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New Colorimetric Methods for the Determination of Indapamide and its Formulations

Hanna M. Saleh[1,*], **Alaa S. Amin**[2], and **Magda El-Mammli**[1]

[1] Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt
[2] Chemistry Department, Faculty of Science, Benha University, Benha, Egypt

Abstract. Two simple and sensitive methods for the determination of indapamide in pure and in dosage forms are developed. These methods are based on the oxidation of indapamide with iron(III) in acidic medium. The liberated iron(II) reacts with 1,10-phenanthroline (Method A) and the ferroin complex is colorimetrically measured at λ_{max} 509 nm against reagent blank. Method B is based on the reduction of Fe(III) by the drug. Iron(II) forms a colored complex (λ_{max} 522 nm) with 2,2′-bipyridyl. Optimization of the experimental conditions is described. Beer's law is obeyed in the concentration range 1.0–12 μg ml^{-1} and 4.0–18 μg ml^{-1} for A and B, respectively. The apparent molar absorptivity and Sandell sensitivity for method A is 3×10^4 L mol^{-1} cm^{-1} and 0.0188 μg cm^{-2}, while for method B is 2.3×10^4 L mol^{-1} cm^{-1} and 0.0159 μg cm^{-2}. The detection and quantification limits are calculated. The developed methods are applied successfully for the determination of indapamide in pure and in tablet form without interference from common excepients.

Key words: Colorimetry; indapamide; pharmaceutical formulations; iron-phenanthroline; iron-bipyridyl complexes.

Indapamide [4-chloro-N(2-methyl-1-indolinyl)-3-sulphamoyl benzamide] is a diuretic agent having a potent anti-hypertensive activity [1, 2]. In the UPS XXIV, supplement, a liquid chromatographic assay for indapamide is reported [1]. A review of the literature of the past two decades reveals that there are a few spectrophotometric methods [3, 4] reported for the assay of indapamide but lack both sensitivity and selectivity. Most analytical techniques for its determination were concerning on GLC and HPLC methods [5–13]. However, all these methods are costly, tedious, time consuming and prior separation of the drug is required.

This paper describes colorimetric methods for the determination of indapamide which can be used in laboratories where modern and expensive apparatus such as that required for GLC or HPLC are not available. However, colorimetric methods are versatile and economical particularly for developing countries. The proposed procedures are also applicable for estimation of indapamide in some pharmaceutical dosage forms without previous separation.

Experimental

Apparatus

UV-Visible recording spectrophotometer Shimadzu-260 with 1.0 cm quartz cuvettes was used for all spectral measurements.

Material and Reagents

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

1. Pure indapamide and indapamide tablets (Natrilix^R 2.5 mg) were supplied by Les Laboratories Servier, France.
2. Preparation of iron(III)-o-phenanthroline reagent [14]. Mix 0.198 g of 1,10-phenanthroline monohydrate (Sigma Chemical Company, St. Louis, U.S.A.), 2.0 ml of 1.0 M HCl and 0.16 g of ferric ammonium sulphate dodecahydrate (Aldrich, Germany) and diluted with water to the mark in 100 ml calibrated flask.
3. Preparation of iron(III)-bipyridyl reagent (Aldrich, Germany): dissolve 0.16 g of 2,2′-bipyridyl in 2.0 ml of 1.0 M HCl and 0.16 g of ferric ammonium sulphate dodecahydrate and diluted to 100 ml with water in a 100 ml calibrated flask.

* To whom correspondence should be addressed

4. Indapamide solutions: prepared by dissolving 100 mg of pure indapamide in 30 ml ethanol and completed to 100 ml with the same solvent to obtain the standard solution of 1.0 mg ml^{-1}. Working solutions were prepared by an appropriate dilution of the stock standard solution.

General Procedure

Aliquots of standard drug solution of indapamide ranging from 10–120 µg for method A and from 40–180 µg for method B, were transferred into a series of 10 ml calibrated flasks. 2.5 ml Fe(III)-o-phenanthroline or 3.0 ml Fe(III)-2,2′-bipyridyl reagent were added. The flasks were then heated on a water bath at 70 °C for 10 min using method A and 20 min using method B. The contents of the flasks were cooled to room temperature (25 ± 1 °C) and the volume is completed to the mark with distilled water. The absorbance was measured at 509 nm for A and 521 nm for B against blank treated similarly.

Procedure for Tablets

Twenty tablets were accurately weighed and powdered. An accurately weighed quantity equivalent to 50 mg indapamide was dissolved in 10 ml ethanol and transferred to a 100 ml calibrated flask. The contents of the flask was shaken for 10 min, and then completed to the mark with the same solvent. The sample was filtered and the procedure was then continued as described above.

Results and Discussion

The methods A and B are based on the tris(o-phenanthroline) or tris(2,2′-bipyridyl)-iron(II) chelate upon the reaction of indapamide with an iron(III)-o-phenanthroline or iron(III)-2,2′-bipyridyl reagent. The reaction proceeds through reduction of iron(III) ion to iron(II) and subsequent formation of an intensive orange-red coloration of the complex.

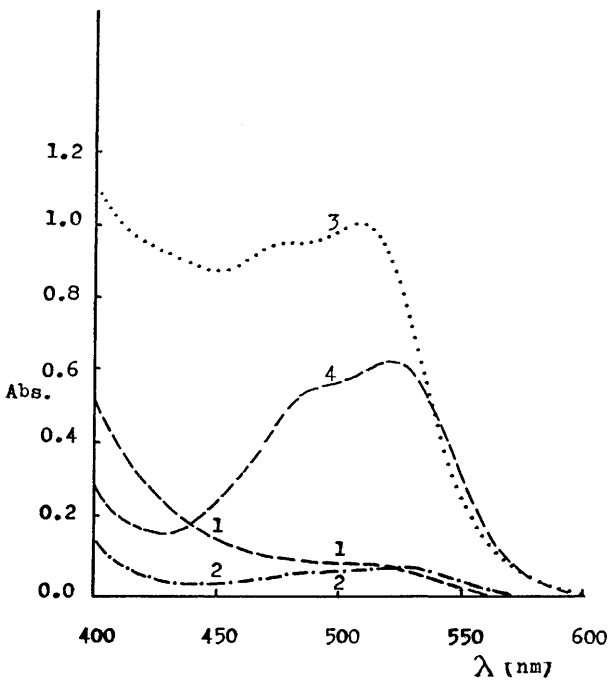

Fig. 1. Absorption spectra of 1-Fe(III)-1,10-phenanthroline, 2-Fe(III)-2,2′-bipyridyl before addition of 12 µg ml^{-1} and 3, 4 after reduction with indapamide, respectively

The absorption spectra of the colored complex species in the proposed methods show characteristic λ_{max} at 509 and 522 nm using method A and B, respectively (Fig. 1). The experimental conditions were established by varying each parameter individually and observing the effect on the absorbance of the colored species. The obtained complex is stable for at least 24 h.

Effect of pH

The pH adjustment is necessary especially in acidic medium because the reaction is affected by the change of the pH in the range of 2.5–6.0. pH 3.5 is the optimum pH value of the final assay solution for both methods A and B for complete oxidation of the drug and obtaining a high color intensity and stability.

Effect of Reagent Concentration

The addition of 2.5 ml of iron(II)-o-phenanthroline or 3.0 ml of iron(III)-2,2′-bipyridyl reagent was required to obtain a maximum and reproducible absorbance. Smaller amounts give incomplete complex formation. Therefore 2.5 ml of iron(III)-o-phenanthroline or 3.0 ml of iron(III)-2,2′-bipyridyl reagent were used throughout the experimental studies.

Effect of Temperature and Heating Time

Figures 2 and 3 show the effect of temperature and heating time on the formation of the colored complex. The reaction of indapamide with both reagents proceeds slowly at room temperature. Higher temperature was used to accelerate the reaction. Maximum absorbance was obtained after heating (at 70 °C) for about 10 min and 20 min for Fe^{2+}-phen and Fe^{2+}-bipyridyl colored complexes, respectively. Therefore, development times of 10 min at 70 °C (method A) and 20 min at 70 °C (method B) were adopted in subsequent investigations.

Sensitivity, Accuracy and Precision

Under the optimum conditions described above, Beer's law holds well over the concentration range 1.0–12 µg

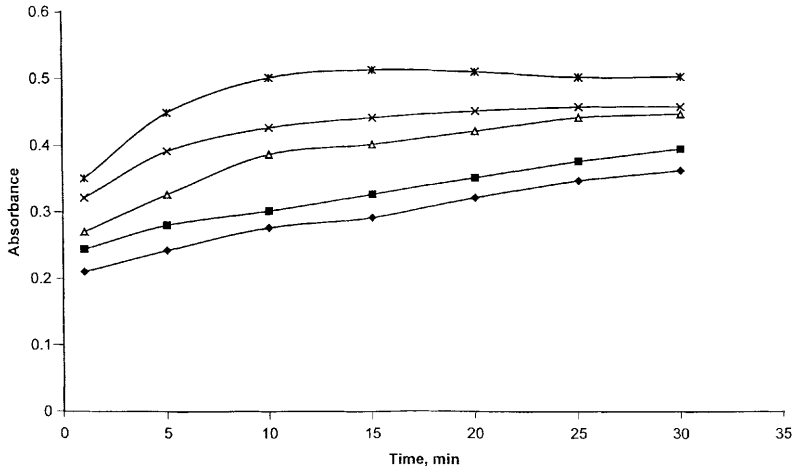

Fig. 2. Effect of temperature and heating time on absorbance of the colored product from method A. ⊸ at 25 °C; ⊸ at 40 °C; ⊸ at 50 °C; ⊸ at 60 °C; ⊸ at 70 °C

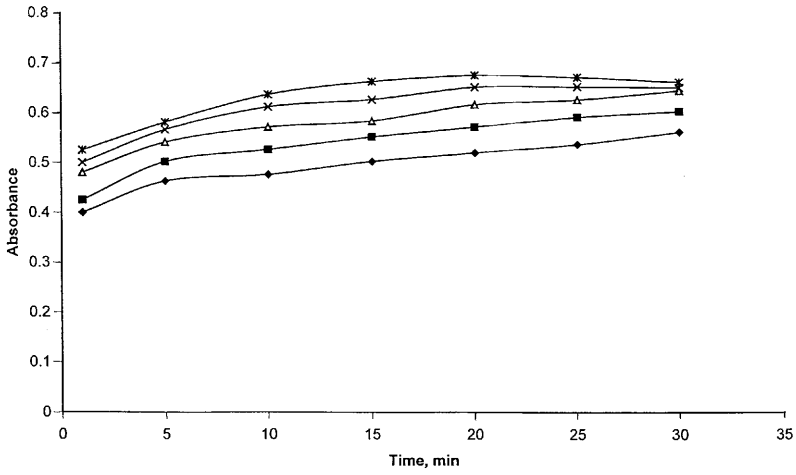

Fig. 3. Effect of temperature and heating time on absorbance of the colored product from method B. ⊸ at 25 °C; ⊸ at 40 °C; ⊸ at 50 °C; ⊸ at 60 °C; ⊸ at 70 °C

ml^{-1} using method A and 4.0–18 $\mu g\,ml^{-1}$ of indapamide using method B. The apparent molar absorptivity and Sandell sensitivity were found to be 3×10^4 L $mol^{-1}cm^{-1}$ and 0.0188 $\mu g\,cm^{-2}$ using method A, whereas for method B were 2.3×10^4 L mol^{-1} cm^{-1} and 0.0159 $\mu g\,cm^{-2}$. The optimum concentration ranges of indapamide that can be measured accurately, as evaluated from Ringbom plot are 2.0–11 and 4.5–16.6 $\mu g\,ml^{-1}$ using methods A and B, respectively. The relative standard deviation for nine replicate determinations of 7.0 $\mu g\,ml^{-1}$ is 0.76 and 0.80% using method A and B, respectively. The regression equation using Fe^{2+}-phen is $A = 0.003 + 0.078\,C$, whereas using Fe^{2+}-bipyridyl is $A = 0.007 + 0.055\,C$ (where C is the concentration in $\mu g\,ml^{-1}$). The standard deviations of the absorbance measurements obtained from a series of 13 blank solutions for each reagent were calculated (Table 1). The limits of detection (K = 3) and of quantification (K = 10) of the methods were established according to IUPAC definition [15].

Table 1. Quantitative parameters for the proposed methods A and B

Parameter	Method A	Method B
λ_{max}/nm	509	522
Beer's law/$\mu g\,ml^{-1}$	1.0–12	4.0–18
Ringbom concentration range/$\mu g\,ml^{-1}$	2.0–11	4.5–16.6
Molar absorptivity (ε)/L mol^{-1} cm^{-1}	3×10^4	2.3×10^4
Sandell sensitivity/$\mu g\,cm^{-2}$	0.0188	0.0159
Detection limit/$\mu g\,ml^{-1}$	0.25	0.90
Quantification limit/$\mu g\,ml^{-1}$	0.83	3.1
Regression equation*		
Intercept/a	0.003	0.007
Slope/b	0.078	0.055
Correlation coefficient/r	0.9997	0.9994
Relative standard deviation (S_r)/%	0.764	0.804

* A = a+bC where (C) is the concentration in $\mu g\,ml^{-1}$ and (A) is the absorbance unit.

The proposed methods are more sensitive compared with Agrawal et al. [3] method using ammonium molybdate as reagent (molar absorptivity is 6.1×10^3 L mol^{-1} cm^{-1} with Sandell sensitivity of 0.059 μg cm^{-2}. Comparison of the recovery obtained with the

proposed methods with that of Agrawal et al. [3] showed a high accuracy of the present methods. Moreover, the proposed methods could be used for the routine determination of indapamide in pure or in dosage forms.

Interferences

No interferences were observed in the determination of indapamide in presence of gum acacia, propylene glycol, calcium lactate, calcium hydrogen phosphate, reserpin, zinc stearate, carboxymethyl cellulose, fractose, glucose, lactose, magnesium stearate and starch when present in 120 fold molar excess. Also, there was no interference from the thermal and hydrolytic degradation products of indapamide. In preliminary experiments, these compounds were found not to reduce iron(III) to iron(II) and therefore do not interfere in the determination. The results suggest that the methods would be useful for quality control of indapamide in their pharmaceutical preparations.

Analytical Applications

The proposed methods were successfully applied to determine indapamide in its dosage forms using the standard addition method in which variable amounts of the pure drug were added to the previously analysed portion of pharmaceutical dosage forms. Results are shown in Table 2 and confirm that the proposed methods are not liable to interference by tablet fillers usually formulated with indapamide. Therefore they could be used easily for the routine analysis of pure indapamide and its dosage forms.

The performance of the proposed methods was assessed by calculation of the t-test (for accuracy) and F-value (for precision) compared with the official method for 95% confidence level with five degrees of freedom [16]. The results showed that the t-values were 1.37 and 1.78 using method A and B, respectively, whereas the critical value is 2.57. The F-values were 2.85, and 3.41, respectively, while the critical value is 5.05. These results indicated that there was no significant difference between the proposed and official methods.

Table 2. Determination of indapamide in pharmaceutical preparations applying the standard addition technique

Dosage form	Taken $\mu g\,ml^{-1}$	Added $\mu g\,ml^{-1}$	Found* $\mu g\,ml^{-1}$		
			A	B	Official
Natrilix(R)	2.50	–	2.48	–	2.55
		2.50	5.04	4.97	5.20
		5.00	7.45	7.60	7.35
		7.50	10.10	9.93	9.80
	2.00	–	1.99	–	1.95
		2.00	3.95	3.99	4.15
		4.00	6.07	5.93	6.20
		6.00	8.03	8.08	7.80
	3.50	–	3.47	–	3.40
		1.50	5.04	4.95	5.15
		3.00	6.55	6.45	6.65
		4.50	7.94	8.10	8.20
		6.00	9.60	9.40	9.30

* Average of six determinations.

Conclusion

The proposed method is simpler, less time consuming and more sensitive than the official method (based on liquid chromatographic assay). Whereas the color developments of Fe^{2+}-phen and Fe^{2+}-bipyridyl complexes at room temperature required 60 and 90 min using method A and B[2] by raising the temperature to 70 °C. The proposed methods are suitable for the determination of indapamide in pure and in dosage forms without interference from excipients such as starch and glucose or from common degradation products, suggesting applications in bulk drug analysis.

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[2] This can be shortened to 10 and 20 min

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