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Short communication

Two derivative spectrophotometric determinations of indapamide in pharmaceutical dosage forms

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Abstract

Simple, fast and reliable derivative spectrophotometric methods were developed for determination of indapamide in bulk and pharmaceutical dosage forms. The solutions of standard and the sample were prepared in methanol. The quantitative determination of the drug was carried out using the first-derivative values measured at 252.8 nm ($N = 6$) and the second-derivative values measured at 260.4 nm ($N = 9$). Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of indapamide using peak to zero 1.00–30.00 μg ml^{-1} for first-derivative and 1.00–35.00 μg ml^{-1} for second-derivative spectrophotometric method. The developed methods were successfully applied for the assay of pharmaceutical dosage forms which do not require any preliminary separation or treatment of the samples. The details of the statistical treatment of analytical data are also presented. The results obtained from two derivative spectrophotometry were compared with a spectrophotometric method reported in literature and no significant difference was found statistically. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Indapamide; Derivative UV spectrophotometry; Pharmaceutical dosage forms (tablet, capsule, drage)

1. Introduction

The chemical formula of indapamide is 3-(aminosulfonyl)-4-chloro-*N*-(2,3-dihydro-2-methyl-1*H*-indol-1-yl)-benzamide (Fig. 1). Indapamide is an oral antihypertensive diuretic agent indicated for the treatment of hypertensive and edema [1]. Indapamide inhibits carbonic anhydrase enzyme [2]. A significant reduction in blood pressure can be achieved with daily oral dose of 2.5 mg [3]. It differs chemically from the thiazide diuretics in that it does not posses the thiazide ring system and contains only a sulfonamide groups [4].

Several methods have been reported for the determination of indapamide in biological fluids and in pharmaceutical preparations including spectrophotometry [5], fluorimetry [6,7], gas chromatography–mass spectrophotometry [8–10], high performance liquid chromatography [3,4,11–18], thin layer chromatography [19], capillary electrophoresis [20,21], and electrochemical analysis at carbon paste electrode [22].

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There is no derivative spectrophotometric method for the analysis of indapamide in pharmaceutical preparations has been reported in literature. The aim of this work was to investigate the utility of derivative spectrophotometry in the assay of indapamide in pharmaceutical preparations without the necessity of sample pre-treatment. In this study, two derivative UV spectrophotometric methods are developed and validated for the determination of indapamide. The developed methods were applied to three different commercial preparations as tablet, capsule and drage. The results obtained by these two approaches were compared. The results obtained from two derivative spectrophotometry were compared with those obtained by using spectrophotometric method in literature [23].

2. Experimental

2.1. Apparatus

A Shimadzu UV-160 recording double-beam UV–visible spectrophotometer with a data processing system was used. UV spectra of reference and sample solutions were recorded in 1 cm quartz cells at scan speed of 50 nm min^{-1} and a fixed slit width of 3 nm. First- ($N=6$, $\Delta\lambda=21.0$ nm) and second-order derivative ($N=9$, $\Delta\lambda=31.5$ nm) curves were recorded over the range of 200–350 nm.

2.2. Reagents and solutions

Indapamide standard was obtained from the Central Institue of Hygiene of Turkey. It was tested for purity by controlling its melting point, UV and infrared spectra and no impurities were found. Analytical grade of methanol was purchased from Merck.

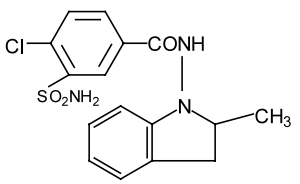

Fig. 1. Chemical formula of indapamide.

Working standard solutions were daily prepared by diluting stock solutions at the concentrations of 1, 2, 5, 10, 15, 20, 25, 30 and 35 μg ml^{-1} in methanol and methanol was used as a blank solution.

2.3. Sample solutions

A total 10 tablets or drages of indapamide accurately weighed and powdered. An amount corresponding to one tablet or drages content was weighed and transferred in a 25 ml volumetric flask. Ten milliliter of methanol was added and the flask was sonicated for 30 min, then filled up to the volume with methanol. Appropriate dilutions were made in the range of 1.00–35.00 μg ml^{-1} with methanol. UV spectra were recorded against methanol.

The contents of 10 capsules were weighed and powdered. The average of one capsule content was calculated and a sample equivalent to one capsule was weighed and transferred in to a 25 ml volumetric flask and same procedure made as tablet and drage forms.

3. Results and discussion

3.1. Optimization of conditions

The solvent, the degree of derivation, the wavelength range and N values were chosen in order to optimize the conditions. Optimum results were obtained in the measuring wavelength range 200–350 nm, $N=6$ ($\Delta\lambda=21.0$) for first-order derivative and $N=9$ ($\Delta\lambda=31.5$) for second-order derivative spectrophotometry. UV spectrum of indapamide in methanol gives two broad shouldered peaks at 211.6 and 241.2 nm (Fig. 2). These two shouldered peaks were separated by using derivative spectrophotometric method (Figs. 3 and 4).

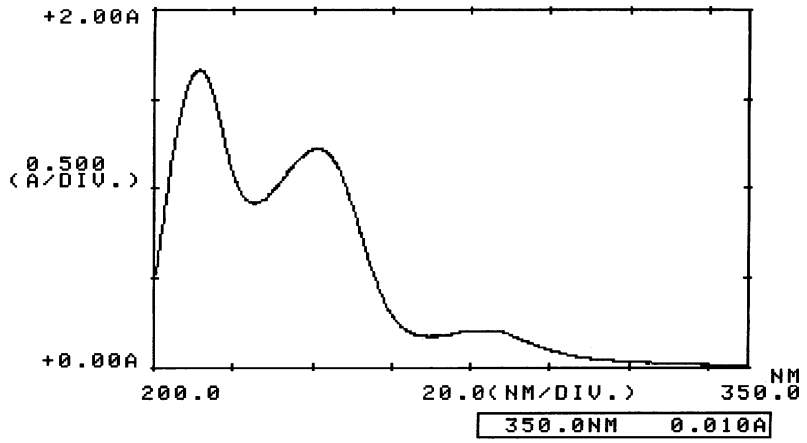

Fig. 2. UV spectrum of 20 μg ml^{-1} standard indapamide solution in methanol.

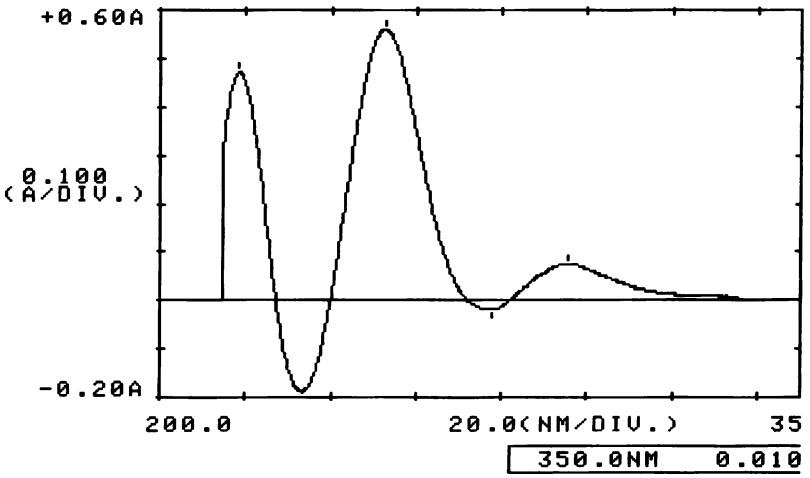

Fig. 3. First-derivative spectrum of 20 μg ml^{-1}standard indapamide solution in methanol.

3.2. Linearity range

Under the experimental conditions described the graphs obtained by plotting D^1, D^2 values versus concentration (in the range stated in Table 1) show linear relationships. Regression analysis using the method of least-squares was made for the slope, intercept and correlation coefficient values (Table 1). The regression equations (with standard error of intercept and slope) of calibration curves were $y = (0.0271 \pm 5.1430 \times 10^{-5})x + (0.0036 \pm 3.3832 \times 10^{-4})$ and $y = (0.0401 \pm 4.4594 \times 10^{-5})x + (0.0130 \pm 5.7368 \times 10^{-4})$ for first and second-derivative spectrophotometric method, respectively. The linearity ranges were found to be 1.00–30.00 μg ml^{-1} for first-derivative and 1.00–35.00 μg ml^{-1} for second-derivative spectrophotometric methods.

3.3. Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.13 and 0.43 μg ml^{-1} for first-derivative, 0.22 and 0.73 μg ml^{-1} for second-derivative method. The determinations

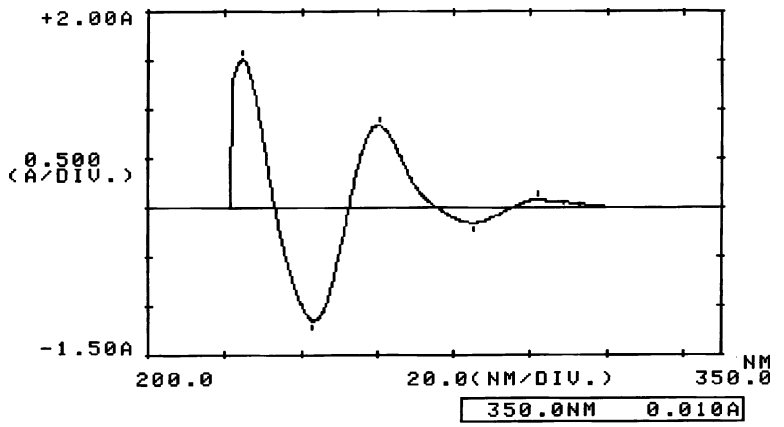

Fig. 4. Second-derivative spectrum of 20 μg ml^{-1} standard indapamide solution in methanol.

of different concentration levels were carried out for each drug to test sensitivity, quantitation and reproducibility and of the D^1 and D^2 values [24–26] (Table 1).

3.4. Specifity and selectivity

Comparison of the original, first and second-derivative spectrum of indapamide in standard and drug formulation solutions show that the wavelength of maximum absorbance did not changed.

In order to evaluate the effect excipients in these methods, the standard addition method was applied. The regression equation (with standard error of intercept and slope) of this method were found as $y = (0.0243 \pm 3.7081 \times 10^{-4})x + (0.5509 \pm 5.5314 \times 10^{-3})$ for first-derivative and $y = (0.0367 \pm 5.9442 \times 10^{-4})x + (0.8166 \pm 7.7745 \times 10^{-3})$ for second-order spectrophotometry. The amount of indapamide in the drugs was calculated using calibration and standard addition curve method. Since the slopes of the calibration and standard addition curves were identical (Table 1). According to the results obtained by interference study, the derivative spectrophotometric method is able to access the analyte in the presence of excipients and hence, it can be considered specific. It has been concluded that there was no spectral interaction in the analysis of pharmaceutical preparation of indapamide. Therefore, calibration curve method was chosen for analysis of drug.

Table 1
Optical characteristic, precision and accuracy ($n = 12$)

Parameters	Derivative spectrophotometry	
	First	Second
Wavelength (λ) (nm)	252.8	260.4
Regression equation of calibration curve method (y)[a]	$y = 0.0271x + 0.0036$	$y = 0.0401x + 0.0130$
Standard error on slope	5.1430×10^{-5}	4.4594×10^{-5}
Standard error on intercept	3.3832×10^{-4}	5.7368×10^{-4}
Regression equation of standard addition method (y)[a]	$y = 0.0243x + 0.5509$	$y = 0.0367x + 0.8166$
Correlation coefficient (r)	0.9997	0.9995
Linearity range (μg ml^{-1})	1.00–30.00	1.00–35.00
Limit of quantitation (μg ml^{-1})	0.43	0.73
Limit of detection (μg ml^{-1})	0.13	0.22

[a] $y = bx + a$ where x is the concentration in μg ml^{-1}, y is amplitude for first- and second-derivative.

Table 2
Assay results for the determination of indapamide in laboratory synthetic mixture and commercial tablet, drages and capsule ($n = 12$)

Sample	Amount[a] ($X \pm$ SE, RSD%)[b], (CI)[c]	
Synthetic mixtures	First-derivative spectrophotometric method	Second-derivative spectrophotometric method
Coated-tablet	$X = 2.51 \pm 0.009$, 0.80% CI = 2.49–2.53 $t_c = 10 > t_t = 2$, $P > 0.05$	$X = 2.53 \pm 0.02$, 0.79% CI = 2.48–2.58
Drage A	$X = 2.52 \pm 0.01$, 1.19% CI = 2.50–2.54 $t_c = 3.5 > t_t = 2$, $P > 0.05$	$X = 2.54 \pm 0.01$, 1.18% CI = 2.52–2.56
Drage B	$X = 2.53 \pm 0.02$, 1.58% CI = 2.48–2.58 $t_c = 12 > t_t = 2$, $P > 0.05$	$X = 2.53 \pm 0.008$, 0.79% CI = 2.51–2.55
Capsule	$X = 2.52 \pm 0.01$, 1.19% CI = 2.50–2.54 $t_c = 18 > t_t = 2$, $P > 0.05$	$X = 2.52 \pm 0.01$, 1.19% CI = 2.50–2.54
Commercial coated-tablet[d]	$X = 2.53 \pm 0.005$, 0.79% CI = 2.52–2.54 $t_c = 26.25 > t_t = 11$, $P > 0.05$	$X = 2.52 \pm 0.005$, 0.79% CI = 2.51–2.53
Commercial drage A[e]	$X = 2.53 \pm 0.009$, 1.19% CI = 2.51–2.55 $t_c = 18 > t_t = 14$, $P > 0.05$	$X = 2.56 \pm 0.005$, 0.78% CI = 2.55–2.57
Commercial drage B[f]	$X = 2.56 \pm 0.005$, 0.78% CI = 2.55–2.57 $t_c = 22 > t_t = 14$, $P > 0.05$	$X = 2.58 \pm 0.003$, 0.39% CI = 2.57–2.59
Commercial capsule[g]	$X = 2.44 \pm 0.004$, 0.41% CI = 2.43–2.45 $t_c = 21 > t_t = 2$, $P > 0.05$	$X = 2.42 \pm 0.005$, 0.83% CI = 2.41–2.43

[a] Each one tablet, drage and capsule was labeled to contain 2.5 mg of indapamide.
[b] X, mean; RSD, relative standard deviation; SE, standard error.
[c] CI, confidence intervals (at % 95); t_c, calculated t values; t_t, tabulated t values.
[d] Fludin®-coated-tablet (Saba A.Ş).
[e] Fludex® drage (Servier A.Ş).
[f] Flubest® drage (Ali Raif Ilaç Sanayi A.Ş).
[g] Indamid® capsule (İlsan İltaş A.Ş) are the products of indapamide in Turkey.

3.5. Application of methods to the pharmaceutical preparations

The present methods were applied for the determination of the commercial tablet, drages and capsule (Table 2). The results show the high reliability and reproducibility of two methods. The results obtained were statistically compared using the Wilcoxon test (Table 2). As shown from the table, indicating no significant difference between the two methods.

3.6. Accuracy and recovery

Recovery experiments were conducted to determined the accuracy of the proposed method. Recovery studies were performed at a concentration of 10 μg ml^{-1} standard indapamide standard solutions in methanol ($n = 12$). The mean recovery and relative standard deviation (RSD) were found to be 101.60 and 0.97% for first-derivative, 101.70 and 0.54% for second-derivative spectrophotometric method, indicates very good reproducibility of these methods.

The other recovery study was performed on the synthetic mixtures. Interfering compounds were lactose, magnesium stearate, talc, corn starch and titan dioxide in tablet, lactose, talc, corn starch and laque coccine in drage A, saccharose, corn starch and Brilliant Scarlet 4 R lake and titanium dioxide in drage B, corn starch, quinoline yellow and indigotine in capsule. 2.5 mg indapamide was added to the excipient mixture and these synthetic mixtures were assayed using procedures. The results obtained using the above methods were precise, accurate and selective (Table 2).

3.7. Precision

To determine the precision of the method, indapamide solutions at a concentration of 10 μg ml^{-1} in methanol were analyzed 12 times and the mean indapamide value were found as 10.16 ± 0.03 for first-derivative and 0.17 ± 0.02 for second-derivative spectrophotometric methods. The SD was found as 0.10 and 0.06 for first and second-derivative spectrophotometric methods, respectively and the developed methods have a good precision.

3.8. Repeatability

Repeatability is given as inter- and intra-day precision and accuracy where evaluated by analyzing three different concentration of indapamide. Accuracy of the method was checked for 3 days at three concentration levels at 5, 15 and 25 μg ml^{-1} each in triplicate. Solutions for the standard curves were prepared fresh every each day. The results are given in Table 3. The precision of the assays is demonstrated by RSD of lower than 1.37%.

3.9. Robustness and ruggedness

For ruggedness and robustness of analytical methods the tests mentioned below were carried out. The robustness of presented methods was tested changing parameters such as the degree of derivation, wavelength range and N value and optimum parameters were chosen for this study. First-derivative UV spectrophotometric determination of indapamide was carried out by two analysts and in two different instruments with the same standard. The results showed no statistical differences between different operators and instruments suggesting that the developed methods were robust and rugged (Table 4).

3.10. Stability

Stock solutions of indapamide were prepared at a concentration of 1000 μg ml^{-1} in methanol and kept at +4 °C. Stability of indapamide stock

Table 3
Intra- and inter-day precision and accuracy of indapamide ($n = 12$)

Intra-day				Inter-day			
Added (μg ml^{-1})	Found (μg ml^{-1}) (X)[a]	Precision	Accuracy[b] (%)	Added (μg ml^{-1})	Found (μg ml^{-1}) (X)[a]	Precision	Accuracy[b] (%)
First-derivative spectrophotometric method							
5	5.01	SD = 0.06 RSD = 1.20%	0.20	5	5.04	SD = 0.06 RSD = 1.19%	0.80
15	15.38	SD = 0.21 RSD = 1.37%	2.53	15	15.32	SD = 0.14 RSD = 0.91%	2.13
25	25.12	SD = 0.09 RSD = 0.36%	0.48	25	24.93	SD = 0.13 RSD = 0.52%	−0.28
Second-derivative spectrophotometric method							
5	4.99	SD = 0.05 RSD = 1.00	−0.20	5	5.01	SD = 0.05 RSD = 0.99%	0.20
15	15.32	SD = 0.05 RSD = 0.33%	2.13	15	15.35	SD = 0.12 RSD = 0.78%	2.33
25	25.57	SD = 0.05 RSD = 0.20%	2.28	25	25.44	SD = 0.11 RSD = 0.43%	1.76

SD, standard deviation; RSD, relative standard deviation.
[a] X, mean values represent 12 indapamide standard solutions for each concentration.
[b] Accuracy (% relative error) = (found − added/added) × 100.

Table 4
The results of analysis from pharmaceutical preparation and standard of indapamide by two different analysts and instruments ($n = 6$)

	Different analyst					
	X	SD	RSD (%)	X	SD	RSD (%)
Standard of indapamide (10 μg ml^{-1})	9.99 ± 0.02	0.04	0.44	10.14 ± 0.04	0.09	0.87
Coated-tablet (2.5 mg indapamide)	2.52 ± 0.01	0.03	1.36	2.54 ± 0.01	0.03	1.24

Table 5
Comparison of the results from first- and second-derivative spectrophotometry and spectrophotometric methods ($n = 7$)

	First-derivative method	Second-derivative method	Reference method [26]
Coated-tablet	$X = 2.51 \pm 0.01$ $t_c = 9.5$	$X = 2.52 \pm 0.01$ $t_c = 8$	$X = 2.50 \pm 0.01$
Drage	$X = 2.52 \pm 0.01$ $t_c = 6$	$X = 2.51 \pm 0.02$ $t_c = 12.5$	$X = 2.51 \pm 0.01$
Drage	$X = 2.54 \pm 0.01$ $t_c = 6.5$	$X = 2.55 \pm 0.01$ $t_c = 5$	$X = 2.51 \pm 0.01$
Capsule	$X = 2.45 \pm 0.02$ $t_c = 7.5$	$X = 2.44 \pm 0.02$ $t_c = 11.5$	$X = 2.49 \pm 0.01$

t_c, calculated t values; t_t, tabulated t values ($t_t = 2$ for $n = 7$). H_o hypothesis: no statistically significant difference exists between two methods. $t_c > t_t$; H_o hypothesis is accepted ($P > 0.05$).

solutions were tested during 3 months and results show that indapamide solutions in methanol were stable in this period.

No detectable changes were observed in the drug solutions when those were exposed to sunlight and UV light (254 nm), different pH (acidic and basic pH), temperatures (50, 70 and 100 °C). It cannot be said anything about the methods to use as stability-indicating procedures, because of not decomposing the drug solutions.

3.11. Comparison of the methods

A spectrophotometric method in literature [23] was employed as a comparison to evaluate the validity of the developed methods. Table 5 gives the results obtained by two derivative methods and a spectrophotomertric method in literature for the determination of indapamide in pharmaceutical preparations. The results were compared by the Wilcoxon test and there was no significant difference between these methods. The linearity ranges of the proposed methods had more extensive than the spectrophotometric method in literature [23]. The LOD and LOQ values for proposed methods were lower than this method. Additionally, these methods do not involve procedural steps as a compared spectrophotometric method in literature.

4. Conclusion

No derivative procedures have been described for the assay of indapamide, therefore two new derivative spectrophotometric methods were to develop for routine determination of indapamide. That is more sensitive than already existing derivative assays of indapamide [5], also less complex and faster than reported which high performance liquid chromatography and capillary electrophoresis assays. Derivative spectrophotometry offers greater selectivity than UV–visible spectrophotometry in the determination without

previous chemical separation and there was no need for extraction processes to eliminate the excipients. Since tablet, drages and capsules of indapamide yielded turbid solutions, first and second-derivative spectrophotometry presents an advantage for determination of indapamide in these solutions.

The developed spectrophotometric methods are concluded as accurate, sensitive, precise, reproducible and can be easily and directly applied to the pharmaceutical preparations.

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