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Simultaneous Estimation of Metolazone and Spironolactone in Combined Tablet Dosage Form BY UV Spectroscopy.

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Abstract: Three simple, rapid, precise and reproducible UV spectroscopic methods for simultaneous estimation of two component drug mixture of Metolazone(METO) and Spironolactone(SPIR) in combined dosage form have been developed. First method employs simultaneous equation method using 236.5 nm (λ max of METO) and 242.5 nm (λ max of SPIR) as two wavelengths for estimation. The second method involves absorbance correction method, the wavelength used were 242.5nm (λ max of SPIR) and 345nm (second λ max of METO it is zero for SPIR). The third method involves first derivative spectroscopy using 266 nm and 289 nm as zero crossing points for METO and SPIR respectively. For the entire three methods methanol followed by 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid was used. Linearity was observed in the concentration range of 0.5 - 2.5µg/ml for METO and 5-25µg/ml for SPIR. Accuracy and precision of the method was determined by performing intra day and inter day studies results found were satisfactory and statistical validation reveals that they can be applied to marketed samples. Method showed good reproducibility and recovery, this is evident from % RSD which is less than 2%.The methods were successfully applied for determining the amount in marketed formulation.
Key words: Metolazone, Spironolactone, simultaneous equation, first order, zero crossing, absorbance correction.

Introduction

Metolazone (METO) is an Antihypertensive and Diuretic agent chemically it is 7-chloro-1, 2, 3, 4-tetrahydro-2-methyl-4-oxo-3-o-tolyl-6-quinazoline sulfonamide[1-2]. Few HPLC and other methods have been reported for its estimation[3-4]. Spironolactone (SPIR) is a Diuretic drug (Aldosterone antagonist). Chemically it is 7α-acetyl thio-3-oxo-17α pregn-4-ene-21, 17 β-carbolactone[5]. Few estimations in body fluids, bulk in combination with other drugs and in single dosage forms have been reported[6-7]. Both these drugs are available in combined tablet dosage form, as a diuretic agent. The extensive literature survey revealed that numbers of methods are reported for the individual drugs but no method is so far reported for the simultaneous estimation of both the drugs in combined pharmaceutical dosage forms. So the present article discusses the attempts made to develop three simple, sensitive and reproducible methods for the simultaneous estimation of METO and SPIR in table formulation, using simultaneous equation, absorbance correction and first derivative[8].

Developed spectroscopic methods are for simultaneous estimation of METO and SPIR from combined tablet dosage form. Proposed methods are found to be simple, rapid, precise, accurate and reproducible. These methods can be applied successfully for quality

control testing of drugs from combined tablet dosage form, without prior separation.

Experimental

Materials and Methods

Standard bulk drug samples of METO and SPIR were provided by Centaur Pharmaceuticals Mumbai. Tablets of combined dosage form were procured from the local market (METOLACTONE-5). All other reagents used were of analytical grade. Shimadzu UV/ visible spectrophotometer, model 1700 and 1cm matched quartz cells was used. Spectra were recorded using program having following specifications, spectral bandwidth 1 nm, wavelength accuracy ± 0.5 nm, and wavelength readability in 0.1 nm increments.

Method I: Simultaneous Equation Method:

Accurately weighed drug samples of both METO and SPIR (50 mg each) were transferred to a suitable standard volumetric flask dissolved and diluted to mark with methanol. Both the drug solutions were diluted so as to get 10 mcg/ml by using 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid. These solutions were scanned in the UV region of 200-400 nm. From the overlain spectra of METO (10 µg/ml) and SPIR (10 µg/ml) in 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid (**Fig 1),** wavelengths 236.5 nm (λ max of METO) and 242.5 nm (λ max of SPIR) were selected for the formation of Simultaneous equation method. From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 0.5 – 2.5 µg/ml of METO and 5-25 µg/ml of SPIR. Absorbances of these solutions were recorded in the said wavelengths. Linearity was found to obey in the said range for both the drugs in both the wavelengths with correlation coefficient value not less than 0.999. Optical and regression characteristics are found out. E (1%, 1cm) determined for METO at 236.5 and 242.5 nm were 128.22 and 102.7 while respective values for SPIR are 402.06and 426.33.These values are the mean of six independent determinations.

The simultaneous equations formed were,

At λ_1 $A_1 = a\,x_1bc_x + ay_1bc_y$ ------ (1)

$A1 = 128.22\,C_X + 402.06\,C_Y$ ------ (2)

At λ_2 $A_2 = a\,x_2bc_x + ay_2bc_y$ ------- (3)

$A2 = 102.7\,C_X + 426.33C_Y$ ------ (4)

Where A_1 and A_2 are the absorbances of sample solution at 236.5 and 242.5 nm respectively. Cx and C_Y are the concentration of METO and SPIR respectively (µg/ml) in sample solution.

The absorbances of the sample solution ($A_{1\&}$ A_2) were recorded at 236.5 and 242.5nm respectively and concentration of both the drugs were calculated using above mentioned equation (2&4). Precision of the method was determined by carrying out Intra-Day (n = 3) and Inter Day (n = 3) studies.

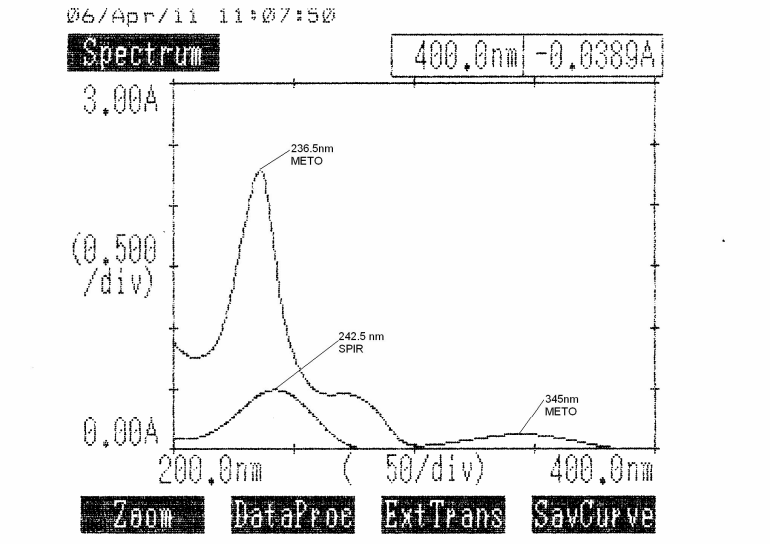

Fig 1. Overlain spectra of zero order spectrum of METO and SPIR

Method II : Absorbance Correction Method:

The standard stock solutions METO and SPIR were further diluted with 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid to get the concentration of 10 µg/ ml of each and the solutions were scanned between the range 200 - 400 nm in 1cm cell against 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid as blank and the overlain spectra was recorded. From the overlain spectrum of METO and SPIR in methanol, it was observed that SPIR have zero absorbance at 345 nm, where as METO has substantial absorbance. Thus METO was estimated directly at 345 nm without interference of SPIR. For estimation of SPIR, the absorbance of METO was measured at 242.5 nm using standard solution of METO (10 µg/ ml). The contribution of METO was deducted from the total absorbance of sample mixture at 242.5 nm. The calculated absorbance was called as corrected absorbance for SPIR. To estimate the amount of SPIR, the absorbance of METO were corrected for interference at 242.5 nm by using absorptivity values. A set of two equations were framed using absorptivity coefficients at selected wavelengths.

$$cx = \frac{A1}{ax1}$$

$$cy = \frac{A2 - ax2\ cx}{ay2}$$

Where,

A1 and A2 are absorbance of sample solution at 345 nm and 242.5 nm, respectively.

ax1 and ax2 absorptivity coefficients of METO at 345 nm and 242.5 nm, respectively.

ay1and ay2, absorptivity coefficients of SPIR at 345 nm and 242.5 nm, respectively.

From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 0.5 – 2.5 µg/ml of METO and 5-25 µg/ml of SPIR. Absorbances of these solutions were recorded in the said wavelengths.

Method III: Derivative Spectroscopy determination:

UV spectrum of both the drugs (METO and SPIR) were derivatised to first order with $\Delta\lambda = 1$ for the entire spectrum. Zero crossing points for METO and SPIR was found to be 266 and 289 nm respectively (**Fig 2).** From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 1 – 7 µg/ml of METO and 10-70 µg/ml of SPIR and the readings were taken in the first order mode at the selected wavelengths. Optical and regression data were calculated. Accuracy of the method was checked by preparing five mixed standards containing different concentration, absorbance was measured at respective zero crossing points in first order UV spectrum and amount present in the sample was calculated from their respective calibration curve. Precision of the method was determined by performing Intra Day (n = 3) and Inter Day (n = 3).

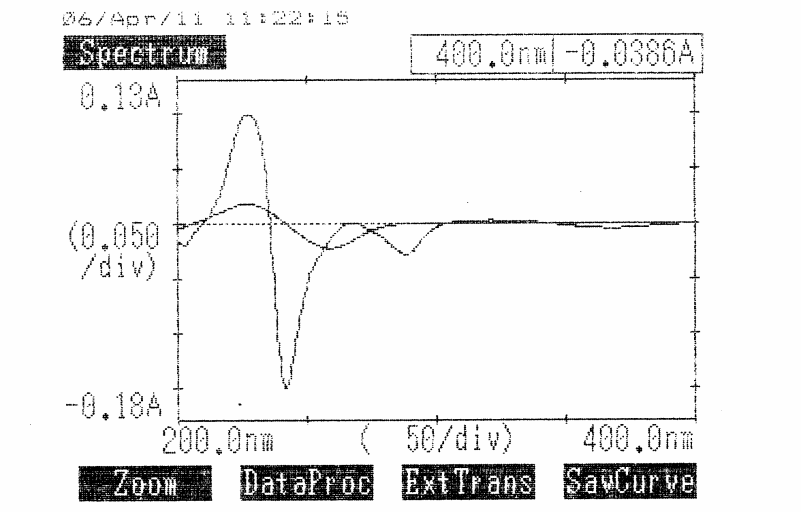

Fig 2. Overlain spectra of first order spectrum of METO and SPIR

Analysis of Commercial Formulations:

Twenty tablets were weighed and average weight per tablet was determined. Tablets were grounded to fine powder and accurately weighed the tablet powder equivalent to 75 mg of METO transferred to the flask, sufficient methanol was added sonicated for 5 min and diluted to the mark with 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid. It was filtered through Whatman Filter paper no: 41, filtrate was suitably diluted to get final concentration with 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid, so as to get the mid concentration of the linearity. Absorbances were measured at the said wavelengths,236.5 and 242.5 nm in Simultaneous Equation method, 266 & 289 nm in First order spectrum for derivative method and at 242.5 and 345 for absorbance correction method and amount present was calculated using simultaneous equation, first order derivative methods and absorbance correction method. Findings are tabulated in table 3.

Validation of methods

The methods were validated with respects to linearity, LOD (Limitof detection), LOQ (Limit of quantitation), precision and accuracy[9].

Recovery studies

To study accuracy, reproducibility and precision of the proposed methods, recovery studies were carried out by standard addition method. Results of recovery studies were found to be satisfactory and presented in Table 4. Precision of the method was determined by performing Intra Day (n = 3) and Inter Day (n = 3) refer the results in table 2.

Table: 1 Spectral and linearity characteristics data of both drugs in Zero Order Spectra.

Parameters	Simultaneous equation method				Asorbance correction method
	236.5nm		242.5 nm		345nm
	METO	SPIR	METO	SPIR	METO
Linearity range(µg/ml)	0.5 -2.5	5 -25	0.5 -2.5	5-25	0.5 -2.5
Correlation coefficient (r^2)	0.9998	0.9999	0.9995	0.9999	0.9999
Molar absorbitivity L mol^{-1} cm^{-1}	46448.87	16782.83	36809.60	17749.80	8093.600
Sandell's Sensitivity (µg/cm^2/0.001A.U)	0.007885	0.024829	0.0100613	0.023654	0.045643
Slope (m)	0.1268571	0.040395	0.1004990	0.0425457	0.022137
Intercept (c)	0.0010785	-0.001104	0.0011761	0.0006063	-0.0001380
LOD(µg/ml)	0.0489834	0.356395	0.076456	0.6907166	0.0530679
LOQ (µg/ml)	0.1484345	1.079985	0.231685	2.093654	0.1608119
Standard error	0.0003785	0.000692	0.0004739	0.0016894	3.82133E-05

Table: 1a Spectral and linearity characteristics data of both drugs in First Order Spectra

Parameters	First derivative method	
	289nm	266nm
	METO	SPIR
Linearity range(µg/ml)	1 -7	10 -70
Correlation coefficient (r^2)	0.9998	0.9999
Molar absorbitivity L mol^{-1} cm^{-1}	682.4295	350.1093
Sandell's Sensitivity (µg/cm^2/0.001A.U)	0.5370689	1.2193285
Slope (m)	0.0018646	0.0008202
Intercept (c)	6.94444E-06	0.0002013
LOD(µg/ml)	0.0276462	0.4184765
LOQ (µg/ml)	0.0837765	1.2681107
Standard error	7.0918E-05	0.00015477

Table: 2 Results of Intraday and Inter day studies

DRUG	Simultaneous equation method	Absorbance correction method
	Average % Found □ S.D,%RSD	Average % Found □ S.D,%RSD
METO	**INTRA DAY STUDIES (n = 3)**	
	99.866□ 0.050, 1.002	100.533 □ 0.045, 0.913
	100.466 □ 0.048, 0.964	100.333 □ 0.045, 0.914
	100.566□ 0.058, 1.169	100.033 □ 0.039, 0.793
SPIR	100.516 □ 0.102, 0.203	99.83 □ 0.099, 0.199
	100.396□ 0.104, 0.209	100.106 □ 0.096, 0.192
	100.426□ 0.117, 0.234	100.283 □ 0.105, 0.210
METO	**INTER DAY STUDIES (n = 3)**	
	100.366 □ 0.057, 1.151	100.166 □ 0.047, 0.956
	100.166 □ 0.051, 1.021	100.3 □ 0.048, 0.966
	100.333 □ 0.054, 1.089	100.1 □ 0.032, 0.641
SPIR	100.446 □ 0.115, 0.228	100.06 □ 0.094, 0.188
	100.54 □ 0.124, 0.248	99.766 □ 0.125, 0.251
	100.39 □ 0.115, 0.229	100.12 □ 0.085, 0.174

DRUG	First order derivative method
	Average % Found □ S.D,%RSD
METO	**INTRA DAY STUDIES (n = 3)**
	100.433□ 0.070, 1.399
	100.233 □ 0.063, 1.264
	100.433□ 0.070, 1.399
SPIR	99.786□ 0.155, 0.310
	100.013□ 0.155, 0.309
	99.836 □ 0.153, 0.307
METO	**INTER DAY STUDIES (n = 3)**
	99.666□ 0.064,1.298
	100.3 □ 0.067, 1.360
	100.2□ 0.063, 1.274
SPIR	100.093 □ 0.163, 0.326
	99.9461 □ 0.419, 0.840
	99.973 □ 0.155, 0.310

Table: 3 Results of Analysis of Formulation**

Methods	Drug	Label Claim	% Label claim found *	S.D *	% RSD *
Simultaneous equation method	METO	5 mg	100.366	± 0.057	1.151
	SPIR	50 mg	100.446	± 0.115	0.2289
Absorbance correction	METO	5 mg	100.166	± 0.047	0.9568
	SPIR	50 mg	100.06	± 0.094	0.1887
First Order Derivative	METO	5 mg	100.2	± 0.063	1.2749
	SPIR	50 mg	99.973	± 0.155	0.3100

*Average of six determinations

Table: 4 Results of Recovery Studies

Method	Drug	Amount in µg/ml		% RECOVERY	S.D*	% RSD*
		Added*	Recovered*			
Simultaneous Equation method	METO	0.8	0.7973	99.67	0.0015	0.1948
		1	0.9995	99.953	0.0026	0.2632
		1.2	1.2031	100.256	0.0063	0.5285
	SPIR	8	7.9531	99.413	0.0101	0.1281
		10	10.032	100.32	0.0174	0.1743
		12	12.1634	101.36	0.0157	0.1294
Absorbance correction	METO	0.8	0.7996	99.95	0.0068	0.8596
		1	1.0029	100.29	0.0068	0.6853
		1.2	1.2001	100.006	0.0025	0.2164
	SPIR	8	8.05	100.626	0.0231	0.28711
		10	10.0535	100.533	0.0207	0.2067
		12	12.0625	100.52	0.01	0.0832
First Order Derivative	METO	2.4	2.4221	100.923	0.0309	1.2776
		3	2.9942	99.806	0.0536	1.7901
		3.6	3.602	100.056	0.0309	0.8591
	SPIR	24	24.0371	100.15	0.0703	0.2927
		30	30.0921	100.306	0.1219	0.4052
		36	36.1065	100.293	0.0703	0.1949

*Average of three determinations

Results and Discussion

Three simple simultaneous estimation methods were successfully developed for the estimation of METO and SPIR in raw material and combined dosage form.

Linearity

Calibration curves were prepared for both the drugs at the selected analytical wavelengths are summarized in Table1 and 1a.This shows that METO obeys Beer's law in the concentration range of 0.5-2.5 µg/ml and SPIR obeys Beer's law in the concentration range of 5-25 µg/ml for simultaneous equation method and absorbance correction method. Whereas METO obeys Beer's law in the concentration range of 1-7 µg/ml and SPIR obeys Beer's law in the concentration range of 10-70 µg/ml for First order Derivative spectroscopy.

LOD and LOQ

LOD and LOQ were calculated, in accordance with ICH guidelines, as 3.3σ/S and 10σ/S, respectively, where σ is the standard deviation of the response(y-intercept) and S is the slope of the calibration plot.

Accuracy

The accuracy of the method was determined by investigating the recovery of METO and SPIR, three levels ranging from 80, 100 &120% of the nominal concentration by standard addition technique.

The results as shown in Table 4 indicate excellent recoveries.

Precision &repeatability

The precision and repeatability of the method was studied by repeating the proposed method three times in a day and the average percentage and RSD values were tabulated and when the experiment was repeated on three different days the average percentage RSD values for determination was tabulated in Table 2. The results confirm the intra day and inter day precision of the method.

All the three methods are suitable for the reliable analysis of commercial formulations containing combinations of METO and SPIR. The methods are simple, precise, rapid and accurate. High percentage recovery shows that the method is free from the interference of excipients used in the formulation.

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