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*Original Article*

# Chlorpromazine-HCl Determination *via* Its Oxidation with Sodium Nitrite in Sulfanilic Acid Medium *via* CFIA Technique through Long Distance Chasing Photometer NAG-ADF-300-2

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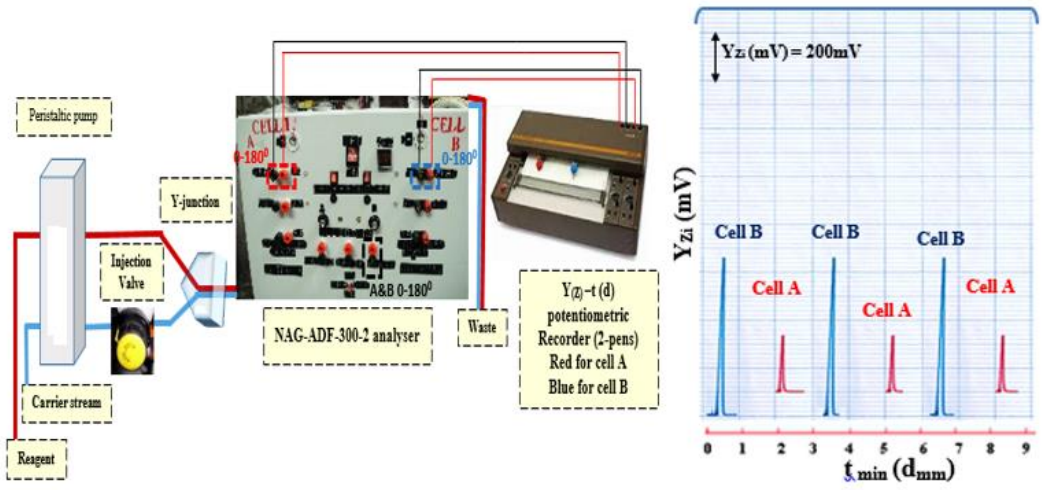
**ABSTRACT**

In this work, precipitation reaction with a new photometer NAG-ADF-300-2 analyzer was used to detect chlorpromazine hydrochloride by attenuation of incident light (White Snow Light Emitting Diode) at two steps, the first 110 mm and the second 60 mm with a separation distance of 100 mm of the chlorpromazine-hydrochloride reaction with a mixture of two reagents of sodium nitrite and sulfanilic acid form a yellowish-white precipitate. The attenuation of this precipitate by incident light was measured in a highly repeatable and reproducible way from a relative standard deviation percent (RSD %) of less than 0.3% at a variable concentration. The linear dynamic graph ranges from 0.5 to 45 mmol.L$^{-1}$ for Cell A and 1-43 mmol.L$^{-1}$ for Cell B, with a limit of detection (L.O.D) 0.9984 µg and 49.9239 µg at 281 µL sample, and a correlation coefficient (r) of 0.9994, 0.9993 for Cell A and Cell B, respectively.

**GRAPHICAL ABSTRACT**

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## Introduction

Chemically, chlorpromazine-hydrochloride is [3-(2-chlorophenothazine-10yl) propyl] dimethylamine hydrochloride, with a molecular formula $C_{17}H_{19}ClN_2S.HCl$ is one of the best medications in the phenothiazine derivatives family (Figure 1) [1]. Chlorpromazine-hydrochloride is used as an antiemetic in nausea therapy, as an antipsychotic in the mental illness treatment, and to enhance the analgesic, narcotic, and tranquillizing effects of other medications [2]. A review of the literature exposes that several analytical methods for determining chlorpromazine. HCl have been reported, including spectrophotometry [3,4], HPLC [5], turbid-metric method [6], and chemiluminescence [7].

The estimate of chlorpromazine hydrochloride was the focus of this research. Development of a turbidimetric method for the quantitative determination of chlorpromazine hydrochloride is based on forming a turbid, yellowish white ion-pair association complex in closed flow cell system as a result of a reaction between the drug and sodium nitrite and sulfanilic acid which was used as a precipitation reagent using a homemade photometer for long distance chasing (NAG-ADF-300-2) the output of response was represented by Yz (mV) – t min. (d mm) recorder.

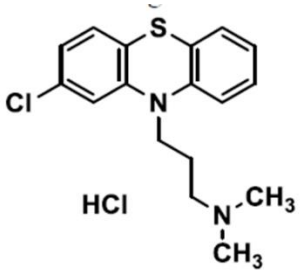



**Figure 1:** Structure of chlorpromazine-HCl

## Chemicals and Methods

*Reagents and chemicals*

The chemicals that were used as a solvent were high purity, supplied by SDI, BDH and Fluka companies. A stock solution of chlorpromazine-HCL ($C_{17}H_{19}ClN_2S.HCl$, 355.33 $g.mol^{-1}$, SDI, 100 $mmol.L^{-1}$) was prepared by dissolving 8.8833 g in 250 mL of distilled $H_2O$. A stock solution of sodium nitrite ($NaNO_2$, 68.9953 $g.mol^{-1}$, BDH, 100 $mmol.L^{-1}$), by dissolving 0.6899 g in 100 mL of distilled water. A stock solution of sulfanilic acid ($NH_2C_5H_4SO_3H$), 173.19 $g.mol^{-1}$, Fluka, 100 $mmol.L^{-1}$) had been made by dissolving 1.7319 g in 100 mL of distilled water.

*Sample preparation of Chlorpromazine hydrochloride*

Twenty tablets were weighted then crushed and grinded. Tablets containing 25 mg of chlorpromazine hydrochloride 1.1964 and 1.1295 g were weighted (equivalent to 0.2665 g of active ingredient, 15 $mmol.L^{-1}$) for Largektil-nexus-Germany- 25 mg and Largactil - Oubari pharma-Syria-25 mg respectively, dissolved in as a little water, followed by filtration to get rid of undissolved materials and completed the volume to 50 mL with distilled water.

*Apparatus*

The novel photometer NAG-ADF-300-2 instrument is a multi-purpose photometric device that includes the offer of multiple measurements individually or concurrently, combined or separated, whether 0-180 or 0-90, The NAG-ADF-300-2 photometer is built entirely at home (homemade) 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 cell number one (flow cell A), with a 110 mm length, has eleven sources of WSLED white snow LED facing 0-1800 two 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° to measure the scattering of light or the divergent or even fluorescence.

The cell number two (flow 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 degrees on another solar cell. Passing through

the face of 20 mm × 20 mm, a 4 mm whole that will represent the flow tube (made of quartz) 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 entire multifunction system (Figure 2) for determining chlorpromazine hydrochloride by precipitating a yellowish white colored precipitate, which is an ion-pair between the medicine and the precipitating agent (mixture of sodium nitrite and sulfanilic acid). The system is Consists of 2 lines, the line number one is supplied with distilled H2O as a conveyor stream (3 $ml.min^{-1}$) carrying the sample section (281 µL for both cells) of 40 $mmol.L^{-1}$ chlorpromazine hydrochloride to the injection valve, while the line number two supplies the mixture of sodium nitrite (20 $mmol.L^{-1}$) and sulfanilic acid (16 $mmol.L^{-1}$) at a flow rate (3 $ml.min^{-1}$ ). At the Y-junction, the two lines merge and lead to the measuring cell. Brown yellowish particles from the ion pair complex are the reaction result. The measuring technique is based on the light signal from the weakened the incident light by the precipitating particles in the flow cell to the detector location at angle 0-180°.

A suggested reaction pathway for the formation of the product of chlorpromazine hydrochloride-sodium nitrite-sulfanilic acid ion–pair complex formation in aqueous solution [9,10] is presented in scheme 1. Figure 3 shows the excitation and emission spectroscopic of the azo bridge group resulting from adding sodium nitrite (0.05 $mmol.L^{-1}$) to sulfanilic acid (0.01 $mmo.L^{-1}$). These azo bridge groups in sulfanilic acid derivatives exhibited a maximum excitation at 332 nm wavelength. These bands observed below 400 nm can be attributed to the π-π* transitions. The emission wavelength was observed to be above 400 nm ($\lambda_{max}$= 430 nm). The large stocke's shift and single energy level (is often highly desirable for fluorescence measurements) of sulfanilic derivatives showed a bathochromic shift (red shift) approximately between 90-100 nm compared with sulfanilic acid. This situation can be explained by the enhancement of electron-charge transfer (*i.e.* n- π* transition) due to longer conjugation by azo bridge group.

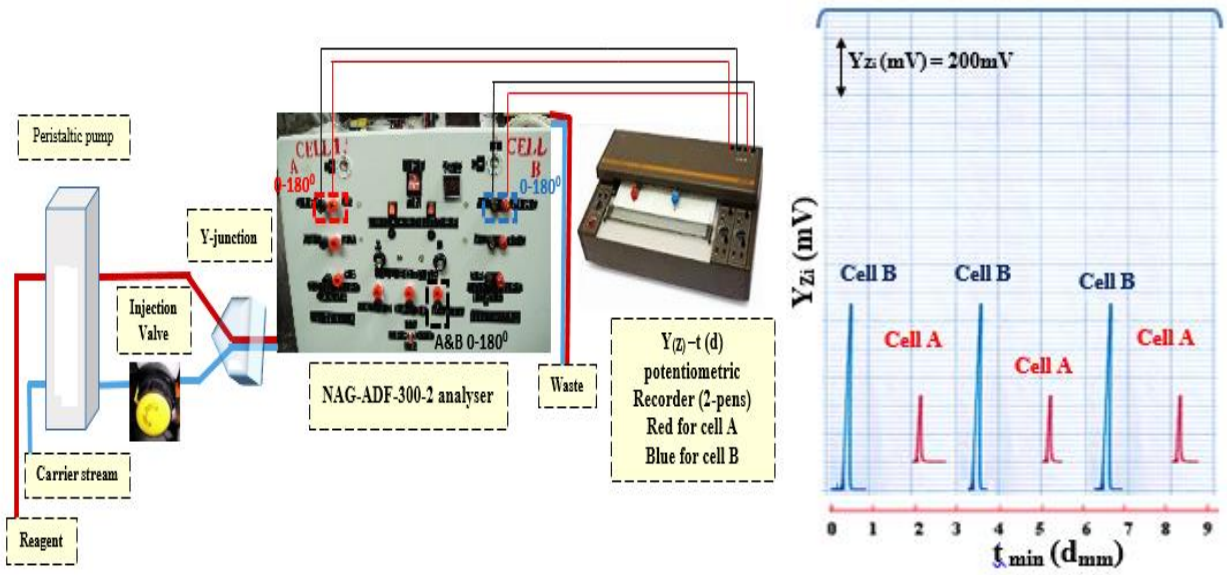
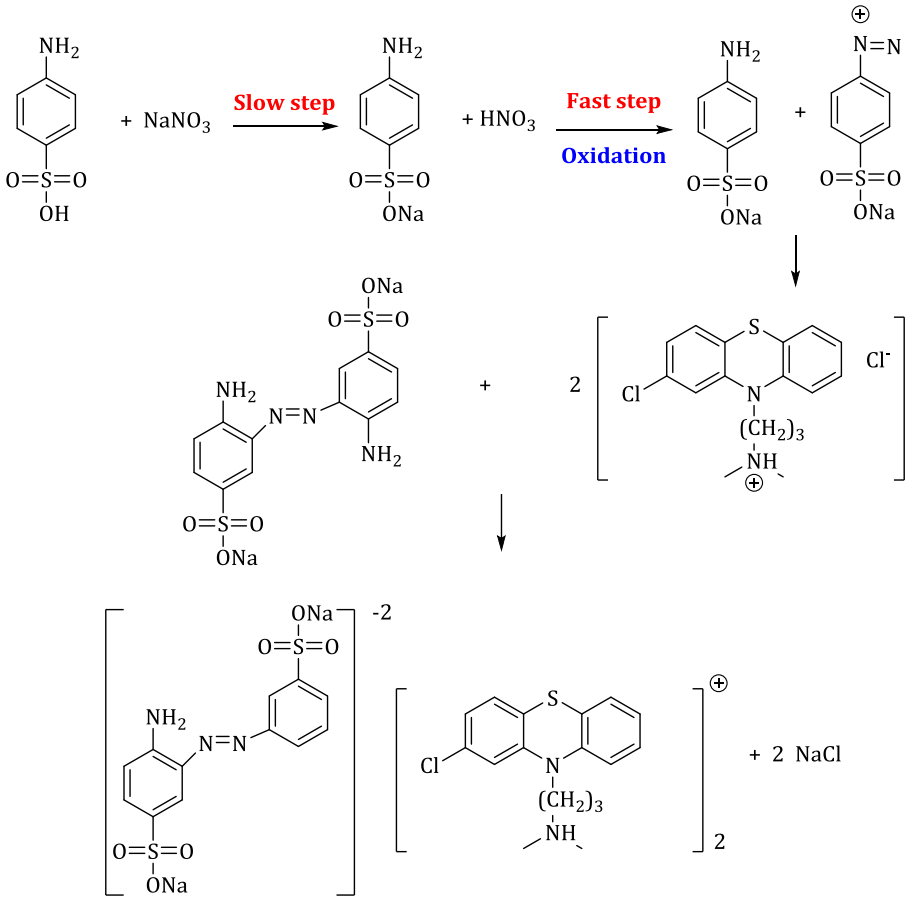



**Figure 2:** Flow gram of manifold system consist of two lines of Chlorpromazine-HCl determination




**Scheme 1:** Proposed mechanism of Chlorpromazine hydrochloride–PMA formation of ion pair complex

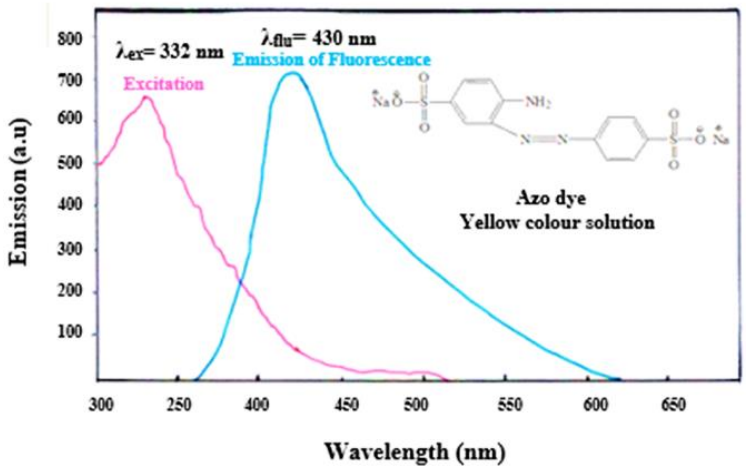



**Figure 3:** Excitation and emission spectrum of azo dye compound

## Results and Discussion

*Chemical variables*

*Effect of sodium nitrite [NaNO2] concentration*

A sequence of the $NaNO_2$ solution ranging from 20-200 mmol.L$^{-1}$ concentration mixed with sulfanilic acid (12 mmol.L$^{-1}$) which are represented as a precipitating agent at flow rate 3 ml.min$^{-1}$ for conveyor stream line (Distilled water) and line two (reagents); and 78.5 µL sample segment with 40 mmol.L$^{-1}$ concentration of chlorpromazine hydrochloride as an injected sample. The increase of Sodium nitrite concentration (i.e., > 20 mmol.L$^{-1}$) leads to decrease of peak heights that were obtained from both cells. This might probably cause by the increase of accumulation of particulate, which in turn to preventing the optical fiber phenomenon; that might occur in the measuring flow cell that will increase the light intensity (Figure 4).

Therefore, 20 mmol.L$^{-1}$ had been chosen as the ideal concentration for both cells.

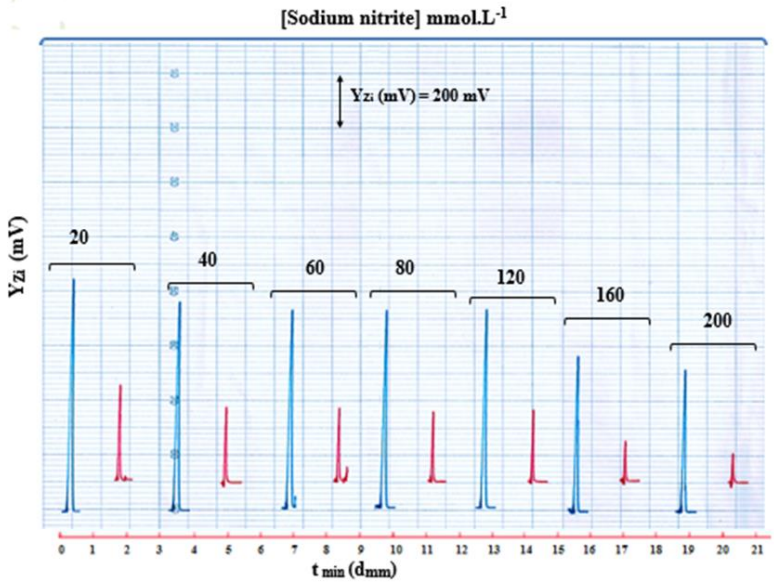



**Figure 4:** The effect of the [$NaNO_2$] on (S/N) energy transducer response vs. $t_{min}$ ($d_{mm}$). Responses were plotted simultaneously but with a time difference expressed by distance equal to 100 mm

*Effect of Sulfanilic acid concentration*

The study was carried out using variable concentrations of sulfanilic acid ranging from 4-40 mmol.L$^{-1}$ were prepared and mixed with 20 mmol.L$^{-1}$ concentration of $NaNO_2$ equally a precipitate reagent at flow rate (3 ml.min$^{-1}$) for both lines. It can be seen that the increase of sulfanilic acid concentration leads to an increase in the peak height expressed as an attenuation of incident light arriving to 16 mmol.L$^{-1}$ obtaining highest sensitivity (Figure 5), this can be attributed to the nature of formed particulate (*e.g.*, colloidal, crystalline, or suspension) and it's surfaces, also it's tendency in obscuring the direct light to the detector. Dealing with higher concentration (i.e., > 16 mmol.L$^{-1}$) leads to decrease of peak heights that were obtained from both cells. This might probably cause by the increase of accumulation of particulate, which in turn to preventing the optical fiber phenomenon; that might occur in the measuring flow cell that will increase the light intensity. Therefore, 16 mmol.L$^{-1}$ was selected as optimum concentration for both cells.

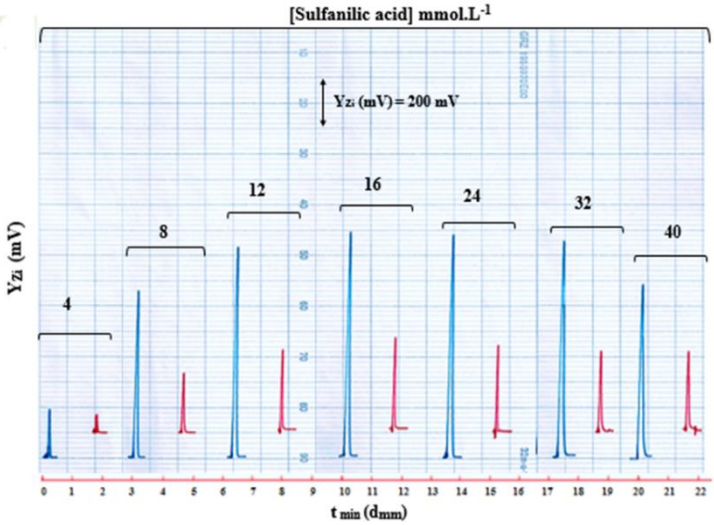



**Figure 5:** Effect of [sulfanilic acid] on (S/N) energy transducer response versus time

*The effect of different media (selected salts and acids)*

A variety medium at 50 mmol.L$^{-1}$ concentration ($Na_2SO_4$, $Na_2CO_3$, NaCl, KCl, K, Na-tartrate, $NH_4Cl$

and $CH_3COONH_4$) as well as the use of 50 mmol.$L^{-1}$ concentration of $C_6H_8O_6$, $C_4H_6O_6$, HCl, $H_2SO_4$, $HNO_3$and $CH_3COOH$ as an acid media in addition to distilled water used as a carrier stream. It can be seen that no responses were obtained when acids were used, this might be attributed to the dissolving of precipitate particulate or a complete dissociation leaving the reactant medium free of solid particulate to the detector response sensitivity, while the studied of salts affect reason for low of S/N- response; This may be attributed to its effect in increasing agglomeration i.e., aggregate density increase and Compactness with each other by increasing the intensity of transmitted light such as there will be more vacant spaces in between agglomerates of particulate which in turn decreases the attenuation of incident light. Distilled water was used as a transferring medium on the first line (carrier stream) to improve the sensitivity of measurement to determine Chlorpromazine-HCL. All the obtained results are summarized in table 1.

**Table 1:** Effect of different medium on the measurement of energy transducer response

| Type of salt (50 mmol.$L^{-1}$) | 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) $\pm t_{0.05/2,\,n-1}\,\sigma_{n-1}/\sqrt{n}$ |
|---|---|---|---|
| Cell A | | | |
| Cell B | | | |
| $H_2O$ | 376 | 0.19 | 376±1.8385 |
| | 880 | 0.12 | 880±2.5838 |
| $Na_2SO_4$ | 296 | 0.30 | 296±2.2111 |
| | 496 | 0.13 | 496±1.6149 |
| $Na_2CO_3$ | 360 | 0.36 | 360±3.1800 |
| | 840 | 0.07 | 840±1.4409 |
| NaCl | 320 | 0.35 | 320±2.7577 |
| | 640 | 0.22 | 640±3.4285 |
| KCl | 312 | 0.32 | 312±2.5093 |
| | 640 | 0.18 | 640±2.7825 |
| K,Na-tartarate | 304 | 0.31 | 304±2.3105 |
| | 488 | 0.33 | 488±4.0247 |
| $NH_4Cl$ | 336 | 0.26 | 336±2.1863 |
| | 728 | 0.16 | 728±2.8322 |
| $CH_3COONH_4$ | 336 | 0.21 | 336±1.7639 |
| | 656 | 0.22 | 656±3.5527 |
| Ascorbic acid, Tartaric acid, HCl, $H_2SO_4$, $HNO_3$ and $CH_3COOH$ | 0 | 0 | 0±0 |

*Physical variables*

*Effect of Flow rate*

Variable of flow rates (0.5 to 5) ml/min for two lines ($H_2O$ as a carrier stream and a mixture of $NaNO_2$ - sulfanilic acid as a reagents line) for cell A and cell B was used at chlorpromazine hydrochloride (40 mmol/L)-$NaNO_2$ (20 mmol/L)-sulfanilic acid (16 mmol/L) system, 78.5 µL sample volume for both cells. It was noticed that at low flow rate there is an increase in S/N- response profile from cell A and cell B and an increase ΔtB (base width) of response, it's may be to an increased opportunity for the crystal that formed to grow up relative. While at high flow rate (i.e.; more than 3 ml/min) for both cells due to not sufficient time is given for growth a particle which means immature or incomplete precipitation, that causing to form a small or semi-transparent particulates (Table 2). On this basis the 3ml/min flow rate for both cells, will the choice. The obtained confidence corresponds to the slope-intercept method for the choice of optimum parameter where the sector a5-a7 is the chosen section due to the increase of value of (a) within it the selected choice of optimum flow rate to obtain a regular response and sensitivity increase.

**Table 2:** The effect of flow rate variation on incident light attenuation

| Pump speed | Flow rate ($ml.min^{-1}$) For both lines | 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 $\Delta t_B$ (sec) | Vadd. To sample volume (ml) at flow cell | Concentration Of Drug ($mmol.L^{-1}$) at flow cell | $D_f$ at flow cell |
|---|---|---|---|---|---|---|---|---|---|
| Cell A | | | | | | | | | |
| Cell B | | | | | | | | | |
| 5 | 0.5 | 152 | 0.59 | 152±2.2111 | 30 | 30 | 0.579 | 5.4231 | 7.38 |
| | | 520 | 0.16 | 520±2.1117 | 42 | 48 | 0.879 | 3.5722 | 11.19 |
| 10 | 1 | 320 | 0.32 | 320±2.5093 | 21 | 24 | 0.879 | 3.5722 | 11.19 |
| | | 792 | 0.19 | 792±3.9254 | 30 | 33 | 1.179 | 2.6633 | 15.02 |
| 15 | 2 | 336 | 0.33 | 336±2.7577 | 15 | 21 | 1.479 | 2.1231 | 18.84 |
| | | 824 | 0.16 | 824±3.2794 | 24 | 24 | 1.679 | 1.8702 | 21.39 |
| 20 | 2.6 | 352 | 0.36 | 352±3.1055 | 12 | 15 | 1.379 | 2.2770 | 17.57 |
| | | 848 | 0.14 | 848±3.0061 | 18 | 21 | 1.899 | 1.6535 | 24.19 |
| 25 | 3 | 376 | 0.30 | 376±2.8322 | 9 | 9 | 0.979 | 3.2074 | 12.47 |
| | | 880 | 0.15 | 880±3.2794 | 12 | 15 | 1.579 | 1.9886 | 20.11 |
| 30 | 3.8 | 304 | 0.42 | 304±3.1800 | 6 | 7 | 0.965 | 3.2539 | 12.29 |
| | | 832 | 0.19 | 832±3.9254 | 9 | 12 | 1.599 | 1.9637 | 20.37 |
| 35 | 4.4 | 288 | 0.32 | 288±2.3105 | 5 | 6 | 0.959 | 3.2742 | 12.22 |
| | | 832 | 0.15 | 832±3.0807 | 7 | 9 | 1.399 | 2.2445 | 17.82 |
| 40 | 5 | 280 | 0.36 | 280±2.5341 | 3 | 5 | 0.912 | 3.4429 | 11.62 |
| | | 816 | 0.24 | 816±4.9191 | 6 | 6 | 1.079 | 2.9101 | 13.75 |

*Effect of Sample volume*

Sample volumes variation (40 to 281) µL were studied at optimum flow rate 3 ml/min for both lines ($H_2O$ line and reagents line), with selected concentration (40 $mmol.L^{-1}$) of chlorpromazine hydrochloride, NaNO2 (20 $mmol.L^{-1}$)- sulfanilic acid (16 $mmol.L^{-1}$) were used. It can be seen from

the reported results in table 3 there is an increase in sensitivity with the increase of sample segment (loop) and obtaining a symmetric response this reflects that the precipitate particles are crystalline in nature. Here the crystals are spherical, in addition to the aid in moving with the carrier stream, it is speed will be higher (more than) and falls within the effect of convection. Therefore, 281 µL was chosen as the most suitable injected volume. In addition, slope-intercept method (figure 6) supports this choice as the segment no.5 in the range of 140-281 µL has got the highest intercept (measurement high of sensitivity taking other factors too). Which shows that 281 µL as the optimum choice the researchers (Table 4; in which the segment was chosen on the basis of the increase in sensitivity represented by the value of a through the increase in the output of the energy transducer response).

**Table 3:** Variation of sample volume on the output of response (mV) at 2.6 ml.min$^{-1}$ flow rate

| Length of Sample Segment Cm r=0.5 mm | Sample Volume µL $V=\pi r^2h$ | 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 $\Delta t_B$ (sec) | Vadd (ml) at flow cell | Concentration (mmol.L$^{-1}$) at flow cell | $D_f$ at flow cell |
|---|---|---|---|---|---|---|---|---|---|
| Cell A | | | | | | | | | |
| Cell B | | | | | | | | | |
| 5.10 | 40 | 232 | 0.31 | 232±1.8136 | 6 | 6 | 0.640 | 2.5000 | 16.00 |
| | | 640 | 0.20 | 640±3.1800 | 9 | 12 | 1.240 | 1.2903 | 31.00 |
| 10 | 78.5 | 376 | 0.27 | 376±2.5093 | 9 | 9 | 0.979 | 3.2074 | 12.47 |
| | | 880 | 0.17 | 880±3.7763 | 12 | 15 | 1.579 | 1.9886 | 20.11 |
| 14.40 | 113 | 408 | 0.22 | 408±2.1863 | 12 | 18 | 1.913 | 2.3628 | 16.93 |
| | | 952 | 0.18 | 952±4.2980 | 15 | 21 | 2.213 | 2.0425 | 19.58 |
| 17.20 | 135 | 432 | 0.28 | 432±3.0558 | 14 | 20 | 2.135 | 2.5293 | 15.81 |
| | | 1008 | 0.19 | 1008±4.7452 | 18 | 24 | 2.535 | 2.1302 | 18.78 |
| 17.90 | 140 | 440 | 0.29 | 440±3.1800 | 15 | 21 | 2.240 | 2.5000 | 16.00 |
| | | 1040 | 0.17 | 1040±4.2732 | 21 | 27 | 2.840 | 1.9718 | 20.29 |
| 19.50 | 153 | 464 | 0.23 | 464±2.7080 | 17 | 23 | 2.453 | 2.4949 | 16.03 |
| | | 1052 | 0.13 | 1052±3.2794 | 23 | 29 | 3.053 | 2.0046 | 19.95 |
| 35.80 | 281 | 1080 | 0.12 | 1080±3.2794 | 19 | 24 | 2.681 | 4.1925 | 9.54 |
| | | 1264 | 0.11 | 1264±3.3291 | 25 | 30 | 3.281 | 3.4258 | 11.68 |

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

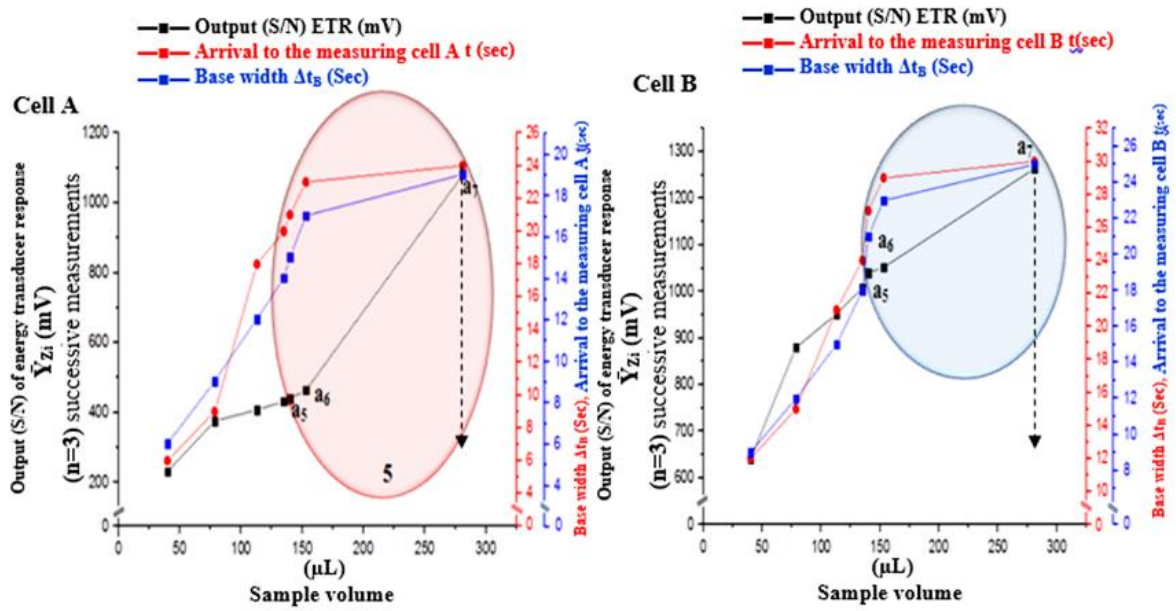



**Figure 6:** Output (S/N) of energy transducer response expressed as an average peak heights in mV ($\bar{Y}Zi$ (mV)) for cell A and cell B using chlorpromazine hydrochloride (40 mmol.L$^{-1}$) -$NaNO_2$ (20 mmol.L$^{-1}$) - sulfanilic acid (16 mmol.L$^{-1}$) system at flow rate of the carrier stream 3 ml.min$^{-1}$, 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

**Table 4:** (S/N) energy transducer response (S/N) output for cell A and cell B with five chosen segments (each three data points) for variable sample volume

| No. of segment | Sample volume (µL) | Segment | Slope (mV/mmol.L$^{-1}$) | Intercept mV |
|---|---|---|---|---|
| Cell A | | | | |
| Cell B | | | | |
| 1 | 40-113 | $a_1$-$a_3$ | 2.43 | 150.65 |
| | | | 4.31 | 491.28 |
| 2 | 78.5-135 | $a_2$-$a_4$ | 0.98 | 298.08 |
| | | | 2.25 | 701.83 |
| 3 | 113-140 | $a_3$-$a_5$ | 1.16 | 277.07 |
| | | | 3.04 | 606.36 |
| 4 | 135-153 | $a_4$-$a_6$ | 1.79 | 189.75 |
| | | | 2.14 | 728.17 |
| 5 | 140-281 | $a_5$-$a_7$ | 4.65 | 228.27 |
| | | | 1.62 | 809.48 |

*Effect of junction points*

Variable junction points volume: 6.28 µL, 98.125 µL, 1.85 ml, 2 ml and 2.154 ml were studied which is conducted on the use of chlorpromazine.HCl (40 mmol.L$^{-1}$)- $NaNO_2$ (20 mmol.L$^{-1}$) -Sulfanilic acid (16 mmol.L$^{-1}$) system, 281 $\mu L$ sample volume and flow rate 3 ml.min$^{-1}$ for both cells. It was observed quite well that the decrease of responses heights (Y(mV)) in using junction points at various different volumes for both cells which shows and indicate clearly that the reaction product formed instantly and directly while mixing of the complimentary reactant, while suffering from dilution and dispersion with the increase of mixing chamber. Therefore, the junction point that is equivalent to a volume of 6.28 µL is observed as the optimum for table 5 obtaining most satisfaction optimum and in a regular peak profile for both cells.

**Table 5:** Effect of Y-junction points on the attenuation of incident light as average peak heights (mV)

| Type of Y-junction | | Volume $\pi r^2 h$ | Reliability (two tailed) at 95% $\bar{y}(mV) \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$ | $t_{sec}$ | Volume ml | C (mmol.L$^{-1}$) (Df) |
|---|---|---|---|---|---|---|
| | | | Cell A / Cell B | | At junction point | |
| Intersection junction point | 2 mm (ID) 2 mm (thickness) | 6.28 µL | 1080±3.5526 / 1264±2.8014 | 18 | 2.0873 | 5.3849 (7.43) |
| | 5 mm (ID) 5 mm (thickness) | 98.125 µL | 96±2.0620 / 232±2.4350 | 19 | 2.2791 | 4.9318 (8.11) |
| Premix chamber | 14 mm (ID) 12 mm(thickness) | 1.85 ml | 80±2.9813 / 184±2.3350 | 20 | 4.1310 | 2.7209 (14.70) |
| | 14mm (ID) 13 mm (thickness) | 2 ml | 64±2.2110 / 128±1.7140 | 20.5 | 4.3310 | 2.5952 (15.41) |
| | 14 mm (ID) 14 mm (thickness) | 2.154 ml | 24±1.1180 / 32±1.4410 | 21 | 4.5350 | 2.4785 (16.14) |

t: Time (Sec) from injection valve tilling to junction point, t0.05/2,2=4.303, Df= Dilution factor at junction point

*The investigation of the variation of concentration with obtained response results in a linear range*

Using the optimum of chemical and physical parameters; a series of chlorpromazine. HCl solutions ranging from 0.5-60 mmol.L$^{-1}$ for both cells were prepared. This will represent the x-axis (Independent Variable). Figure 6-A and 6-B show the flow gram that was used in conducting this research study for both cells. The attenuation of incident light that was measured gave the following (S/N) energy transducer responses as the Y-axis here represents the dependent variable. The results are tabulated in Table 5.

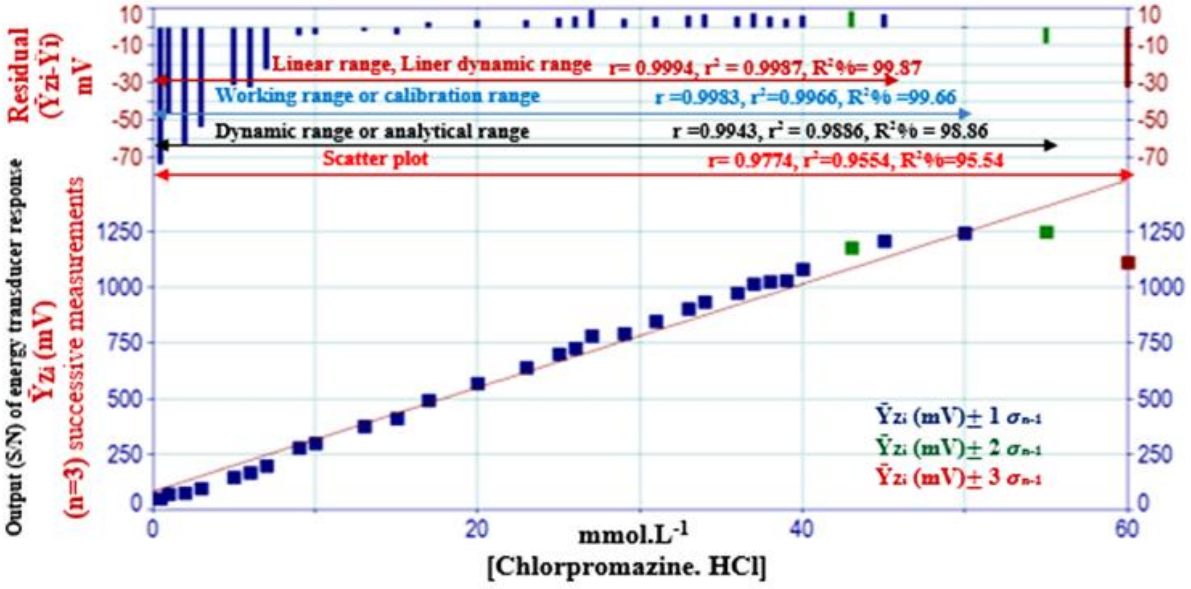
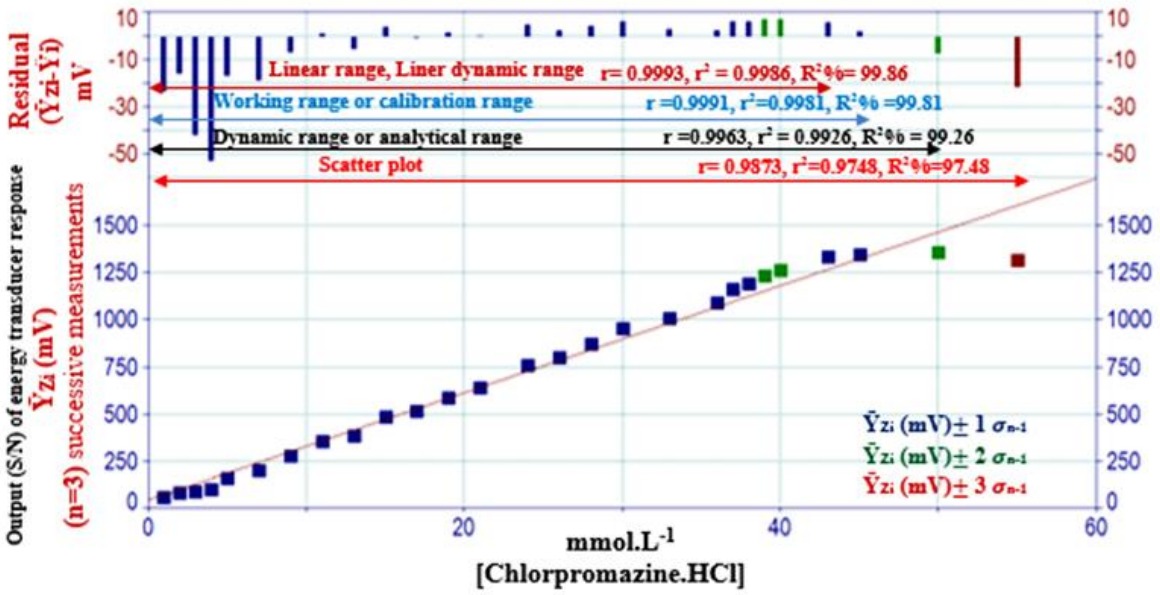



**Figure 6 A:** A calibration graph that is represented a linear dynamic range. For cell A




(B)

**Figure 6 B:** A calibration graph that is represented a linear dynamic range. For cell B

**Table 6:** The summary of linear regression of the proposed method

| Type of mode | Range of [Chlorpromazine.HCl] mmol.L$^{-1}$(n) | $\hat{Y}_{Z\,i\,(mV)}=a_{mV} \pm S_a\,t + b\,(\Delta y_{mV}/\Delta x_{mmol.L^{-1}}) \pm S_b\,t$ Chlorpromazine.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 | 0.5 -45 (28) | 32.1683± 10.0347+26.3829± 0.3843 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9994, 0.9987, 99.87 | 2.056 << 141.175 | |
| | 1-43 (24) | 3.6846±12.8938+31.0451±0.5177 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9993, 0.9986, 99.86 | 2.074 << 124.402 | |
| Working range or calibration range | 0.5-50 (29) | 38.8574±16.3733+25.9309±0.6000 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9983, 0.9966, 99.66 | 2.052<< 88.689 | |
| | 1-45 (25) | 7.1585±14.9142+30.7821±0.5733 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9990, 0.9982, 99.82 | 2.069 << 111.094 | |
| Dynamic range or analytical range | 0.5-55 (30) | 53.4038±29.8461 +25.0256±1.0418 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9943, 0.9886, 98.86 | 2.048<<49.197 | |
| | 1-50 (26) | 20.2122±29.7311+29.8968±1.0879 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9963, 0.9926, 99.26 | 2.064 << 56.723 | |
| Scatter plot | 0.5-60 (31) | 84.2759±57.4067+23.2269±1.9025 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9774, 0.9554, 95.54 | 2.042 << 24.929 | |
| | 1-55 (27) | 45.9136±53.9765+28.2924±1.8719 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9873, 0.9748, 97.48 | 2.060 << 31.132 | |

n: the number of measurements taken, ŶZi (mV); the estimated value 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 $t_{tab} = t_{0.05/2}$

*The Limit of Detection (L.O.D)*

A study was achieved to estimate the limit of detection of Chlorpromazine-HCl by three variation methods [6] as tabulated in table 6 as an injected sample volume of 281 µL.

**Table 7:** Limit of detection (L.O.D) for chlorpromazine hydrochloride at optimal achieved parameters using 281 µl as an injection sample for both cells and flow rate 3 ml.min$^{-1}$ for each line

| Type of cell | Gradual dilution for the minimum concentration in scatter plot (0.5 mmol.L$^{-1}$) for cell A (1 mmol.L$^{-1}$) for cell B | Theoretical based on the value of slope x=3$S_B$/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 | 0.9984 $\mu g$ /sample | 9.4236 $\mu g$ /sample | 158.9527 $\mu g/sample$ | 529.8425$\mu g/sample$ |
| Cell B | 49.9239 $\mu g$ /sample | 6.4646 $\mu g$ /sample | **1**60.3829 $\mu g/sample$ | 534.6097$\mu g/sample$ |

*Repeatability Study*

The relative standard deviation as a percentage (RSD %) is equal to the repeatability of the measurement (RSD% less than 0.3%). A Repeated measurement for eight successive injections were measured at a fixed concentration of chlorpromazine-HCl. Two concentrations (33 and 40 mmol.L$^{-1}$) were used for both cells (cell A & cell B) at optimum parameters. The obtained results are tabulated in table 7.

**Table 8:** Repeatability of Chlorpromazine hydrochloride results

| [Chlorpromazine hydrochloride] mmol.L$^{-1}$ | Output (S/N) of energy transducer response expressed as an average peak heights (n=8) $\bar{Y}_{zi}$ (mV) | RSD% | Reliability (two tailed) at (95%) $\bar{Y}_{zi}$ (mV)± $t_{0.05/2,\,n-1}\sigma_{n-1}/\sqrt{n}$ |
|---|---|---|---|
| Cell A | | | |
| Cell B | | | |
| 33 | 904 | 0.24 | 904± 1.8141 |
| | 1008 | 0.12 | 1008 ±1.0114 |
| 40 | 1080 | 0.11 | 1080 ±0.9934 |
| | 1264 | 0.17 | 1265 ±1.7967 |

*The application of the use of the novel analyzer for the quantitative determination of chlorpromazine hydrochloride in different commercial medicines*

The newly established method (NAG-ADF-300-2) was used for the determination of chlorpromazine hydrochloride in two samples of drugs from two different companies (Largektil, nexus, Germany, 25 mg), (Largactil, Oubari pharma, Syria, 25 mg). The continuous flow injection analysis was used with a homemade NAG-ADF-300-2 which that mean a long distance chasing photometer as a flow cell will have 300 mm as a distance with 2 mm as a path length to track and to accumulate the output results from attenuation of incident light at 0-180° and was compared with two reference methods which includes UV-spectrometric via the measurement of absorbance at $\lambda_{max}$=255 nm and turbidity classical method, the measurements of scattered light at 0-180° for a yellowish white precipitate particles of Chlorpromazine hydrochloride- $NaNO_2$ (20 mmol/L) - sulfanilic acid (16 mmol/L) system were used. A series of solutions were prepared from each pharmaceutical drug via transferring 5 mL of each sample (15 mmol/L) to five volumetric flasks (10 mL) followed by the addition of 0, 1, 1.5, 2 and 2.5

mL from 100 mmol.L$^{-1}$ concentration of stock solution to obtain 0, 10, 15, 20 and 25 mmol/L for novel method. But in UV-detection reference method via transferring of 0.15 mL from 15 mmol.L$^{-1}$ concentration sample solution to five volumetric flasks (10 mL) followed by the addition of 0, 0.1, 0.2, 0.3 and 0.4 mL from 35 mmol/L of standard solution of Chlorpromazine hydrochloride to obtain 0.0, 0.35, 0.7, 1.05 and 1.4 mmol.L$^{-1}$; As for the turbid metric mode by transferring of 1mL from 15 mmol/L of each sample to five volumetric flasks (10 mL) followed by the addition of 0.0, 0.1 , 0.2, 0.3 and 0.4 mL from 100 mmol.L$^{-1}$ concentration of standard solution of Chlorpromazine hydrochloride to get 0, 1, 2, 3 and 4 mmol/L, taking into a consideration that the first flask is for the sample. Three techniques have been used to conduct the measurements. The results were statistically treated [11] for the standard addition method and the results were summed up in the table 9-A and 9-B were shown a practical content of active ingredient at confidence level 95 % and efficiency of determination in addition to t-test, which shows a comparison at two different paths.

Primary test: Individual t-test for compared between of the mean practical weight by newly reputable novel method (homemade analyzer) using two flow cells with cited value ((25 mg of Largektil, and Largactil)

A theory for the active component can be estimated as shown below:

Worthless theory, for two commercial companies ($\underline{w}_i$) with cited value (μ = 25 mg) will be accepted and rejected the alternative hypothesis. These mean, that there was no significant difference between the cited value and founded value since $t_{cal}$ less than $t_{tab}$ (4.303) at 95% confidence interval.

i.e., $H_o$: $\underline{w}_{i(\text{homemade analyzer/Cell A})} = \mu_{(25\text{ mg or }25\text{ mg})}$ or $\underline{w}_{i(\text{homemade analyzer /Cell B})} = \mu_{(25\text{ mg or }25\text{ mg})}$

For: Largektil (Nexus, Germany, 25 mg) and Largactil (Oubari pharma, Syria, 25 mg) commercial companies

In contradiction alternative hypothesis, there is a significant difference between the founded value and cited value

i.e.; H1: $\bar{w}i$(homemade analyzer/Cell A) ≠ μ( 25 mg) or $\bar{w}i$(homemade analyzer/Cell B) ≠ μ( 25 mg)

It was seen that all values of t- calculated are less than the t-tabulated values. Therefore, the worthless theory will be accepted, and we will reject the alternative hypothesis. That is to say that there is no significant difference amidst the cited active ingredient value and the measured value.

Secondary test: based on the one way-ANOVA (F-test) [12], which was carried out at α = 0.05 (95 % confidence interval) for compare between four different methods (i.e., Cell A, Cell B (using NAG-ADF 300-2) and reference methods. This test (i.e., ANOVA-one way) table 8-B summed up the obtained results depends on the calculated F-value for comparing three or more means. The first estimated is called between group variance while second estimated based on the within variance. The assumption statistically is made as follows for two samples:

Ho= Null theory, this means there is no significant difference among all the methods used concerning the obtained results.

i.e., μ (homemade analyzer/cell A) = μ (homemade analyzer/cell B) = μUV-detection= μ Turbidity methode

H1= Alternative hypothesis, this means there is a significant difference between the four methods.

i.e., μ (homemade analyzer/cell A)≠ μ(homemade analyzer/cell B) ≠μUV- detection ≠ μ Turbidity method

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, it was found alternative hypothesis is accepted on behalf drug's active ingredient.

Based on the results of the previous study, which showed that there were no significant differences among the all methods, an ANOVA one-way test for the analysis of two samples of chlorpromazine hydrochloride drugs at 95% confidence interval and it was discovered that another hypothesis was accepted on behalf of the drug's active ingredient.

**Table 8.A:** Results of standard addition method for the analysis of two samples of Chlorpromazine hydrochloride drugs using the novel NAG - ADF- 300 -2 analyzer for flow cell A and flow cell B with two reference methods

| No. of sample | Commercial name, Company Content Country | Type of method: Newly developed methodology; Cell A; Cell B; UV- Sp. Classical method Absorbance measurement at $\lambda_{max}$=255 nm; Turbidimetry (NTU) | | | Chlorpromazine.HCl mmol.L$^{-1}$ | | | | | Equation of standard addition at 95% for n-2 | r, $r^2$, $R^2$% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 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 0.2665 g (15 mmol.L$^{-1}$) of the active ingredient of the active ingredient Wi (g) | Theoretical content for the active ingredient at 95% (mg) $Wi \pm 1.96\sigma_{n-1}/\sqrt{n}$ | 0.0 | 1 ml | 1.5ml | 2 ml | 2.5 ml | $\hat{Y}_{Z\,i} = a \pm S_a t + b\,(\Delta y/\Delta x_{mmoL}L^{-1}) \pm S_b t$ [Chlorpromazine.HCl] mmol.L$^{-1}$ | |
| | | | | | 0.0 | 10 | 15 | 20 | 25 | | |
| | | | | | 0.00 | 0.1ml | 0.2ml | 0.3 ml | 0.4ml | | |
| | | | | | 0.00 | 0.35 | 0.7 | 1.05 | 1.4 | | |
| | | | | | 0.0 | 0.1 | 0.2 | 0.3 | 0.4 | | |
| | | | | | 0.0 | 1 | 2 | 3 | 4 | | |
| 1 | Largektil nexus 25 mg Germany | 0.1119±0.0013 | 1.1934 | 25±0.2904 | 185 | 520 | 680 | 800 | 880 | 216.0811±112.8878+28.3513±6.8703 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9914, 0.9829, 98.29 |
| | | | | | 298 | 698 | 880 | 1090 | 1280 | 299.1892±18.3315+39.2864±1.1156 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9999, 0.9998, 99.98 |
| | | | | | 0.19 | 0.49 | 0.78 | 1.12 | 1.39 | 0.1880±0.0410+0.8657±0.0477 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9995, 0.9991, 99.91 |
| | | | | | 230 | 305 | 441 | 601 | 740 | 200.2000±68.5956+131.600±28.0040 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9934, 0.9868, 98.68 |
| 2 | Largactil Oubari pharma 25 mg Syria | 0.1059±0.0014 | 1.1295 | 25±0.3305 | 180 | 440 | 560 | 696 | 800 | 184.9730±25.8206+25.0162±1.5713 [ Chlorpromazine .HCl] mmol.L$^{-1}$ | 0.9994, 0.9988, 99.88 |
| | | | | | 245 | 608 | 780 | 944 | 1104 | 254.6756±30.7203+34.3946±1.8697 [ Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9996, 0.9991, 99.91 |
| | | | | | 0.15 | 0.41 | 0.76 | 0.94 | 1.21 | 0.1640±0.1053+0.7571±0.1228 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9961, 0.9923, 99.23 |
| | | | | | 210 | 340 | 430 | 588 | 760 | 196.0±68.4951+134.800±27.9634 [Chlorpromazine.HCl] mmol.L$^{-1}$ | 0.9937, 0.9874, 98.74 |

**Table 8.B:** The application results of the proposed method for the determination of Chlorpromazine-HCl in tablets

| No. of sample | Type of method: Newly developed method; Cell A; Cell B; (Classical method) UV- Spectrophotometric at $\lambda_{max}$= 255 nm; Turbidimetry (NTU) | | | Individual t-test for compared between quoted value & practical value ($\bar{w}i$ -μ) $\sqrt{n}$ /$\sigma_{n-1}$ Cell A or Cell B | F- test compared between two drugs with (25 mg) | | |
|---|---|---|---|---|---|---|---|
| | Practically concentration ($mmol.L^{-1}$) in 10 ml / Original sample solution in 50 ml | Practically weight of Chlorpromazine. HCl in weight of sample $\bar{W}_i$ (g) ± 4.303 $\sigma_{n-1}/\sqrt{n}$ / Weight of Chlorpromazine. HCl in tablet $\bar{W}_i$ (mg) ± 4.303 $\sigma_{n-1}/\sqrt{n}$ | Efficiency of determination Recovery % E. O. D | | ANOVA- one way using F-test | | |
| | | | | | Source | SSq (d.f) | MSq |
| 1 | 7.6215 / 15.2430 | 0.2708±0.0105 / 25.3930±0.9870 | 101.57% | 1.7133 << 4.303 | Between | 0.85936 (1) | $S_B^2$= 0.85936 |
| | 7.6156 / 15.2312 | 0.2706±0.0104 / 25.3730±0.9730 | 101.49% | | | | |
| | 0.2172 / 14.4800 | 0.2573±0.0137 / 24.1330 ± 1.2820 | 96.52% | 1.6497 << 4.303 | Within (error) | 1.434 (6) | $S_W^2$= 0.2390 |
| | 1.5213 / 15.2130 | 0.2703±0.0104 / 25.3540±0.9720 | 101.42% | | | | |
| 2 | 7.3940 / 14.7880 | 0.2627±0.0133 / 24.6480±1.0560 | 98.59% | / -1.4342 / << 4.303 | Total | 2.29336 (7) | |
| | 7.4050 / 14.8090 | 0.2631±0.0114 / 24.6820±1.0680 | 98.73% | | | $F_{cal}= S_1^2/ S_2^2$ \| $F_{0.95, 1, 6}$ | |
| | 0.2166 / 14.4400 | 0.2566±0.0211 / 24.0670±1.9820 | 96.27% | / -1.2812 / << 4.303 | $F_{cal}$ 3.5957 < $F_{tab}$ 5.99 | | |
| | 1.4540 / 14.5400 | 0.2583±0.0109 / 24.2340±1.0220 | 96.94% | | No significant difference between four methods | | |

μ: quoted value, n:(no. of sample) = 2, σn-1: standard deviation of different (individual t-test), $\bar{w}i$: practically weight in mg, ttab=t0.05/2,2=4.303 (for individual t-test), SSq: Sum of squares, MSq: Mean squares, S_B^2: between group variance, S_W^2: within group variance, d.f: degree of freedom, ANOVA: Analysis of variance, Ftab=F0.95,υ1,υ2=F0.95,1,6=5.99

## Conclusions

The suggested technique for determining chlorpromazine-HCL 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 the 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 a perfect success, as evidenced by the repeatability of response at varied concentrations within the range of determination.

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## Future research

It is necessary to conduct further studies with larger sample sizes to investigate administering the right sedative for traumatic children who are admitted to the emergency department. It is also

rewarding to use ketamine and midazolam in non-emergency situations where there is enough time to sedate the patient or in emergency departments that are not very crowded.

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**Authors' contributions**
All authors have contributed significantly and met criteria for authorship. All the authors read and approved the final copy of the manuscript.

**Conflict of Interest**
We have no conflicts of interest to disclose.

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