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Original Resarch Article

Promethazine-HCl determination via CFIA-NAG-ADF-300-2 instrument using phosphomolybdic acid as a precipitating reagent

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ABSTRACT

In this work, a highly accurate and sensitive method with low cost analyzer NAG/ADF/(300-2) homemade instrument coupled under continuous flow injection (CFIA) analysis was used to determine promethazine hydrochloride in pure and pharmaceutical drug tablets. This method is dependent on the reaction between promethazine hydrochloride and phosphomolybdic acid (PMA) in the presence of ammonium chloride to form a brownish-yellowish ion-pair complex as precipitate. The turbidity of the formed complex has measured at an angle of 0-180° through the attenuation of incident light by precipitating. The chemical and physical parameters have been studied and optimized to enhance the sensitivity for the developed method of the promethazine hydrochloride-PMA-NH_4Cl system. The calibration curve of the proposed method was linear over the range 0.5-30 mmole.L^{-1} for both cells (Cell no.1 and Cell no.2), the detection limit for cell A = 2.1659 µg/sample and for cell B = 0.4332 µg/sample from the visual evaluation of the lowest concentration at which the analyte can be reliably detected with the correlation coefficient (r) = 0.9984, 0.9997 for cell A and cell B, respectively. For promethazine-HCl concentrations (5 and 10 mmole.L^{-1}) for both cells (n=8), the relative standard deviation percent (RSD%) was lower than 0.5%. The method has been successfully applied for the promethazine HCl determination in two pharmaceutical medicines. A comparison was made using the standard addition curve between the newly proposed method (NAG-ADF-300-2 analyzer) and the reference methods, mainly British Pharmacopoeia (B.P), turbidimetry, and UV-spectrophotometry (λ_{max} =249 nm) using paired t-test. It was noticed that there was no significant difference between the methods at 95% confidence level. The statistical procedures have shown that a homemade NAG/ADF/(300-2) analyzer which contains two identical detections units (cell A and cell B) is the best choice with an excellent prolonged detection, widespread application and extra sensitive.

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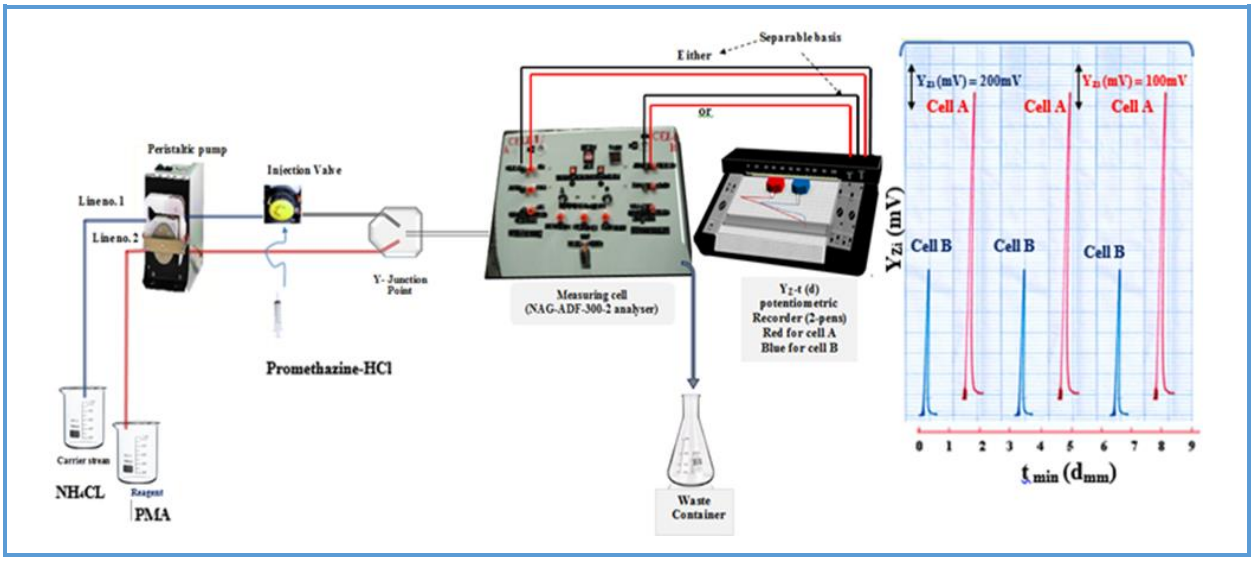
Graphical Abstract



Introduction

Promethazine hydrochloride (PRM.HCl) (Figure 1) is one of the main drugs in the enormous group of the derivatives of phenothiazine group [1]. Promethazine hydrochloride is used as an antiemetic in nausea treatment, as an antipsychotic in mental illness treatment, and to enhance the analgesic, narcotic, and tranquilizing effects of other medications [2].

Promethazine hydrochloride's chemical name is 10-[2-(Dimethyl amino propyl)-phenothiazine monohydrochloride, and its molecular formula is C_{17}

$H_{20}N_2S.HCl$ (320.9 $g.mol^{-1}$). Promethazine hydrochloride is a white or a pale-yellow powder, soluble in water and alcohol, but practically insoluble in acetone and ether [3].

A review of the literature reveals that several analytical methods for determining promethazine hydrochloride have been reported, including spectrophotometry [4], HPLC [5], turbid metric method [6], nephelometric titration [7], chemiluminescence [8], flow injection/stopped-flow technique [9]. Due to the importance of FIA methods, they were combined with many various methods that were based on the turbidimetric principle [10–11].

In this study, using the continuous flow injection analysis method, which measures the attenuated of the incident light (turbidimetric principle) via precipitation of promethazine hydrochloride by phosphomolybdic acid, a brownish yellowish precipitate product was obtained, which was determined at an angle of 0-180° using a homemade analyzer (NAG-ADF-300-2) [12] by integration white snow light emitting diodes as a light source with solar cells as a detector and represents the output of the response by Yz (mV)-tmin (dmm) recorder.

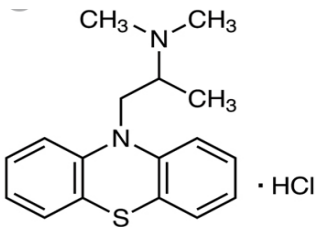


Figure 1. Structure of promethazine hydrochloride

Experimental

Materials and methods

All the chemicals used in this research study were of analytical reagent grade with high purity. A stock solution of (100 mmol.L^{-1}) of promethazine hydrochloride (PRM.HCl) ($C_{17}H_{20}N_2S.HCl$) (320.9 g.mol^{-1}) was daily prepared by weight of 3.2090 g and transferred into 100 mL volumetric flask. A stock solution of phosphomolybdic acid anhydrous ($H_3PMo_{12}O_{40}$), 1825.25 g.mol^{-1}, (Fluka) 12 mmol.L^{-1} by dissolving 5.4758 g in 250 mL of distilled water. A stock solution of ammonium chloride (NH_4Cl, 53.49 g.mol^{-1}, Fluka, 100 mmol.L^{-1}) was prepared by dissolving 1.3373 g in 250 mL of distilled water (D.W).

Sample preparation of Promethazine hydrochloride

The Tablets contain 25 mg and 5 mg of Promethazine hydrochloride (2.1259 g and 3.6009 g) were crushed and weighted (equivalent to 0.4011 and 0.0802 g of active ingredient, 50 mmol.L^{-1} and 10 mmol.L^{-1}) for Histazin ®-United Pharmaceuticals-Jorden-25 mg and Coldin ®-S.D.I-Iraq-5 mg, respectively, dissolved in a small amount of D.W., followed by filtration to remove undissolved materials and completing the volume to 25 mL with the same solvent (distilled water).

Apparatus

The novel photometer NAG/ADF/(300-2) instrument is a multi-purpose photometric device that includes the offer of multiple measurements from absorption or attenuation of incident light at 0-180^o and diverged or fluorescence light at 0-90^o via a flow cell (i.e.; cell A & cell B). The NAG/ADF/(300-2) photometer is built entirely at home (in the house) and used in this study.

This applies to clear solutions as well as colored or precipitated reaction products, whether colloidal or crystalline colored, or white, or even transparent precipitate.

The first measuring cell (cell A), with 110 mm length, has 11 sources of WSLED white snow LED facing 0-180^o tow solar cells to measure the turbidity, attenuation of incident light, or absorbance. In addition to the presence of two solar cells at a 0-90 angle to measure the scattering of light or the divergent or even fluorescence.

The second cell (cell B) has a length of 20 mm* 20 mm and is supplied with 6 WSLED facing at 0-180 degrees on one solar cell and at 0-90 on another solar cell. Passing through the face of 20 mm × 20 mm, a 4 mm hole that will represent the flow tube on each side was used.

For loading and injection, a four-channel peristaltic pump (Switzerland) and a six-port medium pressure injection valve (IDEX Corporation, USA) with a sample loop (1 mm i.e., Teflon, variable length) were employed. An x-t potentiometric recorder (Kompenso Graph C-1032, 1-1500 mV, Siemens, Germany) serves as the system's readout.

Methodology

The whole manifold system as shown in Figure 2 for promethazine hydrochloride determination by producing a turbid, yellow-brownish ion-pair association complex between the drug and phosphomolybdic acid which was used as a precipitation reagent. It consists of two lines. The first line in the manifold system is supplied with a carrier stream which is the ammonium chloride (50 mmol.L^{-1}) (2.6 mL.min^{-1}), that carries the sample segment (135 µL for two cells A & B) through the system of 25 mmol.L^{-1} experimental

concentration of Promethazine hydrochloride, while the second line supplied the phosphomolybdic acid (3 mmol.L^{-1}) with a flow rate of 2.6 mL.min^{-1}. The two lines mix at the Y-intercept and lead to the measurement cell. The product of the reaction is yellowish brown particles of the ion-pair complex. The measurement method is based on the transmission of the light signal from weak incident light by particles deposited in the flow cell to the detector site at an angle of 0°-180°.

A proposed mechanism for the reaction of promethazine hydrochloride (PRM-HCl) with phosphomolybdic acid (PMA) in an aqueous solution to form an ion pair color precipitate is presented in Scheme 1 [13, 14].

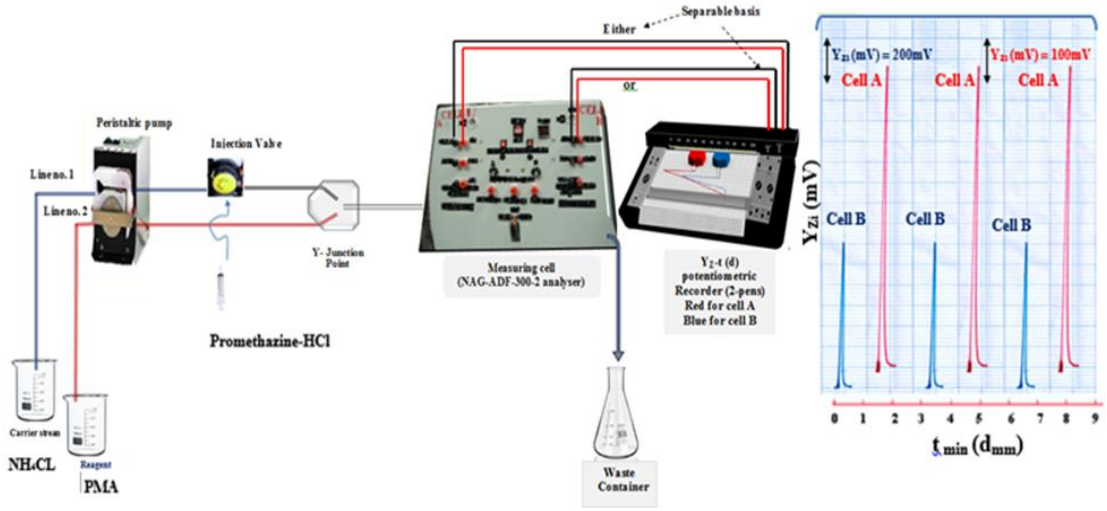
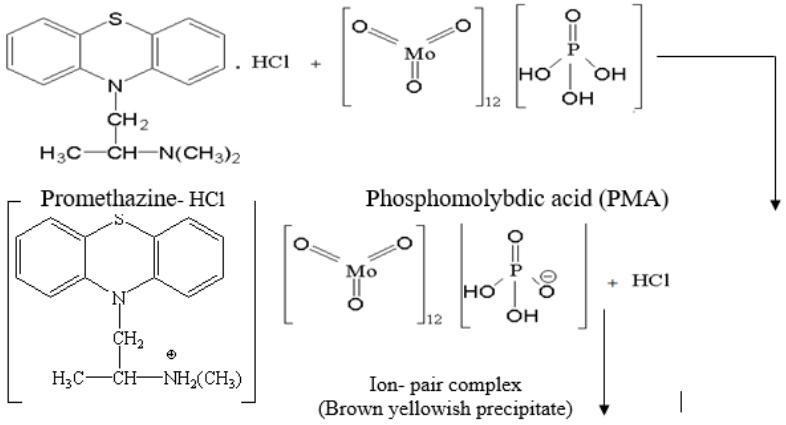


Figure 2. Flow gram of manifold system consist of two lines of PRM-HCl determination



Scheme 1. Proposed reaction between PRM.HCl with PMA forming ion pair complex

Results and Discussion

Variables optimization

The chemical parameters (mainly reagent concentration and carrier current type of promethazine hydrochloride with the phosphomolybdic acid system) as well as physical parameters: sample volume, flow rate, and delay reaction coil were studied using a two-line manifold system as an initial setup to

establish best available parameters to conduct the determination.

Chemical variables

Phosphomolybdic acid (PMA) solutions with variable concentrations ranging from one to twelve mmol.L^{-1} were prepared and used as a precipitating agent in a 135 µL sample volume with a flow rate (3 mL.min^{-1}) for the carrier stream (distilled water) and reagent with a concentration of promethazine hydrochloride 25 mmol.L^{-1} as an injected sample.

From the results obtained, it was noted that with an increase the concentration of the reagent, the precipitated particles increase, which begins to grow and increase in the size of the lamp and may cause a more compact and dense precipitation, which in its new size leads to a restriction and attenuation of the incident light up to three mmol. L^{-1} for both cells.

Using the slope-intercept method [15, 16] of optimization for the most optimum range of variable ranges, it was found that the most sensitive segment with an extended range of concentration was the second segment that extended from a_2-a_4, which represents three to seven mmol.L^{-1}. Within it is the optimum concentration of reagent, i.e., three mmol.L^{-1} for both cells, as shown in Figure 3 and Table 1.

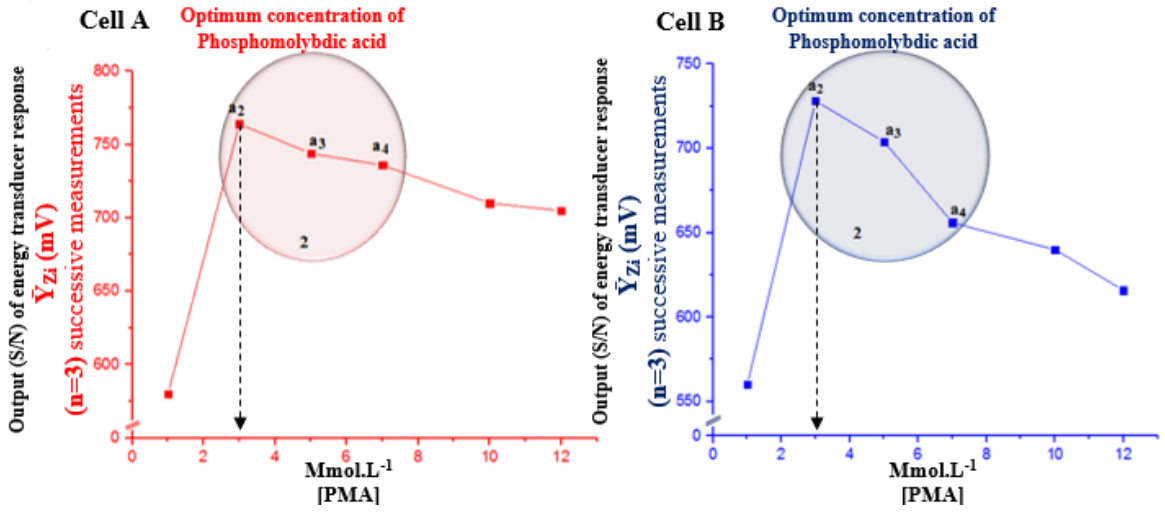


Figure 3. The application of slop-intercept method for choosing the optimum of phosphomolybdic acid concentration. Three data points as one segment of their interaction and choice for both cells.

Table 1. Segmentation pattern for selection of optimum segment of Promethazine-HCl-PMA-system

No. of segment	[PMA] range mmol.L^{-1}	Segment	intercept mV	Slope mV/mmol.L^{-1}
		Cell A Cell B		
1	1-5	a_1-a_3	41.0 36.0	573.0 556.0
2	3-7	a_2-a_4	-7.0 -18.0	783.0 786.0
3	5-10	a_3-a_5	-6.9 -12.2	780.9 756.2
4	7-12	a_4-a_6	-6.4	778.8

	-7.8	712.6

Different aqueous media (salts and acids) solutions effect

The effect of a variety of solutions as a carrier stream during the reaction between promethazine hydrochride (25 mmol.L^{-1}) and phosphomolybdic acid (3 mmol.L^{-1}) in both cells was investigated. Different salt media (50 mmol.L^{-1} concentration ammonium acetate, ammonium chloride, sodium chloride, potassium chloride, and K, Na-tartarate), as well as acid media (50 mmol.L^{-1} concentration tartaric acid, ascorbic acid, hydrochloric acid, and acetic acid), and distilled water as a carrier stream were used.

Figure 4 reveals that while using phosphomolybdic acid as a precipitating agent, an increase in S/N-response from 764, 728 mV to 848, 760 mV in the presence of ammonium chloride for both cells. It could be related to the effect of the formation of tiny solid particulates that cause a decrease in inter-spatial distances and an increase in attenuation of incident light. In general, the ammonium chloride (NH_4Cl) salts and what they possess of (-) ve radical help to reconstruct and accumulate precipitated particulates, which increases the prevention of light and then increases peak height. On the first line (carrier stream), NH_4Cl was utilized as a transferring medium to improve the sensitivity of the measurement to determine promethazine-HCl.

Physical variables

Effect of flow rate

Fixing all pervious experimental parameters concerning chemical variation (Concentrations of complementing components for this reaction) for: Promethazine-HCl (25 mmol.L^{-1})-PMA (3 mmol.L^{-1} for both cells)-NH_4Cl (50 mmol.L^{-1}) system. A variable flow rate study was carried out with flow rates ranging from 0.5 to 5 mL.min^{-1} for each line (carrier streamLine and precipitating reagent solution line). The obtained response profile is shown in figure 5. It can be noticed from Figure 5 that up to 2.6 mL.min^{-1} for both lines and for both cells gave a good S/N peak height and more sensitive. It could be due to the growth of crystals and lead to the formation of a larger particulate, which in turn will be affected by the motion of the peristaltic pump in both cases of relaxed and pressed mode. In the case of pressed mode, the formed precipitated particulate will be pressed, causing an accumulative compactness that leads to the obscurement of the incident light and distortion of the obtained responses. While at a high flow rate, i.e., more than 2.6 mL.min^{-1}, despite obtaining a sharp response and profile (undisturbed profile), the sensitivity is low due to the availability of enough time for growth and accumulation of the precipitated particulate that is quite necessary for obstruction and attenuation of incident light. Also, not enough time was given for the solar cell detector to respond completely to passing segments. On this basis, the 2.6 mL.min^{-1} was chosen as the optimum flow rate for both cells to obtain a maximum response.

Variation of Sample volume in CFIA

At Promethazine hydrochloride of 25 mmol.L^{-1}, phosphomolybdic acid (PMA) of 3 mmol.L^{-1} and 2.6 mL.min^{-1} flow rate for both lines (NH_4Cl line and PMA line), the effect of variable sample volumes (40, 78.5, 113, 135, 140, 153 and 281) µL were studied.

Figure 6 shows the (S/N) energy transduce response, and it can be seen from the reported results in Table 2 that an increase in the sample loop led to a significant increase in sensitivity and more perceptible up to 135 µL which gives

a regular response to the attenuation of incident light by precipitated colour particulate; this indicates that the reaction has been completed.

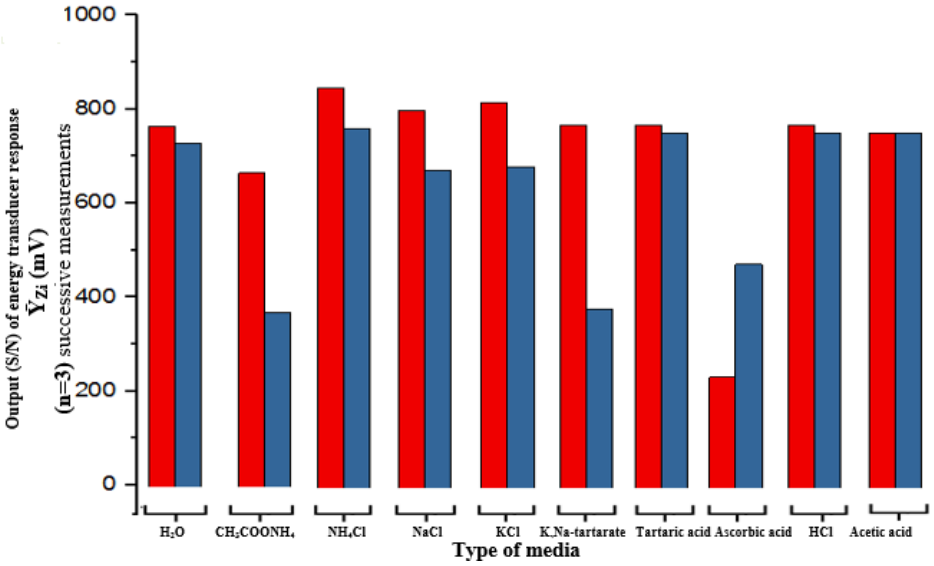


Figure 4. The effect of different aqueous salts and acid solutions as a carrier stream on (S/N) energy transducer response versus time

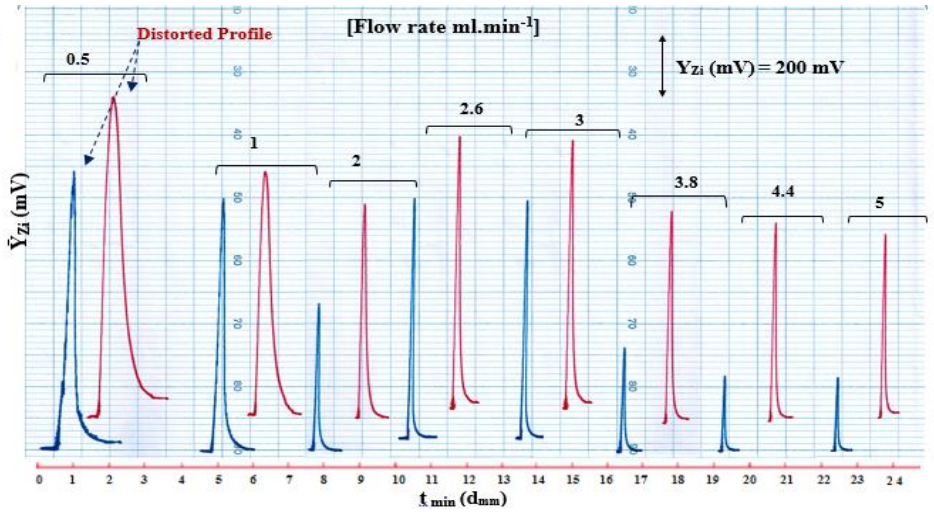


Figure 5. Effect of the variation of flow rate on (S/N) energy transducer response versus t_{min} (d_{mm}). Using PRM.HCl (25 mmol.L^{-1})-PMA (3 mmol.L^{-1}) system, sample volume (135 µL)

When the volume is greater than 135 µL, responses are lower and the width at the apex of the profile and base width are wider, this might due to a greater density of particulate precipitate at the center through the travel of the sample loop. In addition to increased sample, segment might cause an increase in the amount of precipitated particulate on all flow tube distances, which in turn decreases the optical fiber phenomenon that increases the

intensity of incident light, which in turn causes a decrease in the obtained signals and the sensitivity is minimized. On this above basis, and to compromise on the economy of sample usage, 135 µL was the best sample volume for both cells.

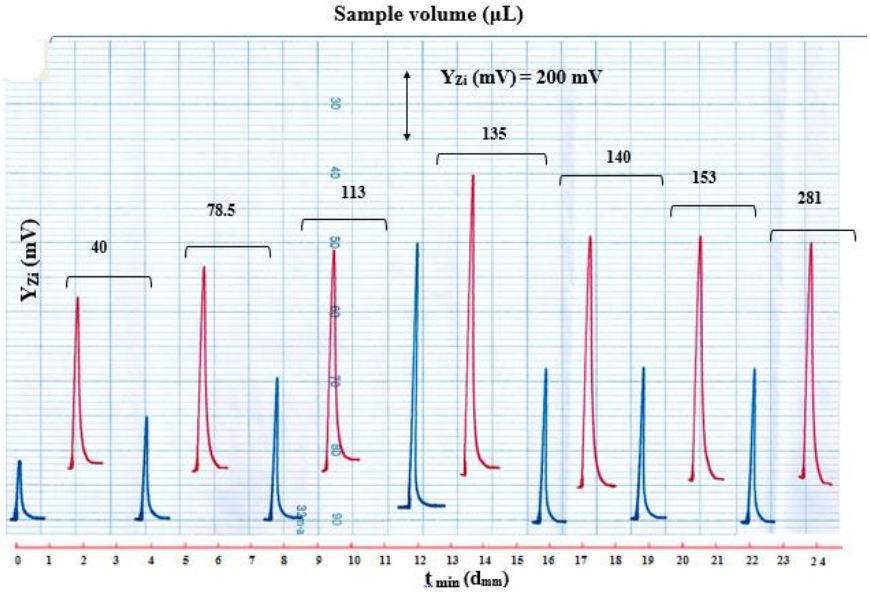


Figure 6. Effect of using variable sample volume on response $\bar{Y}_{Z\,i}$ (mV) versus t_{min} (d_{mm}), chart speed of recorder 60 cm/hr.($60_{sec}/10_{mm}$)

Table 2. Variation of sample volume on the output of response (mV) at a 2.6 mL.min^{-1} flow rate

Length of Sample Segment Cm r=0.5mm	Sample Volume μL $V=\pi r^2 h$	Output (S/N) of energy transducer response expressed as an average peak heights (n=3) $\bar{Y}_{Z\,i}$ (mV)	RSD %	Reliability (two tailed) at 95% $\bar{y}$(mV)$\pm t_{0.05/2,n-1}$ $\sigma n-1/\sqrt{n}$	t (sec)	Base Width Δt_B (sec)	$V_{add.}$ of sample loop (mL) at flow cell	Concentration of Drug (mmol. L^{-1}) at flow cell	D_f at flow cell
				Cell A Cell B					
5.10	40	496 176	0.35 0.65	496±4.2980 176±2. 8571	3 6	30 24	2.640 2.120	0.3788 0.4717	65.99 52.99
10	78.5	592 256	0.33 0.52	592±4.9191 256±3.2794	6 9	36 30	3.199 2.679	0.6135 0.7325	40.75 34.13
14.40	113	632 416	0.21 0.34	632±3.3539 416±3.5279	12 12	39 33	3.493 2.973	0.8088 0.9502	30.91 26.31
17.20	135	852 772	0.18 0.19	852±3.7763 772±3.7515	18 15	45 36	4.035 3.255	0.8364 1.0369	29.89 24.11
17.90	140	720 448	0.23 0.29	720±4.0496 448±3.2049	21 24	48 39	4.300 3.520	0.8139 0.9943	30.72 25.14
19.50	153	704 440	0.24 0.30	704±4.2732 440±3.2794	24 30	51 42	4.573 3.793	0.8364 1.0084	29.89 24.79
35.80	281	680 440	0.23 0.36	680±3.8259 440±3.9005	27 36	54 45	4.961 4.181	1.4160 1.6802	17.66 14.88

t: Departure time lapse from injection valve reaching to measuring cell (sec), Δt_B: Time lapse for the precipitate response within measuring cell or peak base width (sec), $t_{0.05/2,2}$=4.303, Df: Dilution factor

Effect of delay reaction coil (Teflon) for completion of reaction

The variable length of the coil was studied: from zero (direct attachment) to thirty cm. These lengths have a volume ranging from zero (direct attachment) to 942 µL, which is linked directly into the flow system after a Y-junction as shown in Figure 1. While keeping all other variables fixed, i.e., chemical and physical parameters, Figure 7 shows that an increase in delay reaction coil volume for both cells (Cell A and Cell B) lead to a decrease in response height with an increase in base width (ΔtB). This might be attributed to the diffusion and dispersion of precipitate particles, which is causing an increase in the dispersion regions and leading to a decrease in the (S/N) energy transducer response, which in turn gives a lower sensitivity of response. Therefore, the delayed reaction coils are not needed through the flow system.

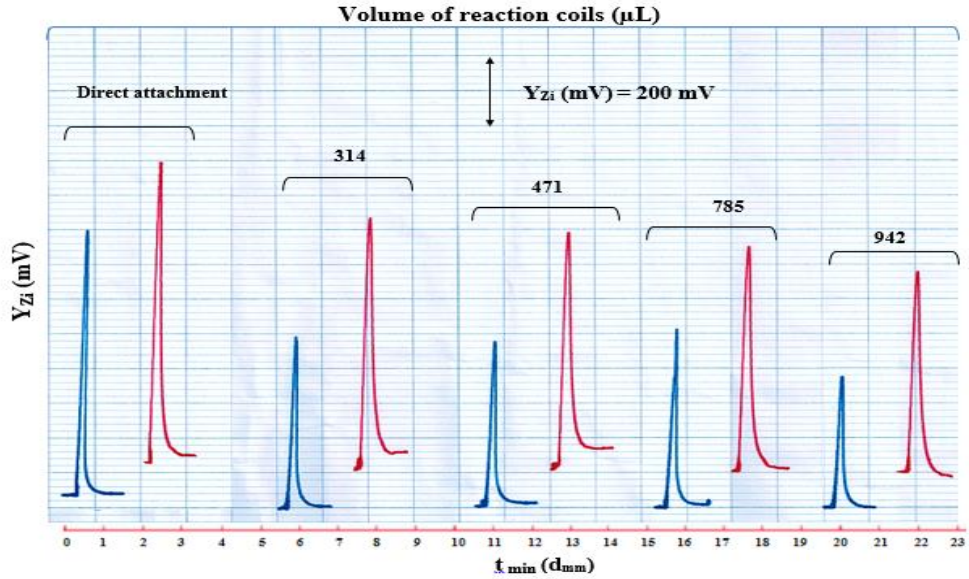


Figure 7. Effect variation of delay reaction coil volume on response YZi (mV) versus $t_{min}(d_{mm})$, chart speed 60 cm/hr. ($60_{sec}/10_{mm}$). Responses were plotted simultaneously (red for cell A & blue for cell B) but with a time difference expressed by distance equivalent to 100mm without any detection

Assessment of using NAG/ADF/(300-2) (0-180°)(0-90°) Analyzer for the proposed method of determination promethazine hydrochloride using phosphomolybdic acid

Using the optimum of chemical and physical parameters; a series of promethazine hydrochloride solutions ranging from 0.5 to 40 mmol.L^{-1} for both cells were prepared, this will represent the X-axis (independent variable). The attenuation of the incident light that was measured gave the following (S/N) energy transducer response as Y-value here represents the dependent variables. The calibration curve was constructed using the X-value (concentrations of promethazine hydrochloride) which represents independent values versus the response Y_{Zi} (mV)) and this falls into the range, i.e., the extent to which or the limit between which variations are possible. i.e., obtaining a peak, which means a mountain with a pointed summit, and avoiding noise (electrical disturbance). A scatter plot diagram (Figure 8A and 8B) shows that a linear range for the variation of the energy transducer response of NAG-ADF-300-2 analyzer with promethazine hydrochloride concentration was ranging from

0.5-30 mmol.L^{-1} for both cells (A & B) with correlation coefficient (r) of Y_Z (mV) versus t_{min} (d_{mm}) = 0.9984 and 0.9997 with coefficient of determination (r^2)=0.9968 and 0.9994 and chosen a linear equation response (R^2)= 99.68% and 99.94% of the obtained results for measuruing cells i.e., cell A and cell B respectively. All results are obtained tabulated in Table 3. *The limit of detection (L.O.D)*

A study to estimate the detection limit of promethazine hydrochloride was carried out by three different methods [16] as shown in Table 4 as an injected sample volume of 135 µL.

Repeatability

The relative standard deviation (RSD %) of Promethazine hydrochloride at selected concentrations (5 and 10 mmol.L^{-1}) for both cells A and B, which is equally to the repeatability and reality of the measurements was studied. All the results are shown in Table 5.

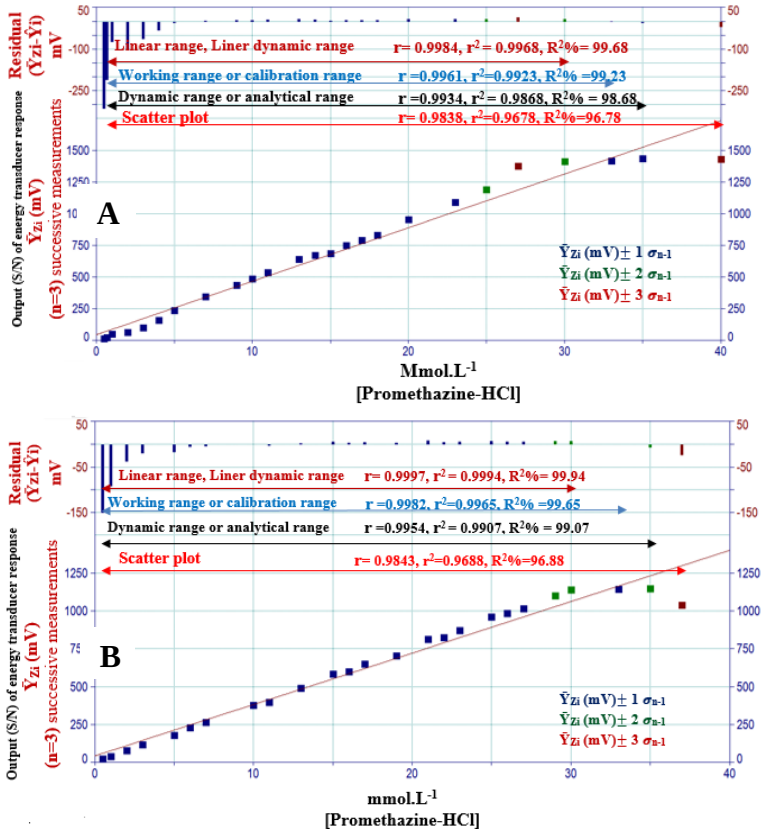


Figure 8. A calibration graph that is represented a linear dynamic range

Table 3. The summary of linear regression of the proposed method

Type of mode	Range of [Promethazine-HCl] mmol.L^{-1}(n)	$\hat{Y}_{Z\,i\,(mV)}$=a $_{mV}$ ± S_a t+ b (Δ y $_{mV}$/Δx $_{mmol.L^{-1}}$) ± S_b t [Promethazine-HCl] mmol.L^{-1} at confidence level 95%, n-2 Cell A Cell B	r,r^2, R^2%	t $_{tab}$ at 95%, n-2	Calculated t-value t_{cal}=/r/√n -2/√1-r^2
Linear range or	0.5 -30 (22)	-14.0692± 19.5270+48.5639± 1.2891 [Promethazine hydrochloride] mmol.L^{-1}	0.9984, 0.9968, 99.68	2.086 << 78.588	

linear dynamic range	0.5-30 (22)	1.0648±7.8534+37.8709±0.4443 [Promethazine hydrochloride] mmol.L^{-1}	0.9997, 0.9994, 99.94	2.086 << 177.764
Working range or calibration range	0.5 -33 (23)	-0.1498±30.7070+46.9409±1.8797 [Promethazine hydrochloride] mmol.L^{-1}	0.9961, 0.9923, 99.23	2.080 << 51.942
	0.5-33 (23)	9.0843±18.5765+37.0679±0.9984 [Promethazine hydrochloride] mmol.L^{-1}	0.9982, 0.9965, 99.65	2.080 << 77.210
Dynamic range or analytical range	0.5 -35 (24)	15.0492±40.5218+45.2530±2.3125 [Promethazine hydrochloride] mmol.L^{-1}	0.9934, 0.9868, 98.68	2.074 << 40.567
	0.5-35 (24)	20.8499±30.0709+35.9561±1.5368 [Promethazine hydrochloride] mmol.L^{-1}	0.9954, 0.9907, 99.07	2.074 << 48.511
Scatter plot	0.5 - 40 (25)	45.5955±62.8748+42.1970±3.3207 [Promethazine hydrochloride] mmol.L^{-1}	0.9838, 0.9678, 96.78	2.069 << 26.291
	0.5-37 (25)	43.3435±53.9512+33.9409±2.6256 [Promethazine hydrochloride] mmol.L^{-1}	0.9843, 0.9688, 96.88	2.069 << 26.743

n Number of measurements
$\hat{Y}_{Zi}$ Estimated value in mV by developed method
r correlation coefficient
r^2 coefficient of determination
$R^2\%$ (percentage capital R-squared): explained variation as a percentage/total variation and $t_{tab} = t_{0.05/2,\, n-2}$

Table 4. The calculated LOD of the proposed method for cell A and cell B using sample volume (135 µl), reagent concentration 3 mmol.L^{-1} and a flow rate for both lines is 2.6 mL.min^{-1}

Type of cell	Gradual dilution for the lowest concentration in the scatter plot (0.5 mmol.L^{-1}) for cell A (0.5 mmol.L^{-1}) for cell B	Theoretical based on the value of slope $x=3S_B/\text{slope}$	Theoretical based on the linear equation $\hat{Y} = Y_b + 3Sb$	Limit of quantitative L. O. Q $\hat{Y}=Y_b+10S_b$
Cell A	0.05 mmol.L^{-1} 2.1659 µg/sample	0.3345 µg/sample	68.2932 µg/ sample	227.6439 µg/sample
Cell B	0.01 mmol.L^{-1} 0.4332 µg/sample	1.2822 µg/sample	32.4079 µg /sample	108.0266 µg/sample

Table 5. Repeatability of Promethazine hydrochloride at optimum parameters with 135 µL sample volume

[Promethazine hydrochloride] mmol.L^{-1}	Output (S/N) of energy transducer response expressed as an average peak heights (n=8) $\bar{Y}_{zi}$ (mV) Cell A Cell B	RSD%	Reliability (two tailed) at (95%) $\bar{Y}_{zi}$ (mV)± $t_{0.05/\,2,\, n-1}\sigma_{\,n-1}/\sqrt{n}$
5	240	0.48	240± 0.9633
	180	0.25	180 ±0.3763
+	488	0.49	488 ±1.9984

380	0.41	380 ±1.3044

$^{(n=8)}$ number of injection, $t_{0.05/2,7}$ =2.365

Application of the newly developed method for determination of promethazine hydrochloride in commercial tablets and comparision the results with the references method

The newly established method was used to determine promethazine hydrochloride in two commercial tablets (Histazin ®United pharmaceutical-Jordan-25 mg) and (Coldin ® S.D.I- Iraq-5 mg). The measurement was conducted by newly method and two reference methods which includes UV-Spectrophotometry at λ_{max}=249 nm as shown in Figure 9 and turbidimetry method. The measurements of scattered light at angle from 0 to180 degree for a brown yellowish precipitate particles of promethazine hydrochloride - PMA (3 mmol.L^{-1})- ammonium chloride (50 mmol.L^{-1}) system were used. A series of solutions of standard addition method were prepared of tablet solution of Histazin drug sample (50 mmol.L^{-1}) and tablet solution of Coldin drug sample (10 mmol.L^{-1}) by transferring a constant volume (1 mL from sample no. 1 and 1.25 mL from sample no. 2) to each of the five volumetric flask (25 mL) followed by transferring 0.0, 2.0, 3.0, 4.0 and 5.0 mL from the standard solution of promethazine hydrochloride (50 mmol.L^{-1}) to obtain concentrations of promethazine hydrochloride 0.0, 4.0, 6.0, 8.0 and 10.0 mmol.L^{-1}, respectively for the developed method.

The three methods measurements NAG/ADF/(300-2) analyzer, and classical methods (UV Spectrophotometry at λmax=249 nm and turbidity) were conducted and the results were mathematically treated for the preparation of standard addition calibration plot, the results illustrated in table 6-A and 6-B at confidence level 95%, which shows a comparison at two different paths.

The first statistical test is individual t-test for compared between of the mean practical weight by the novel method NAG/ADF/(300-2) using cell A and B with quoted value (British Pharmacopia) (25 mg of Histazin ® and 5 mg of Coldin ®).

In order to conduct this procedure, hypothesizes must set up which are:

The null hypothesis (Ho):This hypothesis assumes that there are no significant differences between the population mean of the true value (μ = 25 mg or 5 mg) and the practical samples mean ($\underline{w}_i$) which obtained from the promethazine hydrochloride of (25 mg Jordan and 5 mg Iraq) drugs using developed method.

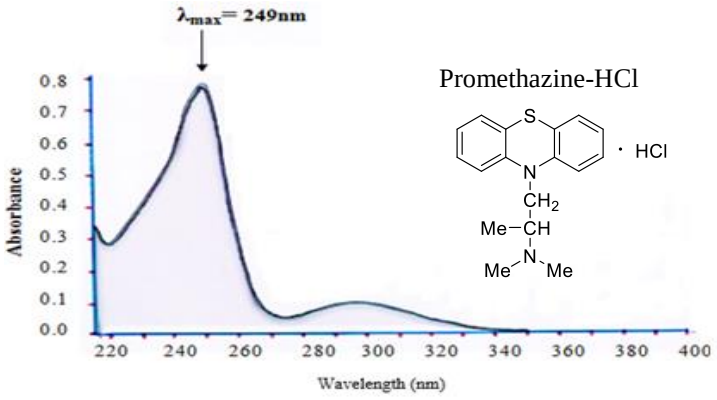


Figure 9. The absorption spectra of Promethazine hydrochloride of (0.03 mmol.L^{-1}) concentration of that shows λ_{max} = 249 nm

Alternative hypothesis (H_1): This hypothesis assumes that there is a significant difference between the population mean of the true value (μ =25 mg or 5 mg) and the practical samples mean ($\bar{w}i$).

The null hypothesis (Ho):μ(25 mg or 5 mg)=$\bar{w}i$(NAG/ADF/(300-2)) at (0-180°)(the measure cell A) or $\bar{w}i$ (NAG/ADF/(300-2)) at (0-180°)(the measure cell B)

The alternative hypothesis (H_1): μ(25 mg or 5 mg) ≠ $\underline{w}_{i(\text{NAG/ADF/(300-2)})}$ at (0-180°)($_{\text{the measure Cell A}} \neq$ or $\underline{w}_{i(\text{NAG/ADF/(300-2)})\text{at(0-180°)(the measure cell B)}}$

Therefore, the purpose of the Individual t-test is to determine if the null hypothesis should be rejected or accepted. Based on the obtained results of the means of two companies, all $t_{cal} < t_{tab}$ (4.303) at 95% confidence interval and 2 as a degree of freedom. This means the null hypothesis (Ho) will be accepted and the alternative hypothesis will be rejected which claims that there is no significant different between the true value (μ) and the practical value ($\bar{w}i$).

Second, Paired sample t-test, this test can be defined as a statistical procedure used to compares the means of two measurements taken from two methods. The comparisons have made between the method (cell A-developed method) and the methods of UV spectrophotometry and turbidity. Also, the developed method (cell B) has also be compared with the classical methods as well. In this test, all the drugs from different companies have the same population which the means the differences amongst the manufacturings are neglectable. In to conduct the Paired sample t-test, the hypothesis theory must be established.

The null hypothesis (Ho): μ(NAG/ADF/(300-2))(0-180°)(the measure cell A)= μ(NAG/ADF/(300-2))(0-180°)(cell B)
μ(NAG/ADF/(300-20)(0-180°)(the measure cell A)= μUV
μ(NAG/ADF/(300-2))(0-180°)(the measure cell B)= μUV
μ(NAG/ADF/(300-2))(0-180°)(the measure cell A)= μ turbid
μ(NAG/ADF/(300-2))(0-180°)(the measure cell B)= μ turbid

The alternative hypothesis (H_1): μ(NAG-ADF-300-2)(0-180°)(the measure cell A) ≠ μ(NAG-ADF-300-2)(0-180°)(the measure cell B)
μ(NAG/ADF/(300-2))(0-180°)(the measure cell A)= μUV
μ(NAG/ADF/(300-2))(0-180°)(the measure cell B)= μUV
μ(NAG/ADF/(300-2))(0-180°)(the measure cell A)= μ turbid
μ(NAG/ADF/(300-2))(0-180°)(the measure cell B)= μ turbid

According to the obtained results, there was no significant differences between the methods due to tcal << ttab (12.706) therefore, the null hypothesis will be accepted and the alternative hypothesis will be rejected which means there is no significant differences between the methods. All the data analysis which mentioned above are shown in Tables 6A and 6B.

Table 6A. Summary of standard addition method results for determination of promethazine hydrochloride in samples from different companies promethazine hydrochloride using two different methods novel NAG/ADF/(300 -2)method for measure cells A& B and two references methods (Turbidimetry and UV Spectrophotometry), using PRM/HCL-PMA system

No. of sample	Commercial Name, Company Content Country	Types of used methods: Newly developed method using NAG/ADF/(300-2) analyzer; The measure Cell A; The measure Cell B; Turbidimetry method (NTU) (Reference method 1); UV Spectrophotometry method (Reference method 2) at λ_{max}=249 nm									
		Confidence interval For the average Weight of tablet $\bar{w}_i \pm 1.96\sigma_{n-1}/\sqrt{n}$ at 95% (g)	Weight of Sample in g equivalent to 0.40113 g (50 mmol.L^{-1}) Sample no.1 and 0.0802 g (10 mmol.L^{-1}) sample no.2 of the active ingredient Wi (g)	Theoretical content for the active ingredient at 95% (mg) Wi $\pm 1.96\sigma_{n-1}/\sqrt{n}$	Promethazine hydrochloride mmol.L^{-1}					Equation of standard addition method at 95% for n-2	r, r^2, R^2%
					0.00	2 mL	3 mL	4 mL	5 mL	$\hat{Y}_{Z\,i} = a \pm S_a t + b\ (\Delta y/\Delta x_{mmoL.L^{-1}}) \pm S_b t$ [Promethazine hydrochloride] mmol.L^{-1}	
					0.00	4	6	8	10		
					0.00	0.5mL	1mL	1.5 mL	2mL		
					0.00	1	2	3	4		
					0.00	0.01mL	0.015 mL	0.02 mL	0.02 5 mL		
					0.00	0.02	0.03	0.04	0.05		
1	Histazin, United pharmaceutical 25 mg, Jorden	0.1325±0.0012	2.1259	25±0.2264	75	275	360	435	520	85.5410±32.5837+44.1892±4.9576 [Promethazine hydrochloride] mmol.L^{-1}	0.9981, 0.9963, 99.63
					64	200	280	340	400	66.7027±19.0290+33.9460±2.8952 [Promethazine hydrochloride] mmol.L^{-1}	0.9989, 0.9978, 99.78
					175	260	355	440	520	176.0000±11.0129+87.0000±4.4999 [Promethazine hydrochloride]mmol.L^{-1}	0.9996, 0.9992, 99.92
					0.83	1.22	1.39	1.61	1.82	0.8227±0.0437+19.6892±1.3311 [Promethazine hydrochloride] mmol.L^{-1}	0.9993, 0.9986, 99.86
2	Coldin, S.D.I, 5 mg, Iraq	0.2245±0.0032	3.6009	5±0.0713	23	155	222	290	380	17.2432±26.1153+35.1351±3.9733 [Promethazine hydrochloride] mmol.L^{-1}	0.9981, 0.9962, 99.62
					20	175	235	320	400	19.0541±19.1120+37.6689±2.9077 [Promethazine hydrochloride] mmol.L^{-1}	0.9991, 0.9982, 99.82

					60	98	188	290	363	40.2000±47.9712+79.8000±19.5839 [Promethazine hydrochloride] mmol.L^{-1}	0.9912, 0.9825, 98.25
					0.78	1.18	1.39	1.54	1.72	0.7957±0.0614+18.7972±1.8736 [Promethazine hydrochloride] mmol.L^{-1}	0.9985, 0.9971, 99.71

Table 6B. The results of the proposed and classical methods for the determination of Promethazine hydrochloride in tablets

No. of sample	Types of used methods			One sample t-test comparision between claim value and practical value (μ=25 mg & 5 mg) $(\bar{w}i - \mu)\sqrt{n}/\sigma_{n-1}$ The measure Cell A or The measure Cell B	Paired t –test Compared between two methods	
	Newly developed method using NAG/ADF/(300-2) analyzer					
	The measure Cell A					
	The measure Cell B					
	Turbidimetry method (NTU) (Reference method 1)					
	UV spectrophotometry method (Reference method 2) at λ_{max}=249 nm					
	Practical concentration (mmol.L^{-1}) in 25 mL Original sample solution in 25 mL	Practical weight of Promethazine hydrochloride in weight of sample(g) $\bar{w}$ i (g) ±4.303 $\sigma_{n-1}/\sqrt{n}$ Weight of Promethazine hydrochloride in tablet(mg) $\bar{w}$ i (mg) ±4.303 $\sigma_{n-1}/\sqrt{n}$	Efficiency of determination Recovery%		t_{cal}= $\bar{X}d\sqrt{n}/\sigma_{n-1}$	t_{tab} at 95% confidence level
1	1.9358 48.3945	0.3882±0.0156 24.1980±0.9730	96.79	/ - 3.5467 / << 4.303	cell A with Tur. $\bar{X}d$ = - 0.6099 σ_{n-1}=0.6790 /-1.2701/ < 12.706 cell A with UV-Sp. $\bar{X}d$ = - 1.1509 σ_{n-1}= 1.0849 /- 1.5001/ << 12.706 cell B with Tur. $\bar{X}d$ = - 0.3521 σ_{n-1}= 0.5274	
	1.9649 49.1240	0.3941±0.0153 24.5630±0.9530	98.25			
	2.0229 50.575	0.40574±0.0166 25.288±1.032	101.15	/- 1.9732 / << 4.303		
	0.0418 52.231	0.4190±0.0156 26.116±0.973	104.47			
2	0.49077 9.81538	0.07874±0.0039 4.9093±0.2430	98.18	/ -1.6061 / << 4.303		
	0.5058 10.1166	0.0812±0.0032 5.0599±0.1980	101.19			

	0.5038 10.075	0.08083±0.0158 5.039±0.987	100.78	/ -1.3018 / << 4.303	/-0.9439/ << 12.706 cell B with UV-Sp. $\bar{X}d$ = - 0.89305 σ_{n-1}= 0.9333 /- 1.3532/ << 12.706
	0.0423 10.582	0.0849±0.0101 5.293±0.632	105.86		

$^{\mu}$ quoted value

$^{\bar{X}d}$ average of difference between two type of method (developed & classical)

^{n} (no. of sample)=2

$^{\sigma n-1}$ standard deviation of different(paired t-test)

$^{\bar{\omega}i}$ practically

$^{ttab=t0.05/2,2}$ 4.303 (for individual t-test)

$^{ttab = t0.05/2,1}$ 12.706 (for paired t-test)

$^{UV–Sp.}$ UV–Spectrophotometric method

^{Tur} Turbidimetry

Conclusion

The suggested technique for determining promethazine hydrochloride involves turbidity measurements using the NAG-ADF-300-2-CFIA-analyzer. It is distinguished by its precision, quickness, and sensitivity. Furthermore, there is no other turbidimetry method in literature that can operate in the same manner as the manifold used. As a result, a novel alternative approach is available with improved linearity and detection limit, as well as easier manipulation and less expensive instruments and reagents. The use of the NAG-ADF-300-2 analyzer was found to be a perfect success, as evidenced by the repeatability of response at varied concentrations within the range of determination.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

Orcid

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