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FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF HYDROCHLOROTHIAZIDE AND FIMSARTAN IN SYNTHETIC MIXTURE

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ABSTRACT
A simple, economical, accurate, precise and less time-consuming First order derivative UV Spectrophotometric method has been developed and validated for simultaneous estimation of Hydrochlorothiazide and Fimasartan in synthetic mixture. In this method Hydrochlorothiazide and Fimasartan are exhibits maximum absorbance (λ max) at 263 nm and 246 nm with methanol as the solvent. The method was validated as per the International Conference on Harmonization (ICHQ2R1) guidelines. Drugs followed the linearity in the concentration range of 6.25-31.25 µg/mL and 30-150 µg/mL with correlation coefficient (r2) of 0.9988 and 0.9988 for Hydrochlorothiazide and Fimasartan respectively. The validity of the proposed method was assessed by applying the standard addition technique where the percentage recovery of the added standard was found to be 98.93 and 100.05 for Hydrochlorothiazide and Fimasartan. The limit of detection and quantification were calculated and found to be 0.1045 µg/mL and 0.3166 µg/mL and 0.2419 µg/mL and 7.98 µg/mL for Hydrochlorothiazide and Fimasartan respectively. The proposed method is recommended for routine analysis of Hydrochlorothiazide and Fimasartan in synthetic mixture in regular quality control testing laboratories.

KEYWORDS: Hydrochlorothiazide; Fimasartan; UV; Validation.

INTRODUCTION
High blood pressure is a coarse condition in the world which increases the risk of stroke and heart disease. Blood forcing against the walls of arteries in the body creates pressure, which generally varies throughout the day. High blood pressure, also called as **hypertension**, is blood pressure that is constantly higher than what is considered normal There are two types of blood pressure measures: **systolic** and **diastolic**. Hypertension is main risk factor for disability and death globally. This is assigned to two main complications of hypertension, cerebrovascular accidents (CVA) and ischemic heart disease.[1-3]

Fimasartan which is 2- (2-butyl-4-methyl-6-oxo-1-{[2′-(1H-1,2,3,4tetrazol-5-yl)-[1,1′-biphenyl] – 4 - yl] methyl } - 1, 6 – dihydropyrimidin – 5 – yl) – N, N dimethylethanethioamide (Figure 1).[4] Fimasartan manufactured by a korean company Boryung Pharmaceutical as an oral antihypertensive drug. In blocking the AT1 receptor, fimasartan gives vasodilation. At the kidney and adrenal gland, excretion of water and salt by the kidneys, which lowers overall blood volume.[5] Accordingly, fimasartain helps to lower blood pressure and reduces hypertensive indications.[6] Fimasartan is a biochemical derivative of losartan in which the imidazole ring has been substitueted. This gives more potency and longer duration of action to this molecule. Fimasartan is selective AT1 receptor antagonist.[7]

Hydrochlorothiazide is a benzothiadiazine that is 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide replaced by a chloro group at 6 and a sulfonamide at 7 (Figure 2). Hydrochlorothiazide is approved by the U.S.FDA to treat hypertension and peripheral edema. Hydrochlorothiazide is a thiazide-type diuretic that blocks sodium resorption in the DCT of the kidney. Hydrochlorothiazide blocks the sodium-potassium ATPase pump's activity by blocking sodium from crossing the tubular lumen. This blocks the movement of sodium and water into the interstitial space. Raised sodium concentration in the collecting duct prompts aldosterone to bind to the mineralocorticoid receptor, starting the transcription of

ion transport protein channels, resulting in natriuresis and diuresis effects. The initial diuresis and natriuresis effect interfered by the kidney causes an initial decrease in blood pressure through volume loss. Additionally, hydrochlorothiazide has displayed the ability to maintain blood pressure reduction.[8-10]

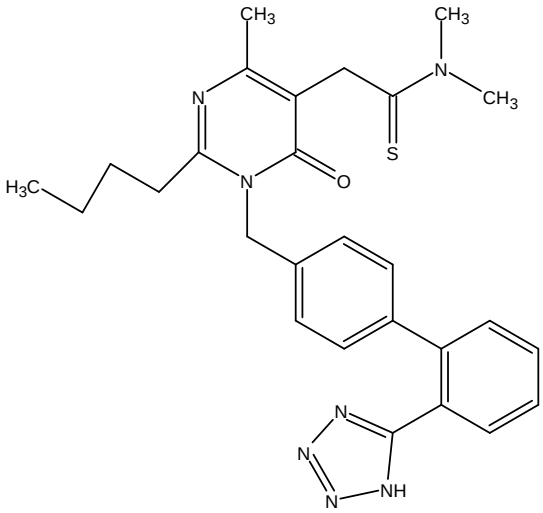

Figure 1: Structure of Fimasartan.

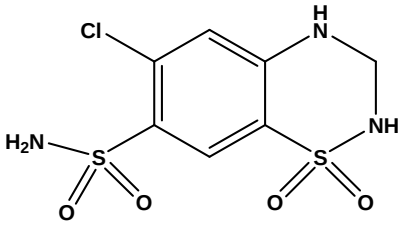

Figure 2: Structure of Hydrochlorothiazide.

The combination treatment of fimasartan and Hydrochlorothiazide achieved better BP control than fimasartan monotherapy, and had comparable safety and tolerance to fimasartan monotherapy.[11]

Literature survey reveals that Hydrochlorothiazide can be estimated by spectrophotometric, Reverse Phase High- Performance Liquid Chromatography (RP-HPLC) and High Performance Thin Layer Chromatography (HPTLC) methods either as a single or in combination with other drugs in pharmaceutical preparations. Analytical methods reported for Fimasartan includes spectrophotometric HPLC and HPTLC either as a single drug or in combination with other drugs. Literature survey reveals that not a single UV method of analysis has yet been reported for simultaneous analysis of Hydrochlorothiazide and Fimasartan. The objective of the present investigations was to develop a rapid, accurate, economical and validated First order derivative Ultra-Violet spectrophotometric (UV) method for the simultaneous estimation so that can play important role in quantification of HCTZ and FIM in synthetic mixture.[12-23]

MATERİALS AND METHODS

INSTRUMENTATION

The instrument used in the present study was UV-Visible spectrophotometer (Lab India-UV 3000+) with spectral band width of 1 nm. All weighing was done on electronic balance (Model Shimadzu AUX -220).

CHEMICALS AND MATERIALS

Pharmaceutical grade of Hydrochlorothiazide (HCTZ) and Fimasartan (FIM) were kindly supplied as a gratis sample by Montage Laboratories Pvt Ltd and Mackur Laboratories. In the present study the UV spectra of ROS and FIM were obtained from different solutions (Methanol, Acetonitrile, Distilled water) were studied. The two drugs were freely soluble in Methanol. At the end of these studies, Methanol was chosen as solvent for studied drugs.

STANDARD STOCK SOLUTIONS

Standard stock solutions of HCTZ (125 µg/ml), FIM (600 µg/ml) were prepared in methanol. For the selection of analytical wavelength solutions of HCTZ (12.5µg/ml), FIM (60µg/ml) were prepared separately by appropriate dilution of standard stock solution with methanol and scanned in the spectrum mode from 200 to 400 nm.

DERIVATIVE SPECTROSCOPY METHOD

Standard solutions of both drugs (12.5 µg/ml and 60 µg/ml) were scanned separately in the range of 200- 400 nm. These spectrums were converted to first order derivative spectra by using derivative mode. For this method, 291 nm and 275 nm were selected as wavelengths of measurements for HCTZ and FIM respectively. There was proportionate increase in amplitude at 263 nm and 246 nm HCTZ and FIM respectively.

LINEARITY AND RANGE

All solutions were scanned between 200-400 nm and they were derivatized to first order derivative spectra.

REPEATABILITY

For adjudging repeatability of method all prepared mixtures were analyzed under optimized conditions for five times and each concentration was monitored for repeatability by RSD.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

LOD and LOQ were determined by statistical method by utilization of repeatability data.

$$LOD = 3.3\ X\ (\frac{\sigma}{S})$$

$$LOQ = 10\ X\ (\frac{\sigma}{S})$$

Where, σ = Standard deviation of intercept
S = mean of slope

INTRADAY AND INTER-DAY PRECISION

Method precision was determined by performing intraday and interday precision.

Mixture that represents overall range (FIMA+HCTZ = 30+6.25, 90+18.75 and 150+31.25) were analyzed on same day at different time interval for intraday precision. (n=3 determinations).

Mixture that represents overall range (FIMA+HCTZ = 30+6.25, 90+18.75 and 150+31.25) were analyzed on different days for interday precision. (n=3 determinations).

ACCURACY

Accuracy was performed by spiking of placebo with standard. Target concentration was 60 µg/mL and 12.5 µg/mL for FIMA and HCTZ respectively.

ASSAY PREPARATION

Sample Stock Solution

Weight about sample (equivalent to 60mg of FIM/12.5mg of HCTZ) into a 100ml volumetric flask. Add 100ml methanol, 5mg Barium sulphate and put this volumetric on water bath at 60°C for 15 minutes then allow to cool at room temperature. Shake for 15 minutes. Make up volume with methanol up to 100ml. Filter this solution with whatman filter paper no-1. (HCTZ-125mcg/ml, FIM-600mcg/ml).

Working Sample Preparation

Take 1ml from sample stock solution into a 10ml volumetric flask and make up with mobile phase. (HCTZ-12.5mcg/ml and FIM-60mcg/ml).

Statistical analysis

All of the samples were analyzed in six replicates, and the RSD values were computed for all of the samples. Ethics committee approval or patient informed consent is not required for the proposed method.

RESULTS AND DISCUSSION

Derivative Spectroscopy method for determination of FIMA and HCTZ

Derivative spectroscopy method was decided to use for quantitative estimation of FIMA and HCTZ from synthetic mixture. In this method D0 spectra of both the drugs are transformed to D1 spectra.

The wavelength at which there is maximum absorbance in D0 spectra shows zero absorbance in D1 spectra and this wavelength is known as Zero Crossover Point (ZCP). Estimation of one drug is carried out at ZCP of another drug (Figure 3 & 4).

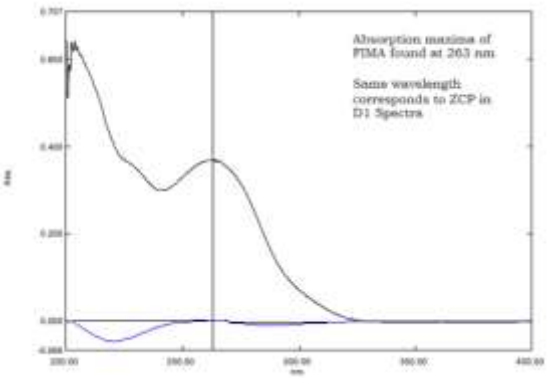

Figure 3: Confirmation of D1 spectra of FIMA (60 µg/mL).

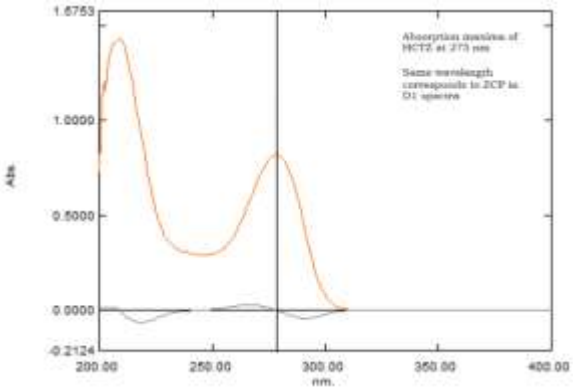

Figure 4: Confirmation of D1 spectra of HCTZ (12.5 µg/mL).

Analytical method development

The method objective was defined based on the assessment of the literature and profile of the analyte. Motivation for selecting First order derivative UV visible spectrophotometric approach was due to its simplicity and speed of analysis compared to more advanced analytical methods. During the analysis of variables such as detecting wavelength, sampling interval, scanning speed, sample integrity, and solvent variation was investigated.

Analytical method validation

A UV-visible spectrophotometer (Shimadzu, UV-6000) operated with spectral bandwidth of 1 nm was used for analytical method development and validation. Validation parameters including precision, linearity and range, accuracy, repeatability, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness were evaluated as per ICH guidelines Q2 (R1).

Selection of Analytical Wavelength

Three different ZCP at 222 nm, 247 nm, 263 nm and 291 nm were observed in overlain D1 spectra of FIMA. It means quantitative determination of HCTZ can be carried out at any of the above wavelength.

Three different ZCP at 245 nm, 275 nm and 316 nm were observed in overlain D1 spectra of HCTZ. It means quantitative determination of FIMA can be carried out at any of the above wavelength.

Therefore, 275 nm and 291 nm were selected as analytical wavelength for quantitative determination of FIMA and HCTZ respectively (Figure 3).

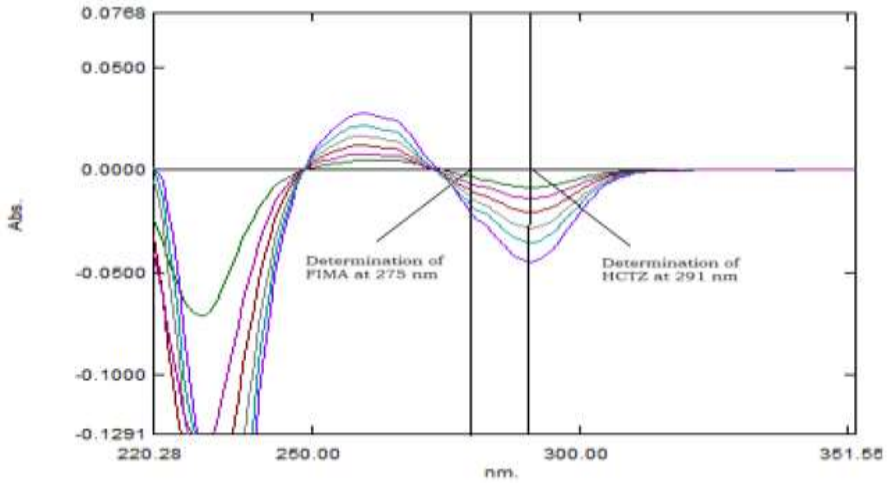

Figure 5. Overlain D1 spectra of mixture of FIMA (30 - 150 µg/mL) and HCTZ (6.25 – 31.25 µg/mL)

Linearity and Range

The linearity study was carried out for both drugs at different concentration levels. The linearity of ROS and HCTZ was in the range of 6.25- 31.25 µg/ml for FIM and 30-150 µg/ml. % RSD of all results were less than 2% (Figure 4 & 5) (Table 1).

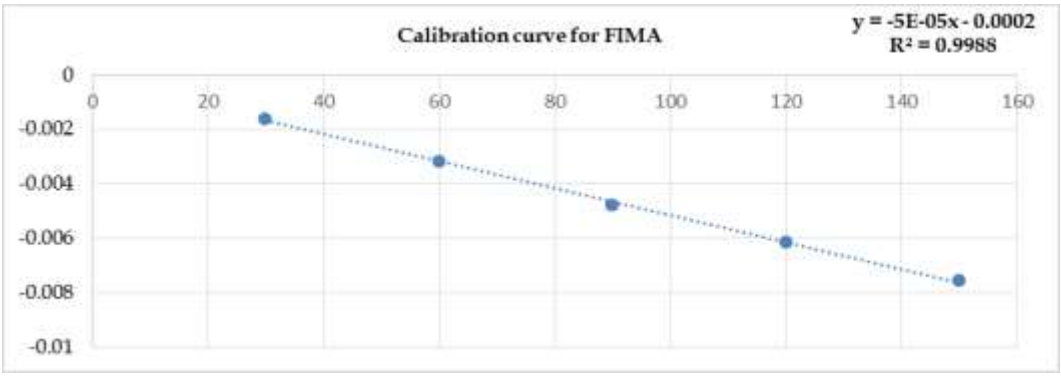

Figure 6: Calibration curve of FIM in UV.

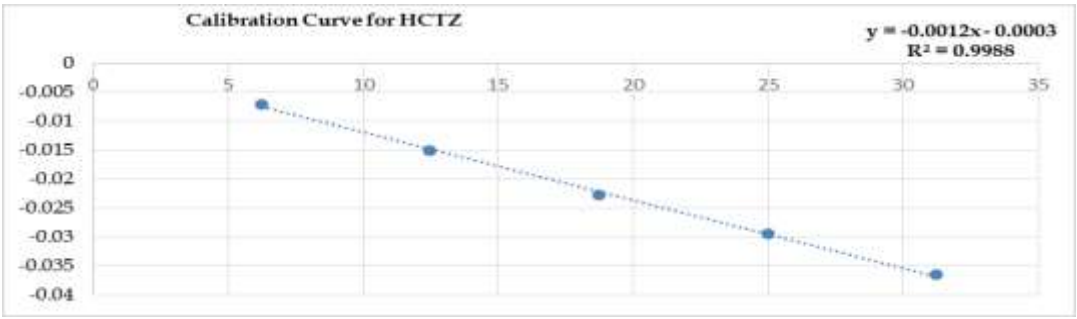

Figure 7: Calibration curve of HCTZ in UV.

Table 1: Linearity data for HCTZ and FIM in UV.

Conc(mcg/ml)		FIM		HCTZ	
FIM	**HCTZ**	**Abs**	**%RSD**	**Abs**	**%RSD**
30	6.25	-0.001598	-0.81	-0.00716	-1.59
60	12.5	-0.003174	-1.32	-0.01516	-0.75
90	18.75	-0.00478	-0.94	-0.02276	-0.50
120	25	-0.00616	-1.85	-0.02944	-0.38
150	31.25	-0.00756	-1.50	-0.03658	-0.44
correlation coefficient		0.9988		0.9988	

LOD and LOQ in UV

The LOD and LOQ of the proposed method were determined to be 0.1045 µg/ml and 0.3166 µg/ml for HCTZ and 0.2419 µg/ml and 7.98 µg/ml for FIM, respectively (Table 2).

Table 2: Result of LOD AND LOQ in UV.

PARAMETERS	FIMA	HCTZ
LOD	0.2419 µg/ml	0.1045 µg/ml
LOQ	7.98 µg/ml	0.3166 µg/ml

Accuracy

The results for accuracy at different levels are listed in Table 3. The percent recovery of HCTZ and FIM was determined to be close to 100%, and the RSD values are less than 2%, which fulfills the acceptance criteria for accuracy as indicated in the ICH guidelines. Hence, the method developed can be considered reliable for analytical application.

Table 3: Accuracy study of UV method.

Drugs	Amount of drugs (µg/ml)	% Of std added	Total amount added	Amount found (µg/ml)	% Recovery (Mean ± SD)	% RSD
HCTZ	12.5 (n=3)	50%	6.25	6.18	98.93 ±0.49	0.49
		100%	12.5	12.49	99.92 ± 0.45	0.45
		150%	18.75	18.76	100.04 ± 0.08	0.07
FIM	60 (n=3)	50%	30	29.59	98.64 ± 0.66	0.66
		100%	60	59.79	99.65 ± 1.33	1.33
		150%	90	88.66	98.51 ± 0.31	0.31

Precision

The results of intraday and interday precision using 6.25, 18.75, and 31.25 µg/mL concentrations of HCTZ and 30, 60 and 90 µg/mL concentrations of FIM are listed in Table 4.

Table 4: Intraday precision of HCTZ and FIM in UV.

	HCTZ			FIM		
Precision	Conc (µg/ml)	(Mean ± SD) (n=3)	% RSD	Conc (µg/ml)	(Mean ± SD) (n=3)	% RSD
Intraday	6.25	-0.007192 + 0.000013	0.18	30	-0.00162 + 0.000019	1.19
	18.75	-0.0294 + 0.00015	0.53	90	-0.0062 + 0.000042	0.67
	31.25	-0.04362 + 0.00025	0.59	150	-0.00932 + 0.000113	1.20
Interday	6.25	-0.007228 + 0.000019	0.26	30	-0.001736 + 0.000021	1.19
	18.75	-0.02942 + 0.00019	0.65	90	-0.0066 + 0.000026	0.38
	31.25	-0.0446 + 0.00035	0.79	150	-0.00966 + 0.000036	0.36

Repeatability (Table 5)

Table 5: Repeatability study of UV method.

HCTZ		FIM	
Mean ± SD (n=6)	%RSD	Mean ± SD (n=6)	%RSD
-0.01516± 0.000101	0.75	-0.003174± 0.00004	1.32

Analysis of Physical mixture

The calculated assay (%) values were in the acceptable range of 95.00% to 105.00% with % RSD lower than 2 listed in Table 6.

Table 6: Analysis of Physical mixture.

Drugs	Amount taken	%Amount of drug found	%RSD
HCTZ (n=3)	12.5 µg/ml	99.95%	0.49
FIM (n=3)	60 µg/ml	99%	0.90

Table 7: Summary of validation parameters.

Parameter	Fimasartan	Hydrochlorothiazide
Linearity		
Regression Equation	y = -5E-05x - 0.0002	y = -0.0009x - 0.0066
Regression Co-efficient (R2)	0.9988	0.9988
Slope	0.00011	0.00016
SD	0.00011	0.00016
Range (µg/ml)	30-150 µg/ml	6.25-31.25 µg/ml
Precision		
Intraday Precision (n=3)	0.43	1.02
Interday Precision (n=3)	0.56	0.64
L.O.D (µg/ml)	0.2419 µg/ml	0.1045 µg/ml
L.O.Q (µg/ml)	7.98 µg/ml	0.3166 µg/ml

CONCLUSIONS

Simple First order derivative UV spectrophotometric methods were developed for the simultaneous determination of Hydrochlrothiazide and Fimasartan in synthetic mixture without any interference from the excipients. To the best of our knowledge, the present study is the first report for the purpose. The present methods succeeded in adopting a simple sample preparation that achieved satisfactory extraction recovery and facilitated its application in coformulated formulation.

The results of our study indicate that the proposed UV spectroscopic methods are simple, rapid, precise and accurate. Statistical analysis proves that, these methods are repeatable and selective for the analysis of HCTZ and FIM. It can therefore be concluded that use of these methods can save much time and money and they can be with accuracy.

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CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest in the manuscript.

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