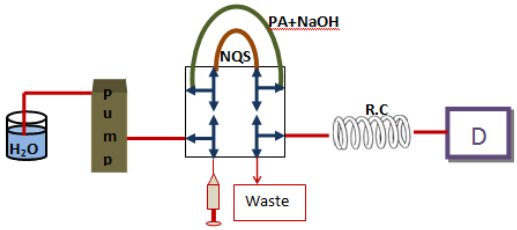

Fig.:5 Designer 3

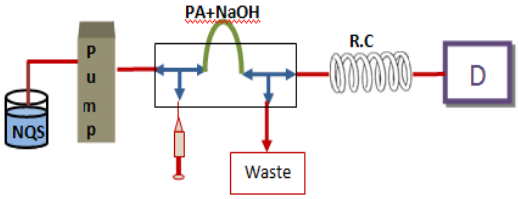

Fig.6 Designer 4

MATERIAL AND REAGENTS

A standard solution of 500 µg.mL^{-1} Penicillamine was prepared by dissolving 0.05 g of Penicillamine in 100 ml of (0.001 M) sodium hydroxide (NaOH) in calibrated flask, sodium-1, 2-naphthoquinone-4-sulfonate (NQS) solution of 0.02% (w/v) was prepared by dissolving 0.2 g in distilled water, transferred into a 1000 mL volumetric flask and diluted to the mark with distilled water and mixed well.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectrum of Penicillamine was recorded against water , it was found that Penicillamine exhibits a maximum absorption peak (λ max) at 198 nm. The reaction between Penicillamine and NQS was performed, and the absorption spectrum of the product was recorded against reagent blank (Fig. 7). It was found that the product is brown colored exhibiting λ max at 498nm, note that the λ max of NQS was 360 nm.

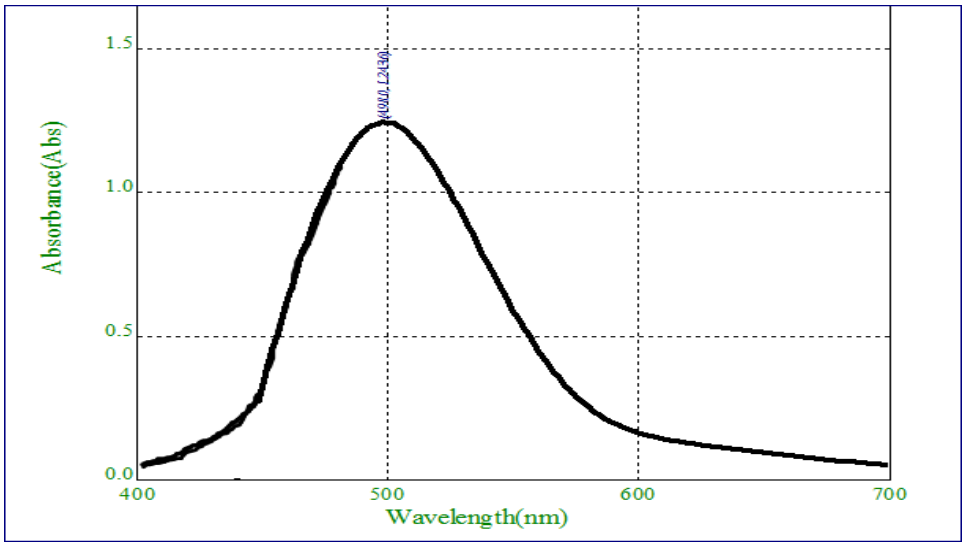

Fig.7: The UV-Visible spectrum of [Penicillamine–NQS] complex

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Study of optimum conditions

After choosing the best design(Designer 4 shown in Fig. 6) which gave the best response, The optimum conditions was studied.

Physical parameters

Effect of the reaction coil length

Table 1 and Fig. 8 shows effect of the reaction coil length on the peak height in the range (without-200) cm it was seen that the suitable reaction coil length was 175 cm, since it provided the greatest sensitivity.

Table 1: Effect of the reaction coil length on the peak height; [PA] = 100 ppm, flow rate (3.5 mL min^{-1}), [NQS] = 0.02%, and (PA) loop (L) = 25 cm.

Reaction coil length (cm)	Peak Height (cm)			Mean $\bar{Y}$	S.D	R.S.D%
without	0.21	0.21	0.21	0.21	0.00	0.00
60	2.30	2.30	2.20	2.30	0.06	2.50
100	2.90	2.90	2.90	2.90	0.00	0.00
125	3.70	3.70	3.70	3.70	0.00	0.00
175	4.70	4.70	4.60	4.70	0.06	1.20
200	4.60	4.60	4.60	4.60	0.00	0.00

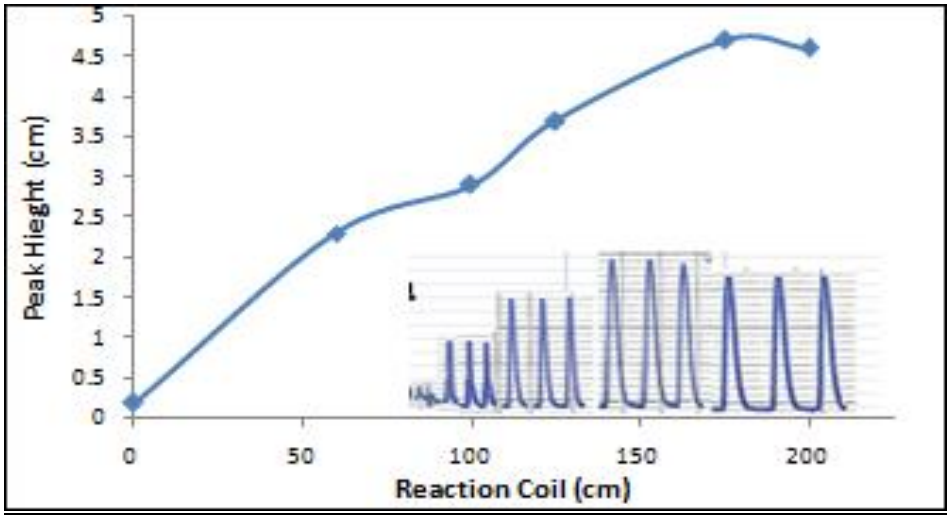

Fig. 8: Change the peak height with the reaction coil length.

Effect of the flow rate

The effect of the flow rate on the peak height was studied in the range of 1.5-5.25 mL min-1 (Table 2 and Fig. 9).It has to be noted increase in the peak height with increasing of flow rate until it reaches a certain amount (3.5 mL min-1),then the peak height decreased with the increasing flow rate. In the lower flow rate that the carrier solution did not sufficiently disperse into the middle of the sample zone[17]. On other hand, the peak height decreased with the increasing flow rate[18].

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Table 2: Effect of the flow rate on the peak height; [PA] = 100 ppm, Reaction coil(R.C.) = 175 cm, [NQS] = 0.02%, and(PA) loop (L) = 25 cm.

Speed of pump round.min^{-1}	Flow rate mL.min^{-1}	Peak height (cm)			Mean $\bar{Y}$	S.D	R.S.D%
20	1.50	4.10	4.10	4.10	4.10	0.00	0.00
30	1.75	4.20	4.40	4.30	4.30	0.06	1.35
40	2.50	4.50	4.40	4.60	4.50	0.07	1.29
50	3.27	4.60	4.60	4.50	4.60	0.06	1.26
60	3.50	4.70	4.70	4.70	4.70	0.00	0.00
70	4.25	3.80	3.80	3.80	3.80	0.00	0.00
80	4.75	3.50	3.50	3.50	3.50	0.00	0.00
90	5.00	3.10	3.10	3.20	3.10	0.05	1.84
100	5.25	3.10	3.10	3.10	3.10	0.00	0.00

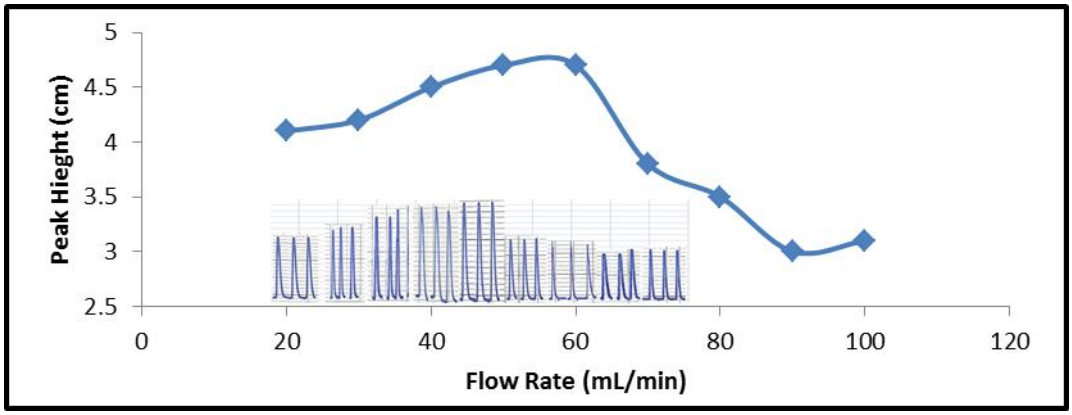

Fig.9: Effect of flow rate (mL.min^{-1}) on the response (cm).

Effect of Penicillamine volume

The influence of the sample volume on the peak height was investigated by injecting different volumes (117.86– 314.28) µL. The peak height increased to the maximum at 235.65 µL and after that volume, the peak height decreased. 235.65 µL was chosen for further work (Table 3 and Fig. 10).

Table 3: Effect of Penicillamine volume on the peak height ; [PA] = 100 ppm, Reaction coil(R.C.) = 175 cm, [NQS] = 0.02%, and flow rate (3.5 mL min^{-1}).

L of PA (cm)	The volume of Penicillamine (µL)	Peak Height (cm)			Mean $\bar{Y}$	S.D	R.S.D%
15	117.75	3.90	3.90	3.9	3.90	0.00	0.00
20	157.00	4.60	4.60	4.60	4.60	0.00	0.00
25	196.25	5.00	5.00	4.80	5.00	0.10	2.30
30	235.50	5.60	5.60	5.70	5.60	0.06	1.00
40	314.28	5.10	5.10	5.10	5.10	0.00	0.00

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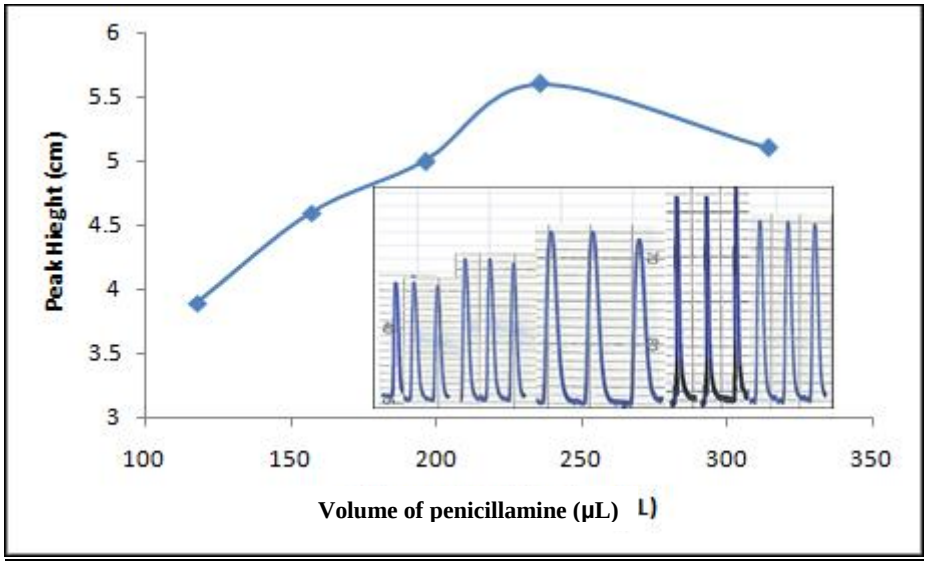

Fig.10: Change of peak height with Penicillamine volume in FIA unit.

Chemical parameters

The effect of NaOH concentration

The formation of the complex was studied in the different of NaOH concentration. The results showed that the best concentration of sodium hydroxide was 0.001M, as, as shown in table:4 and Fig.11.

Table 4: Effect of NaOH concentration on the peak height.

Conc. NaOH (mol / L)	Peak Height (cm)			Mean Ȳ	S.D	R.S.D%
0.0001	0.50	0.50	0.48	0.49	0.012	2.300
0.0003	1.20	1.20	1.20	1.20	0.000	0.000
0.0050	5.60	5.60	5.80	5.67	0.115	2.000
0.0010	10.00	10.00	10.00	10.00	0.000	0.000
0.0030	8.70	8.70	8.70	8.70	0.000	0.000

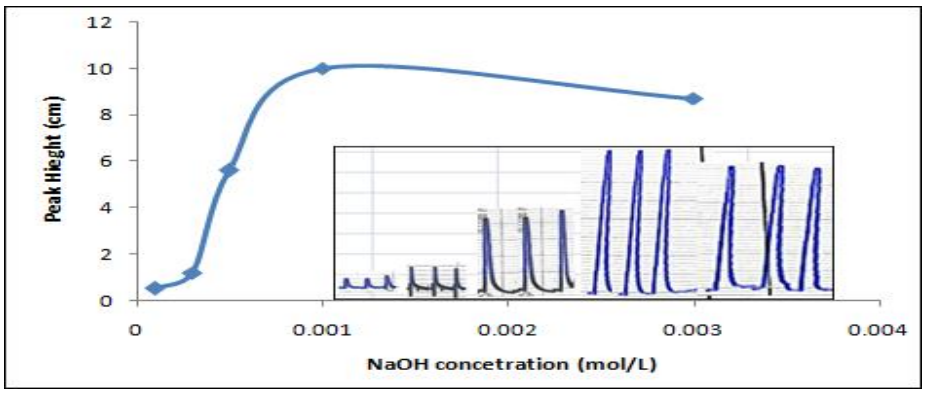

Fig. 11: Change of peak height with [NaOH] in FIA unit.

Effect of the reagent concentration [NQS].

The reagent concentration [NQS] was varied in the range (0.01 –o.o3)% in order to maximize the peak height. Table 5 and Fig. 12 show the effect of reagent concentration on the peak height .The maximum peak height was obtained with 0.02% NQS and therefore, the 0.02% NQS was chosen for further work.

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Table 6: Effect of the reagent concentration [NQS] on the peak height.

[NQS] %	Peak Height (cm)			Mean Ȳ	S.D	R.S.D%
0.010	8.30	8.30	8.30	8.30	0.000	0.000
0.015	8.90	8.90	8.80	8.87	0.058	0.650
0.020	10.20	10.20	10.20	10.20	0.000	0.000
0.025	9.00	8.80	9.00	8.93	0.115	1.290
0.030	8.00	8.00	8.00	8.00	0.000	0.000

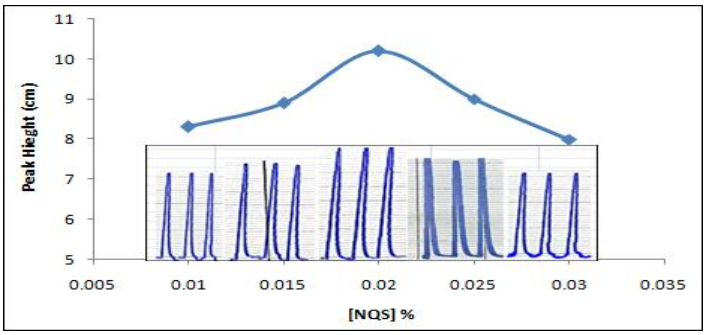

Fig. 12: Change of the peak height with reagent concentration(NQS) in FIA unit.

Study of the dead volume:

To ensure accurate results obtained from this unit, we must be studied. Wherever, the dead volume is small it means a best results. Two experiments were done, in the first the water (H2O) was injected in the loop instead of Penicillamine and there was no response ,in the second experiments the water (H2O) was passed as the carrier instead of reagent [NQS] and there was no response. This shows the efficiency of the system.

Calibration curve in FIA method:

Calibration curve was prepared at the optimum conditions of complexity and change through Penicillamine concentration the result show in Table 7 and Fig.13. The calibration curve is linear in the range of 5 -120 mg. L-1. The detection limit is (5) mg. L-1.

Table 7: Effect of the concentration of Penicillamine con. with peak height (Calibration graph).

Conc. Of PA. µg. mL^{-1}	Peak Height (cm)			Mean Ȳ	S.D	R.S.D %
5	0.70	0.70	0.70	0.70	0.0000	0.0000
10	1.25	1.20	1.20	1.22	0.0289	2.3727
20	2.50	2.50	2.50	2.50	0.0000	0.0000
30	3.50	3.50	3.50	3.50	0.0000	0.0000
40	4.80	4.80	4.80	4.80	0.0000	0.0000
50	5.80	5.80	5.80	5.80	0.0000	0.0000
60	7.40	7.40	7.40	7.40	0.0000	0.0000
70	8.60	8.60	8.60	8.60	0.0000	0.0000
80	9.50	9.50	9.50	9.50	0.0000	0.0000
90	10.80	10.80	10.80	10.80	0.0000	0.0000
100	11.50	11.50	11.50	11.50	0.0000	0.0000
120	13.90	13.90	13.90	13.90	0.0000	0.0000

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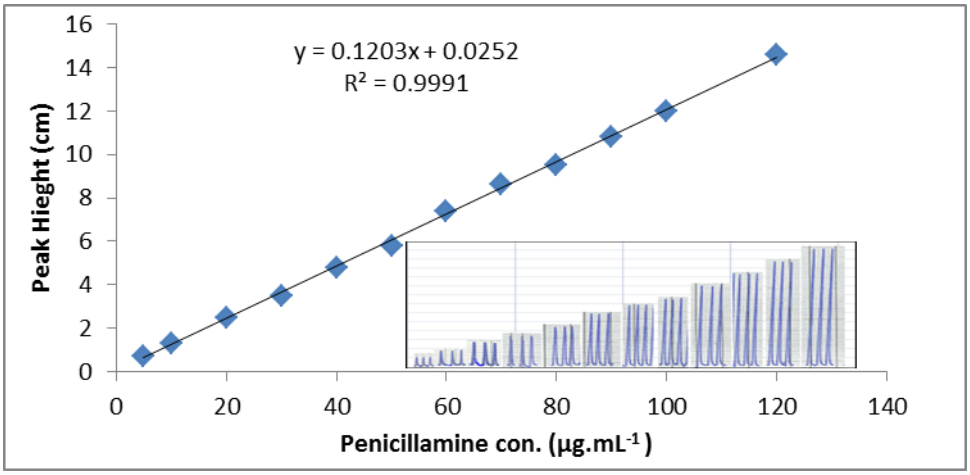

Fig.13: The calibration graph for variable Penicillamine concentrations

Reproducibility

For study Prevision range and effective method in determination of Penicillamine was studied through reproducibility injection and measure for multitudes, using 40 ppm and 80 ppm concentration of Penicillamine, so that amount of standard deviation for (40 mg/L) and (80 mg/L) was n = 6 and amount of relative standard deviation was 0.529% for accuracy and effective system for determination of Penicillamine. The results are shown in Table 8 and Fig. 14.

Table (8) : The repeatability of responses

Conc. of Pen µg.mL^{-1}	Peak Height (cm) n=6						Mean $\bar{Y}$	S.D	R.S.D%
40	4.90	4.90	4.85	4.90	4.90	4.85	4.88	0.026	0.529
80	9.50	9.50	9.50	9.50	9.50	9.50	9.50	0.000	0.000

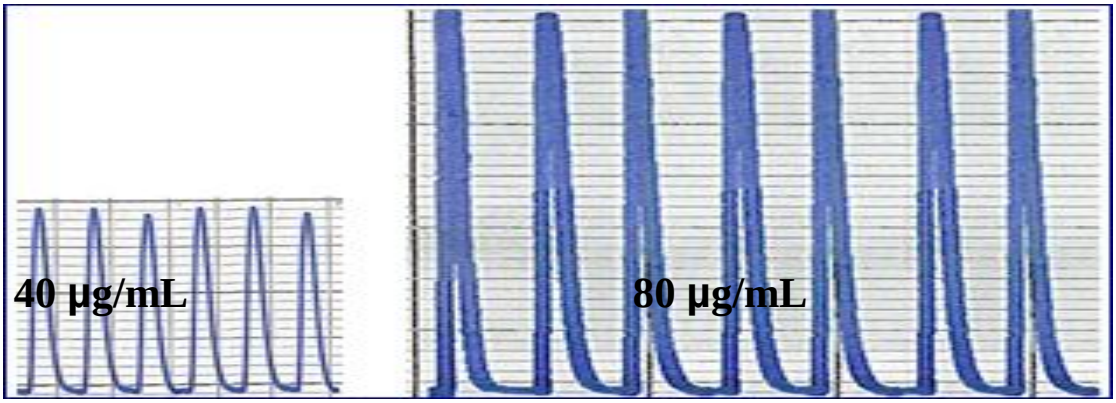

Fig.14:The repeatability of responses.

Determination of dispersion

To measure the dispersion value in different sample zones of 40 and 80 ppm Penicillamine, two experiments were carried out. In the first experiment, after mixing of reactants (Penicillamine and NQS) that passes through manifold unit gives continuous response; this indicates non-existence of dispersion effect by convection or diffusion. This measurement represents (Ho). The second experiment includes injecting different concentration of Penicillamine(40 and 80 ppm) for FIA. The obtained value from this experiment represents intensity response for sample injected (Hmax)[19-22]. The equation used to calculate dispersion (D) is: D ° = Ho/Hmax . These values fall in limit state of dispersion (Table 9 and Fig.15).

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Table 9: Determination of dispersion.

PAConcentration (ppm)	Response (cm)		Dispersion (D)
	H_{max}	H^o	
40	7.50	5.20	1.50
80	11.50	9.50	1.20

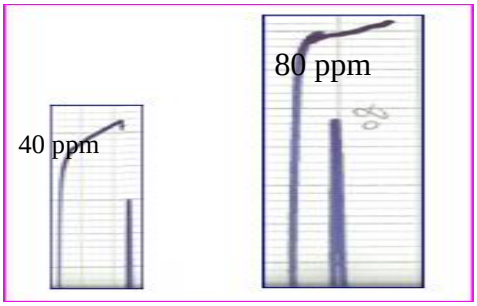

Fig.15: The dispersion for the two concentrations.

Determination of Penicillamine in pharmaceutical product

The proposed method was successfully applied for the analysis of Penicillamine in capsules and prepared solution. The results obtained show good agreement with the labeled information given by the manufacturer , and good agreement between the taken concentration and the recovered amounts of Penicillamine , as shown above in table (10).

Table (10): Penicillamine content found in the prepared solution and analyzed capsule.

Sample	Taken concentration $\mu g.mL^{-1}$	Found concentration $\mu g.mL^{-1}$	S.D	R.S.D %
Prepared solution	30	30	0.00	0.00
	50	50	0.00	0.00
(PA) capsule	30	29	0.35	1.19
	50	48	1.06	2.15

CONCLUSIONS

The new FIA system with single- channel manifold to be efficient for the determination of Penicillamine are selective, low cost , rapidity, simplicity, temperature independent, and applicable without resorting to an extraction step.

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