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Chemometric Optimization of Sequential Injection Spectrophotometric Method for Chlorpheniramine Determination in Pharmaceutical Formulations

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Abstract

A new and fast sequential micro injection analysis (SIA) method for an accurate assay of chlorpheniramine maleate, based on its oxidation by acidified potassium permanganate was optimized. A 2^3 factorial design was applied for both surface plot and factor effect. The experimental conditions were fully optimized so that the method is suitable for industrial application. Some of the optimum parameters were found to be 35µl of 1×10^{-3} mol l^{-1} sulfuric acid, 30µl of 1×10^{-3} mol l^{-1} permanganate, and 30µl of chlorpheniramine maleate with a flow rate of 25µl s^{-1}. Beer' s law was obeyed in the concentration range of 20 – 150 ppm with regression calibration equation (R=0.0016C+0.0064) and correlation coefficient of 0.9998. Chlorpheniramine maleate assay showed an excellent mean recovery and R. S. D. of; ± 1.8% for synthetic sample, ± 1% for Allerfin injection and ± 1.1% for Anallerg tablets. The method was applied to tablet forms as well as injection forms and validated with the British Pharmacopoeia. The developed new SIA method is fully automated, rapid, sensitive, accurate and cost-effective, and therefore suitable for routine pharmaceutical analysis application. Accordingly, it is recommended and proposed to be used in pharmaceutical industries.

Keywords: Sequential injection analysis, Chemometric optimization, Spectrophotometry, Chlorpheniramine maleate,

1. Introduction

Chlorpheniramine maleate (CPM) is chemically named as 3-(4-chlorophenyl) -N-N-dimethyl -3-pyrid -2-ylpro- panamine hydrogen (2)-butenedioate (fig.1). It is a white, crystalline powder soluble in both water and alcohol, but it is slightly soluble in ether [1].

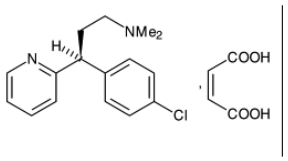

Fig.1. Chlorpheniramine maleate structure

Chlorpheniramine maleate, an alkylamine derivative, is a sedating antihistamine that causes a moderate degree of sedation; it also has antimuscarinic activity. It is used for the symptomatic relief of allergic conditions including urticaria and angioedema, rhinitis and conjunctivitis, and in pruritic skin disorders. It is a common ingredient of compound preparations for symptomatic treatment of coughs and common cold [2].

Many analytical methods have been developed for the assay of chlorpheniramine maleate in pharmaceutical preparations.. Several methods were reported involving official standard methods as well as non-official standard methods. In the British Pharmacopoeia [1] method, chlorpheniramine tablets were assayed spectrophotometrically at a wavelength of 265 nm. This method is lengthy and requires long procedures of extractions and dilutions, hence, lot of reagents are consumed. However, In case of injection form the samples are diluted and the absorbance is measured at the same wavelength.

Several analytical methods have been developed for the determination of chlorpheniramine maleate in pharmaceutical formulations. These methods include, polarography [3], spectrophotometry [4, 5], infra red spectroscopy [6], micellar liquid chromatography [7,8], capillary electrophoresis [9, 10], HPLC and capillary electrophoresis [11]. ion-selective sensors [12], HPLC [13- 15], HPLC and spectroscopy [16] and atomic emission spectrophotometry [17]. Most of the methods mentioned above have some drawbacks. Generally, they require lengthy procedures and consume lot of reagents. It is therefore necessary to find a more effective, economical, automated and specific method as an alternative.

In the current work, sequential micro injection analysis (SIA) was utilized for the development of an oxidation-based spectrophotometric method for the assay of chlorpheniramine maleate (CPM) in tablets and injections forms with chemometric optimization approaches. The newly adopted method has the advantages of being fully automated, reagent –saving (using microliters) and accurate.

2. Experimental

2.1. Reagents

Chlorpheniramine maleate supplied from sigma C3025 was used for preparing a standard solution of 200 ppm. This standard solution together with the working standard solutions were immediately prepared before use.

Tablets were prepared by triturating 20 tablets, and then the required amount of powder was dissolved in water and filtered. The filterate was diluted to the required volume.

Potassium permanganate supplied from Fisher Scientific Co. Fair Lawn, New Jersey 07410 USA was used to prepare a stock solution of 0.01mol l^{-1}. The stock solution was stored in a dark place, covered with an aluminum foil and weekly standardized by sodium oxalate.

Sulfuric acid 95-98 % (W/V^{-1}), 1.84 g l^{-1}, supplied from Fisher Scientific Co., Fair Lawn, New Jeresy USA was used to prepare working standard solutions.

2.2.Instrumentation

The SI analyzer manifold used in this work consisted of a sequential injection analyzer combined with a miniaturized fiber optic spectrophotometer. The SI analyzer system is an Alitea USA/FIALab 3000 (Medina, WA USA). Figure 2 shows the different components of the manifold. The sequential injection analyzer manifold is composed of a syringe pump (SP); a holding coil (HC); a multi-position valve (MPV); a reaction coil

(RC); radiation source (RS); a Z-flow cell (Z) with SMA fiber optic connectors, a pump tubing detector (D) and personal computer (PC). The syringe pump is 24,000-steps stepper motor driven (with an optical encoder feedback and 1.5 seconds to 20 minutes per stroke) of a 2.5ml size. It is > 99% accuracy at full stroke. The MPV used has eight ports with standard pressure of 250 psi (gas) and 600 psi (liquid); zero dead volume and chemically inert. The Z-cell is a 10 mm path length plexiglass compatible with standard SMA terminated fiber optic and contains fused silica windows as wetting surfaces at each fiber optic junction. Pump tubing of a 0.30" ID Teflon type supplied by Upchurch Scientific, Inc. (Oak Harbor, WA USA). The Pump tubing is used to connect the different units of SIA manifold.

The fiber optic spectrometer is composed of a light source, fiber optic connectors, Z-flow cell, windows and a detector. The radiation source used is an LS-1 Tungsten Halogen (Ocean Optics, USA) optimized for VIS-NIR (360 nm -2µm) wavelength range [18]. The detector is The USB2000 fiber optic spectrometer manufactured by Ocean Optics, USA adapted to 200 -1100 nm wavelength range.

FIALab for windows version 5.5 from FIALab (Medina, WA USA) was utilized for programming and controlling SIA system.

OOIbase software version 2.0.1.2, driver version 4.07.00, 2002 supplied from Ocean Optic, Inc. was used for spectrophotometric data acquisition and treatment.

SigmaPlot supplied from Jandel Sceintific Corporation (1994) version 1.02 for windows 3.95 enhanced modes was used for chemometric calculations and for the drawing of three dimensional graphs.

Excel for windows 2003 was used for plotting calibration curves and their related calculations.

2.3. Procedure

An automated and cost-effective SIA method is proposed for the assay of chlorpheniramine maleate in pharmaceutical formulations. The method is based on the oxidation of CPM using potassium permanganate in sulphuric acid medium. The controlled conditions accompanied by chemometric optimization introduce a promising method for pharmaceutical analysis.

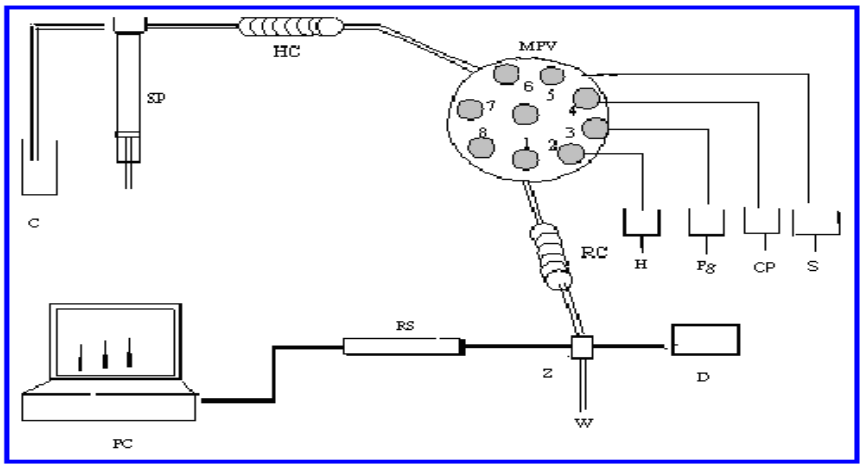

Fig. 1 Fig. (2) Sequential injection analyzer manifold diagram; C: carrier solution (water); syringe pump (SP); holding coil (HC); multi-position valve (MPV); reaction coil (RC); RS: radiation source; Z-flow cell (Z); detector (D); personal computer (PC); H: Sulfuric acid; Pg: Permanganate; CP: Chlorpheniramine; S: Spacer (water) and W: Waste.

The SIA manifold applied for the oxidation of chlorpheniramine maleate,as shown Fig. 2. The steps of applied SIA program are:

1. Water was linked to the syringe pump through in-position mode to push reagents to the required part of SIA manifold.
2. Sulphuric acid, permanganate, chlorpheniramine and water were linked to the selector valve through ports 2, 3, 4 and 5.
3. The syringe was filled with 1500 µl of water by directing the bi-directional valve to position with flow rate of 150 µl s^{-1}.
4. The Teflon tubes were filled with their respective reagents by performing aspirations runs and directing the bi-directional valve to the out-position mode.
5. Same fast flow rate, the syringe was emptied as step 3
6. 35 µl of sulphuric acid, 30 µl of permanganate and 50 µl of water were sequentially aspirated into the holding coil and short reverse strokes were performed three times to allow the mixing of the reagents to take place at flow rate 25 µl s^{-1}.
7. With a flow rate of 50 µl s^{-1}, 250 µl volumes was dispensed to the reaction coil and the mixture was delayed.
8. 1200 µl volume was dispensed with a flow rate of 25 µl s^{-1} to the Z-flow cell passing through the reaction coil and at the same time to the reference and absorbance scan were carried out at wavelength 507, 526, and 546 nm, and the maximum absorbance was recorded as the value a_1.
9. The syringe is emptied and refilled with 1500 µl of water.
10. To ensure the repeatability of dispersion and mixing, steps 6, 7, 8 and 9 were repeated three times and the average of a_1 (last two measurements) was calculated as A_1.
11. 35 µl of sulphuric acid, 30 µl of permanganate, 30 µl of chlorpheniramine and 20 µl of water were sequentially aspirated into the holding coil and short reverse strokes were performed three times to allow the mixing of the reagents at flow rate 25 µl s^{-1}.
12. With a flow rate of 50 µl s^{-1}, 250 µl volumes was dispensed to the reaction coil and the mixture was delayed for the required time to allow the reaction to take place.
13. 1200 µl volume was dispensed with a flow rate of 25 µl s^{-1} to the Z-flow cell passing through the reaction coil and at the same time to the reference and absorbance scan were carried out at wavelength 507, 526, and 546 nm and the maximum absorbance was recorded as the value a_2.
14. The syringe is emptied and refilled with 1500 µl of water.
15. To ensure the repeatability of monitoring the reaction, steps 11, 12, 13 and 14 were repeated three times and the average of a_2 (last two measurements) was calculated as A_2.

The response R of the reaction was calculated using equation

$$R = A_1 - A_2 \quad (1)$$

3. Results and Discussion

The utilization of SIA for the assay of chlorpheniramine in pharmaceutical formulations was by measuring the absorbance

3.1. Optimization of variables

Key parameters that influence the performance of the proposed SIA method were studied in order to establish the optimum physical and chemical working conditions.

3.1.1. Physical parameters

Physical parameters of the SIA system usually control the extent of the reaction and the degree of dispersion. These parameters are responsible for delivering appreciable amount of the product for detection. The response is directly proportional to the color intensity, consequently to the concentration.

3.1.1.1. Wavelength selection

For permanganate spectrum, different wavelengths were tested. It was found that 507nm and 526nm gave poor results. The wavelength of 546nm was found to give the maximum absorbance and was adopted during this work.

3.1.1.2 Time

The reaction between chlorpheniramine and permanganate in acidic medium is based on the oxidation of chlorpheniramine by permanganate. The difference in absorbance between the blank and the analyte was measured. Since this reaction was found to be not fast enough, the time was optimized. Chemometric optimization including the time was performed as shown in Figures 5-8. The optimum time for this reaction was 4.0 min. By increasing time more than 4 min, signal does not increase while by decreasing time less than 2 min the signal was decreasing.

3.1.1.3 Flow rate

The contact period is critical in order to get a significant signal. Therefore, the flow rate was optimized and a value of 50µl s^{-1} was the optimum for the carrier solution. However, the optimium flow rate of the reagents from the multiposition valve to the holding coil was 25µl s^{-1}.

3.1.1.4 Volume

The volumes of the reagents and analyte used throughout the method were kept constant; 30µl chlorpheniramine, 30µl permanganate; 35µl acid and 20µl of spacer solution.

3.1.1.5 Effect of HC and RC dimensions

The dimensions of the holding coil were found to have a limited effect on the generated signal.

3.1.2 Chemical parameters

The proposed SIA system for the determination of chlorpheniramine is based on the oxidation of CP with potassium permanganate in acidic medium. The limit of detection and the linear range of this method were determined by changing the concentrations of each of these chemicals.

3.1.2.1 Effect of sulphuric acid concentration

Preliminary studies revealed that the resulting signal obtained from the oxidation of CP with potassium permanganate depends on the concentration of sulphuric acid. The optimum concentration of this acid which produces a considerable signal ranges between 1×10^{-3} mol l^{-1} and 1×10^{-2} mol l^{-1}. The concentration of 1×10^{-3}mol l^{-1} was adopted and chemometric optimization of the effect of acid is shown in Figures 3-6. By increasing the acid concentration more than 0.01M, no improvement in the signal was observed. Decreasing the acid than 0.001M, lowered the signal.

3.1.2.2 Effect of permanganate concentration

Permanganate has strong oxidizing capabilities and high molar absorptivity. Hence, it has a profound effect on the oxidation process, the concentration of permanganate was optimized. Considerable and reproducible results were obtained when the concentration range was between 5×10^{-4} and 1×10^{-3} mol l^{-1}. Permanganate concentration of 1×10^{-3}mol l^{-1} was employed during the work. The complete chemometric optimization of the permanganate concentration is shown in Fig. 3-8.

3.2. Sequential Chemometric Optimization

Chemometrics is so successful for the optimization of chemical reactions in SIA procedures and found to be efficient when three or more interactive variables are considered. Sequential chemometric optimization was employed to find out the optimum operating conditions of the proposed system and to check the parameters that affect the efficiency of the method [19-23]. Several papers dealing with chemometric optimization of SIA methods for pharmaceutical analysis appeared in the literature. For example, the assay of bromazepam [25], ascorbic acid [26], Perphenazine [27], flouroquinolone antibiotics [28], and promazine [29].

3.2.1 Experimental Design (Factorial Design)

The experimental design approach was employed and 2^k factorial design was considered. In this design, 2 stands for the higher and lower values variable levels and k is the number of factors studied [24]. In this study, three factors were considered including sulphuric acid concentration, permanganate concentration and the delay time. The highest and lowest values were determined and assigned +1 and -1 coded levels respectively, and the matrix was arrayed as shown in Table 1. The lowest and the highest values were determined based on the same criteria mentioned above. Eight experiments as a result of a 2^3 factorial design were performed and the original levels as well as their responses are shown in Table 2.

3.2.1.1. The response surface method

The results obtained from the factorial design were interpolated and plotted using SigmaPlot software package. The surface plot of the response as a function of sulphuric acid concentration and permanganate concentration levels were plotted as in Figures 3 and 4. It is obvious that the surface response increases with an increase in the concentration of permanganate and to some extent with an increase of the sulfuric acid concentration. Figures 3 and 4 indicate that the effect of permanganate concentration on the response is more than that of the acid concentration. Figure 5 shows the surface plot as a function of the sulphuric acid concentration and the delay time. It is obvious that low acid concentrations exhibited a slight effect on the response, however at higher acid concentrations the response increases as time decreases. Figure 6 shows that at high and low acid concentrations the response increases with the time, but the effect on the response was found to be higher at higher acid concentrations. Figures 7 and 8 show the surface plot of the response as a function of permanganate concentration and delay time. The effect of permanganate concentration on the response increases as the delay time increases.

Table 1: Full treatment combinations of the coded values of parameters controlling the assay method of chlorpheniramine

Exp. N	H_2SO_4	$KMnO_4$	Time	Response
1	+1	+1	-1	0.1960
2	+1	+1	+1	0.2285
3	-1	+1	-1	0.1705
4	-1	+1	+1	0.1895
5	-1	-1	-1	0.1045
6	-1	-1	+1	0.1010
7	+1	-1	-1	0.1230
8	+1	-1	+1	0.1145

Table 2: Response obtained with the full treatment combinations of the original values of parameters controlling the assay method of chlorpheniramine

Exp. N	H_2SO_4 (M)	$KMnO_4$ (M)	Time (min)	Response
1	0.01	0.001	2	0.1960
2	0.01	0.001	4	0.2285
3	0.001	0.001	2	0.1705
4	0.001	0.001	4	0.1895
5	0.001	0.0005	2	0.1045
6	0.001	0.0005	4	0.1010
7	0.01	0.0005	2	0.1230
8	0.01	0.0005	4	0..1145

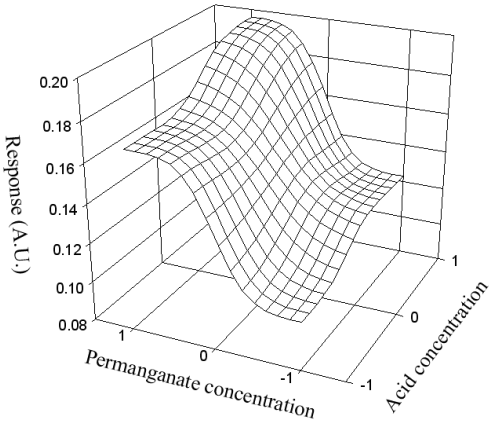

Fig. 3: Response surface plot of acid concentration (mol l^{-1}) against permanganate concentration (mol l^{-1}) for chlorpheniramine (time 2 min)

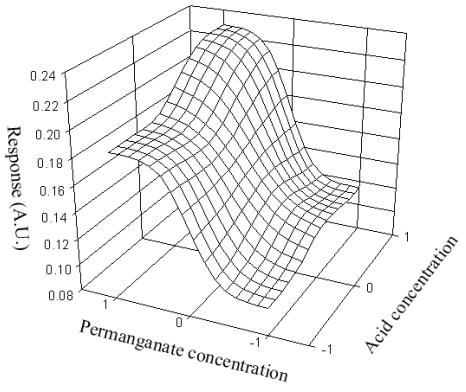

Fig. 4: Response surface plot of acid concentration (mol l^{-1}) against permanganate concentration (mol l^{-1}) for chlorpheniramine (time 4 min)

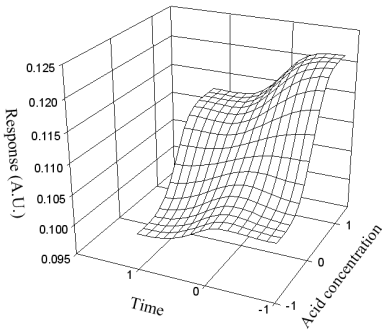

Fig. 5: Response surface plot of acid concentration (mol l^{-1}) against time for chlorpheniramine (permanganate 5×10^{-4} mol l^{-1})

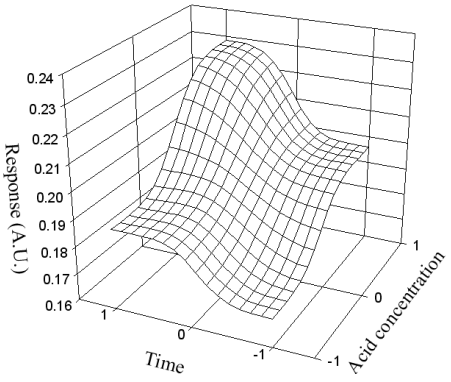

Fig. 6: Response surface plot of acid concentration (mol l^{-1}) against time for chlorpheniramine (permanganate 1×10^{-3} mol l^{-1})

3.2.1.2. The effect factor

Another method of the ANOVA approach was applied i.e. the effect factor (E_f) on the response. The main factor of a variable explains the level of effect on the response of a system. It is calculated as the difference between the average of the response of the highest levels (+1) and the average of the response of the lowest levels (-1) as presented in Eq. (2). Therefore a 2^3 factorial design was adopted to calculate the effect factors. The interaction effect factor explains the level of the interaction effect between variables on the response of a system. In this respect, the encoded levels of each experiment are multiplied and then calculated as in Eq. (2). Table 3 shows a 2^3 factorial design matrix and the multiplication of the encoded levels of the variables. The main and interaction effect factors were calculated and the results obtained were introduced in Table 4. The main effect factors show that permanganate concentration had a significant effect on the response, 0.0853, more than the effect of acid concentration, 0.0242, or delay time, 0.0098. The two-variable interaction effect factor between permanganate concentration and delay time was higher, 0.0158, than that between acid concentration and permanganate concentration or time. The three-variable interactions effect was lower than permanganate concentration and delay time, acid and permanganate concentrations and higher than that of acid and delay time interaction effect. These findings strengthen what was explained by the surface plot that permanganate concentration and delay time strongly interacted with each other than with acid concentration. The finding indicates clearly that the experimental factors have to be optimized with respect to one another to get the highest signal.

$$E_f = \frac{\sum Y(+1)}{n} - \frac{\sum Y(-1)}{n} \quad (2)$$

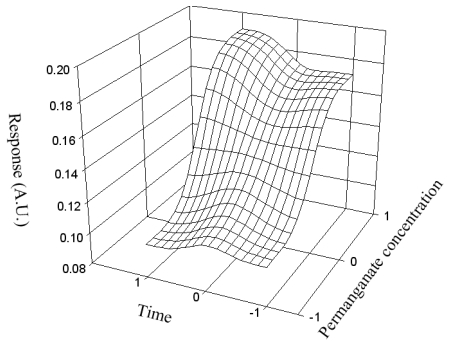

Fig. 7: Response surface plot of permanganate concentration (mol l^{-1}) against time for chlorpheniramine (acid 1×10^{-3} mol l^{-1})

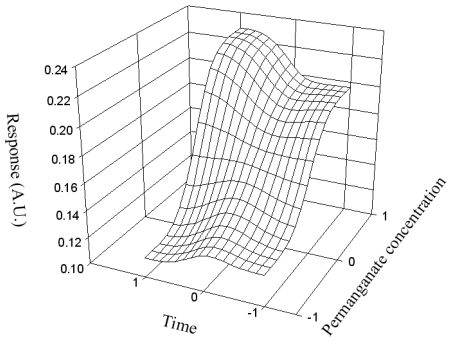

Fig. 8: Response surface plot of permanganate concentration (mol l^{-1}) against time for chlorpheniramine (acid 1×10^{-2} mol l^{-1})

3.3. Analytical Appraisal

The developed method was validated in order to evaluate if adequate linearity, repeatability, recovery, precision and accuracy had been achieved. The linearity of the proposed SIA system for the determination of chlorpheniramine maleate was evaluated under the optimum conditions. A series of standard solutions of chlorpheniramine maleate were prepared and applied. Figure 9 shows the calibration plot of chlorpheniramine maleate by the four standard solutions of 20, 50, 100 and 150 ppm.

Table 3: A 2^3 factorial design matrix and variables interaction

N	A	P	T	AP	AT	PT	APT	Response
1	+1	+1	-1	+	-	-	-	0.1960
2	+1	+1	+1	+	+	+	+	0.2285
3	-1	+1	-1	-	+	-	+	0.1705
4	-1	+1	+1	-	-	+	-	0.1895
5	-1	-1	-1	+	+	+	-	0.1045
6	-1	-1	+1	+	-	-	+	0.1010
7	+1	-1	-1	-	-	+	+	0.1230
8	+1	-1	+1	-	+	-	-	0.1145
A: acid, P: permanganate concentration, T: delay time,								

Table 4: The effect of variables on the response

Effect factor	Variable	Value
Main effect	A	0.0242
	P	0.0853
	T	0.0098
Two-variable interaction	AP	0.0081
	AT	0.0021
	PT	0.0158
Three-variable interaction	APT	0.0046

Beer's law was obeyed for this concentration range. The regression calibration equation obtained under optimum conditions was

$$R = 0.0016C + 0.0064 \quad (3)$$

Where R is the response calculated by the equation (3) and C is the unknown concentration of chlorpheniramine maleate as ppm. The correlation coefficient (r) was found to be 0.9998 indicating good linearity.

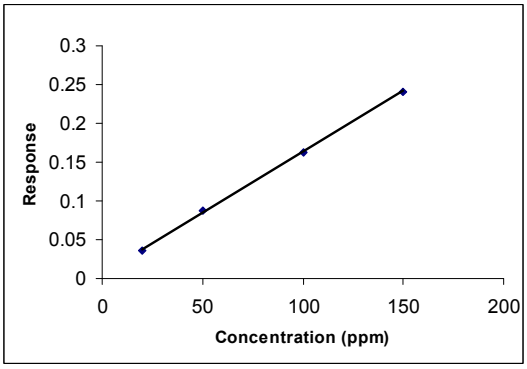

Fig. 9: SIA calibration plot of Chlorpheniramine (1×10^{-3} mol l^{-1}) M H_2SO_4, (1×10^{-3} mol l^{-1}) $KMnO_4$, 4min)

3.4 Application

The optimized SIA method was applied for the assay of chlorpheniramine maleate in pharmaceutical preparations collected from drug stores. Starch as one of the expected additive was examined to make sure that it did not to interfere with the process of determination of the analyte. Blank measurements using only the oxidizing agent which is permanganate and after adding 10 ppm starch were conducted and the absorbance difference was calculated as shown in equation (1). This revealed that the excipient added showed negligible interference with the process. The mean recovery and the RSD% (relative standard deviation) are presented in Table 5. The results obtained showed acceptable accuracy and repeatability.

Acknowledgements

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5. Conclusion

The proposed SIA spectroscopic method was optimized and applied to different pharmaceutical formulas including tablet forms and injection forms. It was validated with the British Pharmacopoeia. The advantages of proposed SIA method is that in addition to the automated ability, it is rapid, sensitive, accurate and cost-effective, and therefore suitable for routine pharmaceutical analysis application. Therefore, it is recommended to be used in pharmaceutical industries.

Table 5 Results obtained by the SIA and BP methods for the analysis of chlorpheniramine maleate synthetic sample, tablets and injections

sample	mg	Recovery %		Official accepted recovery* %	RSD** %
		SIA	BP		
Synthetic	15	99.8	98	98-101	± 1.8
Allerfin	10	90.0	95	90-110	± 1.0
Anallerg	4	97.2	98	92.5-107.5	± 1.1

*The official range of the content of a drug specified by the BP.
**The R. S. D. of n =7

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