DRUG FORMULATIONS AND CLINICAL METHODS

Spectrophotometric Determination of Pipazethate Hydrochloride in Pure Form and in Pharmaceutical Formulations

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Three simple, sensitive, and reproducible spectrophotometric methods (A–C) for the determination of pipazethate hydrochloride (PiCl) in pure form and in pharmaceutical formulations are described. The first and second methods, A and B, are based on the oxidation of the drug by Fe^{3+} in the presence of *o*-phenanthroline (*o*-phen) or bipyridyl (bipy). The formation of tris-complex upon reactions with Fe^{3+}-*o*-phen and/or Fe^{3+}-bipy mixture in an acetate buffer solution of the optimum pH values was demonstrated at 510 and 522 nm, respectively, with *o*-phen and bipy. The third method, C, is based on the reduction of Fe(III) by PiCl in acid medium and subsequent interaction of Fe(II) with ferricyanide to form Prussian blue, which exhibits an absorption maximum at 750 nm. The concentration ranges are from 0.5 to 8, 2 to 16, and 3 to 15 μg/mL for Methods A–C, respectively. For more accurate analysis, Ringbom optimum concentration ranges were calculated. The molar absorptivity, Sandell sensitivity, and detection and quantitation limits were calculated. The developed methods were successfully applied to the determination of PiCl in bulk and pharmaceutical formulations without any interference from common excipients. The relative standard deviations were ≤0.83% with recoveries of 98.9–101.15%.

Pipazethate hydrochloride (PiCl), 10*H*-pyrido[3,2-*b*][1,4]benzothiadiazine-10-carboxylic acid 2-(2-piperidinoethoxy)ethyl ester, is a bronchodilator that suppresses irritative and spasmodic cough by inhibiting the excitability of the cough center and the peripheral neural receptors in the respiratory passage (1; Figure 1).

Pipazethate has been determined using a limited number of techniques including spectrophotometry (2, 3), thin-layer chromatography (TLC; 4), high-performance liquid chromatography (LC; 5), potentiometry (6), and conductometry (7). Also, PiCl and isothipendyl HCl were used as reagents for spectrophotometric determination of Mo(VI) in alloy steels and soil samples (8).

None of these techniques is sufficiently sensitive (2, 3, 7, 8), or they are very laborious and require highly sophisticated instrumentation (5, 6). Zarapker et al. (2, 3) reported extractive spectrophotometric methods for determination of PiCl from pharmaceutical preparations using $Co(SCN)_2$ (2) and BCG, BCP, BPB, BTB, solochrom dark blue, and EBT (3). These methods are not selective, have low sensitivity, take a long time for color development, and require prior extraction with chloroform of the colored product. To date, no work has been performed to use a redox reaction for the determination of PiCl.

o-Phenanthroline (*o*-phen), bipyridyl (bipy), and ferricyanide have been used frequently in the field of pharmaceutical analysis, e.g., in the determination of amlodipine besylate (9), ketoconazole (10), diclofenac sodium and piroxicam (11), indapamide (12), certain dibenzazepine tricyclic antidepressant drugs (13), amoxycillin, ciprofloxacin, and piroxicam (14), amlodipine and felodipine (15), and some phenothiazine drugs (16).

The aim of the present study was to apply redox reactions to develop simple, accurate, sensitive, and reproducible methods of analysis of PiCl in pure form and in pharmaceutical formulations by use of Fe(III) with *o*-phen, bipy, and ferricyanide. This work describes spectrophotometric methods that can be used in laboratories where modern and expensive apparatus, such as those required for TLC or LC, are not available.

Experimental

Apparatus

All absorption spectra were recorded using a Shimadzu (Columbia, MD) Model 260 ultraviolet-visible (UV-Vis) recording spectrophotometer equipped with 10 mm matched quartz cells. An Orion Research (Yonezawa, Japan) Model 601A/digital ionalyzer pH meter was used for checking the pH values of buffer solutions.

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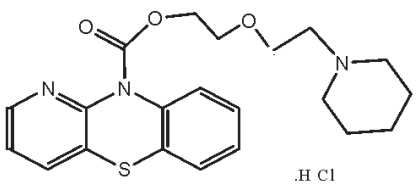

Figure 1. Structure of pipazethate HCl (PiCl).

Materials and Reagents

All chemicals and materials were of analytical grade, and all solutions were freshly prepared in double-distilled water.

(**a**) *PiCl.*—Pure grade, and its pharmaceutical formulations (Selgon, tablets 20 mg and drops 40 mg/mL) were provided by the Egyptian International Pharmaceutical Industries Co. (EIPICO; 10th of Rmadan City, Egypt).

(**b**) *Iron(III)–o-phenanthroline.*—Prepared by mixing 0.198 g 1,10-phenanthroline monohydrate (Fluka, Buchs, Switzerland), 2 mL of 1 M HCl, and 0.16 g ferric ammonium sulfate dodecahydrate (Aldrich, Deisenhofen, Germany) and diluting with double-distilled water to the mark in a 100 mL volumetric flask (17).

(**c**) *Iron(III)–bipyridyl.*—Prepared by dissolving 0.16 g 2,2′-bipyridyl (Fluka), 2 mL of 1 M HCl, and 0.16 g ferric ammonium sulfate dodecahydrate and diluting with double-distilled water to the mark in a 100 mL volumetric flask (17).

(**d**) *Anhydrous $FeCl_3$ (Merck, Darmstadt, Germany) and $K_3[Fe(CN)_6]$ (BDH Laboratory Chemicals, Poole, UK.*—0.2% (w/v) Solutions were prepared in double-distilled water.

(**e**) *Sulfuric acid (10 M).*—Prepared by adding 555 mL concentrated acid (sp. gr. 1.83) to 445 mL double-distilled water with cooling (18).

(**f**) *Acetate buffer solutions.*—Buffers in pH range from 2.56–5.6 were prepared by mixing appropriate quantities of 0.2 M sodium acetate with 0.2 M acetic acid to obtain the desired pH as recommended previously (19).

(**g**) *Stock standard solution of PiCl.*—Prepared by dissolving 100 mg pure PiCl in double-distilled water and diluting to 100 mL with the same solvent to obtain a standard solution of 1 mg/mL. Working solutions were prepared by an appropriate dilution of the stock standard solution.

Recommended Analytical Procedure

(**a**) *Methods A and B.*—Aliquots (0.1–1.6 and 0.4–3.2 mL) of 50 μg/mL PiCl standard solution were transferred for Methods A and B, respectively, into a series of 10 mL calibrated flasks; 2 mL Fe^{3+}-*o*-phen (Method A) or 2.5 mL Fe^{3+}-bipy (Method B) reagent solution and 3 mL acetate buffer solution (pH 4.5) were added and heated on a water bath at 90°C for 10 min. The mixture was cooled to room temperature (25 ± 1°C), and the volume was diluted to the line with double-distilled water. The colored complexes formed were measured at 510 and 520 nm for Methods A and B, respectively, against a reagent blank treated similarly.

(**b**) *Method C.*—Into a series of 10 mL calibrated flasks, different aliquots (0.6–3 mL) of standard PiCl solution (50 μg/mL) were transferred using a microburet, and the total volume was adjusted to 3 mL by adding double-distilled water. Then, 2 mL each of $FeCl_3$ (0.2%) and $K_3[Fe(CN)_6]$ (0.2%) were added to each flask, mixed well, and allowed to stand for 10 min. Finally, 1 mL of 10 M H_2SO_4 was added to each flask, and the solution was diluted to the line with double-distilled water and mixed well. The absorbance of the resulting solution was measured at 750 nm against a reagent blank prepared similarly. A calibration graph was constructed by plotting the absorbance against the concentration of drug, or the regression equation was calculated.

Analysis of Pharmaceutical Formulations

(**a**) *Procedure for tablets.*—Twenty tablets were accurately weighed and powdered. An accurately weighed quantity equivalent to 20 mg PiCl was dissolved in 20 mL double-distilled water and transferred to a 100 mL volumetric flask. The contents of the flask were shaken for 10 min and then diluted to the line with double-distilled water. The general procedure was then followed in the concentration ranges already mentioned above for Methods A–C.

(**b**) *Procedure for drops.*—The contents of 5 bottles (Selgon drops, 40 mg PiCl/mL) were mixed, and the average volume for 1 bottle was determined. An aliquot of the solution equivalent to 40 mg PiCl was quantitatively transferred to a 50 mL volumetric flask and diluted to the line with double-distilled water. The procedure was continued as described above for Methods A–C.

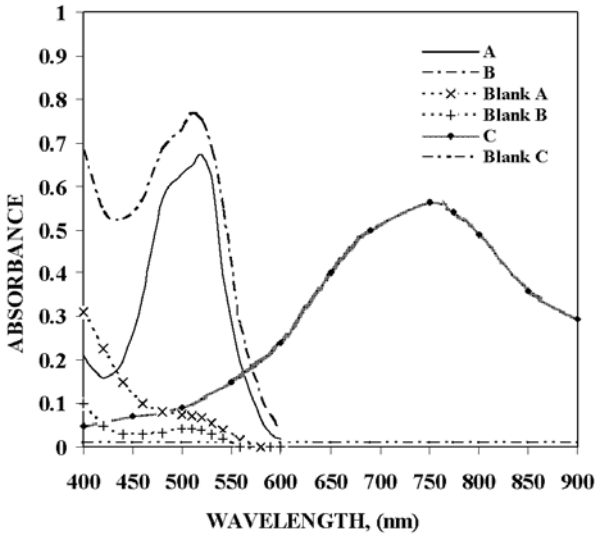

Figure 2. Absorption spectra of Blank A, Fe(III)–1,10-phenanthroline; Blank B, Fe(III)–2,2′-bipyridy; Blank C, Fe(III)-ferricyanide before addition of 8.0 μg/mL pipazethate and A, B, and C after reduction with PiCl.

Table 1. Quantitative parameters for the proposed Methods A–C

Parameter	Method A	Method B	Method C
Media	pH 4.5	pH 4.5	H_2SO_4 (10 M)
λ_{max}, nm	510	520	750
Beer's concn range, μg/mL	0.5–8	2–16	3–15
Ringbom concn range, μg/mL	1–7	3.5–15	4–13.5
Detection limit, μg/mL	0.21	0.45	0.37
Quantitation limit, μg/mL	0.70	1.50	1.23
Molar absorpitivity × 10^4, L/mol cm	7.91	3.24	2.581
Sandell sensitivity, ng/cm^2	5.52	13.5	16.9
Regression equation[a]			
Intercept	0.1521	0.0914	–0.0706
Slope	0.0813	0.0511	0.0694
Correlation coefficient (r)	0.9998	0.9993	0.9990
Relative standard deviation, %	0.83	0.67	0.54
Relative error, %	0.87	0.70	0.57

[a] $A = a + bc$, where c is the concentration in μg/mL.

Results and Discussion

Absorption Spectra

Methods A and B.—1,10-Phenantholine and 2,2′-bipyridyl are organic bases with similar chemical structures and contain the Fe(II)-specific group (20). Methods A and B are based on the formation of tris(*o*-phenanthroline)–Fe(II) or tris(2,2′-bipyridyl)–Fe(II) chelate upon the reaction of PiCl with the Fe^{3+}-*o*-phen or Fe^{3+}-bipy reagent. The reaction proceeds through the reduction of Fe^{3+} to Fe^{2+}, and the subsequent formation of an intensely colored orange-red complex (9–14). The absorption spectra of the colored complex species in the proposed methods at the optimum conditions were scanned in the double-beam mode against a reagent blank in the range of 400–600 nm and showed a characteristic maximum absorbance wavelength (λ_{max}) at 510 and 520 nm using Methods A and B, respectively (Figure 2). The experimental conditions were established by varying each parameter individually (21) and observing its effect on the absorbance of the colored species. All the spectral characteristics and the measured or calculated factors and parameters are summarized in Table 1.

Method C.—PiCl is an amine that reduces Fe(III) to Fe(II), the latter reacting with ferricyanide to form intense greenish-blue (22) colored Prussian blue (PB) having an absorption maximum at 750 nm as shown in (Figure 2). Neither Fe(III) nor ferricyanide solution absorbs at this wavelength. Hence, the use of measured volumes of the reagent solutions and measurement against a corresponding reagent blank give a linear calibration graph for the drug. Therefore, in the present work, we report the formation and application of PB complex in the development of a sensitive spectrophotometric method for the determination of PiCl. The formation of PB complex is a classic qualitative test to detect Fe(II) using hexacyanoferrate(III) reagent (18). The first step is the oxidation of Fe(II):

$$Fe^{2+} + [Fe(CN)_6]^{3-} \rightarrow Fe^{3+} + [Fe(CN)_6]^{4-}$$

The second step is the formation of hexacyanoferrate(II) complex (PB):

$$4Fe^{3+} + 3[Fe(CN)_6]^{4-} \rightarrow Fe_4[Fe(CN)_6]_3$$

The complex formed is highly insoluble ($K_{sp} = 3 \times 10^{-41}$; 23). Using an excess of the complexing reagents, a deep blue soluble compound is formed when Fe(III) is reduced to Fe(II) by products obtained from acidic hydrolysis of the drug.

Optimization of Methods A and B

Effect of pH.—An acetate buffer solution was optimal among those examined (universal, phosphate, thiel, borate, and acetate). pH adjustment is necessary, especially in acidic medium, because the reaction was affected by change of pH in the range of 2.5–5.6. The optimum pH value was 4.5 for both Methods A and B, and 3 mL buffer solution was sufficient for complete color development.

Effect of reagent concentration.—The addition of 2 mL Fe^{3+}-*o*-phen or 2.5 mL Fe^{3+}-bipy reagent was sufficient to obtain the maximum and reproducible absorbance for 8.0 μg/mL PiCl. Smaller amounts gave incomplete complex formation. A larger concentration had no effect on complex formation, although the absorbance increased slightly due to the background of the reagent used. The results are shown in Figure 3.

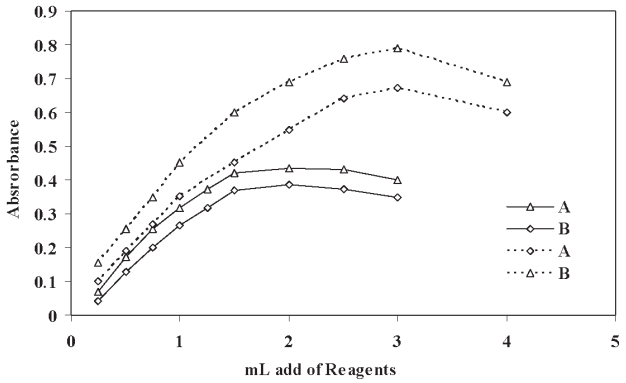

Figure 3. Effects of *o*-phen and bipy concentration on 8.0 μg/mL PiCl.

Effect of temperature and heating time.—The effects of temperature and heating time on the formation of the colored complexes were studied. The reaction of PiCl with both reagents proceeds very slowly at room temperature. Higher temperature was used to accelerate the reaction. Maximum absorbance was obtained after heating for about 10 min with both Fe^{2+}-phen and Fe^{2+}-bipyridyl-colored complexes on a water bath at 90°C. Further heating caused no appreciable change in the color. The obtained complex was stable for more than 12 h. The results are shown in Figures 4A and B.

Optimization of Method C

The optimum conditions were established by variation of such parameters as Fe(III), ferricyanide, and acid concentrations; reaction time; and order of addition of the reactants.

Optimum Fe(III) and ferricyanide concentrations.—When a study on the effect of Fe(III) chloride concentration on the color development was performed, it was observed that the absorbance increased with an increase in the volume of 0.2% Fe(III) solution and reached a maximum when 2.0 mL of the reagent solution was added to 8.0 μg/mL PiCl and 2.0 mL of 0.2% ferricyanide solution in a total volume of 10 mL. These results indicate that a maximum absorbance is obtained when the final Fe(III) chloride concentration is 0.04%. Larger volumes of Fe(III) chloride up to 3.0 mL had no effect on the sensitivity of the reaction. Similar observations were made when varying volumes of 0.2% ferricyanide solution were added to fixed amounts of drug (8.0 μg/mL PiCl) and Fe(III) chloride (2 mL; 0.2%) and diluted to 10 mL after full color development. The results of this study revealed that the concentrations of Fe(III) and ferricyanide reagents are not critical. However, 2.0 mL each of 0.2% reagent solutions in a total volume of 10 mL were used to ensure adequate reagent concentrations for higher drug concentrations. The results are shown in Figure 5.

Effect of nature of acid and its concentration.—The reaction product PB was found to flocculate within 20–30 min of color development. To delay the flocculation, addition of acid after full color development and before diluting to the line was found necessary. Sulfuric acid was found to give a more stable color and more reproducible results compared with HCl. A 1.0 mL volume of 10 M sulfuric acid in a total volume of 10 mL was found to be adequate.

Effect of reaction time and stability of colored species.—The reaction was slow at 30 ± 2°C, but the absorbance increased with time and reached a maximum in 10 min. The developed color remained stable for at least 5 h.

Effect of order of addition of reactants.—After fixing all other parameters, a few other experiments were performed to ascertain the influence of the order of addition of reactants. The order drug, ferricyanide, and Fe(III) followed by sulfuric acid after full development of color gave maximum absorbance and stability, and hence the same order of addition was followed throughout the investigation.

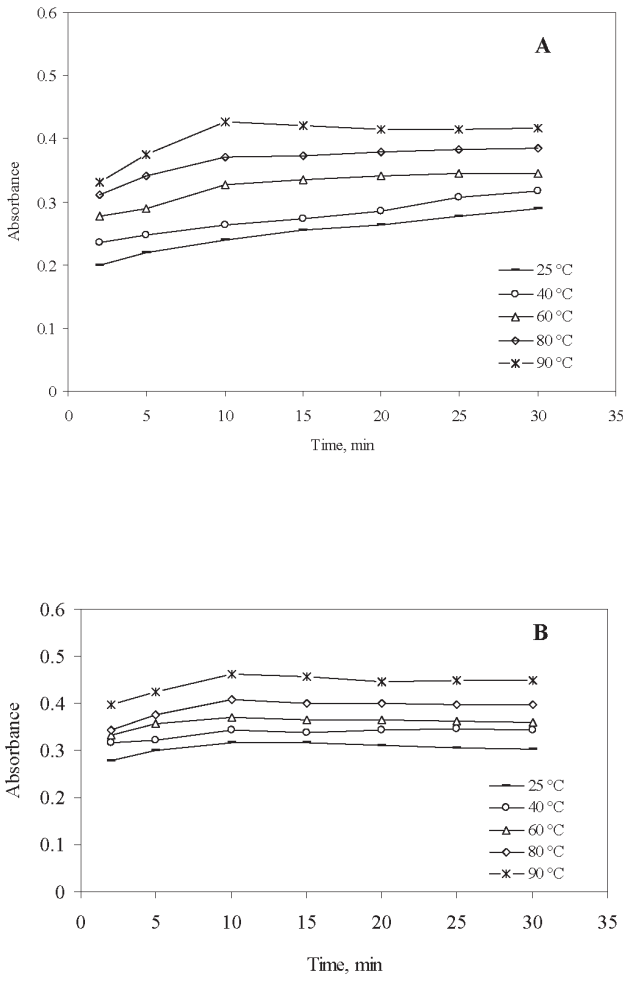

Figure 4. Effects of temperature and heating time on the absorbance of the colored product from (A) Method A and (B) Method B.

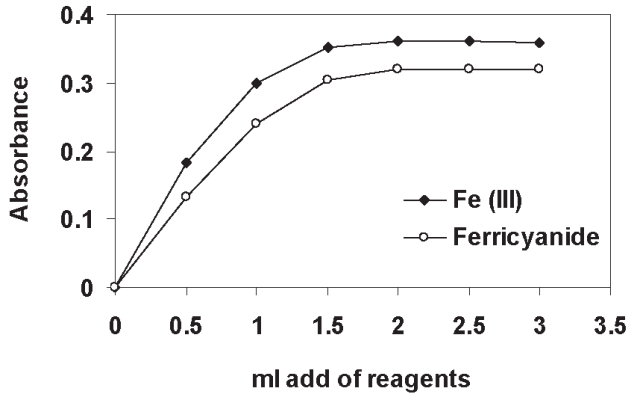

Figure 5. Effect of added Fe(III) chloride and ferricyanide (0.2%) with 8.0 μg/mL PiCl and 1.0 mL of 10 M sulfuric acid per 10 mL.

Analytical Data

Under the optimum conditions described above, Beer's law holds well over the concentration ranges 0.5–8, 2–16, and 3–15 μg/mL PiCl using Methods A–C, respectively. The optimum concentration ranges of PiCl that can be measured accurately, as evaluated from Ringbom plots are 1–7, 3.5–15, and 4–13.5 μg/mL using Methods A–C, respectively. The apparent molar absorptivity and Sandell sensitivity values were found to be 7.91×10^4 L/mol cm and 5.52 ng/cm^2 for Method A, 3.24×10^4 L/mol cm and 13.50 ng/cm^2 for Method B, and 2.581×10^4 L/mol cm and 16.90 ng/cm^2 for Method C. The relative standard deviation (RSD) values for 6 replicate determinations of 8 μg/mL were 0.83, 0.67, and 0.54% using Methods A–C, respectively. The regression equations were:

$$A = 0.1521 + 0.0813C \text{ for } Fe^{2+}\text{-phen},$$

$$A = 0.0983 + 0.0505C \text{ for } Fe^{2+}\text{-bipyridyl, and}$$

$$A = -0.0706 + 0.0694C \text{ for } Fe^{2+}\text{-ferricyanide}$$

where C is the concentration in μg/mL.

Sensitivity

The limit of detection (LOD) values for the proposed methods were calculated using the following equation according to the International Union of Pure and Applied Chemistry (IUPAC) definition (24):

$$LOD = 3s/k$$

where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits obtained for the absorbance were 0.21, 0.45, and 0.37 μg/mL for the PiCl Fe^{2+}-phen, Fe^{2+}-bipyridyl, and Fe^{2+}-ferricyanide methods, respectively.

The limit of quantitation (LOQ) is defined as:

$$LOQ = 10s/k$$

According to this equation, the LOQ was found to be 0.70, 1.5, and 1.23 μg/mL for the PiCl Fe^{2+}-phen, Fe^{2+}-bipyridyl, and Fe^{2+}-ferricyanide methods, respectively.

Table 2. The intra- and interday precision and accuracy data for PiCl obtained by the proposed Methods A–C

		Intraday			Interday		
Method	Added, μg/mL	Found, μg/mL[a,b]	Precision RSD, %[c]	Accuracy Er, %[d]	Found, μg/mL[a,b]	Precision RSD, %[c]	Accuracy Er, %[d]
A	1.5	1.49 ± 0.29	0.72	–0.67	1.52 ± 0.35	0.85	1.33
	3	3.01 ± 0.43	1.04	0.33	2.97 ± 0.27	0.67	–1.00
	4.5	4.49 ± 0.34	0.83	–0.22	4.54 ± 0.38	0.92	0.89
	6	5.95 ± 0.51	1.27	–0.83	6.03 ± 0.43	1.05	0.50
B	3	3.02 ± 0.0.47	1.14	0.67	3.01 ± 0.32	0.77	0.33
	6	6.07 ± 0.40	0.96	1.17	5.99 ± 0.33	0.81	–0.17
	9	8.90 ± 0.32	0.79	–1.11	8.96 ± 0.22	0.54	–0.44
	12	12.04 ± 0.54	1.33	0.33	11.97 ± 0.40	0.97	–-0.25
C	4	3.97 ± 0.58	1.42	–0.75	3.98 ± 0.45	1.12	–0.50
	7	7.05 ± 0.44	1.08	0.71	7.03 ± 0.28	0.69	0.43
	10	9.99 ± 0.38	0.92	–0.10	10.04 ± 0.30	0.73	0.40
	13	13.02 ± 0.36	0.89	0.15	13.01 ± 0.35	0.86	0.077

[a] Average of 6 determinations.
[b] Mean ± standard error.
[c] RSD = Relative standard deviation.
[d] Er = Relative error.

Table 3. Determination of PiCl in the presence of additives or excipients

Material	Amount, mg	Recovery[a] ± SD, %[b]		
		Method A	Method B	Method C
Lactose	50	99.63 ± 0.82	99.20 ± 0.78	100.2 ± 0.51
Glucose	50	98.84 ± 0.67	100.35 ± 1.22	99.55 ± 0.88
Dextrose	50	99.30 ± 0.78	98.70 ± 0.56	98.65 ± 0.46
Magnesium stearate	30	99.25 ± 0.75	99.55 ± 0.89	100.15 ± 1.30
Calcium hydrogen phosphate	50	99.50 ± 0.96	98.95 ± 0.73	99.40 ± 0.84
Talc	40	99.80 ± 0.61	100.40 ± 1.05	100.10 ± 0.95
Starch	50	100.05 ± 1.14	98.80 ± 0.69	99.75 ± 0.62

[a] 8.0 μg/mL PiCl was taken; result is the average of 5 determinations (n = 5).
[b] SD = Standard deviation.

Accuracy and Precision

In order to determine the accuracy and precision of the proposed methods, solutions containing 4 different concentrations of PiCl were prepared and analyzed in 6 replicates. The RSD (precision) and percentage relative error (Er %; accuracy) of the suggested methods were calculated at the 95% confidence level. The percentage relative error was calculated using the following equation:

$$\text{Er, \%} = [(\text{found} - \text{added})/\text{added}] \times 100$$

The inter- and intraday precision and accuracy results are shown in Table 2. The analytical results show that the proposed methods have good accuracy and precision (repeatability and reproducibility).

Interferences

The criterion of absence of interference was an error of not more than ±3.0% in the absorbance. To test the efficiency and selectivity of the proposed analytical Methods A–C for pharmaceutical formulations, a systematic study of additives and excipients (e.g., lactose, glucose, dextrose, talc, calcium hydrogen phosphate, magnesium stearate, and starch) that are usually present in dosage forms was made. Experimentals showed that there was no interference from additives or excipients for the examined Methods A–C as shown in Table 3.

Analytical Applications

The proposed methods were successfully applied to determine PiCl in its pharmaceutical formulations. Therefore, they could be used easily for the routine analysis of pure PiCl and its dosage forms. The performance of the proposed methods was assessed by calculation of the t-test (for accuracy) and a variance ratio F-value (for precision) compared with the official method (1), based on measuring the absorbance of the drug solution in 0.1 mol/L HCl at 252 nm, at the 95% confidence level with 5 degrees of freedom (25). The results showed that the t- and F-values were less than the critical value, indicating that there was no significant difference between the proposed and official methods as shown in Table 4. Because the proposed methods

Table 4. Determination of PiCl in pharmaceutical preparations using the proposed methods

Sample	Statistic	Official method	Proposed methods		
			A	B	C
PiCl pure	Recovery ± SD, %[a]	100.08 ± 1.06	100.40 ± 0.57	99.75 ± 0.81	99.62 ± 0.63
	t[b]		0.59	0.55	0.83
	F[c]		3.46	1.71	2.83
Selgon tablets (20 mg/tablet)	Recovery ± SD, %[a]	99.70 ± 1.16	98.52 ± 0.79	100.65 ± 1.19	100.27 ± 0.92
	t[b]		1.87	0.74	0.86
	F[c]		2.17	1.05	1.60
Selgon drops (40 mg/mL)	Recovery ± SD, %[a]	100.50 ± 1.36	99.95 ± 0.81	100.30 ± 0.96	100.04 ± 1.02
	t[b]		0.78	0.27	0.61
	F[c]		2.82	2.01	1.78

[a] Average of 6 determinations.
[b] Calculated t-value; tabulated t-value for 5 degrees of freedom; at P = 0.05 is 2.57.
[c] Calculated F-value; tabulated F-value for 5 degrees of freedom; at 95% confidence limit is 5.05.

were more reproducible with higher recoveries than the official method, they can be recommended for routine analysis in the majority of drug quality control laboratories.

Conclusions

The proposed methods are simpler, less time consuming, and more sensitive than the official method (1). All of the proposed methods were advantageous compared to other reported visible spectrophotometric (2, 3), potentiometric (6), and conductometric (7) methods with respect to their higher sensitivity, simplicity, precision, accuracy, and stability of the colored species for ≥12 h. In addition, there is no prior extraction with chloroform of the colored product as in the earlier spectrophotometric methods (2, 3). The proposed methods are suitable for the determination of PiCl in pure and in pharmaceutical formulations without interference from excipients such as starch and glucose or from common degradation products, suggesting applications in bulk drug analysis.

References

(1) Parfitt, K. (1993) in *Martindale*, *the Complete Drug Reference,* 30th Ed., The Pharmaceutical Press, London, UK, pp 8140–8141

(2) Zarapker, S.S., Rele, R.V., & Shah, V.M. (1987) *Indian Drugs* **24**, 445–449

(3) Zarapker, S.S., Rele, R.V., & Doshi, V.J. (1987) *Indian Drugs* **24**, 560–564

(4) Revanasiddappa, H.D., & Ramappa, P.G. (1995) *Indian Drugs* **32,** 73–77

(5) Revanasiddappa, H.D., & Ramappa, P.G. (1995) *Indian Drugs* **32,** 534–536

(6) Abdel-Ghani, N.T., Shoukry, A.F., & El-Nashar, R.M. (2001) *Analys*t **126**, 79–85

(7) Issa, Y.M., Shoukry, A.F., & El-Nashar, R.M. (2001) *J. Pharm. Biomed. Anal.* **26**, 379–386

(8) Melwanki, M.B., Seetharamappa, J., & Masti, S.P. (2001) *Anal. Sci.* **17,** 1121–1123

(9) Rahman, N., Singh, M., & Hoda, Md.N. (2004) *Il Farmaco* **59** (**11**), 913–919

(10) Farhadi, K., & Maleki, R. (2001) *Anal. Sci*. **17,** 1867–1870

(11) El-Didamony, A.M., & Amin, A.S. (2004) *Anal. Lett*. **37**, 1151–1162

(12) Saleh, H.M., Amin, A.S., & El-Mammli, M. (2001) *Mikrochim. Acta* **137,** 185–189

(13) Syeda, A., Mahesh, H.R.K., & Syed, A.A. (2005) *Il Farmaco* **60**, 47–51

(14) Nagarali, B.S., Seetharamppa, J., & Melwanki, M.B. (2002) *J. Pharm. Biomed. Anal.* **29**, 859–864

(15) Basavaiah, K., Chandrashekar, U., & Prameela, H.C. (2003) *Il Farmaco* **58**, 141–148

(16) Nagaraja, P., Dinesh, N.D., Gowada, N.M.M., & Rangappa, K.S. (2000) *Anal. Sci*. **16**, 1127–1131

(17) Amin, A.S., Zaky, M., Khater, H.M., & El-Beshbeshy, A.M. (1999) *Anal. Lett.* **32**, 1421–1433

(18) Vogel, A.I. (1979) *Textbook of Macro and Semimicro Qualitative Inorganic Analysis*, 5th Ed., Longman, London, UK

(19) Britton, H.T.S. (1952) *Hydrogen Ions*, 4th Ed., Chapman and Hall, London, UK

(20) Marczenko, Z. (1976) *Spectrophotometric Determination of Elements,* pp 309– 318

(21) Massart, D.L., Vandeginste, B.G.M., Deming, S.N., Michtte, Y., & Kaufmann, L. (1988) *Chemometries, A Textbook,* Elsevier, Amsterdam, The Netherlands, pp 390–393

(22) Pesez, M., & Bartos, J. (1965) *Ann. Pharm. Fr.* **23**, 218–223

(23) Lurie, J. (1975) *Handbook of Analytical Chemistry*, Mir Publishers, Moscow, Russia

(24) International Union of Pure and Applied Chemistry (IUPAC) (1978) *Spectrochim. Acta, Part B* **33**, 242– 245

(25) Miller, J.C., & Miller, J.N. (1993) *Statistics for Analytical Chemistry*, 3rd Ed., Ellis Horwood, New York, NY, pp 53–62