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SPECTROPHOTOMETRIC DETERMINATION OF INDAPAMIDE AND CO-DERGOCRINE MESYLATE

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في هذا البحث تم استخدام طريقتين طيفيتين لتعيين الانداباميد والكوديرجوكرين فى صورهما النقية وفى مستحضراتهما الصيدلية. وذلك بواسطة قياس شدة امتصاص الضوء لناتج تفاعل أى من هذين المركبين مع فانيدات الأمونيوم أو نيترات الصوديوم فى وسط حمضى قوى عند طول موجة ٥٣٠ ن.م. للانداباميد و ٥٥٠ ن.م. للكوديرجوكرين. ولقد تمت دراسة مختلف العوامل التى تؤثر على تركيز اللون الناتج. ووجد أن مدى الاستجابة لقانون بيير هى ١,٨ الى ١٢,٠ ميكروجرام لكل ملليميتر بالنسبة للانداباميد و ١٢ ٤٠ ملليجرام لكل ملليميتر بالنسبة للكوديرجوكرين وباستخدام أى من فانيدات الأمونيوم أو نيترات الصوديوم على السواء. وقد تم اجراء التحاليل الاحصائية لكافة النتائج التى تم الحصول عليها ومقارنتها بنتائج الطرق الدستورية أو المنشورة، الأمر الذى اتضح منه نطابق النتائج ودقتها.

Two simple and sensitive spectrophotometric methods for the determination of indapamide and co-dergocrine in pure form and in pharmaceutical preparations were developed. The methods were based on the interaction of the cited drugs with either ammonium vanadate or sodium nitrite in strongly acidic media. The resulting coloured products were measured at 530 and 550 nm for indapamide and co-dergocrine respectively. The effect of different variables on the involved reactions and the colour development were studied and optimised. Beer's law was obeyed in the range of 1.8-12 $\mu g\ ml^{-1}$ for indapamide using ammonium vanadate and sodium nitrite and 12-40 $\mu g\ ml^{-1}$ for co-dergocrine with both ammonium vanadate and sodium nitrite. The reproducibility and recovery of the methods are excellent. The methods were applied successfully to the determination of some commercially available dosage forms purchased from the local market. The results were comparable to those obtained by official or reported methods.

INTRODUCTION

Indapamide (**I**) is a sulphonamide derivative that possesses diuretic and potent antihypertensive effects.[1] Indapamide has been determined spectrophotometrically[2-4] electro chemically,[5] chromatographically[4,6-10] and volumetrically.[11]

Co-dergocrine (**II**) is a mixture of three hydrogenated alkaloids, dihydroergocristine, dihydroergocornine and dihydroergocryptine (α and β) present approximately in equal ratios.[12] It is used as β-blocker antihypertensive drug. Co-dergocrine has been determined by spectrophotometic,[13] chromatographic[14-15] and radioimmunoassay[16] methods.

Few reports including spectrophotometric methods for the assay of indapamide and co-dergocrine are present in the literature. Most of these methods are tedious and time consuming.

The interaction between some easily oxidized amides and some selected oxidizing agents were investigated previously and developed as a cyclic voltammetric technique for the determination of indapamide.[5] The applicability of this reaction for developing more simpler, sensitive and time saving spectrophotometric procedures has been investigated in our laboratory. As a result of this investigation two simple, rapid, sensitive and reproducible spectrophotometric methods for determination of indapamide and co-dergocrine have been developed.

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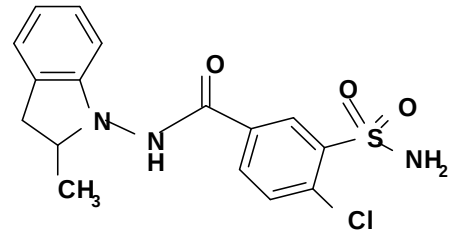
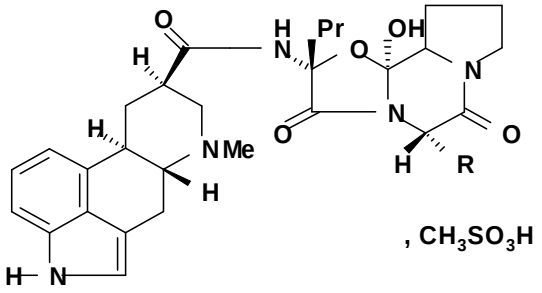
Both methods were applied successfully for determination of the studied drugs in the commercial dosage forms.



(I) Indapamide



(II) Co-dergocrine Mesilate

Dihydroergocristine: R= $-CH_2Ph$
Dihydroergocryptine, α: R= $-CH_2-CH-(Me)_2$
Dihydroergocryptine, β: R= $-CH-Me-CH_2-CH_3$
Dihydroergocornine,: R = $-CH-(Me)_2$

EXPERIMENTAL

Appartus

- Shimadzu UV 1601, UV-VIS spectrophotometer (Kyoto, Japan).
- Shimadzu –IR-470 infrared spectrometer (Kyoto, Japan). IR spectra were recorded as KBr discs.

Chemicals and reagents

All chemicals and solvents used were of analytical grade.

Reagents

Ammonium vanadate solution (0.5% w/v) was prepared by dissolving 0.5 g of ammonium vanadate (VEB Laborchemie, Apolda, Germany) in the least amount of distilled water and diluted to 100 ml with sulphuric acid ,[17] then filtered if necessary.

Sodium nitrite solution (1% w/v) was prepared by dissolving 1.0 g of sodium nitrite (Panreac, Barcelona, Espana) in 100 ml distilled water.

Pharmaceutical Grade Compounds

Indapamide (Amriya Pharm, Alexandria, Egypt), co-dergocrine from (October Pharm, Cairo, Egypt). were obtained and used as working standards without further treatment.

Purity tests were carried out by using reported and official methods[12,18] and were found in the range of the reported value.

Dosage forms

Natrilix® tablets Batch No. 1A134 (Servier, Cairo, Egypt) labelled to contain 2.5 mg indapamide, durix® tablets Batch No. 415107 (Amriya Pharm, Alexandria, Egypt) labelled to contain 2.5 mg indapamide, hypotense tablets Batch No. S10129 (ADCO, Cairo, Egypt) labelled to contain 2.5 mg indapamide and hydergine tablet Batch No. 125 (Novartis Pharm,Cairo, Egypt) labelled to contain 1.5 mg co-dergocrine mesylate.

Synthetic mixtures

Indipamide (2.5 mg) or (1.5 mg) co-dergocrine was mixed well with starch (25 mg), glucose (25 mg), sucrose (25 mg), magnesium stearate (2.5 mg), lactose (25 mg), and gum acacia (2.5 mg) in a morter and used for evaluation of interferences due to common additives and excipients. An amount of the synthetic mixture equivalent to 10 mg of each drug was transferred to a 50-ml volumetric flask, and continued as described under analysis of tablets.

Working standard solutions

An accurately weighed amount of indapamide or co-dergocrine (10 mg) was transferred into 25-ml volumetric flask, 5 ml methanol portions were added and shaked well then completed to volume with methanol. Suitable dilutions of the solution were made with the methanol to obtain concentrations ranging from 15-120 µg ml^{-1} for indapamide and 60-400 µg ml^{-1} for co-dergocrine respectively.

Analysis of tablets

Twenty tablets were weighed and finely powdered after removal of the coloured coat by a piece of cotton wetted with distilled water and the tablets were dried with tissue paper. An accurately weighed amount of the powdered tablets equivalent to 10 mg of either indapamide or co-dergocrine was transferred to 50-ml volumetric flask dissolved in about 20 ml with methanol. The mixture was sonicated well for about 10 minutes and then diluted to the mark with the same solvent and filtered. The first portion of the filtrate was rejected. The clear solution obtained was used for preparation of the working sample solutions.

Isolation of the coloured product

A weight equivalent to 10^{-4} M of indapamide was dissolved in 25 ml methanol, then mixed with 5 ml of 10^{-4} M sodium nitrite previously dissolved in double distilled water. The mixture was stirred for 15 minutes, then stored in a vacuum dessicator over anhydrous calcium chloride until a crystalline solid is formed.

General procedure

Procedure (A)

Into 10-ml volumetric flask, 5-ml perchloric acid followed by 0.1 ml sodium nitrite solution was added to 1.0 ml of either standard or sample drug solution, mixed well, allowed to stand for 10 min at ambient temperature (25 ± 2^{o}) and then diluted to volume with 35% perchloric acid. The absorbance was measured at 530 and 550 nm for indapamide and co-dergocrine respectively, against a blank solution treated similarly.

Procedure (B)

Into 10-ml volumetric flask, 5 ml perchloric acid followed by 0.1 ml ammonium vanadate for indapamide and 0.2 ml for co-dergocrine was added to 1.0 ml of either standard or sample drug solution, mixed well, allowed to stand for 10 min at ambient temperature and then diluted to volume with 35% perchloric acid. The absorbance was measured at 530 and 550 nm for indapamide and co-dergocrine, respectively, against a blank solution treated similarly.

RESULTS AND DISCUSSION

The absorption spectra for the coloured products of the studied drugs from reaction with either ammonium vanadate or sodium nitrite in acid medium exhibit maxima at 530 and 550 nm for indapamide and co-dergocrine, respectively (Figures 1 and 2).

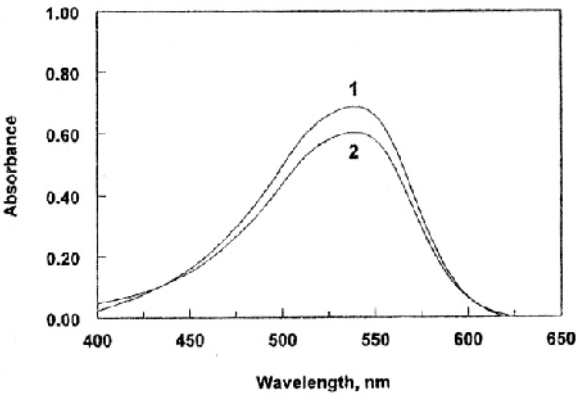


Fig. 1: Absorption spectra of indapamide (8 μg ml^{-1}) with ammonium vanadate (1) and sodium nitrite (2).

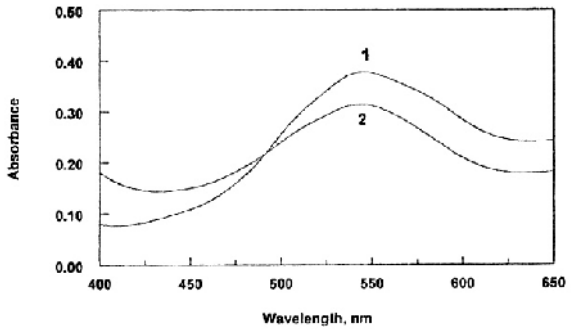


Fig. 2: Absorption spectra of co-dergocrine (15 μg ml^{-1}) with ammonium vanadate (1) and sodium nitrite (2).

Optimization of reaction variables

Investigations were carried out in order to establish the most favourable reaction conditions that affect the assay procedures. It was found that, both reactions occurred only in a strong acidic medium. Several acids in different concentrations were tested. Results showed that both oxalic and citric acid gave no colours, while acetic, nitric and hydrochloric acids gave very weak coloured products. Sulphuric and perchloric acids give more intense violet colours that can be measured spectrophotmetrically. Perchloric acid was

preferred because it gives the most intense and stable coloured products. In addition, the reaction products with perchloric acid exhibited a single and distinct maximum in all studied drug concentrations Table 1.

Different concentrations of perchloric acid were tested in order to select the most suitable one for determinations. It was found that, maximum colour intensity was obtained with 70% perchloric acid. Therefore, different volumes of this acid were tested in order to select the most suitable quantity for determinations. It was found that 4-7 ml of perchloric acid gave the most intense and stable coloured products in all cases. Thus 5 ml of 70%. perchloric acid was selected for the subsequent work.

Dilution of the coloured products by different solvents and acid solutions showed that, there was no or slight effect on the position of absorption maxima but absorption intensities were influenced. Table 2 indicates that 35% perchloric acid (1:1) is the most suitable diluting solvent because it gives the highest and most stable absorption intensity.

Table 1: Effect of the type of the acid on the absorption spectra of indapamide and co-dergocrine.

Acid	Co-dergocrine (15 µg ml^{-1})				Indapamide (8 µg ml^{-1})			
	Amm. vanadate		Sod. nitrite		Amm. vanadate		Sod. nitrite	
	Absorb.	λ_{max}	Absorb.	λ_{max}	Absorb.	λ_{max}	Absorb.	λ_{max}
Perchloric	0.386	550	0.345	550	0.660	530	0.612	530
Sulphuric	0.135	550	0.247	550	0.420	530	0.385	530
Hydrochloric	0.145	430	0.045	405	0.125	530	0.102	530
Acetic	0.078	430	0.055	405	-	-	-	-
Nitric	-	-	-		0.075	530	0.056	530

Table 2: Effect of solvents and perchloric acid solutions on the colour intensity of the reaction products of indapamide and co- dergocrine with ammonium vanadate and sodium nitrite.

Solvent	Co-dergocrine (15 µg ml^{-1})		Indapamide (8 µg ml^{-1})	
	Amm. vanadate	Sod. nitrite	Amm. vanadate	Sod. nitrite
	Absorb.	Absorb.	Absorb.	Absorb.
Ethanol	0.300	0.258	0.619	0.575
Methanol	0.315	0.256	0.571	0.550
Acetonitrile	0.258	0.269	0.516	0.275
Acetone	0.360	0.300	0.619	0.215
Isopropranol	0.300	0.254	0.555	0.446
35% Perchloric acid	0.386	0.327	0.653	0.606

The effect of sodium nitrite or ammonium vanadate concentrations on the cited drugs was studied using concentrations in the range of 0.1- 0.6% w/v for ammonium vanadate and 1-5% w/v for sodium nitrite. In case of sodium nitrite the highest absorbance intensity was obtained by using 0.1 ml of 1.0% w/v sodium nitrite solution. For ammonium vanadate the suitable volume is 0.1 ml of 0.5% w/v for indapamide and 0.2 ml for co-dergocrine (Tables 3 and 4). The coloured products were found to be stable for more than 24 h without any change in absorbance readings at room temperature (Figures 3 and 4).

Table 3: Effect of reagents concentrations on the colour intensity of the reaction products of co-dergocrine (15 μg ml^{-1}) with ammonium vanadate and sodium nitrite.

Amm. vanadate %	Absorb.	λ_{max}	Sod. nitrite %	Absorb.	λ_{max}
0.05	0.280	550	0.5	0.296	550
0.1	0.310	550	1	0.340	550
0.2	0.318	550	2	0.332	550
0.3	0.330	550	3	0.320	550
0.4	0.342	550	5	0.312	550
0.5	0.380	550	6	0.302	550
0.6	0.360	550			

Table 4: Effect of reagents concentrations on the colour intensity of the reaction products of indapamide (8 μg ml^{-1}) with ammonium vanadate and soduim nitrite.

Amm. vanadate %	Absorb.	λ_{max}	Sod. nitrite %	Absorb.	λ_{max}
0.05	0.360	530	0.5	0.590	530
0.1	0.400	530	1	0.606	530
0.2	0.453	530	2	0.574	530
0.3	0.530	530	3	0.531	530
0.4	0.553	530	5	0.413	530
0.5	0.653	530	6	0.380	530
0.6	0.467	530			

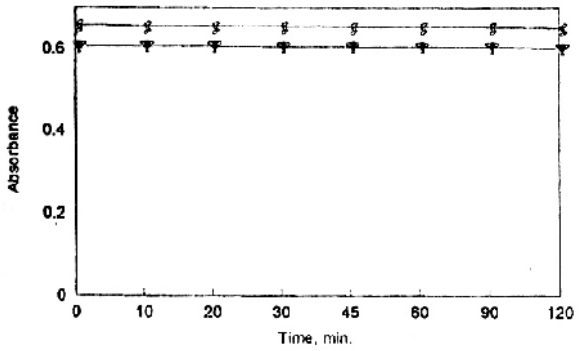


Fig. 3: Stability time of indapamide (8 μg/ ml) with (a) ammonium vanadate and (b) sodium nitrite .

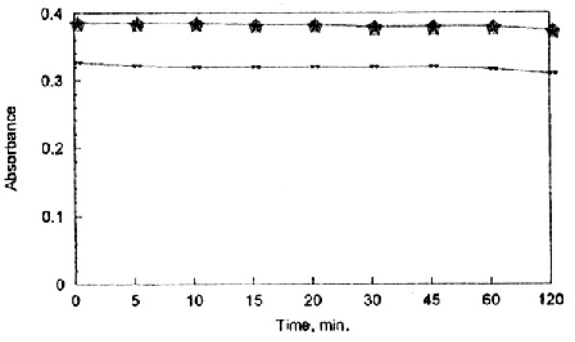


Fig. 4: Stability time of co-dergocrine (15 μg/ml) with (a) ammonium vanadate and (b) sodium nitrite.

Statistical analysis

Under the optimum reaction conditions, Beer's law was obeyed. Table 5 shows the linear calibration ranges, regression parameters, and limits of detection and determination for the proposed methods. Relative standard deviation values of the slope and intercepts of the calibration graphs indicated the high reproducibility of the proposed methods.

The proposed methods were applied for the determination of indapamide and co-dergocrine as the drug entity in some pharmaceutical formulations. Recovery experiments were carried out for each drug in its pharmaceutical formulations. The results were compared with those obtained by the official or reported methods.[2,14] As shown in Table 6 the results are in good agreement with those of the official methods, and the recovery experiments indicated the absence of interferences from the frequently encountered excipients and additives.

Table 5: Quantitative parameters and statistical data for indapamide and co-dergocrine.

Drug	Calibration range (μg/mL)	Intercept ± SD	Slope ± SD	Correlation coefficient (r)	LOD* (μg/ml)	LOQ** (μg/ml)
Procedure A						
Indapamide	1.8-12	0.0327 ± 0.0146	0.0860 ± 0.0020	0.9990	0.51	1.70
Co-dergocrine	12-40	0.0616 ± 0.0187	0.0157 ± 0.0007	0.9970	3.56	11.86
Procedure B						
Indapamide	1.5-12	0.0263 ± 0.0130	0.0929 ± 0.0020	0.9995	0.42	1.41
Co-dergocrine	6-40	0.0183 ± 0.0111	0.0202 ± 0.0005	0.9990	1.66	5.52

* Limit of detection.
** Limit of quantitation.

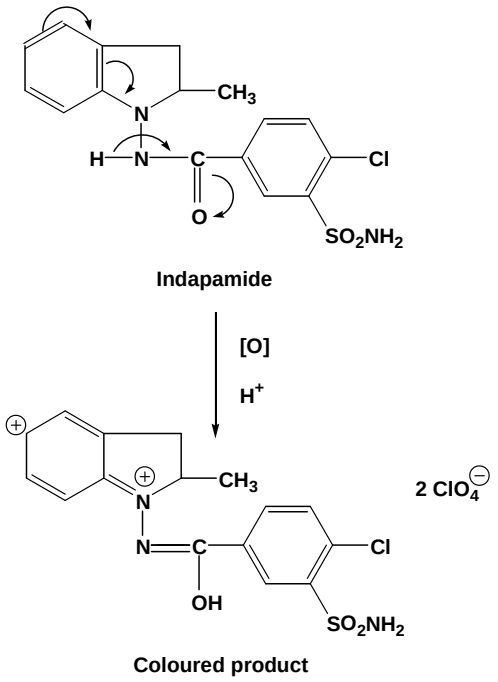
Table 6: Determination of indapamide and co-dergocrine in different formulations by different proposed and reported methods.

Formulation claimed (mg/tablet)	Amm. vanadate (% found ± SD)	Sodium nitrite (% found ± SD)	Reported (% found ± SD)
Natrilix® (2.5)	100.9 ± 0.6[a] t= 0.374 F= 4.54	102.3 ± 0.7 t= 1.574 F= 3.04	101.1 ± 1.2[b]
Diurex® (2.5)	99.5±1.1 t= 2.048 F= 1.057	99.1±0.7 t= 1.605 F= 2.395	98.1 ± 2.0 [b]
Hypotense (2.5) Batch No. S10129	101.3 ± 1.7 t=2.289 F=1.078	100.7 ± 1.7 t=1.692 F=1.104	98.7 ± 1.7 [b]
Hydergine (1.5)	101.8 ± 1.7 t=1.408 F=1.161	101.2 ± 1.2 t=1.764 F=2.606	100.1± 1.9[c]

[a]Average of five determinations.
[b]Reference 2 and [c] reference 14.
Theoretical values of F and t at 95% confidence limit are 6.39 and 2.776.

Reaction pathway

Scheme 1 shows the possible reaction pathway as predicted from the literature reports and the results of the present work. Radi[5] reported the stripping voltametry for indapamide and two anodic peaks were observed due to the oxidation of the amide group. The oxidation of the amide group will result in the formation of the structure given in the scheme. There is no positive IR evidence for the formation of –OH band or disappearance of –NH band of amide group, due to the presence of $–NH_2$ bands of the sulphamoyl group which overlaps with them, but the absence of the characteristic C=O stretching band at 1660 cm^{-1} is indicated.



Scheme 1

Interference study

The influence of frequently encountered excipients such as starch, sucrose, glucose, gum accacia and magnesium stearate on the assays of the both drugs was investigated. No interference was observed even in case of oxidation of some of these excipients.

Conclusion

The proposed methods are simple, rapid and time saving for analysis of the studied drugs without interference from additives and excipients. The methods also could be successfully used for the routine analysis of the studied drugs in pure forms and in different formulations.

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