TELMISARTAN AND ROSUVASTATIN:A REVIEW ON THE ANALYTICAL METHODS FOR THE INDIVIDUAL AND COMBINED DOSAGE FORMS.

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Abstract:

Rosuvastatin calcium belongs to the class "statins", which is indicated for dyslipidemias and decreases the level of bad cholesterol .Telmisartan is an oral antihypertensive agent that acts by blocking angiotensin receptors.Rosuvastatin calcium and telmisartan , is a fixed drug combination given to treat hypertension and dyslipidemia. The article gives glimpses of published analytical methods reported so far in the literature for the determination of Rosuvastatin calcium and telmisartan, individually and/or in combinations present in pharmaceutical formulations and biological fluids by various methods like Spectrophotometry, High Performance Liquid Chromatography using Ultra Violet and Flourimetric detection, Liquid chromatography coupled with Tandem mass spectrometry, Immunoassays, and Electrochemical methods such as Cathodic absorptive stripping voltammetric method.

Index terms:Rosuvastatin calcium, Telmisartan,Analytical methods

1.TELMISARTAN: A REVIEW OF ANALYTICAL METHODS

Introduction[2]:

Telmisartan is an angiotensin II receptor antagonist (AT_1) used in the management of hypertension. It selectively antagonizes angiotensin II binding to the AT1 subtype receptors. Inhibition of AT1 receptors leads to vasodilation and inhibits the angiotensin II mediated aldosterone production which in turn leads to decrease in sodium and water excretion and also increases potassium excretion and thereby causes a reduction in blood pressure. Chemically 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-propyl-1H-1,3benzodiazol-1-yl] methyl}phenyl)benzoic acid.

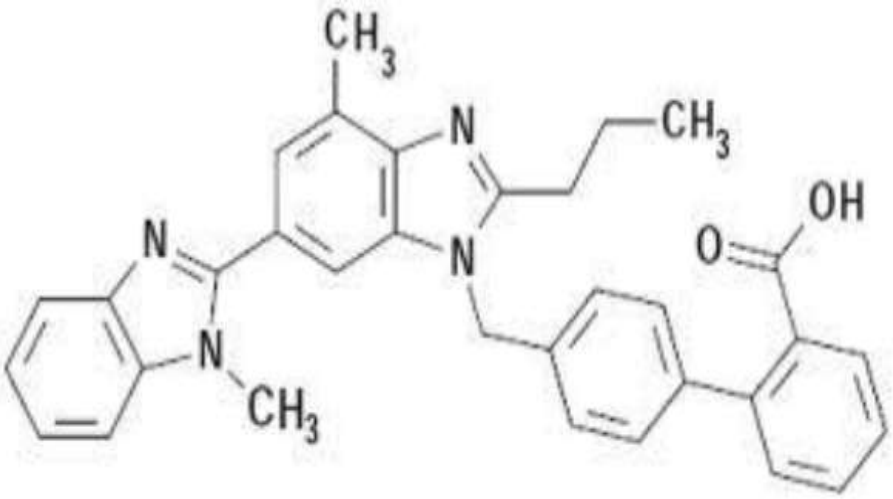

Fig:1.1 –Telmisartan

It is a white slightly yellowish solid with a molecular formula C_{33} $H_{30}N_4O_2$. The molecular weight was found to be 514.6g/mol. It is insoluble in water[2.8×10-6mgL at 25 degree C], sparingly soluble in strong acid and soluble in strong base. It is commonly administered through oral route.An overview of analytical methods used for the estimation of telmisartan is given in Chart1.2.

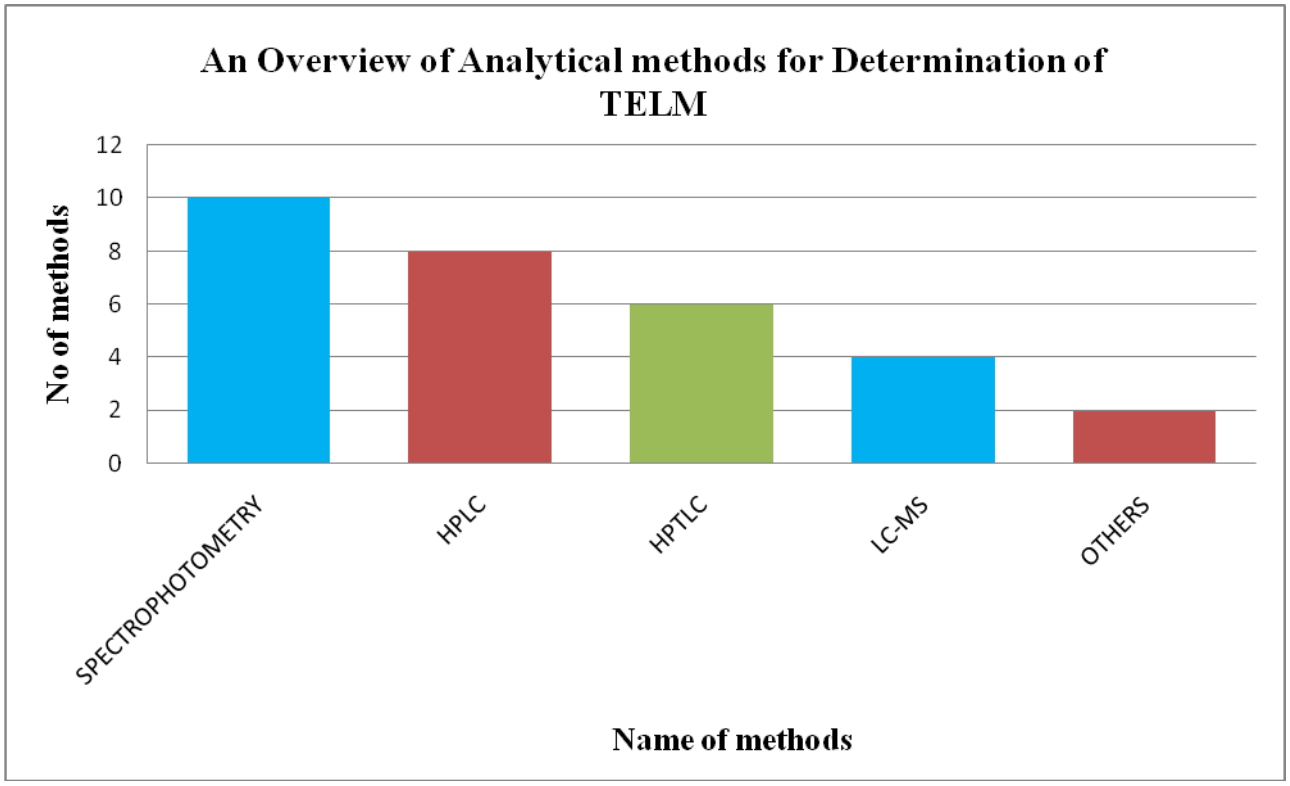

Chart: 1.2 Analytical methods for Determination of Telmisartan

Sample preparation:

Solubility:

According to Biopharmaceutical Classification System (BCS) telmisartan is a class II drug that has extremely low water solubility but it is freely soluble in strong alkali solutions. Because of this characteristic behavior Supercritical Anti-Solvent [SAS] process was used. DMF is used as a good solvent, other solvents used are methanol,ethanol, chloroform etc.

Sample preparation strategies[48] :

In most of the experiments 95% methanol and $NaHCO_3$ are used as the diluents. Samples are prepared from biological fluids like plasma, serum and urine by protein precipitation with acetonitrile. Then micro-extraction using poly monolithic capillary and solid phase extraction procedure with a pre-column packed with 25µm C_{18} alkyl-diol support (ADS) and solution of 2% methanol in phosphate buffer are also used.

ANALYTICAL METHODS[1];

Chromatography:

Biological samples: samples are prepared from various biological matrices by solid phase and liquid-liquid extraction, hollow fiber liquid phase microextraction, protein precipitation using acetonitrile and acetonitrile-naproxan mixtures are used.

Pharmaceutical samples:

Commonly used mobile phase include phosphate buffer and methanol in the ratio 40:60v/v. Commonly used diluents is a mixture of water and methanol in the ratio 60:40v/v. other mobile phases used include methanol and acetonitrile in the ratio 70:30v/v, methanol and ammonium acetate solution in the ratio 35:65v/v.

Lakshmi KL et al[3]. developed and validated RP-HPLC method for the simultaneous estimation of telmisartan and hydrochlorothiazide in bulk and tablet dosage form.Column used was Agilent C18 (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase used was methanol:ACN in the ratio 70:30v/v.Detection wavelength was 247nm. WATERS HPLC Auto Sampler,Separation module 2695,photo diode array detector 996, was used. Retention times were found to be 1.866 min and 2.496 min and percentage purity of HCL and TELM was found to be 99.87% and 100.27% respectively. Linearity was found in the concentration range50-250µg and 15-55µg for HCL and TELM respectively.

Surekha LM et al[10]. developed and validated a simple, precise , rapid and reproducible RP-HPLC method for the estimation of telmisartan in bulk and tablet dosage form.Column used was Zorbax-SB-18(150×4.6mm). Mobile phase used was methanol:water in the ratio 75:25 and acetonitrile was used as the diluent. Detection wavelength was found to be 230nm using UV visible detector. The concentration 40-20µg/ml was found to be the linear range. LOD and LOQ was found to be 0.2515µg/ml and 0.6623µg/ml.

R N Rao et al[4]. developed and validated a rapid and sensitive HPLC method for the estimation of telmisartanin pharmaceutical dosage forms. The HPLC column used for the study was C18 column. Methanol:acetonitrile:buffer (pH 2.8) in the ratio 20:70:10v/v was the solvent used, at a flow rate of 1ml/min. Detected at wavelength 290 by UV detector. Linearity was observed when the concentration range 10-1000ng/ml was used.Mean recovery from the solution containing 50ng/ml was found to be 96.6±1.13%.

Table 1.Estimation of Mixtures by HPLC:

Author	Method	Stationary Phase	Mobile Phase	Detection Wavelength	Linear Range	Result
V P Kurade et al[5].	RP-HPLC estimation of ramipril and telmisartan in tablet dosage form	Genesis C18 column (4.6×250mm) particle size; 5µm	KH2PO4:methanol:acetonitrile (15:15:70V/V/V).	210nm	Ramipril-3.5-6.5µg/ml. Telmisatan 28-52µg/ml	Rt of ramipril-3.68min. Rt of telmisartan 4.98min
Leena R et.al[6]	RP-HPLC estimation of telmisartan and hydrochlorthiazide in pharmaceutical formulation	Rabeprazol was used as the internal standard	Methanol:acetonitrile(70:30)	270nm		TEL:1.79±0.01 HCT:2.80±0.01 RAB:3.19±0.01
M AkifulHaque[7]	RP-HPLC method for determination of telmisartan and hydrochlorthiazide in bulk and tablet dosage form	Agilent C18 column(4.6×150 mm)	Methanol:ACN (70:30v/v)	270nm	HCT:50-250µg TELM:15-55µg	HCT:1.86 TELM:2.496

HPTLC:

V A Patel et al[11]. developed and validated thin layer liquid chromatography for the simultaneous estimation of telmisartan and ramipril in combined dosage form. Stationary phase used was TLC plate pre-coated with silica gel, mobile phase used was acetone:benzene:ethylacetate:GAA in the ratio 5:3:2:0.03v/v/v/v. Rf value for telmisartan and ramipril was found to be 0.673 and 0.353 respectively.The concentration range of telmisartan and ramipril was found to be 100-1800ng/spot and 300-1800ng/spot respectively.

NJ Shah et al[13]. performed simultaneous estimation of telmisartan and hydrochlorthiazide in tablet dosage form using TLC plate pre-coated with silica gel and a mixture of chloroform:Methanol:toluene(2:5:5v/v/v) as mobile phase. The concentration range was found to be 250-500ng/spot for telmisartan and 200-700ng/spot for hydrochlorothiazide.

AniruddhaR Chabukswaret.al[12] developed a HPTLC method for the simultaneous estimation of telmisartan and amlodipine besylate in tablet dosage form. Precoated silica gel 60 F_{254} on aluminium sheets was used as the stationary phase. Ethyl acetate:1,4 Dioxane:Methanol:25%Ammonia was used as the mobile phase in the ratio15:1.5:3:1.5v/v. R_f value was found to be 0.16 and 0.33 for TELM and AMLO respectively. Linearity was observed between

100µg/ml -500µg/ml for TELM and 200-1000µg/ml for AMLO. LOD and LOQ were found to be 0.025µg/ml, 0.0747µg/ml and 0.0236µg/ml, 0.0714µg/ml respectively for TELM and AMLO .Average recovery was found to be 100.38% and 100.24% for TELM and AMLO respectively.

LC-MS:

Kiran R Patil et al[14]. developed liquid chromatography- mass spectrometry method for the quantitative simultaneous estimation of telmisartan and ramipril in combined dosage form. Mobile phase used was buffer-acetonitrile in the ratio 55:45v/v. This method shows linearity over the concentration range 20-400µgmL^{-1} for telmisartan and 2.5-50µgm/L for ramipril

VasuBabu Ravi et al[15]. developed a rapid and sensitive liquid chromatography-tandem mass spectrometric (LC-MS/MS) assay method for the simultaneous quantification of telmisartan and amlodipine in human plasma. Linearity was observed over the concentration range 2.01-400.06ngm/ml for telmisartanand 0.05-10.01ng/ml for amlodipine.

Spectrophotometry:

Most common and widely used method is UV spectrophotometry. Commonly used solvents are NaOH, methanol , phosphate buffer and distilled water.

D S Rathod et al[16]. developed a simple precise and accurate UV spectrophotometric method for the estimation of telmisartan in bulk and tablet dosage form and the proposed method was validated by ICH guidelines. The solvents used were 0.1N NaOH and distilled water (20:80) λ_{max} was found to be at 234nm.linear concentration range was found to be at 2-10µg/ml.

Ajithpandey et al[8]. developed and validated UV spectrophotometric method for the estimation of telmisartan in bulk and tablet dosage form. λ_{max} of zero order spectra of telmisartan in 0.1N NaOH was found to be at 234nm. Linearity was observedover the concentration range of 4-24µg/ml.The developed method was validated by ICH guidelines.

D N Chivate[17] developed uv spectrophotometric method for the estimation of telmisartan as a pure API. The oposed method was validated according to ICH guidelines. The solvents used were 60% ethanol(95%) and 40% of $NaHCO_3$. λ_{max} was observed 240nm. Linear range lies between 2-14µg/ml, beer's law was obeyed in this concentration ge.

Table 1.2: Spectroscopic methods for Telmisartan with other Drugs

Author	Method	Solvent	λ_{max}	Linearity
Ilango K , Shijikumar[18]	Simultaneous equation method	Methanol	TELM-296nm HCT-270nm	TELM:2-10-5g/ml HCT:4-20-5g/ml
Asha B Thomas et al[9].	Simultaneous spectrophotometric estimation of amilodipinbesylate and telmisartan in tablet dosage form by 1. Simultaneous equation method 2. Area under curve method 3. Multicomponent mode method	Methanol	1) AMLO:364.5nm TELM:254.5nm. 2) AMLO:362.5-366.5nm TELM:252.5-256.5nm. 3) AMLO:364.5nm TELM:254.5nm	AMLO : 1-50µg/ml TElM:10-80µg/ml
U P Patil et.al[19]	Simultaneous determination of atorvastatin calcium and telmisartan in tablet dosage form by 1. Absorbance correction method 2. First order derivative spectroscopic method 3. Duel wavelength method	Methanol and distilled water	1. TELM:328nm ATV: nil 2. ATV:297nm TELM:241.8nm 3. ATV: difference in absorbance at 258 and 291nm. TELM: difference in absorbance at 225 and 252nm	Both the drugs obey Beer's law in the concentration range 5-30µg/ml.

ELECTROCHEMICAL METHODS:

Xu M, Song J et.al[20] developed an electrochemical method for the rapid determination of telmisartan in pharmaceutical preparations and serum by linear sweep polarography. Supporting electrolyte used was 0.8mol/L NH_3 .H_2O-NH_4Cl. The reduction peak was obtained at ca.-1.30V, correspondent to a catalytic hydrogen wave. Linear range was observed over 2×10^{-7} to 3×10^{-6}mol/L. Detection limit was found to be at 1×10^{-7}mol/l.

Tasdemir H I, et.al[21] developed a rapid electrochemical method for the assay of telmisartan in pharmaceutical dosage forms and biological fluids by voltammetric behavior of telmisartan and cathodic adsorptive stripping voltammetric method. Electrochemical behavior of telmisartan and suitable conditions for its assay were determined by using cyclic voltammetry and square –wave voltammetry. Both of the studies were based on quasi-reversible and adsorption –controlled electrochemical reduction signal of TS at about -1.50V vs Ag/AgCl at p^H 10 in Britton-Robinson buffer. Linearity of peak current was observed over the concentration range 0.87µg/L to 14.15µg/L. LOD and LOQ were found to be 0.54µg/L and 1.79µg/L respectively.

IMMUNE ASSAY:

C M Hempen et.al[23] developed a novel and rapid analytical method, an enzyme linked immuno sorbent assay(ELISA) for the estimation of telmisartan in human blood plasma. The assay method was based on a conversion of 4-(N-methylhydrazino)-7-nitro-2,1,3-benzooxadiazole(MNBDH) to 4-(N-methylamino)-7-nitro-2,1,3-benzooxadiazole(MNBDA) and this was detected by fluorescence spectroscopy. LOQ and LOD were 0.3ng/ml and 0.1ng/ml respectively.

2. ROSUVASTATIN CALCIUM : A REVIEW OF ANALYTICAL METHODS

Introduction[2]:

Rosuvastatin calcium is chemically,(E)-(3R,5S)-7-(4-(4-flurophenyl)-6-isopropyl-2-[(methyl(methanesulfonylamino)] pyrimidine-5-yl)-3,5-dihydroxyhepten-6-oic acid calcium. Normally statins are HMGCoAreductase inhibitors that are found to be effective in the reduction of total cholesterol and LDL cholesterol and hence it is indicated for dyslipidemias. In addition to it, rosuvastatin also possess pleiotropic effects which include improvement in endothelial function, anti-inflammatory, antithrombotic and anti-oxidant effects. Structure of rosuvastain is given below;

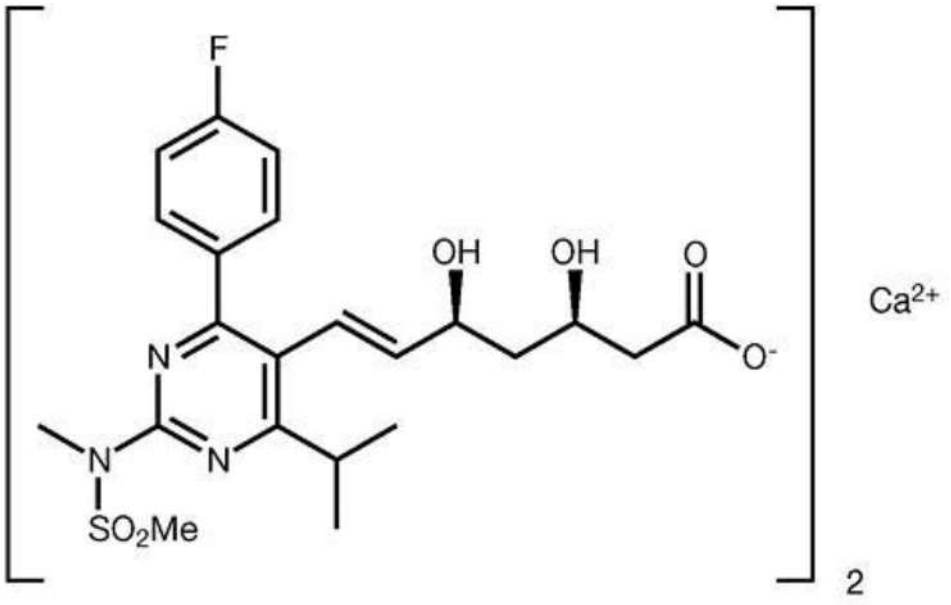

Fig:2.1- Structure of Rosuvastatin Calcium

Mechanism of Action:

Rosuvastatin is a competitive inhibitor of HMG CoA reductase enzyme, an enzyme which is responsible for the conversion of HMG CoA to mevalonic acid in the cholesterol biosynthetic pathway, which is the rate limiting step in cholesterol synthesis. Rosuvastatin decreases hepatic sterol synthesis which in turn cause a decrease in hepatocellular cholesterol. Hepatocytes respond to this decreased intracellular cholesterol concentration by the increased synthesis of LDL receptors to enhance hepatic LDL reuptake from circulation, thereby decreasing the serum LDL cholesterol and total cholesterol.

This study includes a literature review of analytical methods for the quantification of rosuvastatin in pharmaceutical dosage forms and biological fluids. The major analytical methods mentioned in this study includes spectrophotometry, high performance liquid chromatography(HPLC) coupled to ultraviolet detection, liquid chromatography with tandem mass spectrometry(LC-MS/MS), high performance thin layer chromatography(HPTLC), thin layer chromatography and immunoassays like ELISA. An overview of analytical methods used for the estimation of rosuvastatin is shown in the bar chart given below

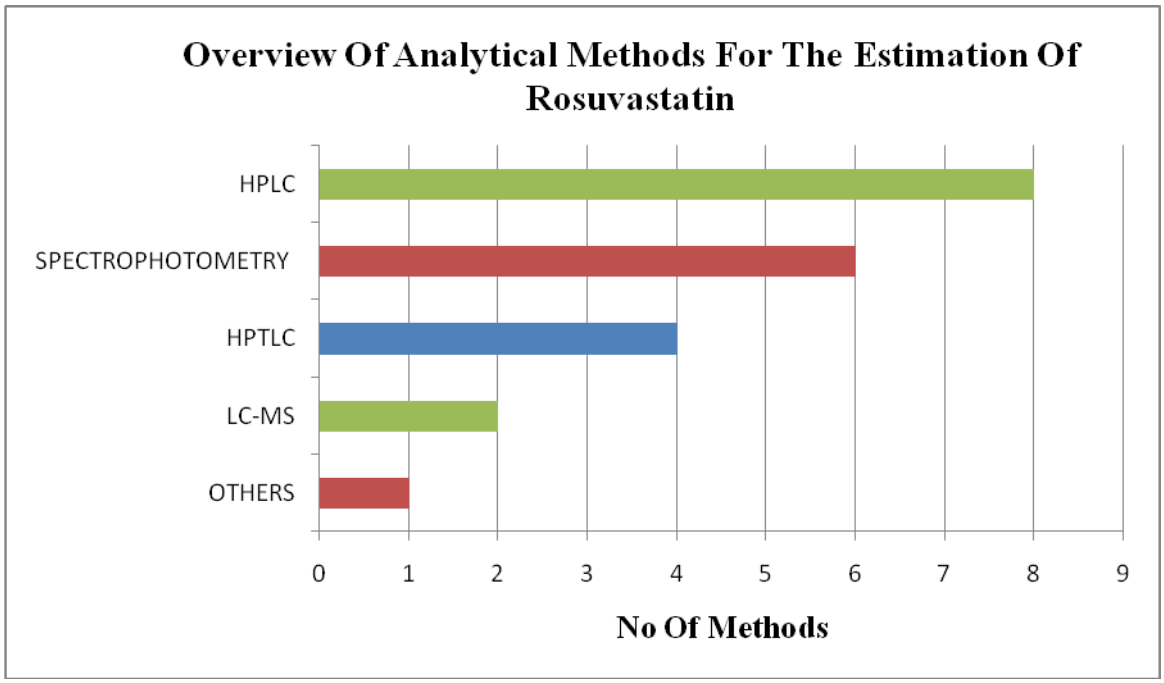

Chart 2.1 Analytical Methods For The Estimation Of Rosuvastatin

Sample preparations:

Solubility:

According to BCS classification rosuvastatin comes under class 1drug. Solubility studies were carried out in propylene glycol, polyethylene glycol 200, polyethylene glycol 400, distilled water, phosphate buffer. Highest solubility was found in polyethylene glycol 200.

Sample preparation strategies[48]:

In spectrophotometry, methanol(MeOH) is the commonly used solvent for the preparation of both standard and test solution series. In HPLC with UV detection methods employed C18 analytical column as the stationary phase and methanol and/or acetonitrile(ACN) as organic solvents and water or buffer with pH adjusted to 3.0 is widely used. LC-MS/MS methods were performed using mobile phase composed of MeOH/ACN as organic solvents and water with volatile components like formic acid and ammonium acetate, which were added to improve the sensitivity of the developed method.C18 analytical column was used as the stationary phase in most of the studies.

Analytical Methods:

Chromatography:

HPLC:

H O Kaila et.al[27] developed a new improved RP-HPLC method for assay of rosuvastatin calcium in tablets. YMC C8 column was used as the stationary phase. The mobile phase employed was acetonitrile: water(40:60v/v)pH adjusted to 3.5 with phosphoric acid in isocratic mode. The compound obeyed Beer's law over the concentration range of 0.5-80µg/ml. LOD and LOQ were found to be 0.1 and 0.5 µg/ml respectively. Proposed method were validated as per ICH guidelines.

Chirag B Pandyaet.al[46] developed and validated a simple, rapid, accurate, specific and precise RP-HPLC method for the quantification of rosuvastatin in the pharmaceutical dosage forms. Stationary phase employed was Thermo hypersil reversed phase C18 (5µm) column in gradient mode. The mobile phase used contained HPLC grade ACN: Potassium dihydrogen orthophosphate (50:50v/v) at a flow rate of 0.5ml/min. LOD and LOQ were found to be 0.14 and 0.46µg/ml. The chromatogram showed major peak at retention time 3.333±0.004 min.

Hemant Kumar T et.al[28] performed a rapid, sensitive and specific RP-HPLC method and the proposed method has been validated as per ICH guidelines. Study was done using Enable C18G (250×4.6mm) column as stationary phase and degassed mixture of ACN:Water in the ratio 75:25%v/v as mobile phase at flow rate of 0.6ml/min. Detection wavelength was found to be 252 nm. The developed method was linear over the concentration range 5-40µg/ ml. Main peak was observed at Rt 3.097min.

Table: 2.1 Estimation Of Mixtures by HPLC

Author	Method	Stationary Phase	Mobile Phase	Detection Wavelength	Linear Range	Result	Ref
Mohammed IshaqBeludariet. al[29]	RP-HPLC method for the simultaneous estimation of Rosuvastatin and Ezetimibe in tablet dosage form	Water's C18 analytical column Dimension: 250×4.6mm, particle size: 5µm	ACN: Water: 0.02M phosphate buffer pH 8 (40:10:50v/v) Flow rate:1.0 ml/min	230 nm	30-90µg/ml for both RSV and EZE	Rt of RSV:2.8min Rt of EZE: 4.7min	
Yasar Shah et.al[30]	Simultaneous determination of rosuvastatinand atorvastatin in human serum by RP-HPLC/UVdetection	Brownlee analytical C18 Dimension:150×4.6mm Particle size:5µm column	MeOH: Water, pH adjusted to 3.0 with trifluoroacetic acid. Ratio:68:32 v/v Flow rate:1.5ml/min	241nm	RSV:2.0-256ng/ml ATV:3.0-384ng/ml	Rt of ATV and RSV is less than 7.0 min.	
Murthy TGK et.al[31]	RP-HPLC method for the Simultaneous determination of metformin hydrochloride and rosuvastatin calcium in bulk and in-house formulation	Phenomenex C18 column Dimension: 250×4.6mm Particle size:5µm	Phosphate buffer(pH2.8): ACN with pH adjusted to 3.8. Ratio: 65:35v/v Flow rate:1ml/min	252nm	MET:5-30µg/ml RSV:0.4-2.4µg/ml	Rt of MET:2.147 min Rt of RSV: 3.80min	

HPTLC:

S U Devi et.al[26] developed and validated a simple, specific, accurate and precise HPTLC method for the estimation of rosuvastatin calcium in bulk and pharmaceutical dosage form. In this method aluminium plates coated with silica gel 60 F254 were used as stationary phase and ethyl acetate: toluene: methanol in the ratio 6:2:2v/v/v as the mobile phase. The RF value of ROS was found to be 0.32±0.05. Linearity was observed over the concentration range 500-2500ng/spot.

Rani S Potawaleet.al[25] performed and validated HPTLC method for the simultaneous determination of rosuvastatin and fenofibrate in bulk and pharmaceutical formulation. Mobile phase used were a mixture of ethyl acetate: acetic acid in the ratio 20:0.2v/v and aluminium pre-coated plates of silica gel G F_{254} were used as the stationary phase. The method was found to be linear over the concentration range 50-800ng/band for both drugs. R_f values were 0.31±0.01 and 0.76±0.01 for ROS and FENO respectively.

B G Chaudhariet.al[32] developed and validated a simple and reproducible HPTLC method for the determination of simvastatin, pravastatin and rosuvastatin calcium in tablet dosage forms. Stationary phase used was pre-coated silica gel $60F_{254}$ and a mixture of chloroform: methanol: toluene was used as mobile phase in the ratio 6:2:2v/v/v. R_f value of SMV, PRV and RSV were found to be 0.59, 0.45 and 0.53 respectively. Linear ranges were 200-1000ng/spot,100-1000ng/spot and 100 to 600ng/spot for SMV, PRV and RSV.

Varghese S J et.al[33] developed and validated HPLC and HPTLC method for the simultaneous estimation of rosuvastatin and ezetimibe in a combined tablet dosage form. In RP-HPLC Stationary phase used was Chromolith C18 column and mobile phase used was orthophosphoric acid solution with ACN at a flow rate of 1ml/min. Detection wavelength was found to be 245nm. Linearity was observed over the concentration range of 0.5-10µg/ml. In HPTLC, aluminium-backed sheet of silica gel 60F(254) layers were used as stationary phase and n-butyl acetate: chloroform: GAA in the ratio 1:8:1v/v/v as mobile phase. Linear range was found to be 0.1-0.9µg/spot for ROS and EZE.

LC-MS/MS:

Dong-Hang Xuet.al[34] performed a simple and sensitive method for the quantitative determination ofrosuvastatin in human plasma by liquid chromatography with electrospray ionization tandem massspectrometry. The drug was extracted by one step liquid/liquid extraction with ether. Atlantis C18 column (2.1×150mm,5µm) was used as the stationary phase. Mobile phase employed was 0.2% formic acid: methanol in the ratio 30:70v/v at a flow rate of 0.2ml/min. The proposed method obeyed Beer's law over the concentration range 0.2-50ng/ml.

Ravi Kumar Trivediet.al[l][35] developed and validated a simple, sensitive and specific LC-MS/MS with electrospray ionization method for the simultaneous estimation of rosuvastatin and fenofibric acid in human plasma. X- Terra MS C18 column (4.6×50mm) was used as the stationary phase and mobile phase used consist of 0.05M formic acid :ACN in the ratio 45:55v/v at a flow rate 0.40ml/min. $R_t$of ROS, FFA and IS were found to be 2.35min, 4.70min and 2.32min respectively. Linearity was established over the range 1-50ng/ml and 0.5-20µg/ml for ROS and FFA respectively.

Caroline K Hull et.al[36] performed an assay method for the quantification of rosuvastatin in human plasma by automated solid-phase extraction using high performance liquid chromatography with positive ion Turbolonspray tandem mass spectrometric (LC-MS-MS) detection. Linear range were found to be 0.1-30ng/ml.

Spectrophotometry

In spectrophotometry, only few methods were developed for the estimation of rosuvastatin alone either in pure form or in pharmaceutical formulations whereas there were many methods developed for the simultaneous estimation of rosuvastatin and other drugs from combined dosage form. Among them most sited studies are discussed in the table:4

Alka Gupta et.al[24] developed a simple UV spectrophotometric determination of rosuvastatin calcium in pure form and in pharmaceutical formulations. Solvent used was methanol and the λ_{max} was found be at 244nm. This method was validated as per ICH guidelines and the method was found to be linear over the range 2-18µg/ml.

Table : 2.2 Estimation Of Mixtures By Spectrophotometry

Author	Method	Solvent	λ_{max}	Linear range
GajjarAnuradha K and Shah Vishal D[45]	1. Q absorption ratio method 2. Dual wavelength method 3. First order derivative technique	Methanol	EZE:232.4nm Rosu:237nm Eze:226.4nm 239.2nm ROS:232.6nm 252.4nm EZE:232.4nm ROS:223.4nm	1-25µg/ml
NarayankarSavita M et al[44].	1. Simultaneous equation method 2. Absorbance ratio method	Water	ROS:241nm Niacin:261nm	5-40µg/ml
Namdeo G Shinde et al[43].	1. Vierort method 2. Absorbance ratio method	Methanol	ROS:243nm PROP HCl: 289nm	ROS:2-42µg/ml PROP:4-40µg/ml
R RSevdaet al[42].	Calibration curve method	Methanol	ROS:244nm FENO:286.7nm	ROS:1-10µg/ml FENO:2-20µg/my

IMMUNOASSAY:

Ibrahim A Darwish et.al.[37] developed and validated a highly sensitive ELISA for the determination of rosuvastatin in plasma at picogram level. A polyclonal antibody that specifically recognizes ROS with high affinity and ROS conjugate of bovine serum albumin (ROS-BSA) immobilized onto microplate wells were employed as the solid phase. The assay method was on the competitive binding reaction between ROS in plasma sample and the immobilized ROS-BSA for the binding sites on anti-ROS antibody which were limited in number. The bound anti –

ROS antibody were estimated with horseradish peroxidase labeled second anti-rabbit IgG antibody and 3,3′,5,5′-tetramethylbenzidine as a substrate for the peroxidase enzyme. The amount of ROS in the sample was quantified by its ability to inhibit the binding of anti-ROS antibody to the immobilized ROS-BSA and also by the colour intensity in the assay wells. The developed method was validated as per ICH guidelines.

3. ROSUVASTATIN-TELMISARTAN COMBINATION: REVIEW ON ANALYTICAL METHODS

Introduction:

Rosuvastatin calcium and telmisartan is a fixed dose combination used in the coronary heart disease. This combination contains rosuvastatin 10mg as a lipid lowering agent and telmisartan 40mg as a antihypertensive agent. Rosuvastatin is chemically bis[(E)-7-[4(4-fluorophenyl)-6-isopropyl-2[methyl (methylsulfonyl) amino] pyrimidin-5-yl](3R,5S)3,5- dihydroxyhept-6-enoic acid] calcium salt. Telmisartan is chemically4'-[(1,4'-dimethyl-2'-propyl [2,6'-bi-1H-benzimidazol]-1'-yl) methyl]-[1,1'-biphenyl]-2-carboxylic acid. Pharmacologically,Rosuvastatin is a competitive inhibitor of HMG CoA reductase inhibitor which causes the reduction of LDL cholesterol and total cholesterol. Pharmacologically, telmisartan antagonizes angiotensin II binding to the AT1 subtype receptor in the vascular smooth muscles and adrenal gland. This antagonism results in the vasodilation and angiotensin II mediated aldosterone production, which in turn leads to a decrease in sodium and water as well as an increase in potassium excretion thereby leads to a reduction in BP.

By combining the HMG CoA reductase inhibitor with ARB leads to the synergistic vascular protective effects. So telmisartan androsuvastatin combination is widely used to treat hypertension and dyslipidemia. This combination could improve the patient compliance by reducing the pill burden.

Major Rosuvastatin and telmisartan combination brands are given below:

Table: 3.1 Rosuvastatin and telmisartan combination brands

Brand name	Amount of telmisartan	Amountofrosuvastatin
ROZUTION T	40mg	10mg
TELLZY RS	40mg	10mg
TELROSE	40mg	10mg

Literature survey suggests that there are only few HPLC methods, few spectroscopic methods and very few HPTLC methods were developed for the simultaneous estimation of rosuvastatin and telmisartan in combined dosage forms.Pie chart given below shows an overview of analytical methods on ROS and TELM combinations;

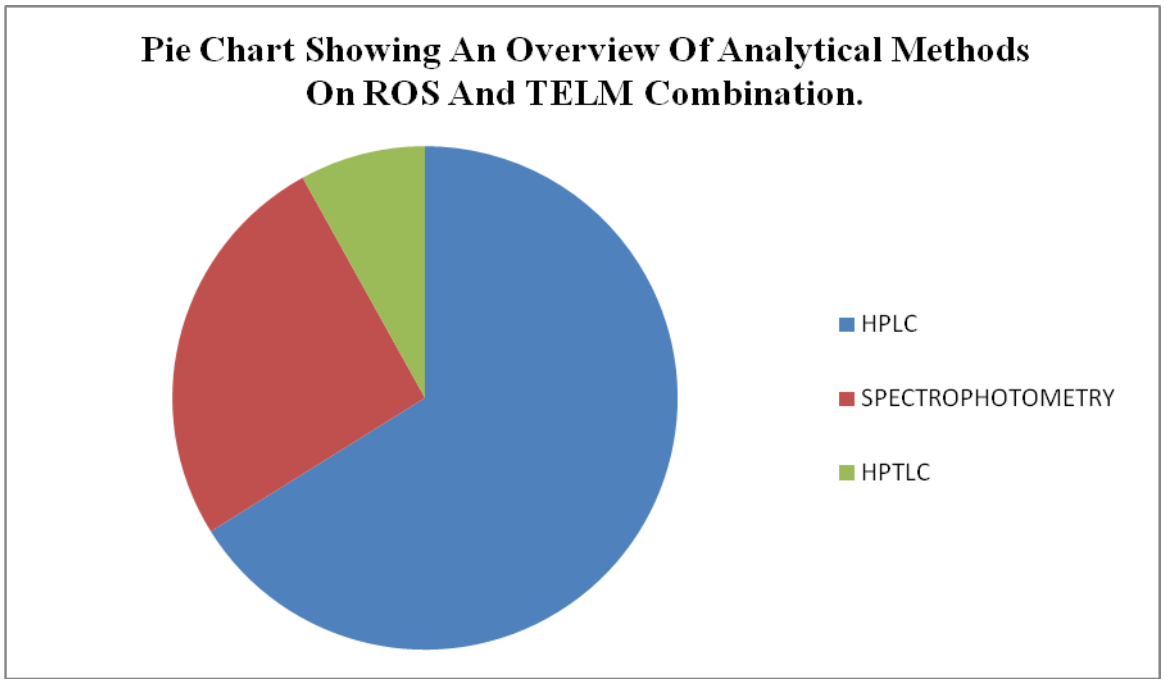

Chart:3.1 Analytical methods for the combined formulations

Sample preparations:

Solubility:

Solubility was discussed under telmisartan and rosuvastatin.

Sample preparation strategies:

Common studies includes HPLC and UV spectroscopy. According to literature review commonly used mobile phase in HPLC were methanol: acetonitrile: phosphate buffer in varying ratios based on the mode of elution, either gradient or isocratic mode. In UV spectroscopy, methanol was the most widely used solvent because its absorbance may not interfere with the study results.

Analytical methods for the combined formulations

Chromatography:

HPLC

M Eswarudu, K venupriya et al[22]. developed and validated a simple,precise isocratic novel reversed-phase HPLC method for the simultaneous estimation of rosuvastatin and telmisartan in tablet dosage form. The chromatographic separation was carried out by using Thermo-C18 column. Mixture of acetonitrile and potassium dihydrogen phosphate buffer in the ratio 60:40(v/v) at flow rate of 1ml/min was used as the mobile phase. Detection wavelength was found to be at 231nm.linearity was observed in the concentration range of 2.52-15µg/ml and 10-60µg/ml for rosuvastatin and telmisartan respectively. Retention times were found to be 3.57min and 5.35min for rosuvastatin and telmisartan respectively. LOD and LOQ for rosuvastatinwas found to be 0.097µg/ml and 0.07µg/ml respectively and 0.0295µg/ml and 0.02µg/ml for telmisartan.

KetemaG[39] developed and validated RP-HPLC method for the simultaneous estimation of rosuvastatin calcium and telmisartan in bulk and pharmaceutical formulations. Stationary phase used was Phenomenex Luna C18 (250mm×4.6mm,5µm). mobile phase used was a mixture of 0.02M phosphate buffer at pH 4.8, acetonitrile and

methanol in the ratio 35:35:30(v/v/v). LOD and LOQ was found to be at 1.132 and 3.43µg/ml.and for telmisartan it was found to be at 0.727 and 2.204µg/ml. Linear concentration range were found to be 50-400µg/ml for rosuvastatin and 16-410µg/ml for telmisartan.

A simple , accurate and precise RPHPLC method for the simultaneous estimation of rosuvastatin calcium and telmisartan in marketed formulation was developed and validated Doshi N et al[38]. stationary phase employed was Inertsil ODS 3V C18 column (250×4.6mm,5µm). Mixture of ammonium dihydrogen phosphate (pH-3) buffer solution and methanol in the ratio 65:35v/v at a flow rate of 1.5ml/min. Detection wavelength was found to be at 298nm by using a UV detector. linearity was observed over the concentration range of 6-18µg/ml for rosuvastatin and 24-72µg/ml for telmisartan. Retention times were found tobe at 6.1min and 16.2min for rosuvastatin and telmisartan respectively.

HPTLC:

NirjuPatel[47] developed and validated a rapid, sensitive and specific thin layer chromatographic method for the simultaneous estimation of rosuvastatin calcium and telmisartan in a combined dosage form. HPTLC method was used for the simultaneous estimation ofrosuvastatin calcium and telmisartan in a fixed dose combination. TLC method were carried out using precoated silica gel on aluminium plate 60F 254 (10cm×10cm, prewashed with methanol and activated at 60℃ for 5min prior to chromatography). The solvent system used was toluene:methanol: glacial acetic acid in the proportion 8.5:1.5:0.1(v/v/v/v) and the R_f value for rosuvastatin and telmisartan was 0.29 and 0.39 respectively. The linearity regression analysis for rosuvastatin calcium and telmisartan was 0.996 and 0.998 respectively. And the linear concentration range was 4-20ng/band for rosuvastatin and 16-80ng/ml for telmisartan.

Spectrophotometry:

Few methods developed for the simultaneous estimation of rosuvastatin and telmisartan by UV spectroscopy are discussed in the table given below;

Table:3.2 Spectrophotometric method for the estimation of rosuvastatin and telmisartan

Author	Method	Solvent	λ_{max}	Linear range
Shah Binal B et al[40].	Difference spectrophotometric method	Methanol	ROSU:248nm TELM:309nm	20-60µg/ml
NamanDoshi et al[41].	spectrophotometric absorption factor method	Methanol	ROSU:244nm TELM:296nm	Rosu:2-7µg/ml Telm:4-14µg/ml.

CONCLUSON:

In this review, we have discussed a broad range of techniques for the analysis of Rosuvastatin and Telmisartan both individually and in combinations present in biological matrices and pharmaceutical formulations. A considerable number of methods are available for the quantification of ROS, either individually or in combinations. Various methods were reported for the estimation of TELM, either alone or in combinations present in pharmaceuticals and biological fluids. Among these methods spectrophotometric methods with UV-Visible detection and HPLC with UV detection is applicable for analysis of ROS and TELM or its combination in pharmaceutical formulations as these methods provide accurate results and low cost compared to more advanced detection techniques. HPLC-MS/MS method can be recommended for the quantification of ROS and TELM or its combination in biological samples, because of its greater selectivity and sensitivity when compared to other techniques.

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