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DEVELOPMENT AND VALIDATION OF RATIO DERIVATIVE SPECTROPHOTOMETRIC FOR SIMULTANEOUS ESTIMATION OF METOLAZONE AND SPIRONOLACTONE IN PHARMACEUTICAL DOSAGE FORM

Arati Chaudhary*, K.R. Vadalia and Punam Thummer

Department of Quality Assurance, Shree H. N. Shukla Institute of Pharmaceutical Education and Research, Rajkot-360002, Gujarat, India

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Correspondence to Author:

Arati M. Chaudhary

Ambaji Sheri , At-Dhinoj, Ta-chansma, Dist-Patan(Pin code-384225), Gujarat, India

E-mail: artichaudhary1912@gmail.com

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ABSTRACT

In the present work for the simultaneous estimation of Metolazone (METO) and Spironolactone (SPI) Ratio Derivative Spectrophotometric method have been developed. In this method the overlapping spectra of Metolazone and Spironolactone were well resolved by making use of the first-derivative of the ratios of their direct absorption spectra. Methanol:water (1:4) was used as solvent for the method. Amplitude at 241.0nm and 263.0 nm were selected in the ratio derivative spectra to determine METO and SPI, respectively. Beer's law was obeyed in the concentration ranges of 1-5µg/mL and 5-25 µg/ mL METO and SPI, respectively in the method. The % assay for commercial formulation was found to be in the range 98.00% – 102.00 % for METO and 99.00% – 102.60 % for SPI by the proposed method. Recovery was found in the range of 99.74 – 100.76 % for METO and SPI in the Formulations. The results of analysis have been validated statistically and recovery studies confirmed the accuracy and reproducibility of the proposed methods which were carried out by following ICH guidelines.

INTRODUCTION: Metolazone is an oral diuretic agent chemically it is 7-chloro-1, 2, 3, 4-tetrahydro-2-methyl-4-oxo-3-o-tolyl-6-quinazoline sulfonamide (**figure 1**).

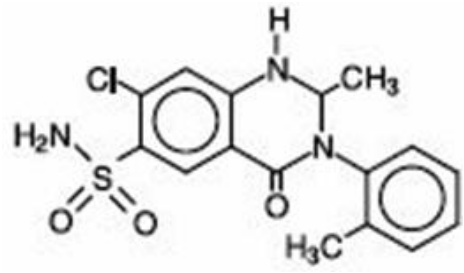
It is primarily used to treat congestive heart failure and high blood pressure. A quinazoline-sulfonamide is considered a thiazide-like diuretic which is long-acting so useful in chronic renal failure. Spironolactone is a potassium sparing diuretic agent Chemically it is 7α-acetyl thio-3-oxo-17α pregn-4-ene-21, 17 β-carbo-lactone. It is used mainly in the treatment of refractory edema in patients with congestive heart failure, nephrotic syndrome, or hepatic cirrhosis [1-5].

Literature survey revealed that Spectrophotometric and HPLC methods [6-12] are available for estimation of METO and SPI individually and in combination with other diuretics in different formulation.

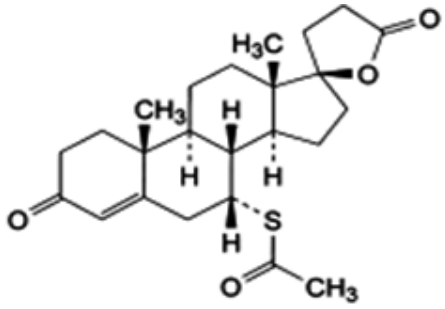
The combination of the both drugs is not official in any pharmacopoeia; hence, no official method is reported for simultaneous estimation of TOR and SPI in formulations.

Aim of present work was to develop simple, economical, rapid, accurate and precise Ratio Derivative spectrophotometric methods for determination of these drugs in fixed dose combination.

The proposed methods were optimized and validated as per the International Conference on Harmonization (ICH) guidelines [13].



(A)



(B)

FIGURE 1: CHEMICAL STRUCTURES OF (A) METOLAZONE AND (B) SPIRONOLACTONE

MATERIALS AND METHODS:

Apparatus: All spectral measurements were made on Shimadzu 1800 UV-Visible spectrophotometer with 1cm matched quartz cells were used. All instruments and glass wares of class-A were calibrated.

Reagents and Materials: Pure drug of Metolazone was obtained as gift sample from Centaur pharmaceutical Pvt Ltd, Mumbai and Pure drug of Spironolactone was obtained as gift sample from RPG Life Science Ltd, Mumbai and commercial formulations were procured from local market. All the chemicals used were of analytical grade.

Preparation of Standard Stock solution: Standard stock solution (1000 µg/mL) of METO and SPI were prepared by dissolving 50 mg of drug each in 50ml methanol. The working standard solutions of these drugs were obtained by dilution of the respective stock solution with Methanol:water to obtain 100µg/ml of each drug. Further, mixed working range dilutions (1-5 µg/mL for Metolazone and 5-25 µg/mL for Spironolactone) were also prepared with Methanol: water. All measurements were made at room temperature. The quality control samples were prepared in the range of the calibration curve at different concentration levels in triplicates.

The absorbances of these solutions were then put in the calibration curve to calculate the accuracy and precision of the method.

Method Development: The method involves dividing the spectrum mixture by the standardized spectra of each of the analyte to get ratio spectra and first derivative of ratio spectrum was obtained, which was independent of concentration of divisor. The derivative spectra of these ratio spectra were recorded by using digital differentiation (Convolution method) with a derivative wavelength difference (Δλ = 4) and scaling factor is 10 for the range of 400-200 nm. The concentration of active compounds are then determined from calibration graph obtained by measuring amplitude at points corresponding to minima or maxima. Using appropriate dilutions of standard stock solution, the two solutions were scanned separately.

The ratio derivative spectra of different METO standards at increasing concentrations were obtained by dividing METO+SPI scans with the stored spectrum of the standard solution of SPI (10µg/ mL) (**Figure 3**). Wavelength 241.0 nm was selected for the quantification of METO in METO+SPI mixture. The ratio and ratio derivative spectra of the solutions of spi at different concentrations were obtained by dividing METO+SPI scans with the stored standard spectrum of the METO (3 µg/ mL) (**Figure 3b**). Wavelength 263 nm was selected for the quantification of SPI in METO+SPI mixture. Measured analytical signals at the selected wavelengths were proportional to the concentrations of the drugs. Calibration curves were prepared from the measured signals at the selected wavelength and concentration of the standard solutions.

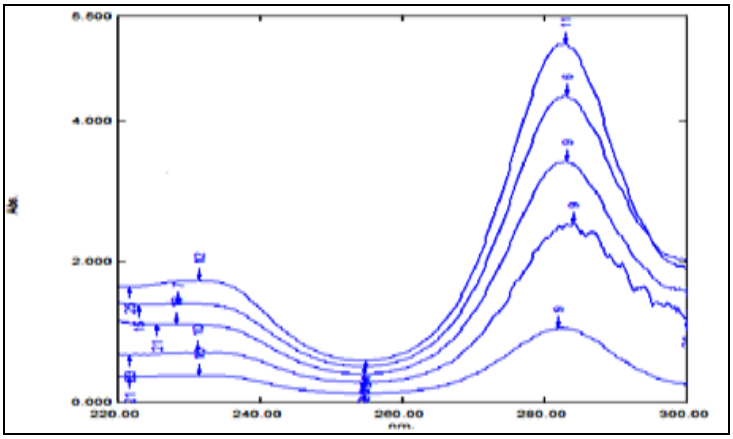


FIGURE 2: OVERLAIN ZERO ORDER RATIO SPECTRA OF STANDARD METO (SPI 10 µg/ml used as divisor)

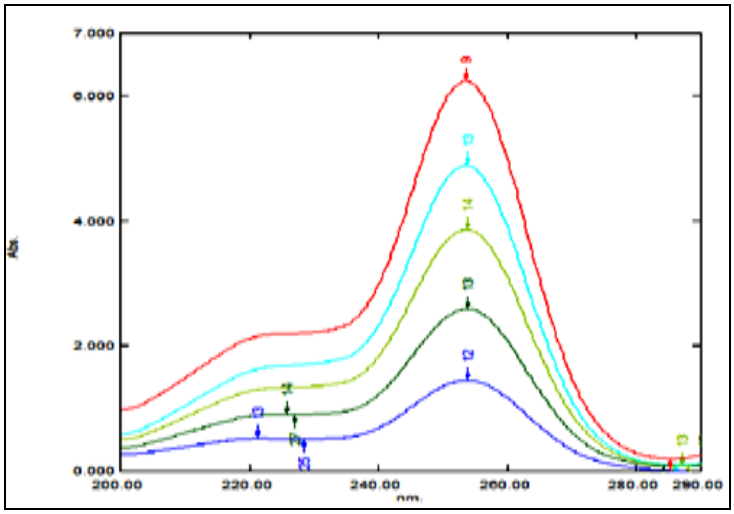


FIGURE 3: OVERLAIN ZERO ORDER RATIO SPECTRA OF STANDARD SPI
(METO 3µg/ml used as divisor)

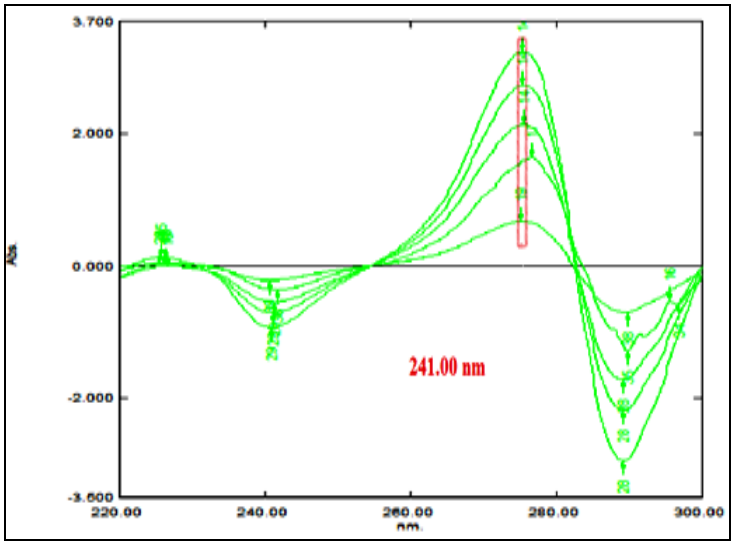


FIGURE 4: OVERLAIN 1ST DERIVATIVE OF RATIO SPECTRA OF STANDARD METO
(SPI 10µg/ml used as divisor)

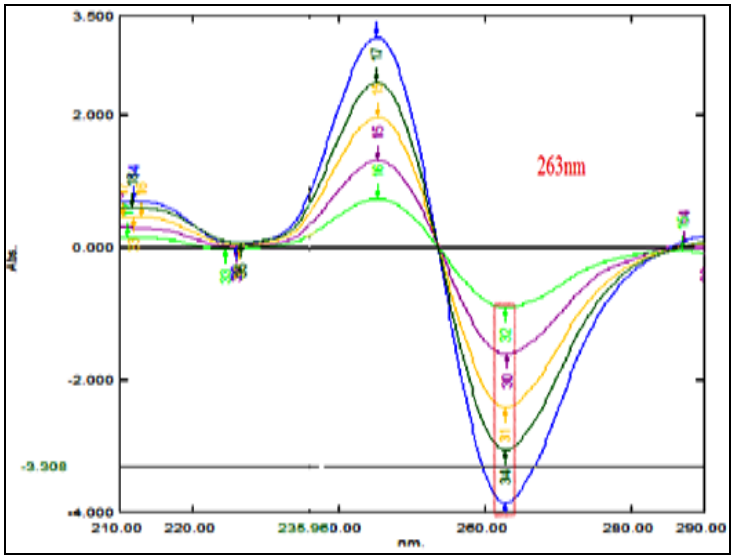


FIGURE 5: OVERLAIN 1ST DERIVATIVE OF RATIO SPECTRA OF STANDARD SPI
(METO 3µg/ml used as divisor)

Method Validation:

Linearity: The methods were validated according to international conference on harmonization Q2B guidelines for validation of analytical procedures in order to determine the linearity ,sensitivity ,precision and accuracy for each analyte [14, 15].

Calibration curves were generated with appropriate volumes of working standard solutions for Ratio derivative Spectrophotometric method. In case of Spectrophotometric method the range was optimised at 1-5µg/mL for Metolazone and 5-25µg/mL for Spironolactone. The linearity was evaluated by the regression analysis. The corresponding regression equation was computed and found to be for Metolazone and Spironolactone:

Metolazone:

$y = -0.1389x - 0.0135$; $r^2 = 0.9947$

Spironolactone:

$y = -0.1384x - 0.1360$; $r^2 = 0.9990$

Where x is the concentration, y is the peak amplitude of the ratio first derivative

Curve and r^2 is the correlation coefficient

Precision and Accuracy: Both precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicates at different concentration levels covering the entire linearity range. Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the assay was determined by intraday and inerday and reported as %R.S.D. The interday precision was determined as standard deviation and %R.S.D. Accuracy is the percent of analyte recovered by assay from a known added amount. The intraday of the method was determined by assaying minimum nine replicates of sample solutions by taking three test concentrations in the calibration range for both the methods.

Analysis of Commercial Formulations: Twenty tablets were powdered.Powder equivalent to 2.5 mg of METO and 50 mg of SPI was weighed and transferred into a 100 mL of volumetric flask ,volume adjusted up to

mark with Methanol. The mixture sonicated for 10 minutes and filtered through Whatman filter paper no. 42, discarding first few mL of filtrate. 1 mL of this filtratediluted to 10 mL with Methanol: water.

Ratio spectra of the resulting solution were obtained by dividing the spectrum with METO (3 µg/mL) and SPI (10 µg/mL). The first derivative of these spectra traced at the interval of Δλ = 4 nm at selected wavelengths for determination of METO and SPI. The concentration of each drug was calculated using calibration curve equation. Absorption at selected wavelength substituted into regression equation for content of tablet and % recovery of standard added calculated.

RESULTS AND DISCUSSION: Under experimental condition described, calibration curve, assay of tablets, precision and recovery studies were performed. The drugs obey beer's law in the concentration range of 1-5 µg/mL for METO and 5-25 µg/mL for SPI with good correlation coefficient > 0.9950.

Results of precision studies are presented in **Table 1 and Table 2**. Results of recovery studies are shown in **Table 3**. The accuracy is reproducibility is evident from the data as results are close to 100 % and low standard deviation. The results of analysis of tablet formulation are presented in **Table 4**.

Ratio derivative spectrophotometric method is simple, economical, rapid, precise, and accurate. Hence these can be used for routine analysis of METO and SPI in tablet formulation.

TABLE 1: INTRADAY STUDIES

Sr. No.	Assay level	Tablet content taken equivalent to (mg)		% Estimated	
		METO	SPI	METO	SPI
1	80%	2	40	97.78	98.45
2	100%	2.5	50	99.89	99.80
7	120%	3	60	100.68	101.11
	Average			99.45	99.78
	SD			1.629	1.593
	% RSD			1.626	1.589

TABLE 2: INTERDAY PRECISION STUDIES

Sr. No.	Assay level	Tablet content taken equivalent to (mg)		% estimated	
		METO	SPI	METO	SPI
1	80%	2	40	98.72	98.08
2	100%	2.5	50	100.40	100.55
3	120%	3	60	101.72	99.85
	Average			100.28	99.49
	SD			1.655	1.756
	% RSD			1.652	1.752

TABLE 3: RECOVERY STUDIES

Sr. No	Assay level (n=3)	Tablet content equivalent to (mg)		Standard added (mg)		Total drug recovered (mg)		% Recovery of standard added	
		METO	SPI	METO	SPI	METO	SPI	METO	SPI
1	0	5	20	0	0	4.98	20.10	-	-
2	80	5	20	2	40	6.95	59.82	98.67	99.32
3	100	5	20	2.5	50	7.52	70.26	101.60	99.96
4	120	5	20	3	60	8.04	80.05	102.00	99.91

TABLE 4: ANALYSIS OF MARKETED FORMULATION

Tablet	Label claim mg/tablet		Assay (% of Estimated value)	
	METO	SPI	METO	SPI
METOLACTONE- tab	2.5	50	99.60	100.51

CONCLUSION: Unlike the HPLC method, our study shows that the UV spectrophotometric techniques enable the use of not only simple sample preparation but also affordable instrument and cheap solvent readily available in the laboratory. The developed ratio spectra first-order derivative has proved to be suitable for the simultaneous determination of Metolazone and Spironolactone commercial tablets. These techniques are free from interference by common excipients as confirmed by their recovery study. In conclusion, they are quite reproducible, simple and accurate which can be used for the routine quality control of pharmaceutical formulations.

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