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Sensitive spectrophotometric method for determination of some phenothiazine drugs

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Abstract   A new simple and sensitive spectrophotometric method for some pheno-thiazine derivatives has been developed. The proposed method is based on the reaction of phenothiazine derivatives promethazine hydrochloride, chlorpromazine hydrochloride, triflupromazine hydrochloride, prochlorperazine, and trifluoperazine with potassium iodate followed by reaction of liberated iodine with leuco crystal violet (LCV) and measurement of the color of the oxidized LCV at 598 nm. The method showed a good linearity in the ranges 0.05–4.0, 0.02–2.0, 0.05–5.0, 0.1–8.0, and 0.05–2.0 µg mL^{-1} respectively. The optimum conditions and other analytical parameters were evaluated. The proposed methods have been applied successfully to the analysis of phenothiazine derivatives in pure form and in their dosage forms, and no interference was observed from common excipients present in pharmaceutical formulations.

Introduction

Phenothiazines are a very significant class of organic compounds with potent physiological activity (Fig. 1) [1]. Phenothiazines are neuroleptics used for the

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treatment of moderate and severe mental and emotional conditions. They are also used as antipsychotics, anticholinergics and antihistamines [2]. Many phenothiazine derivatives and their formulations are officially in British pharmacopoeia [3] and Indian pharmacopoeia [4]. The increasing use of phenothiazine derivatives in medicine has boosted the development of several methods for their determination in pure form and in pharmaceutical formulations. The methods used for their determination include spectrofluorometry [5, 6], conductometry [7], fluoroimmunoassay [8], HPLC [9, 10], HPTLC [11] polarography [12], GLC [13], chemiluminescence [14], and capillary zone electrophoresis [15]. The official methods presented in the British pharmacopoeia for phenothiazines consist of non-aqueous potentiometric titrimetry or spectrophotometry in the ultraviolet region, depending upon the derivative [5]. The first method is time consuming and care must be taken with the second one since many organic compounds absorb in this region of the spectrum.

Many spectrophotometric methods have already been reported for the determination of phenothiazines. They are generally based on redox reaction [16, 17], ion pair complex formation [18, 19], binary complex formation [20], ternary complex formation [21], diazo-coupling [22], and oxidative coupling [23, 24].

HPLC, GC, and HPTLC methods which are widely used for pharmaceutical analysis are accurate and precise with good reproducibility, but the cost of analysis is quite high owing to expensive instrumentation, reagents and expertise. Hence, it is worthwhile to develop simpler and cost-effective methods for simultaneous estimation of drugs for routine analysis of formulations. Spectrophotometric methods fulfil such requirements [25–30].

Some of the spectrophotometric methods found in the literature are reasonably rapid and selective, but suffer from a number of disadvantages such as low sensitivity, narrow dynamic range, heating step, extraction step, poor stability of the colored species, and instrumental or procedural complications.

The 2-substituted and 10-substituted phenothiazine derivatives exhibit many chemical properties, apart from interesting medicinal qualities [31]. Phenothiazine and its derivatives are characterized by low ionization potentials [32]. They are easily oxidized by different chemicals, and by electrochemical, photochemical, and enzymatic methods [33]. Oxidation is interesting for the determination of the phenothiazines, because it improves the spectroscopic properties for UV–Visible and fluorescence detection. General approaches for the oxidation of the phenothiazines are based on either photochemical [34] or chemical oxidation [35–38, 44].

The present investigation aims to develop a more sensitive and cost-effective method for the determination of phenothiazine in pure form and pharmaceutical formulations. The method employs potassium iodate as an oxidizing agent and leuco crystal violet (LCV) as a chromogenic reagent. The proposed methods have been demonstrated to be superior to the reported methods with respect to their speed, simplicity, sensitivity, and cost-effectiveness.

Fig. 1 Structure of the basic
phenothiazine molecule
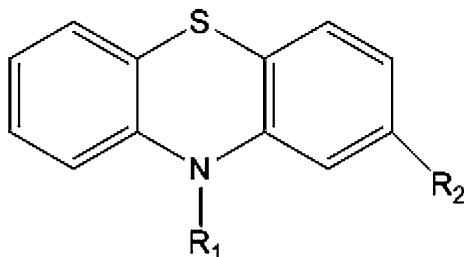
Experimetal

Apparatus

A Varian Carry 50 Bio UV spectrophotometer with 1-cm matched quartz cell was used for all absorbance measurements, and a Systronics type 331- pH meter was employed for the pH measurements.

Reagents and solutions

All chemicals used were of analytical reagent grade and double-distilled water was used throughout the experiment.

Standard drug solution

A standard solution of 1,000 µg mL^{-1} of phenothiazine derivatives was prepared by dissolving accurately weighed 100 mg of pure drug in distilled water, diluted to 100 mL. The stock solution was diluted stepwise to get working concentrations.

Potassium iodate solution: A 5×10^{-2}-M aqueous solution was prepared in deionized water.

Leuco crystal violet (LCV) [Eastman Kodak Co]:   An amount of 250 mg of LCV was dissolved in 200 mL distilled water containing 3 mL 85 % phosphoric acid (Merck) and the volume was made up to 1 L with distilled water and stored in an amber-colored bottle away from sunlight.

Sodium hydroxide solution: A 0.1-M aqueous solution was prepared.

Hydrochloric acid solution: A 2-M aqueous solution was prepared.

Procedure

Preparation of calibration curve

Different aliquots of phenothiazine derivatives containing promethazine (0.05–4.0 µg mL^{-1}), chlorpromazine (0.02–2.0 µg mL^{-1}), triflupromazine (0.05–5.0 µg mL^{-1}), trifluperazine (0.05–2.0 µg mL^{-1}), and prochlorperazine (0.1–8.0 µg mL^{-1}) were
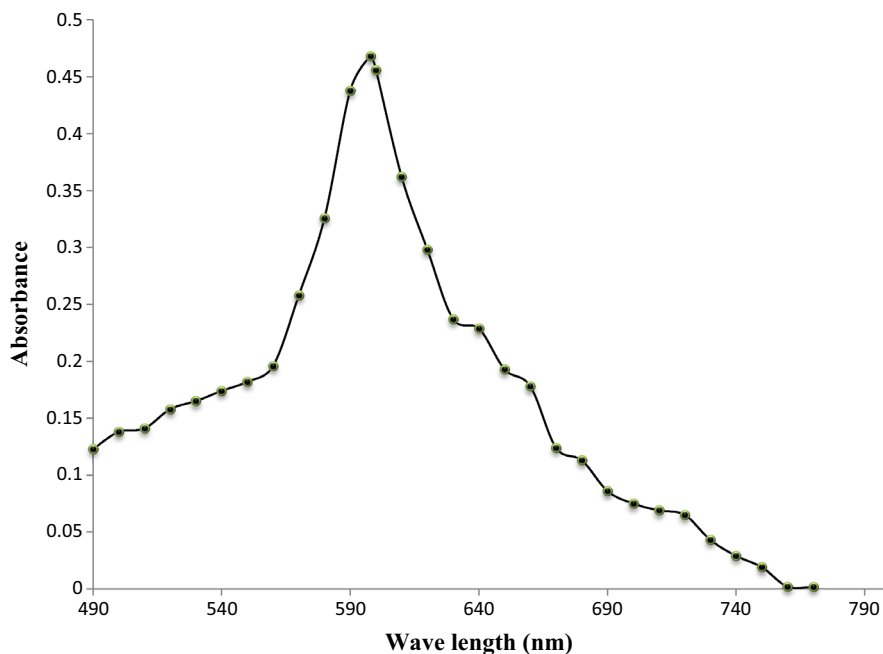
Fig. 2 Absorption maximum for the colored product

accurately measured and transferred into a series of 25-mL standard flasks and the volume was adjusted to 5.0 mL by adding distilled water. To each flask, 1 mL of 1 M HCl, 1 mL of LCV and 1 mL of potassium iodate was added. The contents were mixed well and flasks were allowed to stand for 10 min with occasional shaking, then the pH of each mixture was adjusted with sodium hydroxide solution. The volume was diluted to the mark with water, mixed well, and the absorbance was measured at 598 nm against reagent blank (Fig. 2, 3).

Procedure for tablet

Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 50 mg of the phenothiazine salt was transferred into a 100-mL calibarated flask and diluted to volume with water. Using a mechanical stirrer, the powder was completely disintegrated and the solution was filtered. A suitable aliquot of this solution in the individual phenothiazine working range was treated as described in the recommended procedure.

Results and discussion

The proposed method is based on the oxidation of phenothiazine derivatives with KIO_3 in acidic medium. Phenothiazine derivatives are reported to be oxidized to
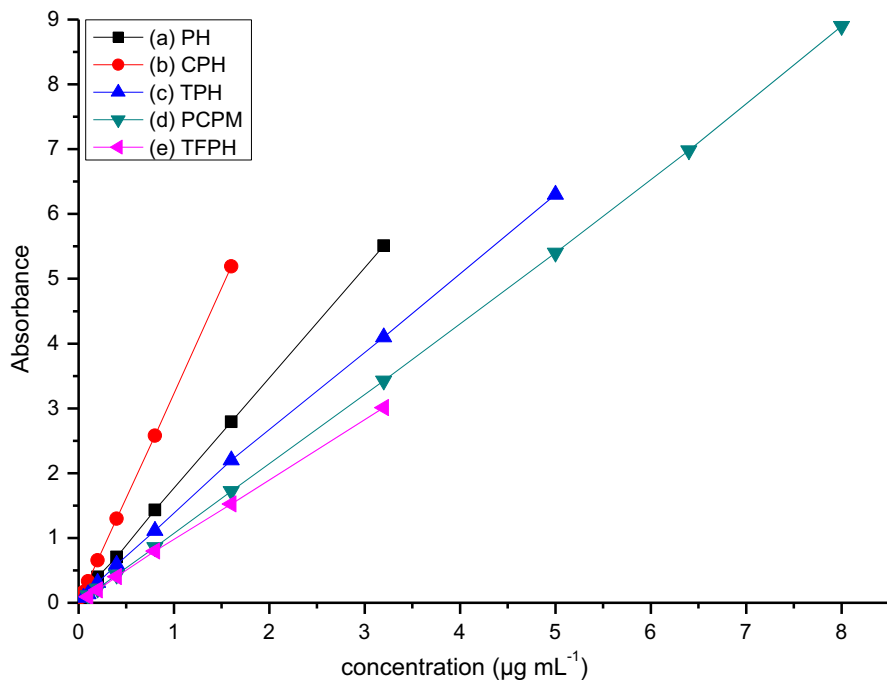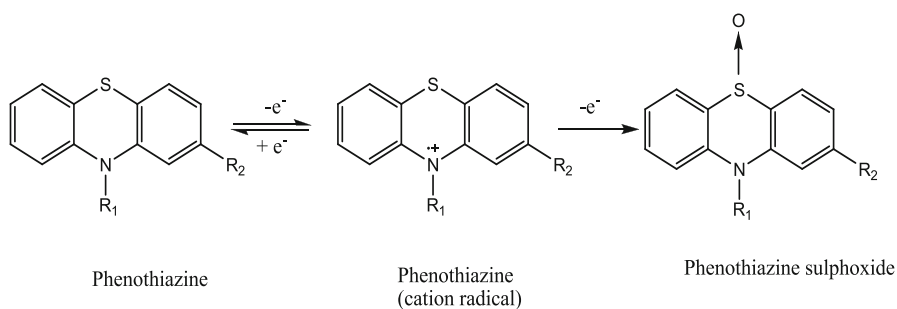
Fig. 3 Calibration graph for **a** chlorpromazine, **b** promethazine, **c** proclorpromazine, **d** trifluoperazine, **e** triflupromazine
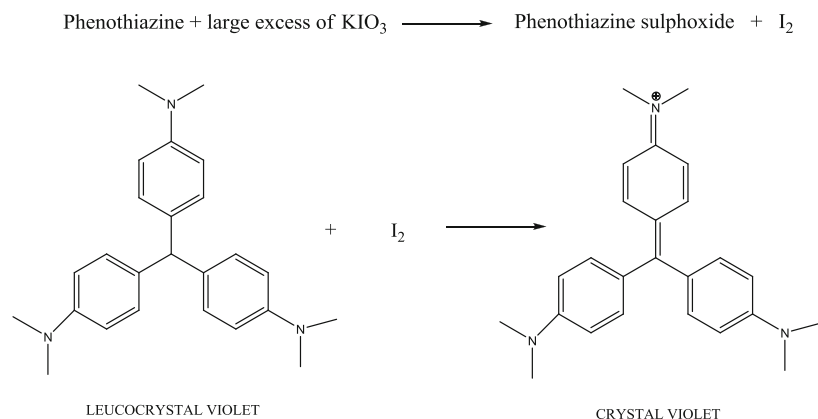

Scheme 1 Mechanism of oxidation of phenothiazine

form sulfoxides. The process proceeds in two steps; the first is reversible electron abstraction from phenothiazine to the colored semiquinol cationic radical. The radical is stable for a certain period of time depending upon phenothiazine substitution, pH and buffer used [37]. The second irreversible step leads to the generation of the colorless sulfoxide [36, 38] (Scheme 1).

To optimize the detection system, the UV–Vis spectra of the phenothiazines were recorded; similar spectroscopic properties were observed for all analytes. The respective data are presented in Table 1. The phenothiazines and their

Table 1 Structure and spectroscopic data of the relevant phenothiazine and their derivatives

Name of phenothiazine derivatives	R_1 substitution	R_2 substitution	λ_{max} (UV, nm) sulphide	λ_{max} (UV, nm) cationic radical	λ_{max} (UV, nm) sulfoxide
Promethazine	$-CH_2-\overset{H}{\underset{CH_3}{C}}-N(CH_3)_2$	H	250	520	333
Chlorpromazine	$-\overset{H_2}{C}-CH_2-\overset{H_2}{C}-N(CH_3)_2$	–Cl	256	529	340
Triflupromazine	$-\overset{H_2}{C}-CH_2-\overset{H_2}{C}-N(CH_3)_2$	–CF$_3$	258	500	347
Prochlorperazine	$-\overset{H_2}{C}-CH_2-\overset{H_2}{C}-N\!\!\diagup\!\!\diagdown\!\!N-CH_3$	–Cl	256	525	340
Trifluperazine	$-\overset{H_2}{C}-CH_2-\overset{H_2}{C}-N\!\!\diagup\!\!\diagdown\!\!N-CH_3$	–CF$_3$	258	500	347

Phenothiazine + large excess of KIO_3 $\longrightarrow$ Phenothiazine sulphoxide + I_2

LEUCOCRYSTAL VIOLET + I_2 $\longrightarrow$ CRYSTAL VIOLET

Scheme 2 Color reaction

corresponding sulfoxide are characterized by similar UV–Vis spectroscopic properties, with a slightly red shifted absorption maximum for the sulfoxide, e.g., at 340 nm for chlorpromazine. The radical cation exhibits an additional absorption maximum at 529 nm with small molar absorptivity.

In the proposed method, when KIO_3 and the phenothiazines are mixed in solution, the formation of red color is observed, which is associated with the additional absorption band as described above. This color quickly fades away. As a result of the reaction between KIO_3 and phenothiazines with sulfoxides, I_2 is liberated. The liberated iodine is treated with LCV to form CV dye which gives an absorption maximum at 598 nm. The intensity of the color of the dye is directly proportional to the concentration of phenothiazines (Scheme 2).

Optimization of reaction variables

Effect of reagent concentrations

For method A, the influence of iodate and LCV concentrations on the colored reaction has been checked. Optimization revealed that the 4.5×10^{-2}–6×10^{-2} M of KIO_3 gives the maximum and stable absorbance and that the intensity decreases with the increase in iodate concentration. Hence, 1 mL of 5×10^{-2} M KIO_3 solution was used for the studies (Fig. 4).

The concentration of LCV on its absorbance showed that maximum absorbance was found after the addition of 0.8 mL of LCV and it remained constant at higher concentrations; hence, 1 mL of LCV was used for further work (Fig. 5).

Effect of time, temperature and pH

The reasonable reaction time was 10 min and delay up to 30 min had no effect on the absorbance, while the developed color remained stable for several days (Fig. 6). The oxidation of phenothiazine derivatives with iodate was studied at the
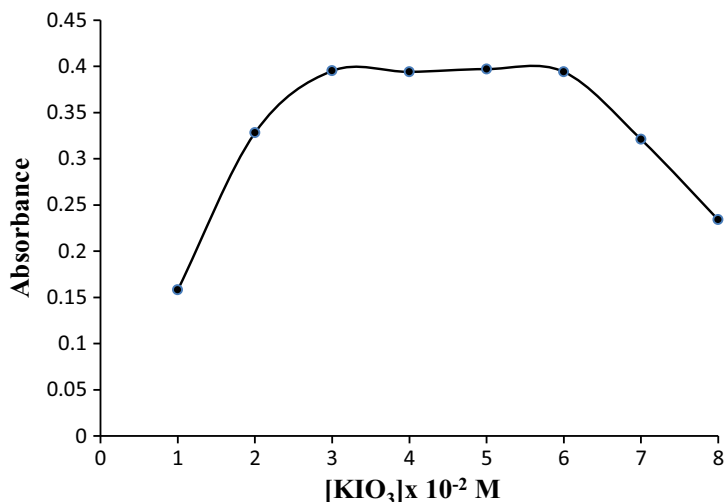
Fig. 4 Effect of potassium iodate concentration on oxidation of phenothiazine
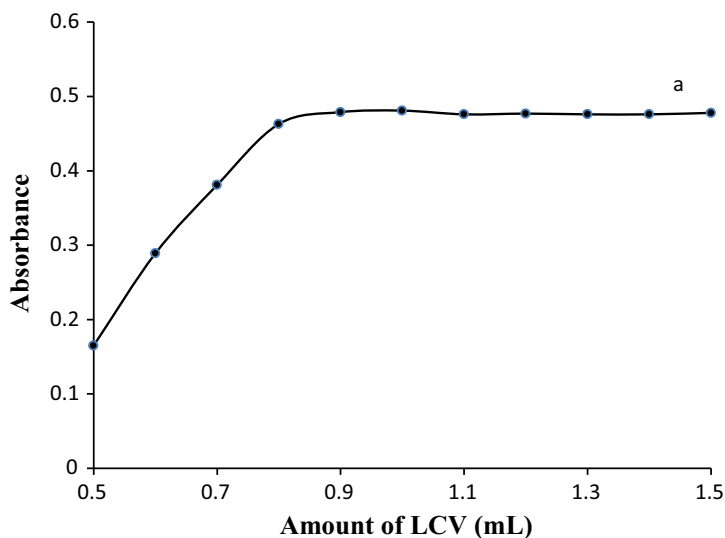
Fig. 5 Effect of the amount of LCV on the color reaction

temperature range between 20 and 40 °C. The rate of oxidation decreased slightly with increasing temperature. Therefore, working at room temperature, i.e. 25 °C, was adopted (Fig. 7).

The effect of various acids like HNO_3, H_2SO_4, HCl, $HClO_4$, etc. were checked and the best results were found in HCl medium. A pH 2–3 was required for liberation of I_2 which gives a blue color with LCV at pH 4–4.5. After pH 5, the solution becomes turbid. Initial pH was maintained by 1 mL of 1 M HCl; later on, the pH was adjusted up to 4 by using 0.1 M NaOH solution (Fig. 8).
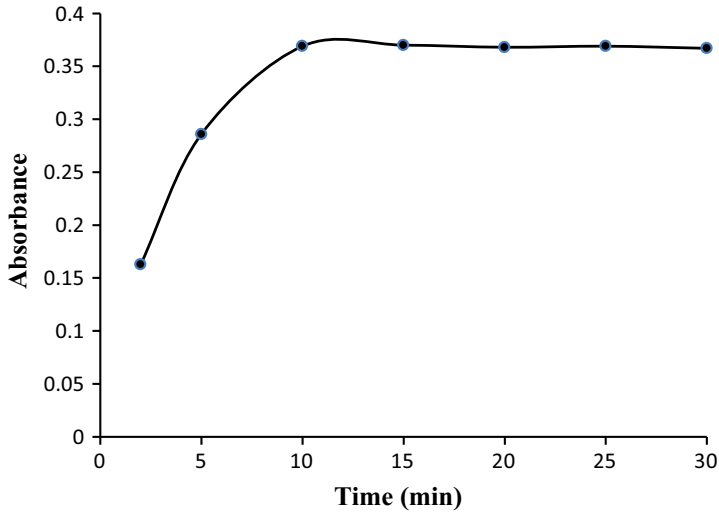
Fig. 6 Effect of time on the reaction

Fig. 7 Effect of temperature on the oxidation of phenothiazine

Method validation

The absorbance versus concentration was plotted and a linear correlation was found (Fig. 3). Beer's law range, the molar absorptivity and Sandell's sensitivity are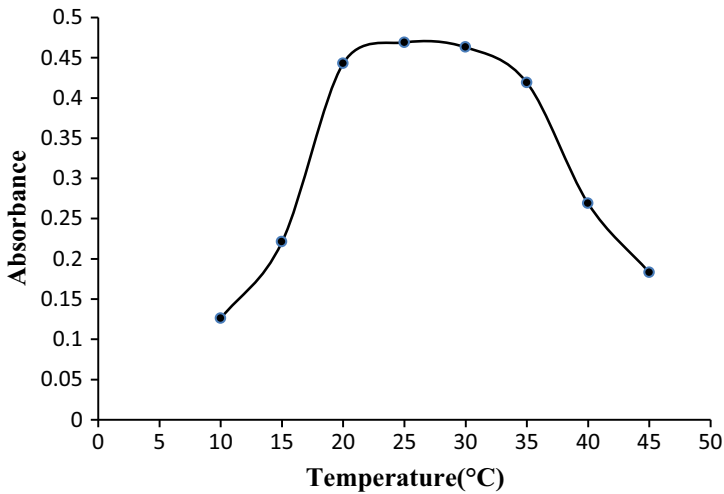 given in Table 2. The limits of detection, limits of quantification, regression equation, and correlation coefficients values are also given in Table 2.
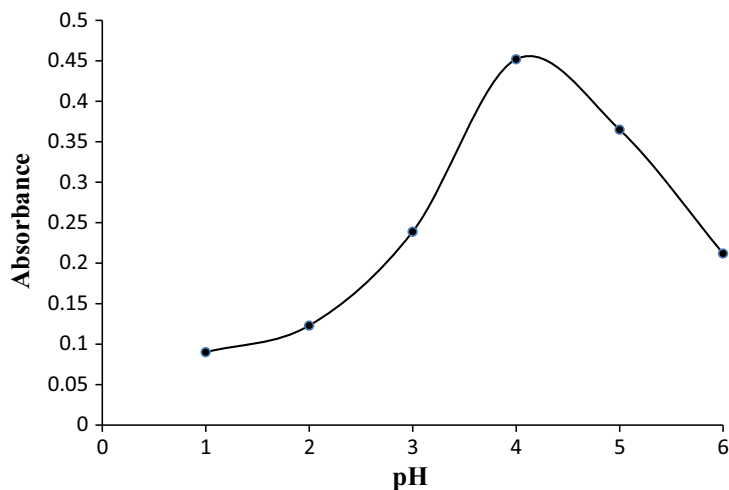
Fig. 8 Effect of pH on the color reaction

The accuracy and precession of the analytical method have been evaluated. For accuracy, the percentage relative error between the measured concentrations and the chosen concentration was calculated. The precision of the method was calculated in terms of intermediate precision (intra-day and inter-day). The SD and RSD values of intra-day and inter-day studies showed that precision was good (Table 3).

Selectivity

To determine the selectivity of the proposed method, the analytical placebo was analyzed by the proposed methods. It was confirmed that the change in the absorbance with respect to the blank was caused only by the change in analyte concentration. To identify the interference by common tablet excipients, a synthetic mixture with the composition of phenothiazine derivatives (50 mg), talc (80 mg), starch (160 mg), calcium gluconate (80 mg), lactose (80 mg), sodium alginate (40 mg) and magnesium stearate (40 mg) was prepared and subjected to analysis by the proposed methods after solution preparation using the procedure described for tablets. The percentage recoveries suggesting no interference by the excipients in the assay of phenothiazine under the described optimum conditions (Table 4).

Application to formulations

The proposed method was applied to the determination of phenothiazines in tablets and injections. The results in Table 4 show that the method is applicable for the determination of phenothiazines and that the excipients in the dosage forms do not interfere. A statistical comparison of the results by the proposed methods and a literature method [3] showed that the results agree well with the claim and are also in agreement with the results obtained by the literature method. Statistical analysis

Table 2 Performance characteristic of the existing spectrophotometric methods and the proposed methods

Parameters	PH	CPH	TPH	PCPM	TFPH
λ_{max} (nm)	598	598	598	598	598
Beer's law limits (μg mL^{-1})	0.05–4.0	0.02–2	0.05–5	0.1–8	0.05–2
Molar absorptivity (L mol^{-1} m^{-1})	4.41×10^5	1.46×10^6	6.4×10^5	3.57×10^5	7.87×10^5
Sandell's sensitivity (μg cm^{-2})	0.00065	0.00024	0.00015	0.00105	0.00052
Limit of detection (μg mL^{-1})	0.019	0.012	0.016	0.032	0.016
Limit of quantification (μg mL^{-1})	0.058	0.036	0.049	0.096	0.049
Regression equation (Y= bX*+a)					
Slop (b)	1.549	4.213	1.844	0.939	1.824
Standard deviation of slop (S_b)	0.0907	0.196	0.050	0.0494	0.103
Intercept (a)	0.019	0.007	0.027	0.018	0.006
Standard deviation of intercept (S_a)	0.0165	0.0301	0.009	0.013	0.014
Variance (S_a^2)	2.7×10^{-4}	9.1×10^{-4}	8.1×10^{-5}	1.7×10^{-4}	1.96×10^{-4}
Correlation coefficient	0.999	0.995	0.998	0.998	0.999

Table 3 Inter-day and intra-day precision and accuracy studies for method A

Phenothiazine taken in μg mL^{-1}	Intra-day ($n = 7$)			Inter-day ($n = 5$)		
	Found	Precision[a]	Accuracy[b]	Found	Precision[a]	Accuracy[b]
PH						
1	1.07 ± 0.077	7.19	7	1.02 ± 0.022	2.19	2
2	2.04 ± 0.079	3.87	2.07	2.09 ± 0.019	3.59	4.5
3	3.08 ± 0.14	4.49	2.76	3.10 ± 0.25	7.21	3.33
CPH						
0.5	0.51 ± 0.022	4.26	1.714	0.51 ± 0.019	3.66	2
1	1.02 ± 0.019	1.82	2.14	1.05 ± 0.045	4.27	5.2
1.5	1.51 ± 0.25	1.69	1.14	1.52 ± 0.027	1.77	1.6
TPH						
1	1.02 ± 0.051	5.04	1.71	1.07 ± 0.084	7.87	6.8
2	2.03 ± 0.051	2.53	1.57	2.04 ± 0.12	5.73	2.0
4	4.06 ± 0.14	3.52	1.67	4.15 ± 0.22	3.52	3.75
PCPM						
2	2.07 ± 0.19	9.53	3.38	2.1 ± 0.26	12.37	5.4
4	4.04 ± 0.16	3.93	0.93	4.16 ± 0.21	4.95	4.05
6	6.18 ± 0.25	4.01	2.98	6.08 ± 0.23	3.70	1.43
TFPH						
0.5	0.51 ± 0.021	4.26	1.71	0.52 ± 0.052	10.12	3.2
1	1.02 ± 0.019	1.82	2.14	1.08 ± 0.130	12.00	8.4
1.5	1.52 ± 0.026	1.69	1.14	1.56 ± 0.099	6.40	3.73

[a] Relative standard deviation (%)

[b] Bias%: (found-taken/taken) × 100

Table 4 Determination of CPH[a] in the presence of excipients

S. no.	Material	Amount (mg)	% recovery of CPH ± % RSD[b]
1	Lactose	40	98 ± 1.41
2	Dextrose	40	98.6 ± 1.10
3	Sucrose	30	99.7 ± 1.46
4	Starch	40	100.0 ± 0.76
5	Talc	30	99.6 ± 0.49
6	Stearic acid	20	100.6 ± 0.84
7	Sodium alginate	10	101.2 ± 0.90
8	Ascorbic acid	0.1	99.8 ± 1.60

[a] 1 μg mL^{-1} of CPH taken

[b] Average of five determinations

Table 5 Results of assay of tablets by the proposed methods and statistical evaluation

Tablet brand name	Nominal amount (mg/tab)	Literature method[a]	Proposed method[a]	t test[b] ($\pm$)	F test[b]
PH					
Phenargan	10	10.05 ± 0.32	10.2 ± 0.20	0.886	2.56
Phenargan	25	24.88 ± 0.38	24.92 ± 0.27	0.189	1.98
Avomine	25	25.02 ± 0.33	25.14 ± 0.34	0.575	1.06
CPH					
Megatil	25	24.96 ± 0.31	24.98 ± 0.33	0.098	1.132
Megatil	100	99.01 ± 0.46	99.4 ± 0.58	1.316	1.59
Emetil	100	100.33 ± 0.38	99.8 ± 0.34	1.882	1.25
TPH					
Siquil (tab)	10	9.8 ± 0.23	9.97 ± 0.26	0.5854	1.28
Siquil (inj.)	10	10.03 ± 0.16	9.9 ± 0.11	1.464	2.11
PCPM					
Emidoxine	5	4.82 ± 0.23	4.98 ± 0.34	0.8646	2.18
Stemetil	5	5.36 ± 0.35	4.84 ± 0.52	0.6926	2.21
TFPH					
Trazine	5	4.76 ± 0.24	5.1 ± 0.39	1.607	2.64
Trazine	10	10.02 ± 0.28	10.0 ± 0.37	0.317	1.75
Espazine	25	25.17 ± 0.17	24.96 ± 0.33	1.271	4.74

[a] Mean value of five determinations

[b] Tabulated t value at the 95 % confidence level is 2.78; tabulated F value at the 95 % confidence level is 6.39

of the results using Student's t test for accuracy and F test for precision revealed no significant difference between the proposed method and the literature method at 95 % confidence level with respect to accuracy and precision (Table 5).

Conclusion

In conclusion, a simple, rapid, and cost-effective method for the determination of phenothiazine derivatives has been developed and validated. The proposed method is more sensitive than many existing methods, and is free from such experimental variables as heating or an extraction step (Table 6). The stability of the color system is an advantage over the earlier methods. The method provides sensitivity comparable to that achieved by sophisticated and expensive techniques like HPLC. Thus, it can be used as an alternative for rapid and routine determination of bulk samples and tablets.

Table 6 Comparison with other spectrophotometric methods

S. no	Reagent	Colored species	Drug analyzed	λ_{max} (nm)	Range of determination ($\mu g\ mL^{-1}$)	Ref.
1.	Ferricyanide		PH, CPH, TFPH, FPH, PDM, PCPM	700 and 720 nm	0.1–8	[39]
2.	Hexacyanoferrate(III) and ferriin	Colored complex	CPH, PH, thioridazine hydrochloride, TEPH, PCPM	510	1–12	[40]
3.	Sulfanilic acid	Coupled product	PH	513	4–28	[41]
4.	Pottsium dichromate, iron(II) and 1.10-phenanthroline	Colored complex	CPH, PH, TFPH, PCPM, FPH	510	2.5–50	[42]
5.	Bromate–bromide mixture and methyl orange and indigocarmine dye	unbleached dye	CPM	520, 610	1–6 and 2.5–15	[43]
6.	Potassium iodate Leuco crystal violet	Crystal violet dye	PH, CPH, TPH, PCPM, TFPH	598	0.05–4, 0.02–2, 0.05–5, 0.1–8, 0.05–2	Proposed method

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