J Fluoresc (2010) 20:275–281
DOI 10.1007/s10895-009-0551-2

ORIGINAL PAPER

Spectrophotometric and Spectrofluorimetric Determination of Certain Diuretics Through Ternary Complex Formation with Eosin and Lead (II)

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Received: 6 July 2009 / Accepted: 28 September 2009 / Published online: 13 October 2009

Abstract Simple and sensitive spectrophotometric and spectrofluorimetric methods have been developed for the determination of hydrochlorothiazide (I), indapamide (II) and xipamide(III) based on ternary complex formation with eosin and lead (II) in the presence of methylcellulose as surfactant. The methods do not involve solvent extraction. For spectrophotometric method, the ternary complex showed an absorption maximum at 543 nm. The factors affecting the formation of ternary complex were studied and optimized. The method obeys Beer's law over concentration range of 8–40 μg mL^{-1}. A fluorescence quenching method for the determination of the cited drugs by forming this ternary complex was also investigated for the purpose of enhancing the sensitivity of the determination. The analytical performance of both methods was fully validated, and the results were satisfactory. The methods have been successfully applied for the determination of the studied drugs in their pharmaceutical tablets and the results obtained ware in good agreement with those obtained by the reference method. Common excipients used as additives in tablets do not interfere with the proposed methods.

Keywords Diuretics · Spectrophotometry · Spectrofluorimetry · Ternary complex · Eosin · Lead

Introduction

Thiazide and thiazide-like diuretics are extremely useful in the treatment of edema associated with heart failure, cirrhosis of the liver or nephritic syndrome. These diuretics are primary agents in the control of hypertension either alone or in combination with other drugs depending on its severity. Generally, thiazide and thiazide-like diuretics decrease blood pressure 10 to 15 mmHg within the first 3 to 4 days of continuous treatment [1]. Several methods have been published for determination of these drugs. These methods include spectrophotometry [2–12], fluorometry [7], chromatography [12–22], electrophoresis [23–29] and electrochemical methods [30–34]. Voltammetric, chromatographic and electrophoretic methods usually used expensive instruments that may not be available in some quality control laboratories. The ion pair complex formation technique between organic dyes and different organic pharmaceuticals is considered as one of the available methods for determination of many drugs. Suitable organic dyes such as bromothymol blue, bromophenol blue, bromocresol green, methyl orange, Tropaeolin O, eosin and zincon, are used [35].

These methods are associated with drawbacks such as lower sensitivity and decreased simplicity of the assay procedure; e.g. laborious extraction steps using halogenated solvents which are dangerous to health and environment. The addition of surfactant or water-miscible solvent to avoid any extraction procedure usually causes decomposition of the developed ion-pair complexes, thus lead to impossibility of the determination. The purpose of this work was to determine the cited drugs in bulk powder and in their pharmaceutical tablets without prior extraction by simple, rapid and selective assays for quality control and routine analysis purposes. This led us to study its reaction through ternary complex formation between the cited drug-metal ion [Pb (II) $(drug)_n$] as cation and an organic dye (eosin) as anion in presence of methyl cellulose in order to develop simple and sensitive spectrophotometric and spectrofluorimetric methods for the deter-

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mination of cited drugs in their bulk powder and in their pharmaceutical tablets.

The proposed methods are characterized by being more sensitive than the aforementioned methods and could be applied successfully to pharmaceutical tablets. In addition the reagents used are relatively cheap and are stable for at least 2–3 weeks.

Experimental

Apparatus

* Spectronic™genesys™ ultraviolet-visible spectrophotometer (Milton Roy Co, Westhaven, USA) with matched 1 cm quartz cells was used, connected to an IBM computer loaded with the Winspec™ application software.
* Jennway® 6505, Ultraviolet/Visible spectrophotometer(London, U.K.)
* Perkin-Elmer UK model LS 45 Luminescence spectrometer, equipped with a 150 Watt Xenon arc lamp, gratting excitation and emission monochromators for all measurements. Slit width for both monochromators were set at 10 nm. A 1 cm quartz cell was used, connected to an IBM PC computer loaded with the FL WINLAB™ software.
* Milwakee SM 101 digital pH meter, Portugal.

Materials

Pure samples

Samples of cited drugs were generously supplied by their respective manufacturers and were used without further purification:

1- Hydrochlorothiazide (Kahira Pharmaceutical Co., Cairo, Egypt)
2- Indapamide (Amriya Pharmaceutical Co., Alexandria, Egypt)
3- Xipamide (Egyptian Pharmaceutical Industries Co., [EIPICO] Tenth of Ramadan city, Egypt)

Pharmaceutical formulation

1- Hydretic® tablets (Chemipharm Pharmaceutical Industries, 6th October City, Egypt) labeled to contain 12.5 mg of hydrochlorothiazide per tablet
2- Diurex® tablets (Amriya Pharmaceutical Co., Alexandria, Egypt) labeled to contain 2.5 mg of indapamide per tablet
3- Epitens® tablets (Egyptian Pharmaceutical Industries Co., [EIPICO] Tenth of Ramadan city, Egypt) labeled to contain 10 mg of xipamide and 30 mg of triamterene per tablet

Reagents

All chemicals used were of analytical grade and are used without further purification. They are kept in the refrigerator all the time and warmed up to room temperature before use.

– Eosin G ($C_{20}H_6Br_4Na_2O_5$; 2′,4′,5′,7′-tetrabromo-3′,6′-dihydroxyspiro[isobenzofuran-1(3*H*),9′-[9H]xanthen]-3-one disodium salt; yellow soda; (S.d.Fine. Chem. Ltd, Boisar, India) 2×10^{-3} M, and 2×10^{-5} M aqueous solutions, were prepared for spectrophotometric and spectrofluorimetric methods, respectively. These solutions were found to be stable for about 2–3 weeks.
– Lead acetate (Merck, Darmstadt, Germany) 2×10^{-3} M, and 2×10^{-5} M aqueous solutions were prepared for spectrophotometric and spectrofluorimetric methods respectively. These solutions were found to be stable for about 2–3 weeks.
– Methylcellulose (1500 CPS, Aldrich) 0.5% *w/v* was prepared by dissolving the appropriate amount in hot water (80 °C) with stirring for 10 min, then chilling to 5 °C for 30 min.
– Buffer solution of pH 3 was prepared by mixing 0.2 M acetic acid and 0.2 M sodium acetate solution and its pH was checked periodically. The prepared buffer was used over a period of 2–3 weeks.
– Freshly distilled water was used.

Preparation of standard solutions

Stock solution containing 0.4 mg mL^{-1} of each drug was prepared by dissolving 20 mg of each drug in 5 mL methanol and the volume was completed to 50 mL with distilled water, and working standard solutions containing 80–400 µg mL^{-1} and 0.5–2.5 µg mL^{-1} for spectrophotometric and spectrofluorimetric methods, respectively were prepared by suitable dilution of the stock solution with distilled water.

Procedures

Spectrophotometric assay

Caliberation curves Into a series of 10 mL volumetric flasks, an aliquot volume of working standard solution of the studied drugs was transferred. 1.5 mL of 0.5% *w/v* methylcellulose solution, 2 mL of acetate buffer pH 3 followed by 1 mL of eosin solution and 0.7 mL of lead acetate solution were added. The content of the flask were

mixed well and heated up at about 70 °C for about 20 min. in a thermostatically controlled water-bath. The reaction mixture was cooled down to room temperature, about 25 °C, and completed to the mark with distilled water. The resultant absorbance was measured at 543 nm against reagent blank treated similarly. The measured absorbance vs., the final concentration was plotted to get the calibration curve. Alternatively the corresponding regression equation was derived.

Spectrofluorimetric assay

Calibration curves The same procedure described for spectrophotometric procedure was followed except that the working solution was diluted quantitatively to obtain 0.5 μg mL^{-1}–2.5 μg mL^{-1}. The difference of the relative fluorescence intensity was measured at 545 nm emission with excitation at 462 nm against reagent blank.

Procedure for tablets

Twenty tablets of each drug were powdered and quantity of the powder equivalent to 20 mg was dissolved by shaking with 5 mL methanol followed by 30 mL of water. The solution was filtered through filter paper into a 50 mL volumetric flask and then diluted to volume with water. Then the procedure was continued as described under calibration curve. The content of tablets was determined using regression equation derived from constructed calibration curve.

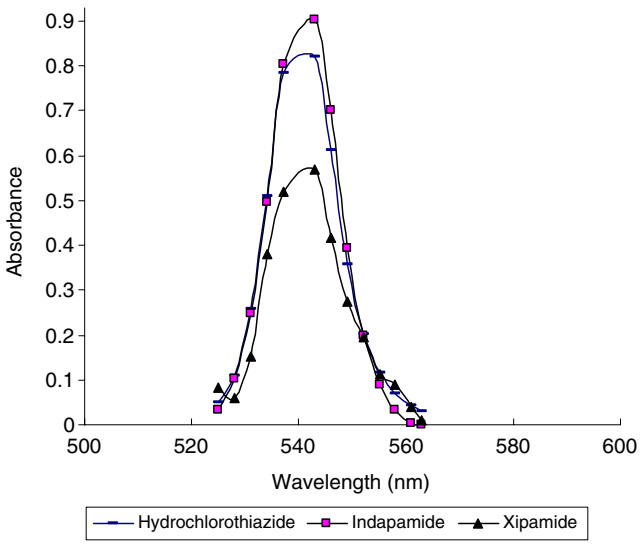

Fig. 1 Absorption spectra of ternary complex of indapamide (38 μg ml^{-1}), hydrochlorothiazide (40 μg ml^{-1}) and xipamide (20 μg ml^{-1})

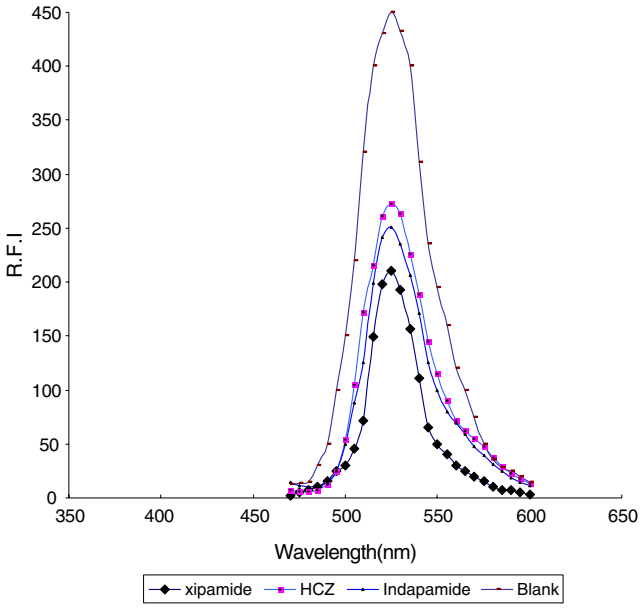

Fig. 2 Emission spectra of eosin–lead and quenching effect of ternary complex of the studied drugs (0.05 μg mL^{-1})

Results and discussion

The main purpose of this study was to establish simple spectrophotometric and spectrofluorometric methods for the determination of certain diuretics in their pharmaceutical formulations without prior extraction. Ternary complexes of general formula ($L_N M_X S_Y$) have been widely used in spectrophotometric and spectrofluorometric analysis [36–39]. For the ternary complexes dealt with in this paper is that the main ligand *L* is the cited drugs, the second ligand *S* is eosin and *M* is lead (II) metal, respectively.

The proposed methods are based on ternary complex formation of cited drugs with eosin and lead (II). The ternary complex formed between the metal ion: electronegative ligand and organic base often have higher values of molar extinction coefficient than binary complexes of the same components. The formation of ternary complexes improves not only the sensitivity of the method but also the selectivity as well.

In the present study, the cited drugs were found to form a ternary complex with each of eosin and lead (II) in the presence of methyl cellulose and at pH 3 producing a red color with maximum absorbance value at 543 nm.

i) Spectrophotometric assay

To optimize the assay variables, the effects of concentration of surfactant, pH, reaction time, eosin, lead (II) chloride and temperature on the absorbance of the ternary complex formed were studied. The effect of concentration of surfactant (methyl cellulose) on the absorbance of the solution of the ternary complex at 543 nm was studied.

Table 1 Analytical parameters for the analysis of the studied drugs using proposed methods

Drugs	Procedure	Linear range μgML−1	a	b	r	LOD μgmL−1	LOQ μgmL−1
Indapamide	1	8–40	0.136	0.830	0.9993	0.046	0.138
	2	0.05–0.25	161	396	0.9998	0.013	0.039
Hydrochlorothiazide	1	8–40	0.204	0.620	0.9995	0.041	0.123
	2	0.05–0.25	153	324	0.9997	0.014	0.044
Xipamide	1	8–32	0.0004	1.123	0.9990	0.035	0.107
	2	0.05–0.25	203	416	0.9998	0.012	0.039

1: Spectrophotometric method

2: Spectrofluorimetric method

Also addition of methyl cellulose was found to be necessary for enhance complex stability and sensitivity and prevention of precipitate formation. The effect of pH on the absorbance of the ternary complex was also studied. The absorbance of the drug–lead (II)– eosin complex solution was investigated over the pH ranges 2–4. The optimum absorbance was achieved at pH 3. The effect of concentration of the reagents, eosin and lead (II) chloride, on the absorbance of the ternary complex was studied. Maximum colour intensity was produced when the amount of the reagents mentioned in the construction of the calibration graphs have been used (Fig. 1). Higher concentration of reagents did not affect the colour intensity. In order to examine the effect of temperature and reaction time on the absorbance of the ternary complex, the above-mentioned procedure was carried out at different temperatures (room temperature, 50, 60, 70 and 80 °C) using thermostatic water bath. Maximum and constant absorbance was obtained at 70±5 °C after 20 min from the addition of the reaction contents, excessive heat decrease the absorbance sharply. The colour formed under the above-mentioned optimum conditions was stable for at least 1 h.

ii) Fluorometric assays

It was found that due to ternary complex formation reduction of fluorescence may occur. Thus, a fluorescence quenching method for the determination of (I), (II) and (III) was also studied. On the addition of the drug to the solution mixture, the relative fluorescence intensity decreased significantly compared with blank (Fig. 2), the magnitude of the decrease was proportional to the concentration of the drug.

Validation of the proposed methods [40, 41]

Linearity

Under the above experimental conditions, the calibration curves were constructed by plotting concentration versus absorbance or ΔRFI for spectrophotometric and spectrofluorimetric methods respectively. Linear calibration graphs were obtained by plotting the absorbance or relative fluorescence intensity of the studied drugs versus the drug

Table 2 Evaluation of accuracy of the analytical procedure of the studied drugs using spectrophotometric method

	Indapamide			Hydrochlorothiazide			Xipamide		
	Taken	Found	% Recovery	Taken	Found	% Recovery	Taken	Found	% Recovery
1	8	7.93	99.10	8	7.92	99.05	8	7.95	99.39
2	16	15.80	98.76	16	15.99	99.92	12	12.01	100.05
3	24	24.05	100.20	24	24.03	100.11	16	16.01	100.06
4	32	31.94	99.83	32	31.94	99.80	20	20.03	100.17
5	40	39.92	99.79	40	40.01	100.03	24	24.17	100.70
Mean			99.54			99.78			100.07
SD			0.588			0.425			0.466
RSD			0.591			0.426			0.466

SD Standard deviation, *RSD* Relative standard deviation

Average of three determinations

Table 3 Evaluation of accuracy of the analytical procedure of the studied drugs using spetrofluorimetric method

	Indapamide			Hydrochlorothiazide			Xipamide		
	Taken	Found	% Recovery	Taken	Found	% Recovery	Taken	Found	% Recovery
1	0.05	0.049	98.33	0.05	0.049	98.55	0.05	0.05	100.12
2	0.1	0.098	98.34	0.1	0.099	99.05	0.1	0.099	99.06
3	0.15	0.148	98.43	0.15	0.148	98.70	0.15	0.148	98.70
4	0.2	0.198	98.88	0.2	0.2	100.10	0.2	0.198	98.99
5	0.25	0.25	100.01	0.25	0.25	100.05	0.25	0.248	99.11
Mean			98.79			99.29			99.20
SD			0.714			0.739			0.540
RSD			0.723			0.744			0.544

SD Standard deviation, *RSD* Relative standard deviation

Average of three determinations

concentration within the specified range. Linearity was studied for both methods indicated by the values of correlation coefficient (r) (Table 1).

Sensitivity

The limit of detection (LOD) and the limit of quantitation (LOQ) for the proposed methods were calculated using the following equation

$$\mathrm{LOD} = 3.3\sigma/\mathrm{s} \qquad \mathrm{LOQ} = 10\sigma/\mathrm{s}$$

$\boldsymbol{\sigma}$ is the standard deviation of intercept, $\boldsymbol{s}$ is the slope of calibration curve.

LOQs and LODs for the studied drugs are listed in Table 1.

Specificity and interference

The specificity of the methods was investigated by observing any interference encountered from common excipients of the pharmaceutical formulation such as starch, magnesium stearate, and Talc. It was found that these compounds did not interfere with the results of the proposed methods. However triamterene formulated with xipamide in epitens® tablets can't interfere because it is practically insoluble in methanol [42].

Precision and accuracy

In order to determine the accuracy and the precision of the method, the accuracy was checked by three times analysis

Table 4 Evaluation of precision of the analytical procedure of the studied drugs using spetrophotometric method

Parameter		Indapamide			Hydrochlorothiazide			Xipamide		
		8	16	24	8	16	24	8	12	16
Intraday	1	99.7	98.1	99.0	99.38	99.82	99.11	99.5	100.2	98.5
	2	98.9	99.5	99.5	98.24	99.33	98.97	99.0	98.3	100.3
	3	100.4	99.3	99.7	99.07	100.86	98.16	100.4	99.2	99.9
	Mean	99.67	98.97	99.06	98.89	100.03	98.75	99.63	99.23	99.57
	SD	0.751	0.757	0.404	0.589	0.781	0.513	0.709	0.950	0.945
	RSD	0.753	0.765	0.408	0.595	0.781	0.519	0.712	0.957	0.949
Interday	1	99.6	99.7	98.7	99.6	100.3	99.7	100.3	99.1	99.2
	2	100.4	98.9	98.9	100.3	99.6	99.1	99.1	99.6	99.7
	3	99.3	100.4	100.9	99.8	98.9	100.2	99.8	100.4	98.8
	Mean	99.67	99.67	99.50	99.90	99.60	99.67	99.73	99.70	99.23
	SD	0.568	0.751	1.217	0.360	0.700	0.551	0.602	0.656	0.451
	RSD	0.569	0.753	1.223	0.361	0.702	0.553	0.604	0.657	0.454

SD Standard deviation, *RSD* Relative standard deviation

Average of three determinations

Table 5 Evaluation of precision of the analytical procedure of the studied drugs using spetrofluorimetric method

Parameter		Indapamide			Hydrochlorothiazide			Xipamide		
		0.05	0.1	0.15	0.05	0.1	0.15	0.05	0.1	0.15
Intraday	1	98.7	100.8	98.5	100.83	99.9	99.8	99.82	99.63	99.82
	2	98.9	99.1	100.3	99.73	99.2	99.54	99.33	99.21	99.33
	3	100.9	98.1	99.9	101.34	101.18	98.31	100.86	100.9	100.86
	Mean	99.50	99.33	99.57	100.63	100.09	99.22	100.03	99.91	100.03
	SD	1.217	1.365	0.945	0.822	1.004	0.795	0.781	0.879	0.781
	RSD	1.223	1.374	0.949	0.817	1.003	0.802	0.780	0.880	0.780
Interday	1	100.7	101.5	100.2	98.83	99.22	99.7	100.3	101.34	99.1
	2	99.4	99.7	98.3	101.34	101.3	98.9	99.6	100.44	99.6
	3	101.5	99.3	99.2	98.96	99.4	100.4	98.9	100.12	100.4
	Mean	100.53	100.17	99.23	99.71	99.97	99.67	99.60	100.63	99.70
	SD	1.060	1.172	0.950	1.413	1.152	0.751	0.700	0.633	0.656
	RSD	1.054	1.170	0.957	1.417	1.153	0.753	0.702	0.629	0.657

Average of three determinations SD: Standard deviation RSD: Relative standard deviation

for five different concentrations of pure samples. The results obtained in (Tables 2 and 3) showed the close agreement between the measured and true values indicating good accuracy of the proposed method. Intraday and interday precision were assessed using three concentration and three replicates of each concentration. The calculated relative standard deviation values were found to be very small below 2% indicating good repeatability and reliability of the proposed methods. The results and their statistical analysis were summarized in (Tables 4 and 5).

Robustness and ruggedness

For the evaluation of the method robustness, some parameters were interchanged such as eosin concentration, pH and concentration of surfactant. The capacity remains unaffected by small deliberate variations. Method ruggedness was expressed as R.S.D. % of the same procedure applied by two different instruments on different days. The results showed no statistical differences between different instruments (spectrophotometric method) suggesting that the developed methods were robust and spectrophotometric method was rugged.

Application to pharmaceutical tablets

The proposed methods have been successfully applied to the determination of the studied drugs in commercial tablets. The results obtained are shown in (Table 6). According to the *t*- and *F*-tests, no significant difference were found between the calculated and theoretical values of both the proposed and the reported methods [11] at 95% confidence level. This indicates good level of precision and accuracy.

Table 6 Determination of studied drugs in tablets using proposed methods

Drug	Pharmaceutical Product	Methods (% Recovery ± S.D) (n=5)		
		Reported[11] ± S.D.	1±S.D.	2±S.D
Indapamide	Diurex® tablets	99.40±1.15	99.39±1.23	99.99±1.54
			t=0.011	t=0.694
			F=1.14	F=1.81
Hydrochlorothiazide	Hydretic® tablets	99.64±0.57	99.70±0.68	99.23±1.25
			t=0.151	t=0.655
			F=1.40	F=4.74
Xipamide	Epitens® tablets	99.77±0.73	99.68±0.49	99.50±1.64
			t=0.245	t=0.346
			F=2.21	F=5.10

Each value is the mean of 5 determinations

The tabulated values at the 95% confidence limits are t=2.78 and F=6.39, respectively

1: Spectrophotometric method

2: Spectrofluorimetric method

Conclusion

The proposed methods are accurate, time saving, do not require prior extraction procedure and have the advantages of simplicity, sensitivity and reproducibility.

The proposed methods are sensitive enough to determine small amounts of these drugs, therefore can be used for quality control and routine determination of drugs in pharmaceutical tablets where precision, time and cost effectiveness of analytical methods are important.

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