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SPECTROPHOTOMETRIC DETERMINATION OF PERINDOPRIL IN THE PRESENCE OF ITS ACID - INDUCED DEGRADATION PRODUCTS

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ABSTRACT

Perindopril has been determined in the presence of its acid - induced degradation products using a first derivative (D_1) spectrophotometric method (method I) or colorimetric methods(methods II and III) . The spectrophotometric method is based on D_1 value measurement at 220 nm. The colorimetric methods are based on either the reaction of perindopril with iron (III) chloride in the presence of potassium thiocyanate to form orange complex that is soluble in chloroform with maximum absorbance at 432 nm (method II) or the formation of an ion-association complex with bromothymol blue at pH 5 which is extracted into chloroform and has maximum absorbance at 418 nm (method III) . The three methods have proved to be stability indicating , since plots of log C% versus time were linear . The application to perindopril in tablets gave good results

INTRODUCTION

The non official drug perindopril [tert. butyl-amine salt of (2S-1-R,R) 2α, 3aβ7 aß]-1-[2-(1-ethoxycarbonyl butyl] amino]- oxopropyl, octahydro-1H indole -2 carboxylic acid] is an antihypertensive agent whose de-esterified metabolite is an active inhibitor of angiotensin I- converting enzyme (ACE) [1]. The few reported methods in literature for the determination of perindopril are gas chromatography [2] , gas chromatography mass spectrometry (GC-MS) [3], radioimmunoassay [4] and derivatization - gas chromatography [5]. The colorimetric methods (one* of the authors previous work) using certain π- acceptors [6] or copper sulphate in the presence of eosin to form ternary complex soluble in chloroform [7] were used to determination of perindopril in tablets.

Derivative UV-spectrophotometry has been successfully applied for the determination of some drugs in the presence of their degradation products. These include the determination of procaine in presence of P-aminobenzoic acid [8], some 1,4-benzo- diazepines[9] , cimetidine [10], some sulphonamides [11] in presence of their acid - induced degradation products and some cephalosporines in presence of their degradation products [12-14] ,

In the present work , perindopril is determined in the presence of its acid-induced degradation products using first-derivative (D_1) UV-spectrophotometry (method I). The reaction of perindopril with iron (III) chloride in the presence of potassium thiocyanate to form orange complex (method II) and the formation of an ion -association complex with bromothymol blue at pH 5 (method III).

EXPERIMENTAL

Instrumentation :

Shimadzu 260 UV recording spectrophotometer.

Material and reagent :

Chemicals used were of the highest purity available from their sources. Perindopril and Coversyl tablets containing 4 mg perindopril per tablet from Servier Egypt Industries, Cairo . Iron (III) chloride solution was prepared as 0.1 M solution in distilled water. Potassium thiocyanate IM aqueous solution. Bromothymol blue was prepared as 1 x 10^{-3} M in 0.1 N NaOH . Acetate buffer pH 5 (to 13.6 g sodium acetate , 6 ml glacial acetic acid was added and the volume was completed to 1000 ml with distilled water).

Standard solutions :

i- Standard aqueous stock solution of perindopril 0.25 $mgml^{-1}$ was stable for at least one weak when stored at ≤ 25°C in a dark place.

ii-Acid induced degradation products : an accurate weight of 25 mg of perindopril was dissolved in a 50 ml volumetric flask containing 20 ml of IN HCl, heated in a boiling water bath for 2 h, cooled and completed to volume with water. The solution was transferred into a beaker and neutralized using a pH meter, with IN NaOH solution. The neutralized solution was transferred quantitively to a 100 ml volumetric flask and completed to volume with water.

General procedures :

First derivative method (method I):

Appropriate volumes of the standard solution in the concentration range stated in Table 1 were placed in a series of 25 ml volumetric flasks and made up to volume with distilled water. The first derivative spectra were recorded against water and the peak amplitude was measured at 220 nm.

Colorimetric method (method II) :

This was carried out using iron (III) and potassium thiocyanate. Appropriate volumes of the standard solution in the concentration range stated in Table 1 were placed in 50 ml separating funnels. The volume of each solution was adjusted to 10 ml with

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distilled water. Exactly, 1.5 ml of 0.1 M iron (III) chloride and 4 ml of 1M potassium thiocyanate were added. The complex was extracted with 3x3 ml portions of chloroform. The solution was shaken for 1 min each time and the chloroform layer was passed through a layer of anhydrous sodium sulphate into a 10 ml volumetric flask. The volume of the chloroform layers was made up to 10 ml, and the absorbance was measured at 432 nm against blank in which the drug is omitted.

Colorimetric method (method III) :

This was carried out using bromothymol blue. Appropriate volumes of the standard solution in the concentration range stated in table 1 were placed in 50 ml separating funnels. The volume of each solution was adjusted to 10 ml with distilled water 2 ml bromothymol blue and 4 ml acetate buffer pH 5 were added. The complex was extracted with portions of chloroform (3x3 ml). The solution was shaken for 1 min each time and the chloroform layer was passed through a layer of anhydrous sodium sulphate into a 10 ml volumetric flask. The volume of the chloroform layers was made up to 10 ml, and the absorbance was measured at 416 nm against blank in which the drug is omitted.

Procedure for tablets :

An accurately weighed quantity of powdered tablets equivalent to 20 mg perindopril was placed in a 50 ml volumetric flask 30 ml distilled water was added and the solution was shaken for 5 min to dissolve the drug. The volume was made up to 50 ml and the solution was filtered and analyzed as per general procedures.

RESULTS AND DISCUSSION

Absorption spectra :

Fig. 1(a) shows the zero - order UV-spectra of perindopril and its acid - induced hydrolytic products in distilled water, while Fig. 1 (b) present their

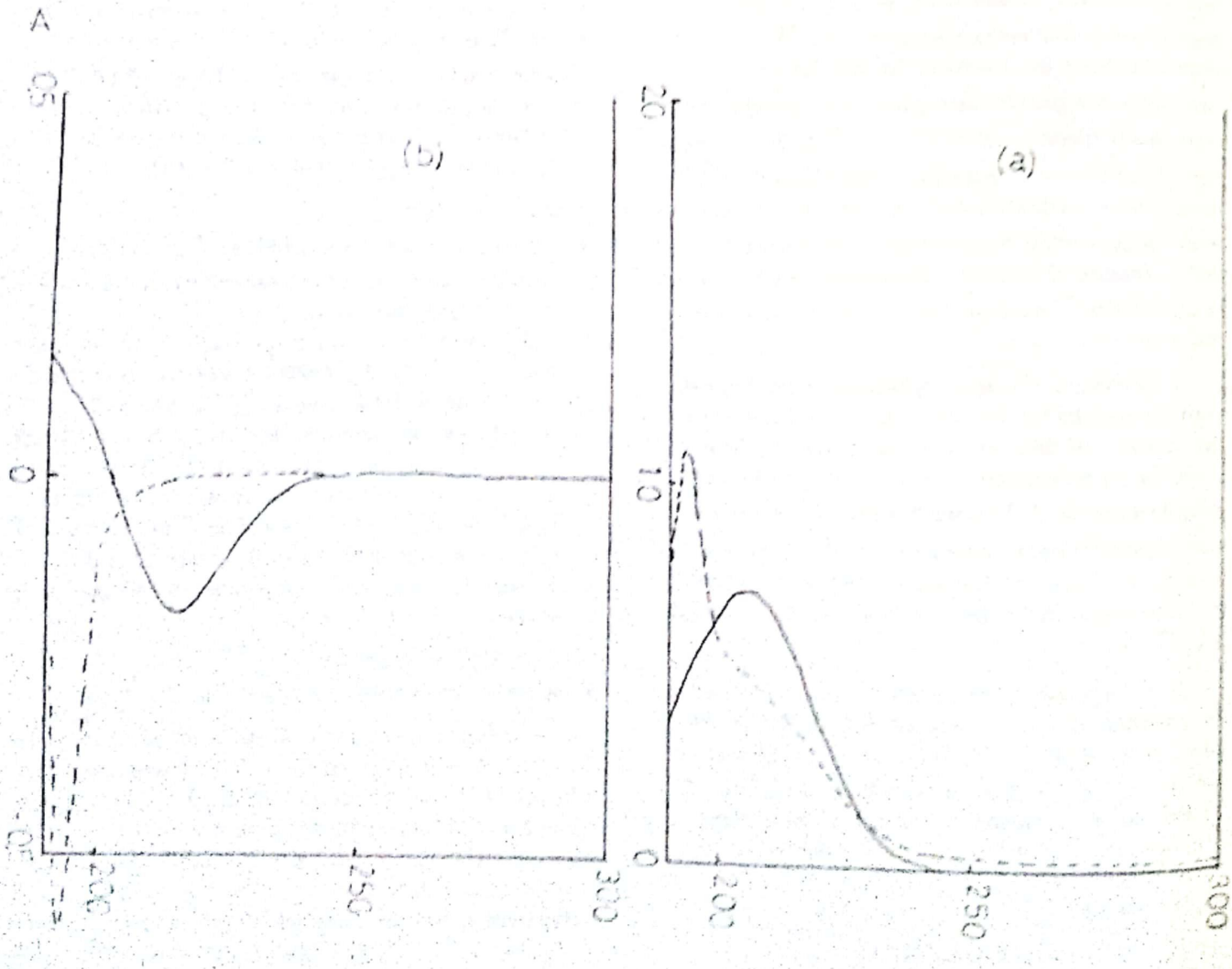

Fig. (1) : Zero order (a) and first derivative (b) spectra of 50 μgml^{-1} of perindopril (_____) and of 100 μgml^{-1} of its degradation products (- - - - -).

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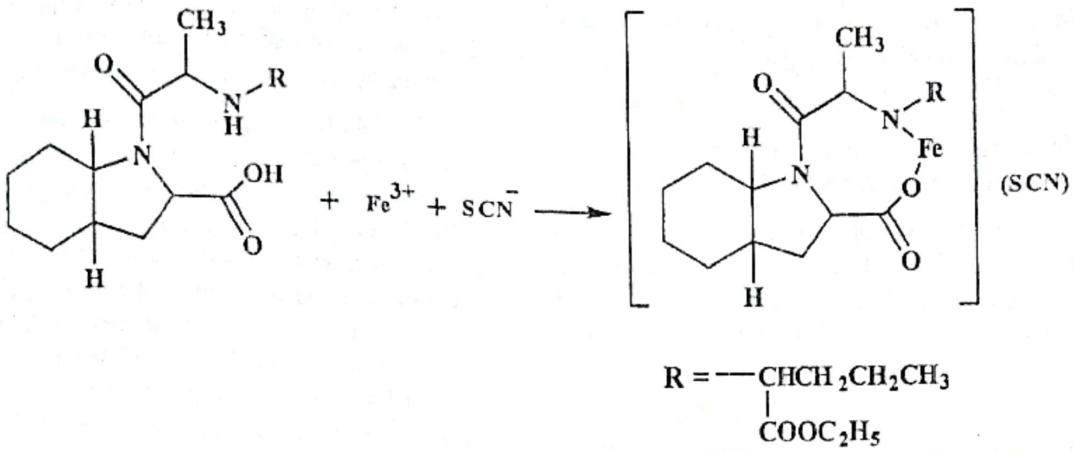
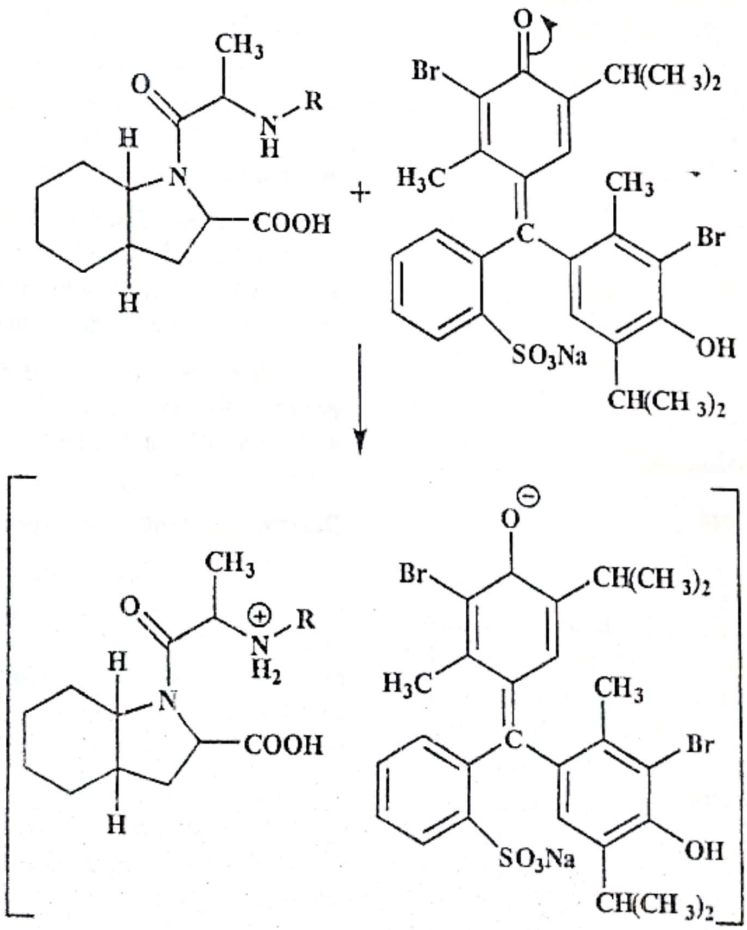


Scheme 1 : Suggested reaction pathway of perindopril with iron (III) in the presence of potassium thiocyanate (ternary complex formation).



Scheme 2 : Suggested reaction pathway of perindopril with bromothymol blue at pH 5 (ion- association complex formation).

corresponding first derivative curve in which the intact perindopril exhibits optimum D_1 peak at 220 nm, while its acid - induced degradation products show almost nil contribution. Accordingly, zero crossing measurement validates the intact perindopril estimation without interference from the degradation products.

The spectra of the coloured reaction products and the correspondent reagent blanks in the methods II and III were shown in Fig. 2. The spectra of the reaction products show characteristic λ max values (432 and 418 nm for method II and III, respectively).

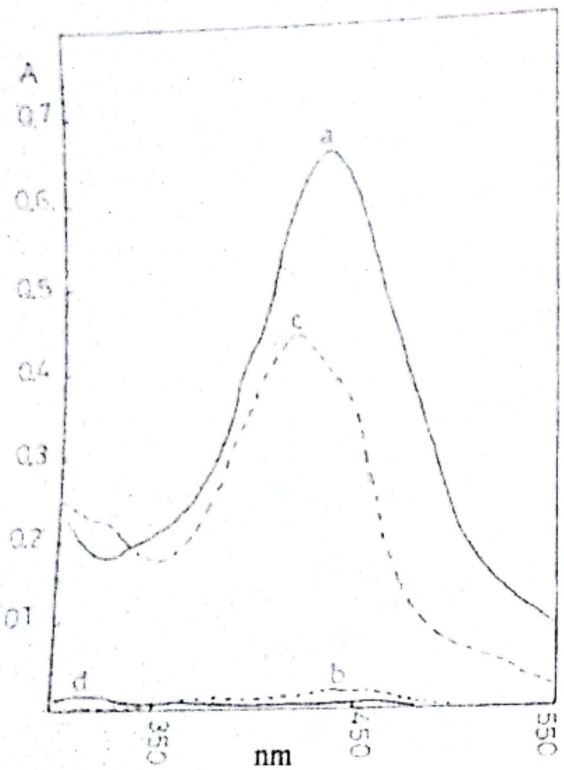


Fig. (2): Absorption spectra of perindopril (200 μgml-1) with iron (III) chloride and potassium thiocyanate (method II) and perindopril (60 μgml^{-1}) with bromothymol blue (method III). **(a)** perindopril - iron (III) chloride , **(b)** chloride blank , **(c)** perindopril-bromothymol blue and **(d)** bromothymol blue blank.

For method II, ternary complexes of general formula (L_n M_x S_y) have been widely used in spectrophotometric analysis [15-20]. The particularity of the ternary complexes dealt with in this paper is that their main Lingand L is the non-official drug perindopril, the second ligand S is thiocyanate and M is iron (III) (scheme 1). This triple complex is extractable with chloroform, whereas the binary systems (Iron- drug and Iron - thiocyanate) cannot be extracted in that way.

The experimental conditions were established by varying one variable and observing its effect on the absorbance of the coloured product : 1-2 ml of 0.1 M aqueous solution of iron (III) chloride and 3-5 ml of 1M aqueous solution of potassium thiocyanate were found optimum to maximize the colour intensity .

Trials have been made to carry out the reaction in acid, base or buffer media. It was found that the addition of alkali such as NaOH, no colour was produced in the chloroformic layer. On the other hand, marked hypochromic effect associated with hypsochromic shift was produced upon addition of acid such as HCL. The influence of buffer on colour development was also studied. 1 ml acetate buffer pH 6.5 was found to be optimal for colour development, but also dilution with distilled water as in procedure for method II gave the same results indicating that there is no need to use buffer .

In method III, perindopril being basic forms an ion-association complex with the acidic dye bromothymol blue (the anion of the acidic dye A reacts quantiatively with the basic perindopril B, to form AB) which is extractable into chloroform from the aqueous phase . The reaction may be respresented by scheme 2.

In order to established an optimum pH range , the perindopril was allowed to react with bromothymol blue solution buffered to pH 4.4 to 6 and the complex formed was extract into chloroform for measurement.

Constant absorbance was obtained over the pH range 4.8 to 5.5 in acetate buffer hence a pH of 5 was used . A 2 ml portion of bromothymol blue 1X10^{-3} M in 0.1 N NaOH solution was found to be optimal .

For both methods II and III, chloroform was preferred for its selective extraction of the perindopril with iron (III) or bromothymol blue for the aqueous solution.

Determination of complexes composition :

The composition of complexes of perindopril with iron (III) ion (in method II) and perindopril with bromothymol (in method III) were determined by Job's method of equimolar solutions. The concentration of aqueous perindopril and iron (III) chloride solution or perindopril and bromothymol blue were 1x10^{-2} or 2x 10^{-4} molml^{-1} , respectively. The plot in each method , reached a maximum value at mole fraction 0.5 which indicates the formation of complex (1:1) for the two complexes (Fig. 3) .

Calibration curves and reproducibility :

The correlation between D_1 at 220 nm or absorbance of the yellow chromogen at 432 or 418 nm (for method II or III, respectively) and perindopril concentration was found to be linear with negligible intercept. Regression analysis using the method of least - squares [21] was made for slope (b), intercept (a) and correlation coefficient (r) for the three proposed methods (Table 1).

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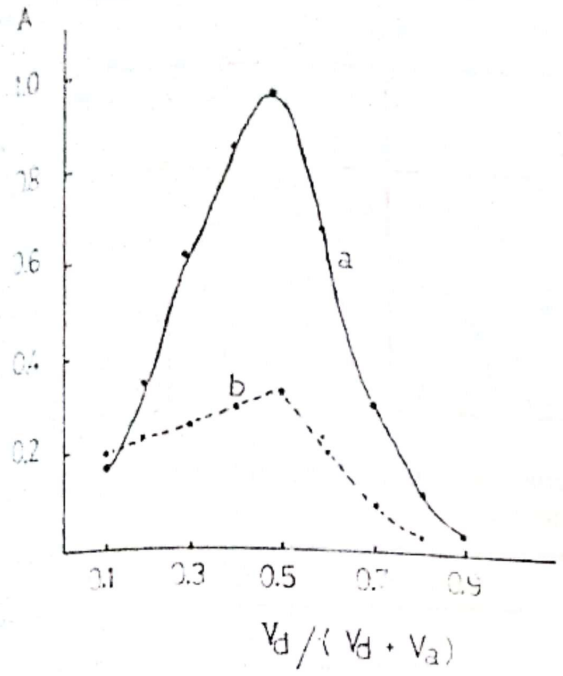

Fig. (3): (a) Job's curve of equimolar solution at 432 nm of [perindopril]= [Fe (III)] = 1 x10^{-2} M. (b) Job's curve of equimolar solution at 418 nm of [perindopril] = [bromothymol blue] = 1 x10^{-4} M at pH 5

Separate determinations at different drug concentration levels were carried out using the three proposed methods to assess their reproducibility. The relative standard deviations (RSD) were found to be <2% , indicating good reproducibility .

To prove the validity and applicability of the three proposed methods , five synthetic mixtures of perindopril and its acid - induced degradation products were prepared with different proportions and assayed for perindopril content using the proposed methods. The concentrations of the intact drug in these mixtures were in the range of 5-40, 50-250 and 20-100 μgml^{-1} in the presence of 5,50 and 20μgml^{-1} of the corresponding degradation products applying methods I, II and III, respectively. The mean percentage recoveries were 100.8 ± 0.97, 100.7 ± 0.42 and 99.8 ± 0.99 for perindopril using methods II, III and I, respectively (Table 2).

For comparison, The A_{max} method was applied to the determination of the intact drug in the above mixtures and the results were unacceptable high owing to the contribution of the degradation products. The error decreased with an increase in the concentration of the intact drug, relative to that of the degradation products.

Stability investigation of perindopril by the proposed methods :

An accurate weight of 100mg of perindopril was dissolved in 25-ml volumetric flask containing 20 ml 0.2 N HCL, completed to volume with water and placed in a constant - temperature water bath maintained at 60°C. It was allowed to equilibrate thermally for 5 min. Aliquots (2ml) were diluted to 25ml with distilled water, transferred into a series of beakers and neutralized using a pH meter, with 0.2 N NaOH solution at zero time and every 30 min over a period of 3 h. The neutralized solution was transferred quantitatively to a 100 -ml volumetric flask and completed to volume with water. The previously mentioned procedures were carried out and the perindopril concentration was calculated after each time intervals, from the calibration graph (Table 1) . The plots of log C% against time gave straight lines indicating that the proposed methods, based on measuring the D_1 values at 220 nm (method I) and the yellow chromogen at 432 or418 nm (for method II or III , respectively) are specific for the intact drug and is independent of degradation products (Fig. 4).

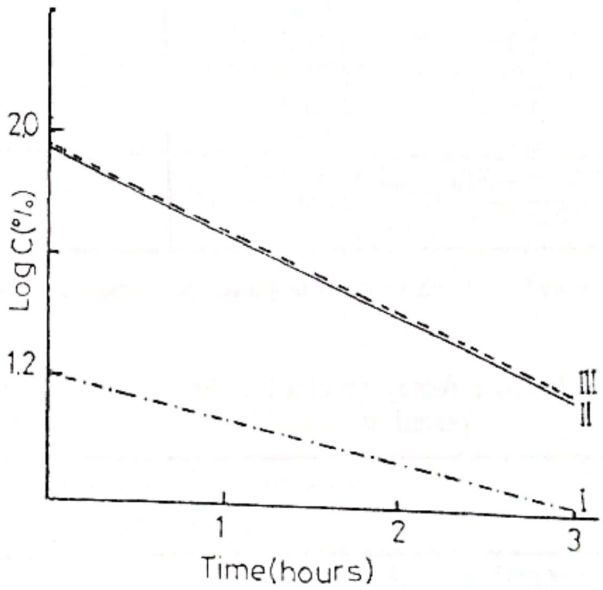

Fig. (4): Stability study of perindopril applying methods I, II and III (16, 80, 80 μgml^{-1}, respectively).

Assay results :

The applicability of the three proposed methods was appraised through the assay of perindopril tablets. The results have compared with the A_{max} method at 208 nm (since perindopril tablets are non - official) using the t-test for accuracy and the F-test for presision assessment. The calculated values did no exceed the corresponding theoretical values, indicating the insignificant difference between the results of the compared methods (Table 3).

Table (1) : Optical characteristics and statistical data of the regression equations for determination of perindopril applying the proposed methods.

Parameters	Spectrophotometric		Colorimetric methods	
	A_{max}	D_1(method I)	(Method II)	(Method III)
Beer's law limits ($\mu g ml^{-1}$))	5-50	5-50	50-250	20-100
Molar absorptivity (mol^{-1} cm^{-1})	-	$-1.707x10^{-3}$	$1.244x10^{-3}$	$3.497x10^{-3}$
Regression equation :				
Slope (b)	0.01919	$-4.465x10^{-3}$	$3.2401x10^{-3}$	$8.6359x10^{-3}$
Intercept (a)	0.0205	-0.0013	0.0108	0.0567
Correlation coefficient (r)	-	0.9991	0.9897	0.9975

Table (2): Spectrophotometric determination of perindopril in presence of its acid - induced degradation products using the proposed methods.

D1 method (method I)		Colorimetric methods			
		Method II		Method III	
Added (μg ml^{-1})	Recovery (%)	Added (μg ml^{-1})	Recovery (%)	Added (μg ml^{-1})	Recovery (%)
5	102	50	100.9	20	99.1
10	101.3	100	100.4	40	98.2
20	101.0	150	101.2	60	99.5
30	100.2	200	101.0	80	99.7
40	99.5	250	100.2	100	98.9
Mean ± SD	100.8±0.97		100.7±0.42		99.8±0.99
RSD (%)	0.96		0.42		0.99

*, + and ++ Each contains degradation products corresponding to 5, 20 and 50 μg ml^{-1} of perindopril, respectively.

Table (3) : Assay results for the determination of perindopril in coversyl tablets by the three proposed methods.

	D_1 method (method I)	Colorimetric methods (Method II)	Colorimetric methods (Method III)	A_{max} method
Coversyl tablets*				
Mean ± SD	100.9±0.98	101.5±1.02	99.1±1.20	100.5±0.58
N	5	5	5	5
Variance	0.96	1.04	1.44	0.34
t- test	0.63 (2.31)	2.2 (2.31)	2.21 (2.31)	
F- test	2.82 (6.39)	3.06 (6.39)	4.24 (6.39)	

* Without degradation products

Since perindopril is non - official drug, conventional A_{max} method results are used for comparison .

Values in parentheses are the tabulated values of t- and F- at P= 0.05.

A_{max} method at 208 nm can not be applied to perindopril assay without prior separation of its degradation products since the spectra of the latter exhibits extensive interference (Fig. 1a). The merits of the proposed methods, however, are in the application of perindopril assay even in the presence of its acid induced degradation products. Accordingly, these methods may find a wide application for perindopril aquality control in many analytical laboratories.

REFERENCES

1- Laubie, M., Vincent, M. and H. Schmitt. **J. Cardio - Vasc. Pharmacol.** 6, 1076-1082 (1984).

2- Sereda, K. M., Hardman, T. C., Dilloway, M. R. and Lant, A. F. **Anal. Proc. (London)** 30 (9), 371-372 (1993).

3- Tasconas, C., Devissaguet, M. and Padieu, P. **J. Chromatogr.** 488, 249-265 (1989).

4- Doucet, L., De Veyrac, B., Delaage, M., Cailla, H. Bernheim, C. and Devissaguet, M. **J. Pharm. Sci.** 79, 741-745 (1990).

5- Lin, S. J., Wu, H. L., Chem, S. H. and Wen, Y. H. **Anal Lett.** 29 (10), 1751-1762 (1996).

6- Abdellatef, H. E. **J. Pharm. Biomed. Anal.** 17 1267-1271 (1998).

7- Abdellatef, H. E., Ayad, M. M. and Taha, E. A. **J. Pharm. Biomed. Anal.** (Under Publication).

8- Korany, M. A., Wahbi, A. M. and Hewala, I. I. **Archs. Pharm. Chem. Sci.** 12, 26-80 (1984).

9- Abdel Hamid, M., Korany, M. A. and Bedair, M., **Acta. Pharm. Jugosl.** 34, 183-190 (1984).

10- Bedair, M. M., Elsayed, M. A., Korany, M. A. and Fahmy, O. T. **J. Pharma. Biomed. Anal.** 9, 291-296 (1991).

11- Abdellatef, H. E., Elhalkany, M. N. and Aboulkheir, A. **J. Pharm. Biomed. Anal.** 7, 571-576 (1989).

12- El-Yazbi, F. A. and Barary, M., **Anal Lett.** 18, 629-633 (1984).

13- Korany, M. A., Elsayed, M. A. and Galal, S. M., **Anal. Lett.** 22, 141 157 (1989).

14- Korany, M. A., Elsayed, M. A. and Galal, S. M. **Anal. Lett** 22, 159-175 (1989).

15- Fujita, Y., Mori, I., Kitano, S., Koshiyama, Y. **Chem. Pharm. Bull.** 33, 242-248 (1985).

16- Mori, I., Fujita, Y., Kawabe, H., Fujita, K., Tanaka, T. and Kishimoto, A. Analyst 111, 1409-1412 (1986).

17- Fujita, Y., Mori, I., Fujita, K., Tanaka, T. Koshiyama, Y. and Kawabe, H. **Chem. Pharm. Bull.** 34, 2236-2238 (1986).

18- Fujita, Y., Mori, I., Fujita, K. T. and Tanaka, Y. **Chem. Pharm. Bull.** 35 865-868 (1987).

19- Issopoulos, B. P. and Economou Fresenius, P. T. **J. Anal. Chem.** 345 (1993) 595-599.

20- El-Walily, A. F. M., Belal, S. F. and Bakry R. S., **J. Pharm. Biomed. Anal.** 14 (1996) 561-569.

21- Mathematics and Statistics for Use in Biological and Pharmaceutical Sciences. 2nd ed. The Pharmaceutical Press (1971).

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التعيين الطيفى لمركب البريندوبريل في وجود نواتج تكسيره فى وسط حامضى

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فى هذا البحث تم دراسة ثلاث طرق طيفية جديدة لتعين مركب البريندوبريل فى وجود نواتج تكسيره فى وسط حامضى . تعتمد الطريقة الأولى على تعيين المركب باستخدام المعامل التفاضلى الأول لمنحنى الأمتصاص (D_1) عند ٢٢٠ نانو ميتر . بينما تعتمد الطريقة الثانية على تعين المركب بعد تفاعله مع عنصر الحديد فى وجود ثايوسيانيدالبوتاسيوم لتكوين معقد تم استخلاصة وقياسة عند ٤٣٢ نانوميتر . بينما اعتمدت الطريقة الثالثة على تكوين معقد ايونى باستخدام كاشف بروموثايمول عند درجة حموضة ٥ وقد تم قياسة عند ٤١٨ نانوميتر هذا وقد تمت مقارنه هذه الطريقة بطريقة الأمتصاص الضوئى Amax حيث أن المركب غير دستورى .

وقد أعطت هذه الطريقة نواتج جيدة عند مقارنتها بطريقة Amax ومن الممكن استخدامها لتعيين المركب فى المستحضرات الصيدلية والمواد الخام.