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EXTRACTIVE SPECTROSCOPIC AND COST EFFECTIVE METHOD DEVELOPMENT FOR THE ESTIMATION OF AZELNIDIPINE AND OMLESARTAN IN COMBINED DOSAGE FORM

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ABSTRACT:-From the above studies, it has been concluded that extractive spectrophotometry techniques and simultaneous estimation methods can be used successfully for the determination of drugs either individually or in combinations. These drugs are recommended as medicines for treatment of various diseases. The advantages of simultaneous estimation method are fast, simple, less time consuming, accurate and sensitive for research purpose where no new method of estimation and analysis has been reported yet.

Hence, the simultaneous estimation of chemical entities using various analytical techniques are very much valuable for the future needs in pharmaceutical as well as other fields of investigation.

The proposed methods are simple, rapid and validated in terms of linearity, accuracy, precision, specificity and reproducibility, and can be used successfully for routine simultaneous estimation of Olmesartan and Azelnidipine in pure and combined dosage forms.

KEYWORDS: - Spectroscopy, Azelnidipine, Azetidin, Olmesartan, Angiotensin, Linearity

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INTRODUCTION: -The Technique of Ultraviolet-Visible spectroscopy is one of the most frequently employed techniques in pharmaceutical analysis. Molecular absorption in ultraviolet & visible region of spectrum is depending on the electronic structure of molecules. Absorption of energy as quantized, resulting in the elevation of electrons from orbital in the ground state to higher orbital in the exited state. The wavelength range of UV radiation starts at the blue end of the visible light and ends at 2000Å.

Principle

Any molecule has either (n) or combination of these electrons. These bonding and nonbonding (n) electrons absorb the characteristics radiation and undergo transition from ground state to exited state. By the characteristic absorption peaks the nature of electrons present and hence the molecular structure can be elucidated. Ultraviolet absorption spectra arise from transition of electron or electrons with in a molecule or an ion from a lower to higher electronic energy level and the ultraviolet emission spectra arise from the reverse type of transition.

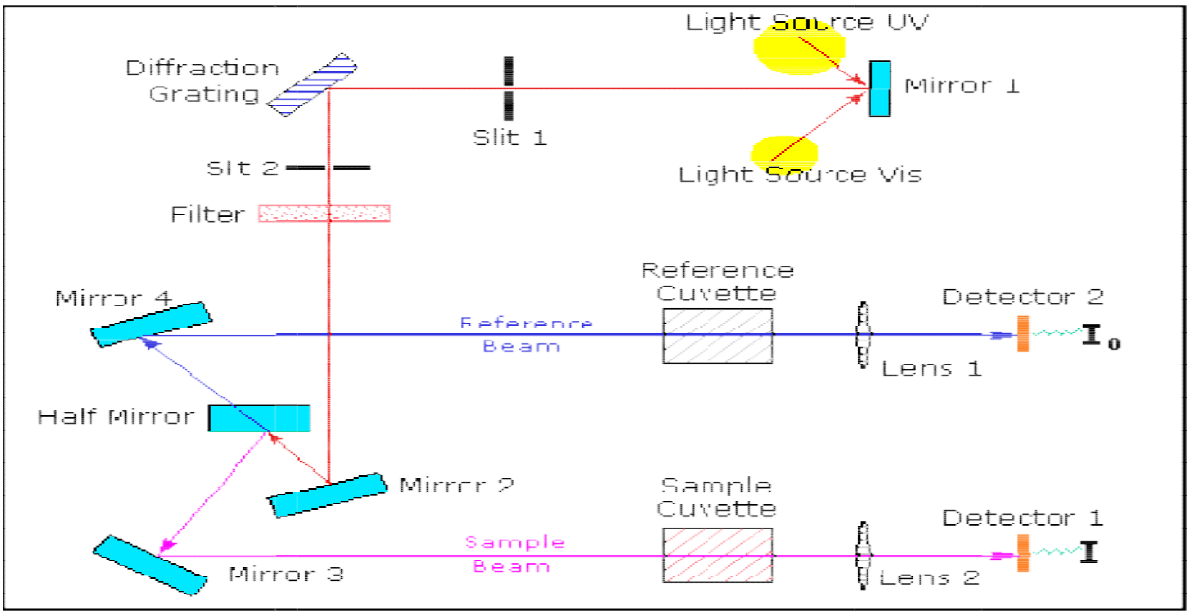

Figure 1.1: Ultraviolet spectrophotometry

The most important characteristic of spectrophotometry are there wide applicability, high sensitivity, and moderate to high sensitivity, good accuracy and convenience. The assay of an absorbing substance may be quickly carried out by preparing a solvent in a transparent solvents and measuring its absorbance at a suitable wavelength.

MATERIALS AND METHOD

DRUG PROFILE

Olmesartan:-

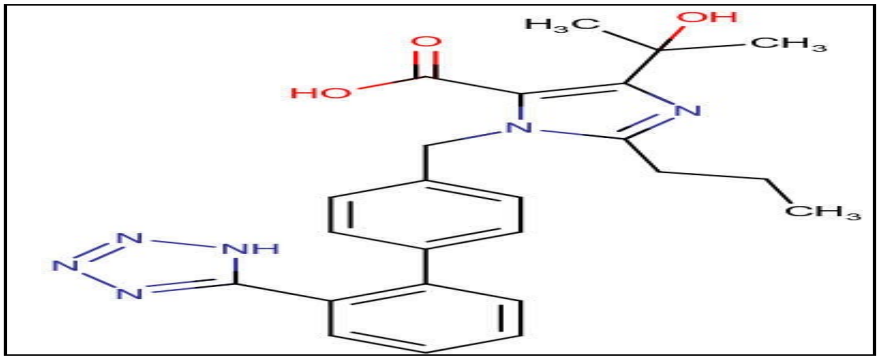

Figure 1.2: Structure of Olmesartan

Olmesartan is an angiotensin receptor blocker (ARB) used in the treatment of hypertension. Olmesartan belongs to the angiotensin II receptor blocker (ARB) family of drugs, which also includes telmisartan, candesartan, losartan, valsartan, and irbesartan.

ARBs selectively bind to angiotensin receptor 1 (AT1) and prevent the protein angiotensin II from binding and exerting its hypertensive effects, which include vasoconstriction, stimulation and synthesis of aldosterone and ADH, cardiac stimulation, and renal reabsorption of sodium, among others. Overall, olmesartan's physiologic effects lead to reduced blood pressure, lower aldosterone levels, reduced cardiac activity, and increased excretion of sodium.

Chemical Formula: $C_{24}H_{26}N_6O_3$

IUPACName: 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2*H*-tetrazol-yl)phenyl]phenyl]methyl]imidazole-4-carboxylic acid

Pharmacology

Indication: Olmesartan is indicated for the treatment of hypertension either alone or in combination with other antihypertensive agents

Pharmacodynamics: Overall, olmesartan's physiologic effects lead to reduced blood pressure, **lower aldosterone levels, reduced cardiac activity, and increased excretion of sodium.**

Mechanism of action: Olmesartan belongs to the angiotensin II receptor blocker (ARB) family of drugs, which also includes telmisartan, candesartan, losartan, valsartan, and irbesartan. ARBs selectively bind to angiotensin receptor 1 (AT1) and prevent the protein angiotensin II from binding and exerting its hypertensive effects. As the principal pressor agent of the renin-angiotensin system, Angiotensin II causes vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation and renal reabsorption of sodium. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle. Its action is, therefore, independent of the pathways for angiotensin II synthesis. Overall, olmesartan's physiologic effects lead to reduced blood pressure, lower aldosterone levels, reduced cardiac activity, and increased excretion of sodium.

Absorption: When taken orally, the prodrug olmesartan medoxomil is rapidly absorbed in the gastrointestinal tract and metabolized to olmesartan. The esterification with medoxomil was created with the intention of increasing olmesartan bioavailability from 4.5% to 28.6%.

Protein binding: Olmesartan is highly bound to plasma proteins. 99% of the administered dose is found in a bound state with no penetration in red blood cells.

Metabolism: Olmesartan medoxomil is rapidly and completely bioactivated by ester hydrolysis to olmesartan during absorption from the gastrointestinal tract. This rapid first-pass metabolism was confirmed by the lack of measurable amounts of olmesartan medoxomil in plasma or excreta. This first-pass metabolism is not driven by cytochrome enzymes and hence it is not expected to interact with other drugs via this mechanism.

Route of elimination: The main elimination route of olmesartan is in the unchanged form through the feces. From the systemically bioavailable dose, about 10-16% is eliminated in the urine.

Half-life: The mean plasma olmesartan half-life is reported to be from 10-15 hours after multiple oral administration.

Uses

Olmesartan is a medicine used to treat high blood pressure. Olmesartan helps prevent future strokes, heart attacks and kidney problems.

Azelnidipine:-

Azelnidipine is a Dihydropyridine calcium channel blocker. It is marketed by Daiichi-Sankyo pharmaceuticals, Inc. in Japan. It has a gradual onset of action and produces a long-lasting decrease in blood pressure, with only a small increase in heart rate, unlike some other calcium channel blockers. It is currently being studied for post-ischemic stroke management.

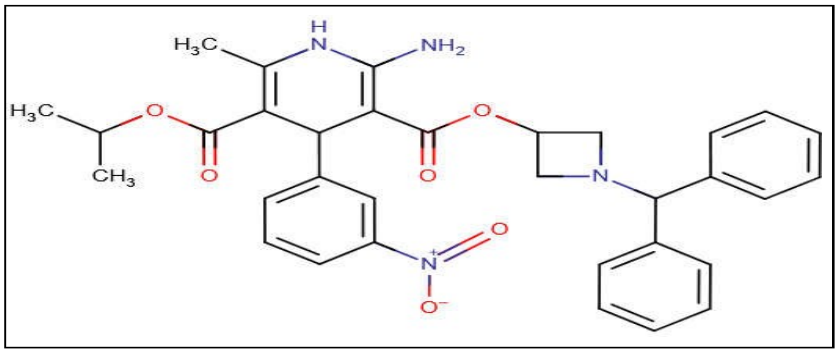

Figure 1.3 - Structure of Azelnidipine

Mol. Weight: 582.657

Chemical Formula: $C_{33}H_{34}N_4O_6$

IUPAC Name: 3-1-Benzhydryl-3-azetidinyl 5-isopropyl 2-amino-6-methyl-4-(m-nitrophenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate

Pharmacology

Indication: For the treatment of hypertension.

Pharmacodynamics: Azelnidipine is a vasodilator that induces a gradual decrease in blood pressure in hypertensive patients. Unlike other members of its drug class, azelnidipine does not induce reflex tachycardia due to vasodilation. This is likely due to the fact that it elicits a gradual fall in blood pressure. It also exhibits a prolonged hypotensive effect and has been shown to have a strong anti-arteriosclerotic action in vessels due to its high affinity for vascular tissue and anti-oxidative activity.

Mechanism of action: Azelnidipine inhibits trans-membrane Ca2+ Influx through the voltage-dependent channels of smooth muscles in vascular walls. Ca2+ Channels are classified into various categories, including L-type, T-type, N-type, P/Q-type, and R-type Ca2+ channels. The L-type Ca2+ Channels. Normally, calcium induces smooth muscle contraction, contributing to hypertension. When calcium channels are blocked, the vascular smooth muscle does not contract, resulting in relaxation of

vascular smooth muscle walls and decreased blood pressure.

Absorption: Oral ingestion of azelnidipine demonstrates rapid and dose-dependent absorption

Protein binding: Azelnidipine is widely bound to human plasma proteins (90%– 91%)

Metabolism: Like most members of its class, azelnidipine primarily undergoes first- pass hepatic metabolism. Azelnidipine is metabolized by hepatic Cytochrome P450 (CYP) 3A4 and has no active metabolite product. It may interact with other drugs or compounds that are substrates for this enzyme. Route of elimination: In one study, following a single 4mg oral dose of 14C- labeled azelnidipine in humans, about 26% of the drug was thought to excreted in the urine and 63% in the feces during the 1 week period post administration

Half-life: 16 –28 hours

Uses

Azelnidipine is used in the treatment of Hypertension (high blood pressure). Azelnidipine is a calcium channel blocker. It regulates the blood pressure by relaxing the blood vessels and reducing the pressure on them, thereby making it easier for the heart to pump more blood throughout the body.

EXPERIMENTAL ANALYSIS AND RESULTS

Standards and Reagents:- Reference standards for the drugs Olmesartan and Azelnidipine, were a kind gift from the Pharmaceutical company, while other chemicals came from the Mumbai-based Merck Chemical Division. Commercial tablets of Azelnidipine, and Olmesartan were purchased from the neighborhood pharmacy.

Apparatus and Instrumentation used in experiment:-

- **Apparatus / Equipments:**

Table-1.1 Apparatus and Instrumentation used in experiment

Components	Volume	Type
Volumetric flasks	10 ml, 25 ml, 50 ml,100 ml	Borosilicate glass type I
Pipettes	1 ml, 2 ml, 5 ml, 10 ml	Borosilicate glass type I
Measuring cylinder	100 ml	Borosilicate glass type I
Beaker	100 ml, 250 ml, 500 ml	Borosilicate glass type I
Whatmann Filter	-	Filter Paper No.41

Instruments:-

- **UV spectrophotometer**

Table-1.2 Instruments used in Experiment

Component	Brand / Model / Software	Manufacturer/ Supplier
UV Visible spectrophotometer	Labindia 3000 Plus	Labindia
Cuvette	Quartz cuvette	Shimadzu Corporation, Kyoto, Japan
Analytical Balance	AUX-200	Shimadzu

- **FTIR spectrophotometer**

Table-1.3 FTIR Spectrophotometer used in Experiment

Component	Brand / Model / Software	Manufacturer/ Supplier
FT IR spectrophotometer	Bruker, alpha	Japan
Analytical Balance	AUX-200	Shimadzu

- **Melting Range**

Table-1.4 Melting Range

Component	Brand / Model / Software	Manufacturer/ Supplier
Melting point Apparatus	Chemiline CL-725	Analab

Identification and characterization of drugs:-

Physical characterization of drugs:-The drugs Olmesartan and Azelnidipine were physically characterized on the beginning of appearance, color and odor. All these parameter were recorded and compared with the literature.

Melting point determination:-The melting point determined used for the strength of mind of melting point of Olmesartan and Azelnidipine by the open capillary methods. The melting point of drug was recorded and compared with literature values. The Melting point of Olmesartan and Azelnidipine was found to be 180-182°C and 193-195°C respectively.

Selection of solvent system:-Azelnidipine and olmesartan scanned in water in the spectrum mode over the UV range (200-400) and was found to be most appropriate because:

- Drug is soluble in it
- Drug is stable in it
- Drug is exhibit good spectral characteristics in it.
- No interference with the λ_{max} of drug.

Linearity range and calibration graph:- Selection of wavelength for linearity:-Solutions of 100g/ml of Azelnidipine and Olmesartan were prepared in 0.1 N HCl, in 3 ml of drug solutions add 1 ml dye and extracted with 3ml chloroform and same manner control also prepared shake both the solution and stand aside for 10 min and compare the colour change compared to control for dye drug reaction .Pipette out the coloured layer of solution and scan between 400 to 800nm as control as blank.

Table-1.5 Selection of solvent

Drug	Dye	Maximum wavelength (λ_{max})
Azelnidipine	Methyl orange	506 nm
Olmesartan	Methyl orange	578 nm

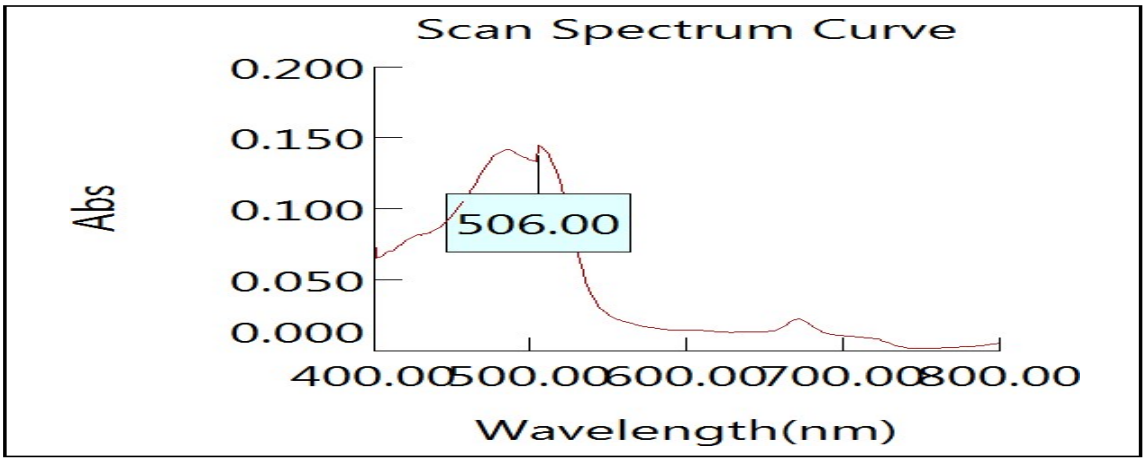

Figure 1.4: Determination of λ_{max} of Azelnidipine

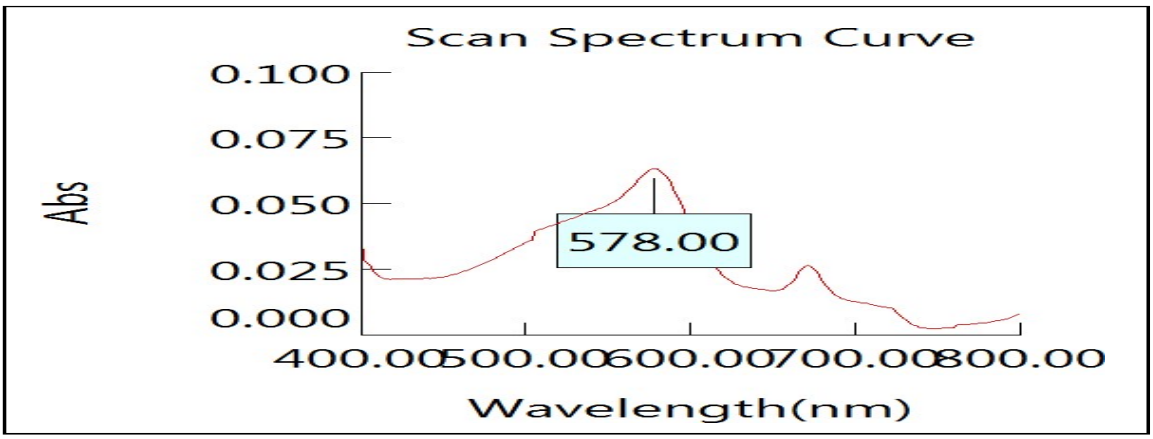

Figure 1.5: Determination of λ_{max} of Olmesartan

Simultaneous equation method

Working standard solution from the standard stock solution prepared in concentration 10µg/ml of AZD and 10µg/ml of OLM were scanned in the spectrum mode over the range of 400-800 nm against blank and the overlain spectra of the two were recorded. AZD showed an absorbance peak at 506.0 nm, whereas OLM at 578.0 nm.

Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method.

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are

506.0 nm and 578.0 nm that are λ_{max} of AZD and OLM respectively.

Analysis of tablet sample

Linearity:-Linearity of analytical procedure is its ability (within a given range) to obtain which are directly proportional to absorbance of analyze in the sample. The calibration plot was contracted after

analysis of five different concentrations (from 5 to 25µg/ml For OLM and 5 to 25µg/ml for AZD) and absorbance for each concentration were recorded three times and mean absorbance was calculated. The response ratio (response factor) was found by dividing the mean absorbance with respective concentration.

Table 1.6: Response ratio data for linearity of OLM

Concentration (□g/ml)	Mean AUC	Response Ratio
5	0.202	0.040
10	0.422	0.042
15	0.614	0.041
20	0.813	0.041
25	1.014	0.041
Mean		0.041
SD		0.001
%RSD		1.773

Table 1.7: Response ratio data for linearity of AZD

Concentration (□g/ml)	Mean AUC	Response Ratio
5	0.058	0.012
10	0.105	0.011
15	0.156	0.010
20	0.195	0.010
25	0.243	0.010
Mean		0.010
SD		0.001
%RSD		7.350

Analysis of tablet sample

Twenty marketed tablets of AZD and OLM were weighed and ground to a fine powder; amount equal to 10mg of OLM was taken in 10 ml volumetric flask. The AZD present in this amount of tablet powder was 4mg. Then 20ml of 0.1 N HCL was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made upto the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with 0.1 N HCL and React with dye and extract with chloroform to get the final concentrations of both drugs in the working

range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from Simultaneous Equation Method. The procedure was repeated for five times.

Table 1.8: Analysis of Tablet Formulation of AZD and OLM

Conc. Present (µg/ml)		Replicate-1				Replicate-2				Replicate-3			
		Conc. Found (µg/ml)		% Conc. Found (µg/ml)		Conc. Found (µg/ml)		% Conc. Found (µg/ml)		Conc. Found (µg/ml)		% Conc. Found (µg/ml)	
OLM	AZD	OLM	AZD	OLM	AZD	OLM	AZD	OLM	AZD	OLM	AZD	OLM	AZD
5	5	4.95	4.99	99.00	99.80	4.85	4.74	97.00	94.80	4.78	4.99	95.60	99.8
10	10	9.95	9.98	99.50	99.80	9.98	9.65	99.80	96.50	9.68	9.78	96.80	97.8
15	15	14.65	14.96	97.67	99.73	14.65	14.85	97.67	99.00	14.95	14.85	99.67	99
20	20	19.95	19.95	99.75	99.75	19.95	19.96	99.75	99.80	19.95	19.96	99.75	99.8
25	25	24.78	24.78	99.12	99.12	24.65	24.65	98.60	98.60	24.88	24.65	99.52	98.6
											MEAN*	98.27	99.00
											SD*	1.936	0.849
											% RSD*	1.970	0.857

DISCUSSION AND CONCLUSION:

From the above studies, it has been concluded that extractive spectrophotometry techniques and simultaneous estimation methods can be used successfully for the determination of drugs either individually or in combinations. These drugs are recommended as medicines for treatment of various diseases. The advantages of simultaneous estimation method are fast, simple, less time consuming, accurate and sensitive for research purpose where no new method of estimation and analysis has been reported yet.

Hence, the simultaneous estimation of chemical entities using various analytical techniques are very much valuable for the future needs in pharmaceutical as well as other fields of investigation.

The proposed methods are simple, rapid and validated in terms of linearity, accuracy, precision, specificity and reproducibility, and can be used successfully for routine simultaneous estimation of Olmesartan and Azelnidipine in pure and combined dosage form.

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