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A New Continuous Flow Injection Analysis Method Coupled with NAG-ADF-300-2 Analyzer for Promethazine-HCl by Cadmium Iodide as a Precipitating Reagent

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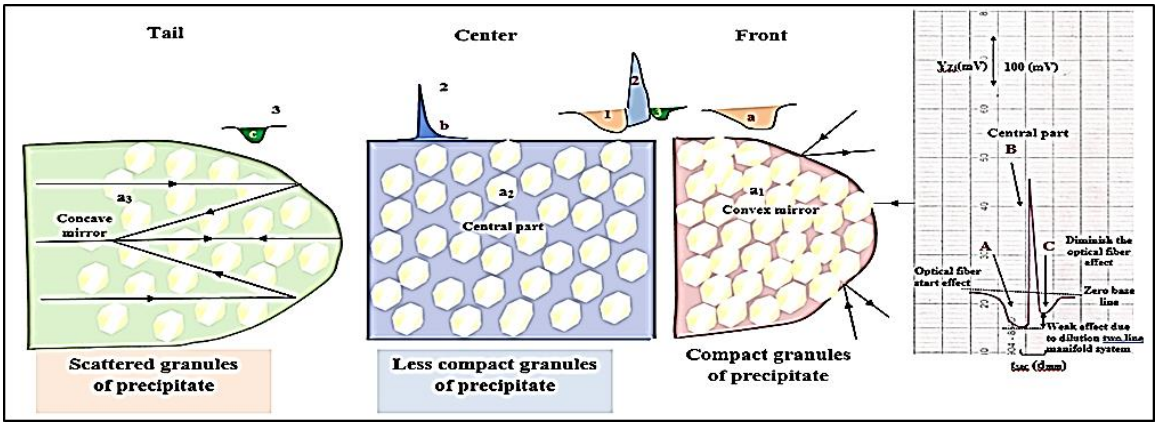
ABSTRACT

A highly accurate, simple, sensitive turbidimetric method was introduced for the determination of promethazine-HCl using a homemade NAG-ADF-300-2 system with continuous flow injection analysis system. This method is based on reaction between promethazine-HCl and cadmium iodide (CdI_2) in the presence of ammonium acetate to obtain a white precipitate for the ion-pair complex. Turbidity was measured via the attenuation of incident light by precipitated at 0-180°. Optimum parameters were studied to increase the sensitivity for newly developed methodology of promethazine-HCl- cadmium iodide system. The linear dynamic range for the instrument response vs. concentration of promethazine-HCL- was 0.25-25 mmol. L^{-1} for cell A and 0.1-20 mmol.L^{-1} for cell B, and limit of quantitative (S/N=10) 360.5574 µg /sample for cell A and 275.5830 µg /sample for cell B respectively with correlation coefficient (r) 0.9961 for cell no. 1 (cell A) and 0.9966 for cell no.2 (cell B); RSD % was lower than 0.6%. A comparison was made between the newly developed method with UV-Spectrophotometric and turbidimetric method as a classical method by using the standard addition method via the use of t-test at 95% confidence level. In addition, F-test was utilized to predict which of the methods was more precise than other method. The comparison of data revealed that long distance chasing photometer (NAG-ADF-300-2) is the choice with excellent extended detection, wide application and more sensitivity.

GRAPHICAL ABSTRACT



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Introduction

Promethazine-HCl (PRM-HCl) is one of the most important drugs in the enormous group of phenothiazine derivatives (Figure1) [1]. The chemical name of promethazine-HCl substance is 10-[2-(dimethyl amino) propyl]-phenothiazine monohydrochloride. It is H_1 antagonist utilized as an antiemetic in motion sickness, an antipsychotic drug for treatment mental disorders and for enhancing analgesic, narcotized and sedative effect of other medicines [2,3].

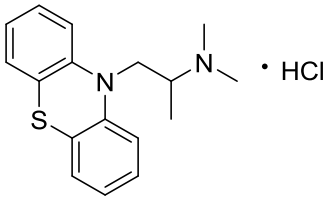


Figure 1: Structure of promethazine hydrochloride

Numerous analytical methods like spectrophotometry [4], HPLC [5], nephelometric titration [6], chemiluminescence [7] have been used. Due to importance of flow injection analysis method, it was combined with many different methods based on the precipitation reaction [8]. During this research work presented, it was determination of promethazine-HCL. Process of determination is based on the attenuation of the incident light (Turbidity measurements) using homemade long distance chasing photometer (NAG-ADF-300-2) [9] and represents the output of response by x-t recorder.

Material and methods

All chemicals were used of analytical reagent grade with highest purity available and all the solutions were dissolved by distilled water. A standard solution of 50 mmol.L^{-1} of PRM-HCl ($C_{17}H_{20}N_2S.HCl$) 320.9 g.mol^{-1} was freshly prepared by weight of 1.6045 g and dissolved in 100 ml conical flask. A series of *cadmium iodide (CdI_2) (BDH)* solutions were prepared from the dilution of standard solution 100 mmol.L^{-1} with distilled water. A stock solution of ammonium acetate (CH_3COONH_4, 77.08 g.mol^{-1}, *BDH,* 100 mmol.L^{-1}*)* was prepared by dissolving 0.7708 g/ 100 ml D.W.

Apparatus

- Peristaltic pump content 4- channels (Switzerland) and six-port medium pressure injection valve (IDEX Corporation, USA) with sample loop (1 mm i.e., Teflon, variable length) was used for loading and injection.
- The response was measured by a homemade homemade NAG-ADF-300-2 instrument is a multi-purpose photometric device including the offer of multi measurement individually or simultaneously or combine or separated whether 0-180° or 0 - 90°.This applies to clear solution or coloured or precipitated reaction product whether colloidal or crystalline coloured or white or even clear precipitate. It is a long-distance chasing photometer as a flow cell will have 300 mm as a distance with 2 mm as a path length to chaise and to accumulate the output resulted from attenuation of incident light 0-180^0 and diverged or fluorescence light at 0-90^0 via a flow cell. The first flow cell (cell A) is of 110 mm length covered with 11 white snow LED (WSLED) followed by uncovered distance of 100 mm length then attached to another with 2 solar cells at each side of (0-180^{0}and 0-90^0), cell (cell B) which is covered by 6 WSLED and a single photo cell (solar) of 60 mm length at each side.
- Potentiometric recorder to estimate the output signals (Siemens, Germany).
- UV-Vis. Spectrophotometric (RF-1501, Shimadzu, Japan) was use for classical methods.
- A Turbidimetry instrument, LUTRON Company (Taiwan), which is used for classical method measurement of turbidity at 0-180°.

Using a manifold of two lines coupled NAG-ADF-300-2 instrument to determine promethazine-HCl via forming a white precipitate an ion-pair between the drug and precipitating reagent (CdI_2) as shown in Figure 2. It is composed of two lines. The first line is the carrier stream of ammonium acetate (3 ml.min^{-1}) flow rate passing through the injection valve to carry the sample segment (135 µL for both cells) of 20 mmol.L^{-1} initial concentration PRM-HCl to mix with second line at flow rate 3 ml.min^{-1} by Y-junction point that carries the reagent of cadmium iodide (35 mmol.L^{-1}) for cell A and cell B before it is introduced to the NAG-ADF-300-2 analyzer, leading to measure

using both cell A and cell B. The reaction product is white particles from the ion pair complex. The obtained signals were resulted from the attenuation of the incident light by the precipitating particles agglomerated at a 0-180°.

Scheme 1 shows the suggested reaction pathway for the reaction product of promethazine-HCl-cadmium iodide ion-pair complex formation in aqueous medium. It was noticed from Figure 3. that those three responses were obtained. The first response (**A**) is represented by frontal surface layer of the precipitate segment, which acts as a convex mirror that will effectively collect the incoming light and increase its intensity causing the lowering of the zero light intensity adjustment. It is deeper than response **C** due to compactness of the precipitate formed. The second response (response **B**) is represented by the central part of the precipitate segment causing the decrease and attenuation of the incident light and consequently leading to decrease of a number of photons reaching the detector. And the third response (response **C**) is represented by the tail of the precipitate segment, which is a form of concave mirror causing collection and its concentration of incident light and leading to fewer number of photon and directed to the light intensity side, i.e., below the zero-background adjustment. It suffers from being unsymmetrical due to increased volume of liquid due to dilution effect of two-line system and its duration. This all will lead to a dispersed non compact medium of precipitated granules.

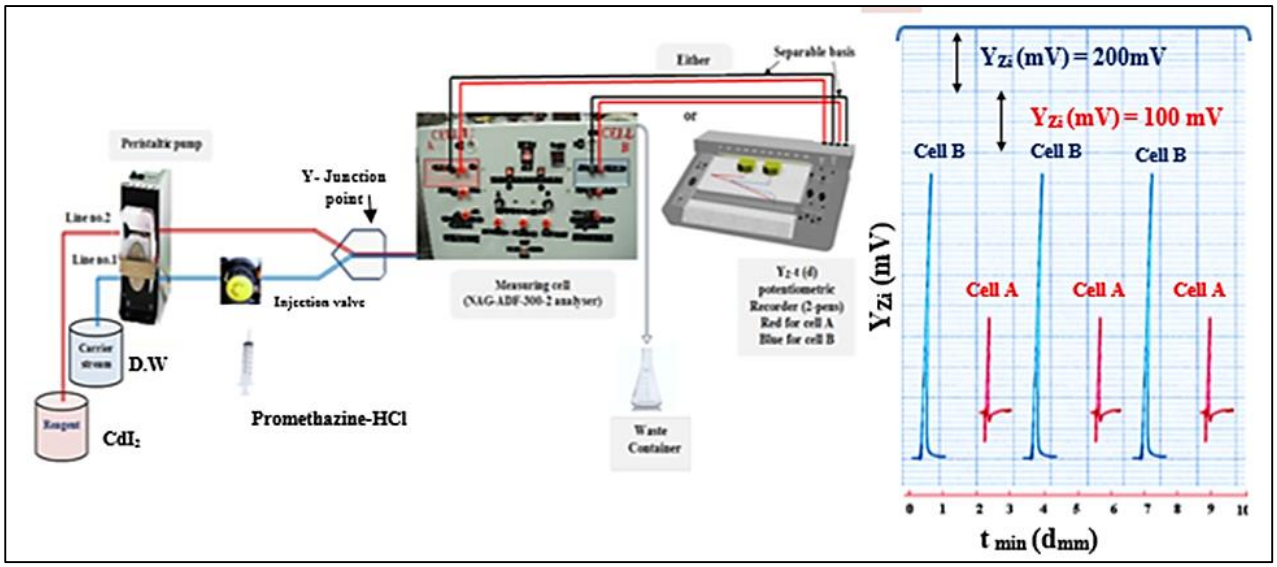
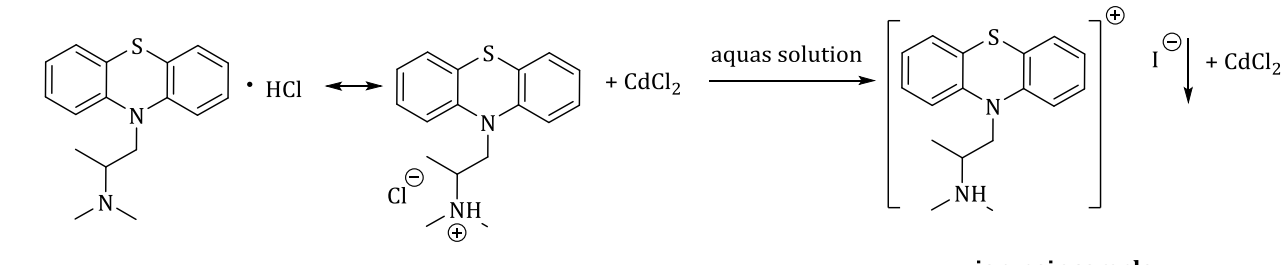


Figure 2: Flow gram system used for the determination of Promethazine-HCl



Promethazine- HCl

ion-pair complex (a gelatinous white precipiatate

Scheme 1: Suggested mechanism of Promethazine- HCl - CdI_2 ion pair complex formation

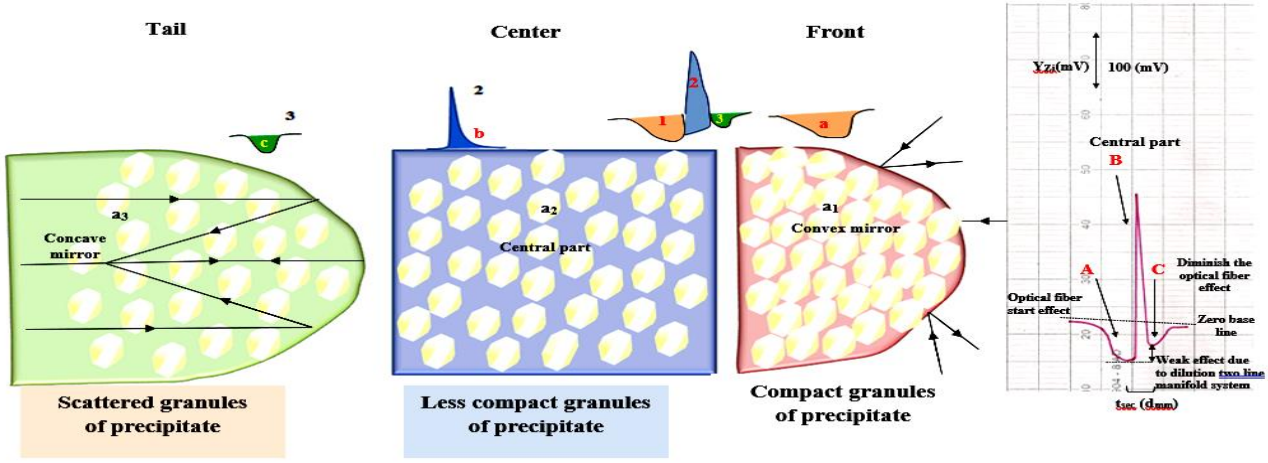


Figure 3: A diagram of the mechanics of what happens in the measuring cells (cell A & cell B)

Result and Dissection

Optimization of variables

Chemical variables

Cadmium iodide (CdI_2) concentration

Variables concentrations of CdI_2 ranging from 20 to 40 mmol. L^{-1} were prepared as a precipitate reagent at 3 ml.min^{-1} flow rate for carrier stream (D.W) and reagent, using 78.5 µL sample volume with 20 mmol. L^{-1} concentration of promethazine-HCl as an injected sample. A decrease of an attenuation of incident light in term of peak height with an increase of CdI_2 concentration up to 20 mmol. L^{-1} was observed. This increase in reagent concentration caused an increase the amount of precipitate and its compactness internally. Based on this, the precipitate formed an opaque partition and causing an increased high response (i.e., toward darkness). At higher concentration (i.e., more than 35 mmol.L^{-1}) for both cells, an introduction and penetration of reagent materials to the soft structural buildup of crystal growth was reported, which caused dispersion of light penetrating to depth of the crystal growth short in peak apex response.

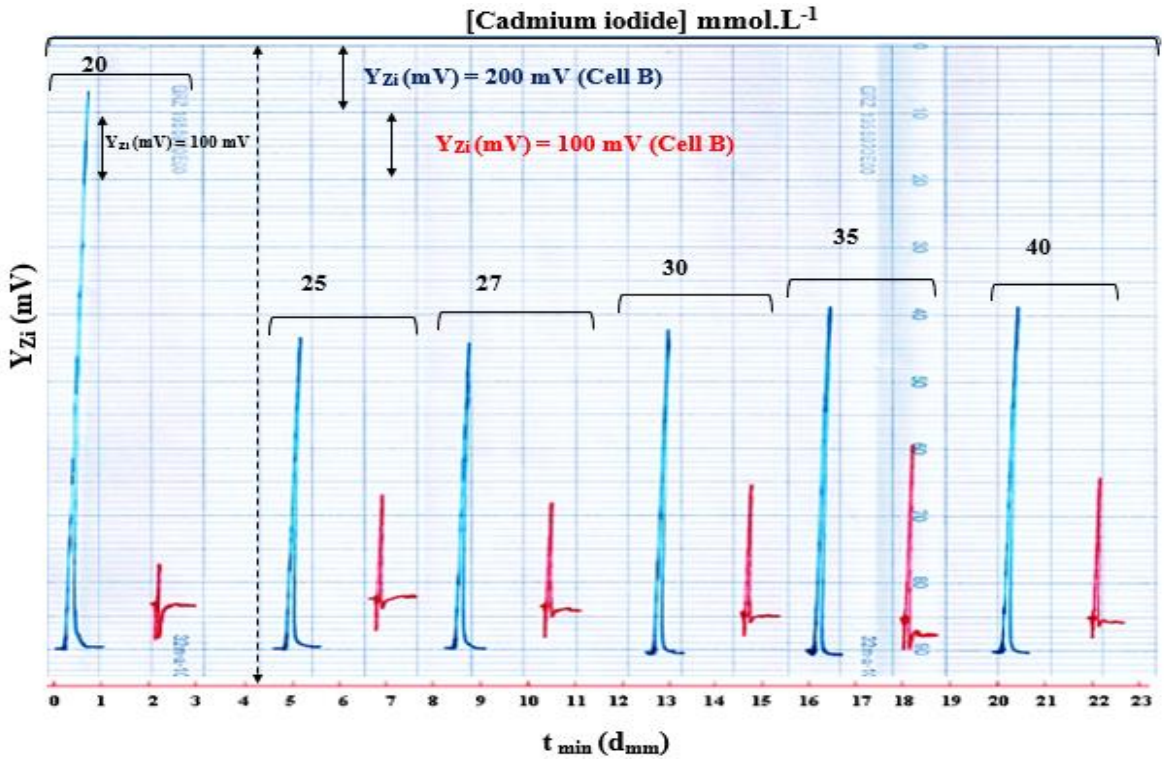


Figure 4: Effect of variable concentrations of CdI_2 on (S/N) energy transducer response and potentiometric scanning speed 60_{sec} (10_{mm}). Responses were plotted simultaneously but with a time difference expressed by distance equivalent to 100 mm

Effect of different media (Selected salts and acids)

The reaction between promethazine-HCl (20 mmol. L^{-1}) and Cadmium iodide (35 mmol. L^{-1}) for both cells to form a precipitate was studied in different media at 50 mmol. L^{-1} concentration (KCl, KNa-tartarate, KI, NaCl, NH_4Cl, CH_3COONH_4, Tartaric acid, Ascorbic acid, Acetic acid & HCl) in addition to aqueous medium as a carrier stream at 3 ml.min^{-1} flow rate for two lines (carrier stream and reagent line). From Figure 5, it can be noticed that an increase in sensitivity of response in salt medium as a carrier stream is compared with the use of aqueous and acid medium that causes a decrease of S/N- response; this might be attributed to an increase in the agglomeration, i.e., increasing the density of aggregates and compactness with each other and then increasing the intensity of transmitted light as there will be more vacant spaces in between agglomerates of particulate. Therefore, CH_3COONH_4 was chosen (as a carrier stream) as the optimum medium for the research work.

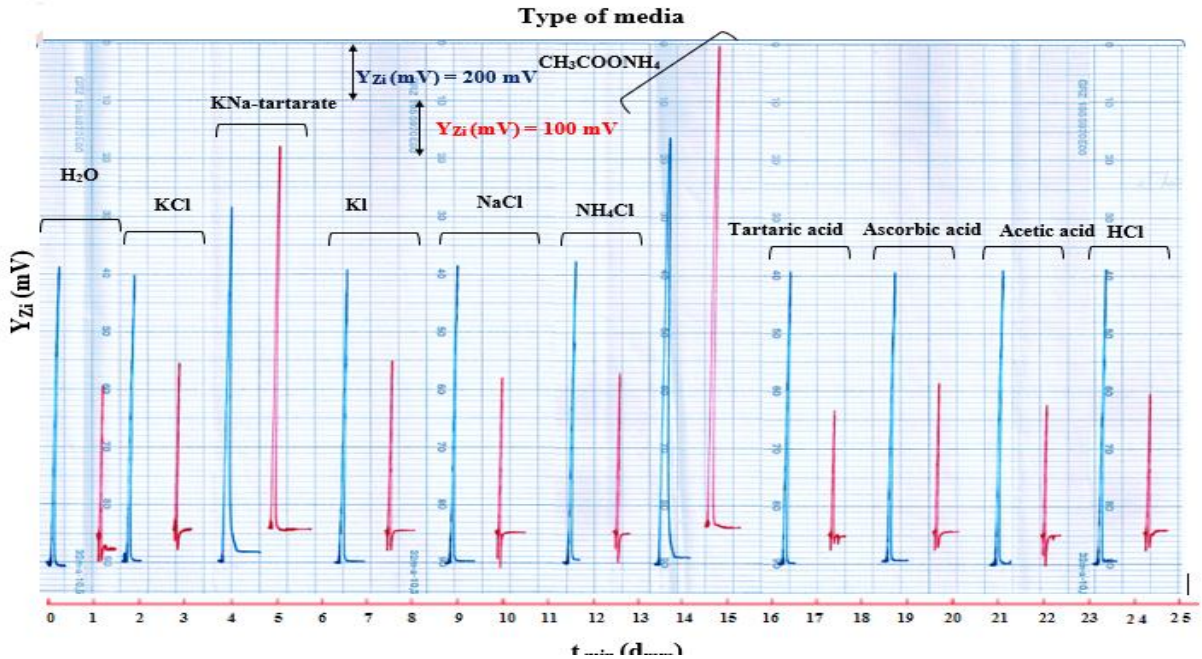


Figure 5: Effect the different media on (S/N) energy transducer response versus time using flow rate for both carrier stream and reagent 3 ml.min^{-1}, irradiation intensity for cell A was I=3 while for cell B was I=2, and 78.5 µL of sample volume was in use and potentiometric scanning speed 60_{sec} (10_{mm}). Responses were plotted simultaneously but with a time difference expressed by distance equivalent to 100 mm

Physical variables

Effect of Flow rate

Variation of flow rates 0.5-5 ml.min^{-1} for both lines (Carrier stream and reagent) were studied and all the previous experimental parameters were fixed, i.e., chemical parameters: promethazine-HCl (20 mmol. L^{-1})- CdI_2 (35 mmol. L^{-1})-CH_3COONH_4 (50 mmol. L^{-1}) system. It was noticed that at low flow rate, there was an increase in S/N- response profile from cell A and Cell B and increase Δt_B (base width) of response. It may be due to an increased opportunity for the crystal that formed to grow up relative flow, while at high flow rate (i.e.; more than 3 ml.min^{-1}) for both cells, insufficient time for the growth of a particle that means immature or incomplete precipitation, led to the formation of a small or semi-transparent particulates (Table 1). Therefore, all the results correspond with slope-intercept account, which is shown in Figure 6, where the choice of the segment can be selected (3–4.4 ml.min^{-1}) for both cells in which the 3 ml.min^{-1} flow rate for both lines within the chosen segment gave rise to obtaining a regular response.

Table 1: Effect of variation of flow rate on output (S/N) of energy transducer response

Pump speed	Flow rate (ml/min) for both lines	Output (S/N) of energy transducer response expressed as an average peak heights (n=3) $\bar{Y}_{Zi}$ (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} (ml) at flow cell	Concentration (mmol.L^{-1}) at flow cell	Df at flow cell
Cell A									
Cell B									
5	0.5	1016	0.12	1106±3.0558	30	120	2.135	1.5808	15.81
		880	0.14	880±3.0061	45	108	1.935	1.7442	14.33
10	1	800	0.15	800±3.0061	24	90	3.135	1.0766	23.22
		800	0.17	800±3.2794	30	66	2.335	1.4454	17.29
15	2	680	0.17	680±2.8074	21	48	3.335	1.0119	24.71
		464	0.27	464±3.0558	18	39	2.735	1.2340	20.26
20	2.6	852	0.21	852±4.4968	18	45	4.035	0.8364	29.89
		772	0.18	772±3.4533	15	36	3.255	1.0369	24.11
25	3	848	0.19	848±4.0496	18	45	4.635	0.7282	34.33
		760	0.19	760±3.6769	15	36	3.735	0.9036	27.67
30	3.8	672	0.26	672±4.2980	15	33	4.315	0.8162	30.63
		328	0.42	328±3.4533	12	27	3.555	0.9494	26.33
35	4.4	624	0.29	624±4.5216	12	30	4.535	0.7442	33.59
		248	0.69	248±4.2732	9	25.5	3.875	0.8709	28.71
40	5	584	0.33	584±4.7701	9	27	4.635	0.7282	34.33
		240	0.64	240±3.8011	6	24	4.135	0.8162	30.63

t*: Arrival time from injection valve reaching to measuring cell (sec), Δt_B: Base width of response (sec), $t_{0.05/2,2}$=4.303, Df: Dilution factor at flow cell.

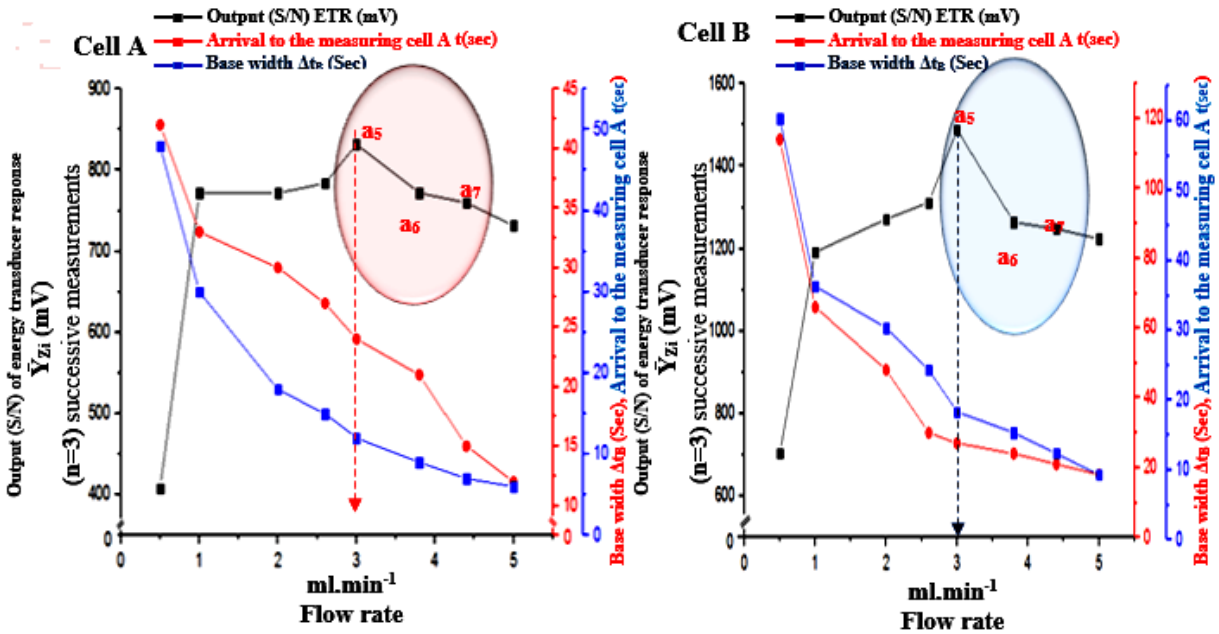


Figure 6: Output (S/N) of energy transducer response expressed as an average peak height in mV ($\bar{Y}_{Zi}$ (mV)) for cell A and cell B by Promethazine-HCl (20 mmol. L^{-1})- CdI_2 (35 mmol. L^{-1}) - CH_3COONH_4 (50 mmol. L^{-1}) system,

78.5 µL of sample volume, the intensity of light expressed as I=3 for cell A and I=2 for cell B; with one segment (three data points) as a chosen segment

Effect of sample volume

The optimum flow rate (3 ml.min^{-1}) for two lines (i.e., carrier stream & reagent), Promethazine-HCl at 20 mmol. L^{-1} concentration and 35 mmol. L^{-1} of CdI_2 and 50 mmol. L^{-1} of CH_3COONH_4 for both cells were used. Variable length of Teflon tube ranging (5.10-35.80) cm with diameter (D) of 1mm, equivalent to (40-281) µL of sample volume, was applied. The obtained results including profile of energy transducer response ($\bar{Y}_{Zi}$(mV)) versus time (t_{sec}(d_{mm})) and average peak heights in mV are shown in Figure 7. It was noticed that in both cases (cell A & cell B) the highest response was obtained at 135µL, while at a sample volume of large volume, i.e., more than 135 µL, it caused a clear decrease in the obtained responses with an increase facing wider Δt_B; most probably due to a higher density of particulate precipitate at the center of travelling sample segment. In addition, a large amount of precipitate formed will enhance the filter affecting the incident light intensity as well as diverged light intensity. On the basis of what stated and in order to compromise with the economy of sample usage, 135 µL was the optimum for both cells as the most convenient sample size level to avoid a broadening as well as disturbed response and high trust ability; therefore, the interference caused by precipitated aggregate was decreased, which might cause a delayed movement of reacting product.

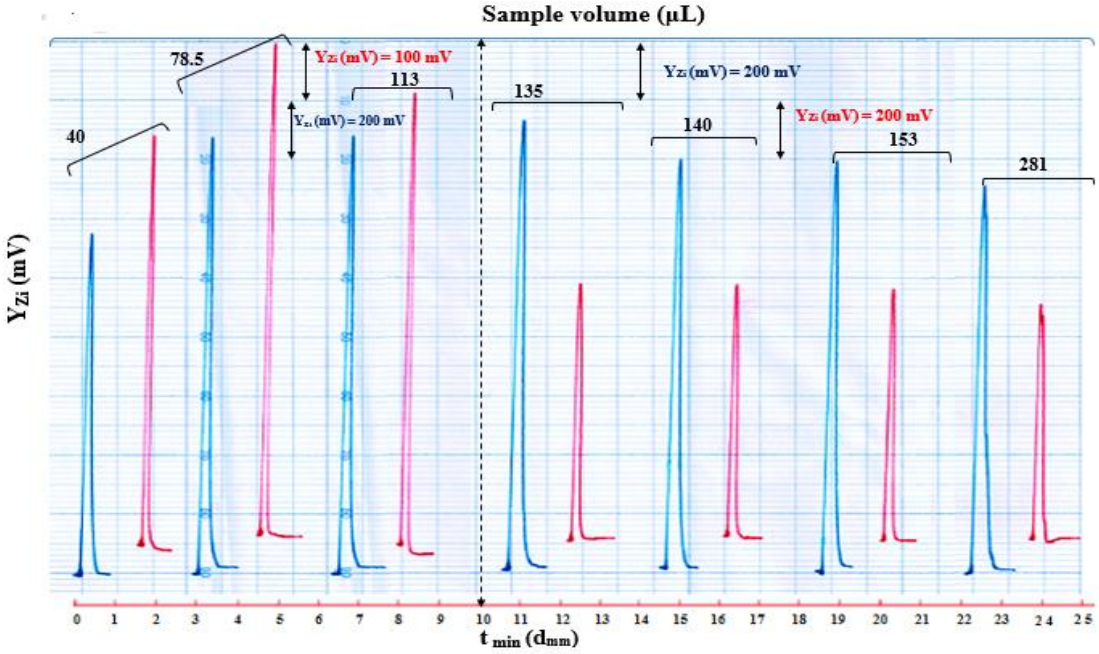


Figure 7: Effect of sample volume on (S/N) energy transducer response versus t_{min} (d_{mm}), potentiometric scanning speed 60_{sec} (10_{mm})

Effect of delay reaction coil

Variable coil length of 0-30 cm was studied. This length comprises a volume ranging from 0 to 942 µL, which is connected after Y-junction directly in flow system. Promethazine-HCl (20 mmol. L^{-1})-CdI_2 (35 mmol. L^{-1})- CH_3COOH_4 (50 mmol. L^{-1} for both cells) system at flow rate of 3 ml.min^{-1} for carrier stream (CH_3COOH_4) and reagent (CdI_2) and the injection volume 135 µL as well as I= 3 for cell A and I= 2 for cell B were used in this study. Table 2 shows that an increase in coil volume leads to the decrease of peak height for either cells (cell A and cell B). In addition, the width of the base is almost constant for cell A but an increase for cell B which is most probable due to agglomeration and condensations of their masses and its difficulty in being moving with the carrier stream flow. we noticed the increase of the of base width by increasing the sample volume Δ t, probably due to a long duration of carrier stream to pass through injection valve causing a restriction of the flow; it led to increase of dispersion of the precipitate

particles segment and increase of base width (Δ t). It was noticed that in both cases (cell A & cell B) the highest response was obtained at 135µL. On the basis the above mentioned statement and in order to compromise with the economy of sample usage, 135 µL was the optimum for both cells as suitable most convenient of sample size level to avoid a broadening as well as disturbed response and hence reach high trust ability to decrease the interference caused by precipitated aggregate that might cause a delayed movement of reacting product. Therefore, the results of measurement show that a direct measurement with no coils in the manifold system will be the best way of the system work.

Table 2: Volume effect of delay reaction coil on output (S/N) of energy transducer response expressed as an average peak height (mV) for determination of Promethazine-HCl

Delay reaction Coil Length (Cm) r=1mm	Volume of delay reaction coil (µL) V_{RC}	Output (S/N) of energy transducer response expressed as an average peak heights (n=3) $\bar{Y}_{Zi}$ (mV)	RSD%	Reliability (two tailed) at 95% $\bar{Y}_{Zi}$ (mV)$\pm t_{0.05}/2$, n-1 $\sigma n\text{-}1/\sqrt{n}$	arrival time to cells unit t^*_{sec}	Base Width Δt_B (sec)	Vadd (ml) at flow cell	Concentration (mmol. L^{-1}) at flow cell	D_f at flow cell
Cell A									
Cell B									
Direct attachment	Direct attachment	872	0.22	872±2.6335	18	30	3.135	0.8612	23.22
		1520	0.08	1520±2.9068	23	33	3.435	0.7860	25.45
10	314	864	0.19	864±4.0242	21	31	3.235	0.8346	23.96
		1520	0.07	1520±2.7577	25	36	3.735	0.7229	27.67
15	471	840	0.19	840±4.0993	23	33	3.435	0.7860	25.45
		1464	0.09	1464±3.2794	27	39	4.035	0.6691	29.89
25	785	840	0.18	840±3.8508	25	35	3.635	0.7428	26.93
		1432	0.12	1432±4.2732	30	40	4.135	0.6529	30.63
30	942	848	0.18	848±3.7018	27	36	3.735	0.7229	27.67
		1464	0.175	1464±6.3601	33	42	4.335	0.6228	32.11

Df: Dilution factor at flow cell.

Study of the applied voltage expressed as an intensity used for supplying the White Snow Light Emitting Diodes (WSLEDs) in NAG-ADF-300-2 analyser

A study was carried out to determine the effect of intensity light of the irradiation sources on the (S/N)- response of the energy transducer response via the selector switch. Using the control of the light intensity knob (they are two), the increase will be maximized clockwise at four stages (level of irradiation: I=1-2-3-4 i.e., 3.0 VDC, 3.2 VDC, 3.4 VDC and 3.5 VDC respectively). In addition to the off position, an indication LED is controlled electrically for intensity operation, i.e., indication lamp for cell A attached with eleven sources of WSLED plus and indication lamp for cell B parallelly attached with the 6 WSLED. As stated earlier, the study was conducted with physical and chemical parameters, which were kept constants for both cells. It was noticed that a selection of position 4 (i.e.; I=4) was a very convenient intensity for cell A (cell no.1) (larger number of the selector switch means more light intensity) while position 2 (I=2) of the selector switch was a convenient intensity for cell B (cell no.2).

Variation of concentration with obtained response leading to linear range

The steps involved in concentration (measured as t_{sec} (d_{mm}) as the x- axis versus the response Y_{Zi}

(mV)), which falls in the range, i.e., the extent to which or the limit between which the variation is possible, i.e., obtaining a peak which means a mountain with a pointed summit, which prevents noise (electrical disturbance). A series of solutions ranging 0.25-33 mmol. L^{-1} for both cells were prepared leading to the scatter plot as shown in Figure 8 A and B, giving a correlation of Y_Z (mV) versus t_{sec} (d_{mm}) of 0.9961 and 0.9966 with coefficient of determination of 0.9922 and 0.9932 and the chosen equation represented 99.22% and 99.32% of the obtained results for cell A and cell B which was fair enough. Therefore, both cells were able to distinguish various ranges of concentration. Table 3 summaries all results for both cells (A & B). Ordinary spectrophotometer will never reach this level of concentration. If under any circumstances higher concentration was required for analyte dilution, it could be used to create the responses with the range available in text above.

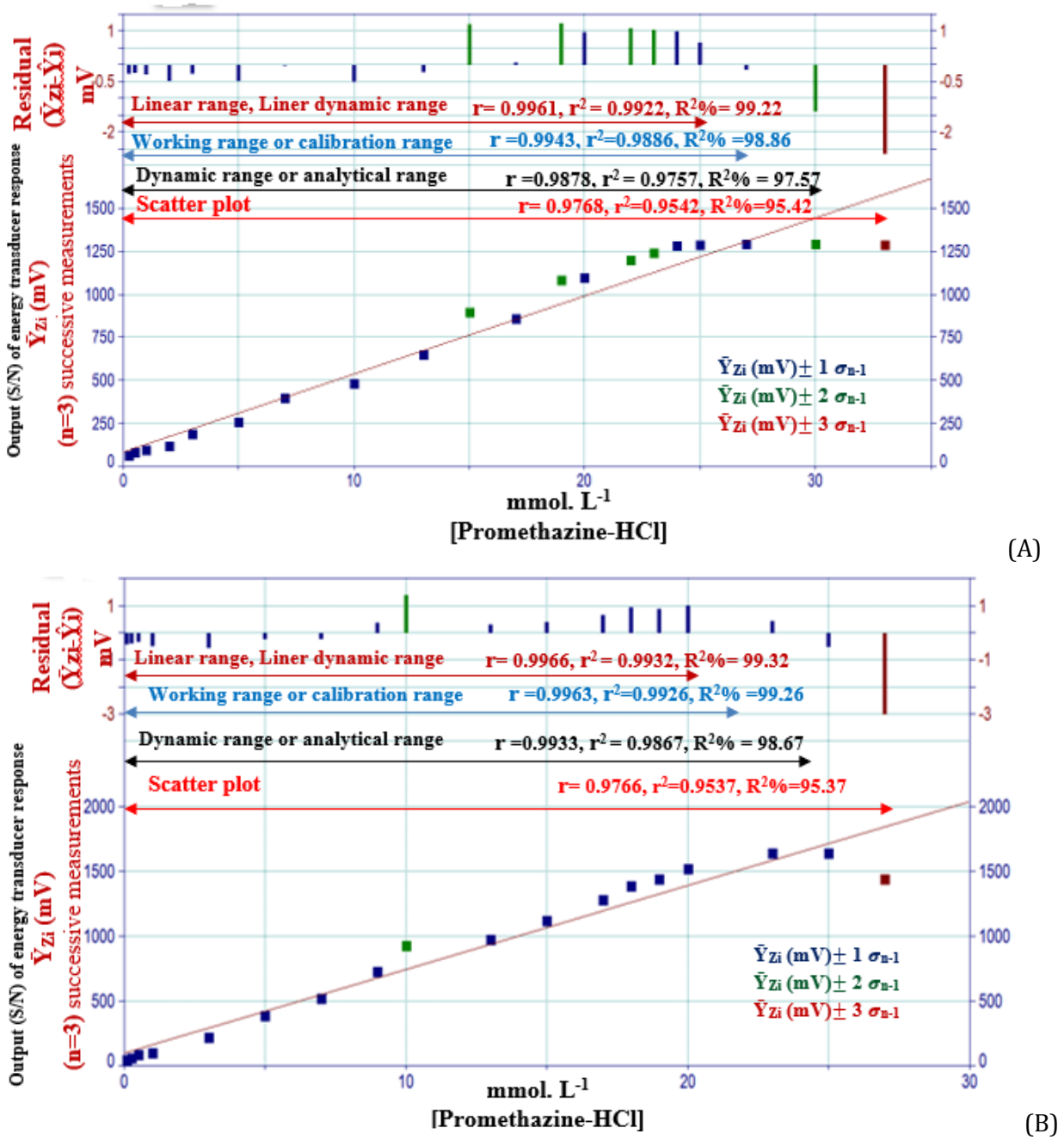


Figure 8: Different range for the effect of promethazine-HCl concentration on attenuation of incident light using NAG- ADF-300-2 analyser. A: For cell A & B: For cell B

n: no. of measurement, $\hat{Y}_{Zi}$ (mV); estimated value of cell A and cell B in mV for developed method, r: correlation coefficient, r^2: coefficient of determination, $R^2\%$ (percentage capital R-squared): explained variation as a percentage / total variation and $t_{tab} = t_{0.05/2, n-2}$.

Three approaches were used to describe the limit of detection (L.o.D). The limit of detection of promethazine hydrochloride were calculated using gradual dilution of the lowest concentration in the scatter plot, and theoretical (slope method) based on the value of slope $X= 3S_B/slope$. Any one of the described definitions could be used depending on the practical research need (Table 4).

Table 3: Summary of results for linear regression for the variation of (S/N) energy transducer response with promethazine-HCl concentration using first degree equation of the form $\hat{Y}=a+ b x$ at optimum conditions.

Type of mode	Range of [Promethazine-HCl] mmol.L^{-1}(n)	$\hat{Y}_{Z\,i\,(mV)}=a_{mV} \pm S_a t + b\,(\Delta y_{mV}/\Delta x_{mmol.L^{-1}}) \pm S_b t$ [Promethazine-HCl] mmol. L^{-1} at confidence level 95%, n-2	r, r^2, $R^2\%$	t_{tab} at 95%, n-2	Calculated t-value $t_{cal}=/r/\sqrt{n-2}\,/\,\sqrt{1-r^2}$
Cell A					
Cell B					
Linear range or linear dynamic range	0.25 -25 (17)	28.3941± 38.1470+52.1852± 2.5359 [Promethazine-HCl] mmol.L^{-1}	0.9961, 0.9922, 99.22	2.131 << 43.849	
	0.1-20 (15)	39.3597±42.8479+74.1502±3.6806 [Promethazine-HCl] mmol.L^{-1}	0.9966, 0.9932, 99.32	2.160 << 43.521	
Working range or calibration range	0.25 -27 (18)	37.6258±46.2054+50.8438±2.8980 [Promethazine-HCl] mmol.L^{-1}	0.9943, 0.9886, 98.86	2.120<< 37.192	
	0.1-23 (16)	47.2407±45.5577+72.7146±3.5993 [Promethazine-HCl] mmol.L^{-1}	0.9963, 0.9926, 99.26	2.145 << 43.326	
Dynamic range or analytical range	0.25 -30 (19)	56.9256±66.3911+48.4186±3.9119 [Promethazine-HCl] mmol.L^{-1}	0.9878, 0.9757, 97.57	2.110 <<26.122	
	0.1-25 (17)	63.9588±61.2151+69.9742±4.4708 [Promethazine-HCl] mmol.L^{-1}	0.9933, 0.9867, 98.67	2.131 << 33.354	
Scatter plot	0.25 - 33 (20)	84.3167±89.1055+45.3367±4.9184 [Promethazine-HCl] mmol.L^{-1}	0.9768, 0.9542, 95.42	2.101 << 19.367	
	0.1-27 (18)	101.2609±110.9269+64.4083±7.5196 [Promethazine-HCl] mmol.L^{-1}	0.9766, 0.9537, 95.37	2.120 << 18.157	

Table 4: Limit of detection (L.o.D) for promethazine-HCl at optimum achieved parameters using 135µl as an injection sample for both cells, and flow rate for each line 3 ml.min^{-1}.

Type of cell	General dilution for the minimum concentration in scatter plot	Based on the value of slope x=3S_B/slope	Linear equation $\hat{Y} = Y_b + 3S_b$	$\hat{Y}=Y_b+10S_b$
Cell A	0.0085 mmol.L^{-1} 24.9418 ng/sample	0.8094 µg/sample	108.1672 µg/sample	360.5574 µg/sample
Cell B	0.01 mmol.L^{-1} 433.2150 ng/sample	1.1393 µg/sample	82.6749 µg/sample	275.5830µg/sample

The assessment evaluation of new developed methodology (i.e.; NAG-ADF-300-2 analyser) for the determination of Promethazine-HCl using Promethazine-HCl-CdI_2 (35 mmol.L^{-1}) – CH_3COONH_4 (50 mmol.L^{-1}) system was compared with the available literature method, namely UV-spectrophotometric method and turbidimetric method, which was based on:

1-Spectrophotometric method: Based on measurements of absorbance for the range of concentration 0.001-0.3 mmol.L^{-1} at λ_{max}= 249 nm [5], (Fig. 9) using quartz cell.

From Figure10, the scatter plot shows the best linear range extend from 0.001-0.08 mmol.L^{-1} with correlation coefficient of 0.9994 and % capital R-square = 99.89 %, n=12 (no. of measurements).

2-Turbidimetry measurement: Based on the reaction of Promethazine-HCl with CdI_2 (6 mmol.L^{-1}) in the presence of CH_3COONH_4 at 7 mmol.L^{-1}concentration, which was already used after being established, as can be seen in Figure 11. The scatter plot (Fig.12) shows the best linear range extend from 0.4-9 mmol.L^{-1} with correlation coefficient of 0.9976 and % capital R-square = 99.52, n= 11(no. of measurements). The detection limit was 0.2 mmol.L^{-1} equivalent to 641.8 µg/ sample.

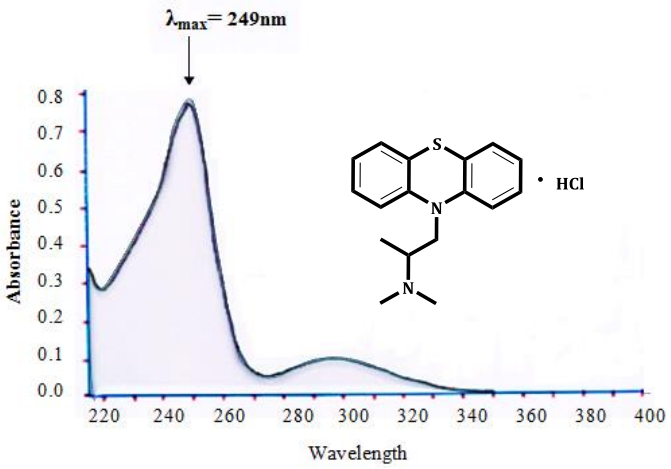


Figure 9: Absorbance of UV- Spectrum of Promethazine-HCl at concentration of 0.03 mmol.L^{-1} that shows λ_{max} = 249 nm

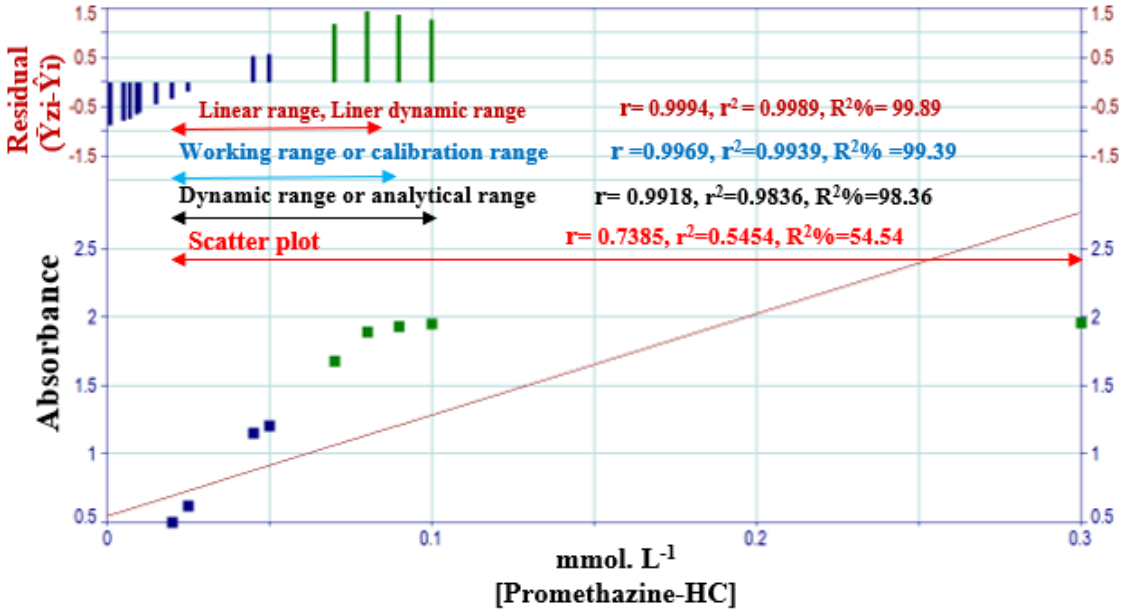


Figure 10: The scatter for Promethazine-HCl using classical method at λ_{max}=249 nm

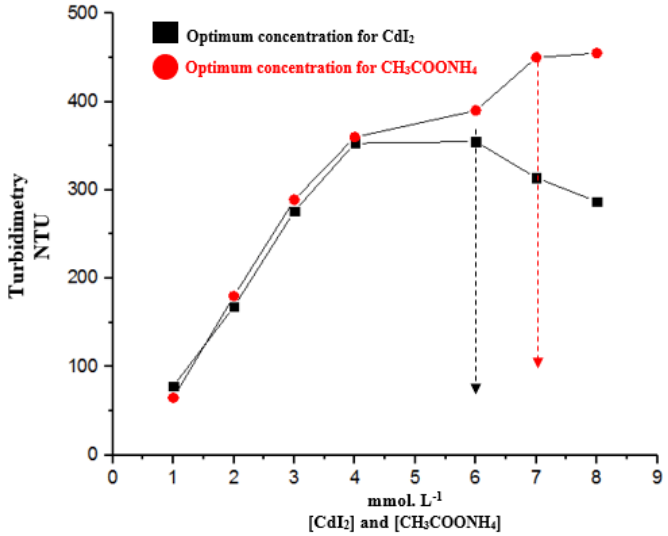


Figure 11: Graphical representation of the optimum concentration of CdI_2 reacting with Promethazine-HCl (5 mmol.L^{-1}) in the presence of the best CH_3COONH_4 concentration of Turbidimetric method

Determination of Promethazine-HCl in drugs using NAG-ADF-300-2 analyser and different classical methods

The newly developed methodology (NAG-ADF-300-2) was used for the determination of Promethazine-HCl in two samples of drugs from two different companies (Histazin, United pharmaceutical, Jordan, 25 mg and Coldin, S.D.I, Iraq, 5 mg). A series of solutions were prepared of sample no.1 by 50 mmol.L^{-1} and sample no. 2 by 10 mmol.L^{-1} via transferring of 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 the addition of 0, 2, 3, 4 and 5 ml from 50 mmol.L^{-1} of standard solution to obtain 0, 4, 6, 8 and 10 mmol.L^{-1} for developed method, while UV- spectrophotometric classical method by transferring of 0.02 ml from 50 mmol.L^{-1} of sample no. 1 and 0.1 ml from 10 mmol.L^{-1} of sample no.2 to each of the five volumetric flask (25 ml) followed by the addition of 0.0, 0.01, 0.015, 0.02 and 0.025 ml from 50 of standard solution of Promethazine-HCl to obtain 0.0, 0.02, 0.03, 0.04 and 0.05 mmol.L^{-1} for the turbid metric method by transferring of 1ml from 50 mmol.L^{-1} sample no.1 and 1.25 ml from 10 mmol.L^{-1} sample no. 2 to each of the five volumetric flask (25 ml) followed by the addition of 0.0, 0.5 , 1, 1.5 and 2 ml from 50 mmol.L^{-1} of standard solution of Promethazine-HCl to obtain 0, 1, 2, 3 and 4 mmol.L^{-1}, taking into a consideration that the first flask was for the sample. The measurements were conducted by three methods. Results were mathematically treated for the standard addition method. Tables 5 A and B show a practical content of active ingredient at 95 % confidence level and efficiency of determination in addition to t-test [10, 11].

The first test: The comparison of newly developed method (NAG-ADF-300-2) analyzer with official quoted value (B.P) (25 mg of Histazin and 5 mg of Coldin), as shown in Table 5 B (column 5) by calculated t-values of each individual company compared with tabulated t-value.

A hypothesis for active ingredient can be estimated as follows:

Null hypothesis: There is no significant difference between the means obtained from two different companies ($\underline{w}_i$) and the quoted value (μ = 25 mg or 5 mg).

i.e., H_o: $\underline{w}_{i(\text{NAG-ADF-300-2/Cell A}} = \mu_{(\text{5 mg or 25 mg})}$

or $\underline{w}_{i(\text{NAG-ADF-300-2/Cell B}} = \mu_{(\text{5 mg or 25 mg})}$

For: Histazin (United pharmaceutical, 25 mg, Jordan), Coldin (S.D.I., 5 mg, Iraq) companies.

Against:

Alternative hypothesis: There is a significant difference between the means and quoted value

i.e.; H_1: $\underline{w}_{i(\text{NAG-ADF-300-2/Cell A}} \neq \mu_{(\text{5 mg or 25 mg})}$

or $\underline{w}_{i(\text{NAG-ADF-300-2/Cell B}} \neq \mu_{(\text{5 mg or 25 mg})}$

All values of t_{cal} less than t-tabulated value. So, Null hypothesis will be accept and rejecting the alternative hypothesis, this means that there is no significant different between the quoted active ingredient value and the measured value.

Table 5.A: Standard addition results for the determination of Promethazine-HCl in two samples of drugs using NAG - ADF- 300 -2 analyser for cell A, cell B and two classical methods

No. of sample	Commercial name, Company Content Country	Type of method: Newly developed methodology; Cell A; Cell B; Turbidimetry (NTU); UV- Sp. Classical method Absorbance measurement 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 equivalent to 0.4011 g (50 mmol. L^{-1}) Sample 1 and 0.0802 g (10 mmol. L^{-1}) sample 2 of the active ingredient 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-HCL mmol. L^{-1}					Equation of standard addition 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 -HCl] mmol. L^{-1}	
					0.00	4	6	8	10		
					0.00	0.5ml	1ml	1.5 ml	2 ml		
					0.00	1	2	3	4		
					0.00	0.01 ml	0.015 ml	0.02 ml	0.025 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	120	360	480	590	710	122.4324±10.1573+58.8514 ±1.5452 [Promethazine-HCl] mmol. L^{-1}	0.9999, 0.9998, 99.98
					165	480	640	810	980	159.0540±19.9009+81.4189 ±3.0277 [Promethazine-HCl] mmol. L^{-1}	0.9998, 0.9996, 99.96
					175	260	355	440	520	176.0000±11.0129+87.0000 ±4.4999 [Promethazine-HCl] 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±1.3 311 [Promethazine-HCl] 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	25	190	270	368	450	21.5405±15.9332+42.6892± 2.4240 [Promethazine-HCl] mmol. L^{-1}	0.9995, 0.9990, 99.90
					65	400	560	720	990	45.2703±35.1185+89.5946± 5.3431 [Promethazine-HCl] mmol. L^{-1}	0.9947, 0.9894, 98.94
					60	98	188	290	363	40.2000±47.9712+79.8000± 19.5839 [Promethazine-HCl] 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±1.8 736 [Promethazine-HCl] mmol. L^{-1}	0.9985, 0.9971, 99.71

Second test: Paired t- test at α=0.05 (2-tailed) was used for the comparison of developed method using NAG-ADF-300-2 analyzer and classical method using shimadzu (UV-1800 double beam) spectrophotometer and turbidimetry via Turbidity- meter, (Taiwan), which depends on the measurements of scattered light at 0-180°, as shown in Table 5 B (column 6).

The assumption is statistically made for the two samples as follows:

H_o hypothesis: There is significant difference between all used methods concerning the output results; i.e., $\mu_{\text{(NAG-ADF-300-2 Analyser cell A)}} = \mu_{\text{(NAG-ADF-300-2 Analyser cell B)}} = \mu_{\text{uv-Sp}} = \mu_{\text{turbidimetry}}$

H_1 (Alternative hypothesis): There is a significant difference between four methods; i.e., $\mu_{\text{(NAG-ADF-300-}}$

2 Analyser cell A)≠ μ(NAG-ADF-300-2 Analyser cell B) ≠$\mu_{uv\text{-}Sp}$ ≠ μ turbidimetry.

The results obtained are displayed in Table 5. B, which shows all the calculated t-values are less than the tabular t, i.e., $t_{cal} << t_{tab}$ (12.706). Therefore, the null hypothesis is accepted and the alternative hypothesis is rejected at 0.05 probability of two tailed type. It can be inferred that there was no significant difference between four methods and two newly developed methods (i.e., NAG-ADF-300-2 analyzer cell A & cell B), which are available with more sensitive and less cost and added to determination of drug in addition to the available classic methods.

Based on the above study which indicated that there is no significant differences between four methods, therefore one way- ANOVA test was used to prove the differences between the two samples regarding the active ingredient.

The assumption of statistical data treatment as follows;

Null hypothesis: there is no significant difference between two samples of drug against the alternative hypothesis; showing a significant difference between the two samples.

From the obtained results as shown in table 5.B (column 7), it was found alternative hypothesis is accepted on behalf drug's active ingredient.

Table 5.B: Summary of the results for practical content, efficiency (Rec %) for determination of Promethazine-HCl in two samples of drugs and t-test, F- test for comparison among four methods

No. of sample	Type of method: Newly developed methodology: Cell A / Cell B: Turbidimetry (NTU): UV- Sp. Classical method Absorbance measurement at λ_{max}=249 nm: Practical concentration (mmol.L^{-1}) in 25 ml / Original sample solution in 25 ml	Practical weight of Promethazine-HCl in weight of sample $\bar{\omega}$ i (g) ±4.303 $\sigma_{n-1}/\sqrt{n}$ / Weight of Promethazine-HCl in tablet $\bar{\omega}$ i (mg) ±4.303 $\sigma_{n-1}/\sqrt{n}$	Efficiency of determination Rec.%	Individual t-test for compared between quoted value & practical value $(\bar{\omega}i - \mu)\sqrt{n}/\sigma_{n-1}$ Cell A or Cell B	Paired t –test Compared between two methods: $t_{cal} = \bar{X}d\sqrt{n}/\sigma_{n-1}$	t_{tab} at 95% confidence level	F- test compared between two drugs with (25 mg & 5 mg): ANOVA-one way using F-test		
							Source	SSq (d.f)	M_{sq}
1	2.080 / 52.009	0.417±0.0158 / 26.005±0.983	104.02	4.3983 >4.303	cell A with Tur. $\bar{X}d$ =0.363 σ_{n-1}=0.5006 1.0255 < 12.706		Between	828.122 (1)	S_1^2=828.122
	1.9535 / 48.838	0.3918±0.0135 / 24.419±0.842	97.68		cell A with UV-Sp. $\bar{X}d$ = - 0.178 σ_{n-1}= 0.0948 /- 2.6554/ << 12.706		Within (error)	1.88607 (6)	S_2^2=0.314345
	2.0229 / 50.575	0.40574±0.0166 / 25.288±1.032	101.15	/- 2.9693/ < 4.303					
	0.0418 / 52.231	0.4190±0.0156 / 26.116±0.973	104.47		cell B with Tur. $\bar{X}d$ = - 0.427 σ_{n-1}=0.6251 /-0.9660/ << 12.706		Total	830.00807 (7)	
2	0.5046 / 10.0918	0.08096±0.0059 / 5.048±0.368	100.95	0.5614 << 4.303			$F_{cal} = S_1^2/S_2^2 = F_{0.95,1,6}$		
	0.5053 / 10.1056	0.08107±0.00548 / 5.054±0.342	101.08		cell B with UV-Sp. $\bar{X}d$ = 0.968 σ_{n-1}=1.0309 /- 1.3279/ << 12.706		2634.43 >> 5.99		
	0.5038 / 10.075	0.08083±0.0158 / 5.039±0.987	100.78	0.6792 << 4.303			Significant difference between two drugs		
	0.0423 / 10.582	0.0849±0.0101 / 5.293±0.632	105.86						

Conclusion

The unchallenged precision, speed, sensitivity and accuracy gained in this research work using NAG-ADF-300-2. showed an attenuation method that can be used to determination of promethazine-HCl by CdI_2 in the presence of ammonium acetate as a carrier stream in a well trusted measurements, which was in complete agreement with the cited values. No other turbidimetric method is available in the literature that can follow some mode of working with the simplicity of the manifold used. Therefore, an alternative method is available with extended linearity and excellent limit of detection.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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