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DEVELOPMENT AND VALIDATION OF A NOVEL ANALYTICAL METHOD FOR ESTIMATION OF TELMISARTAN IN TABLET DOSAGE FORMS USING HYDROTROPIC SOLUBILIZATION

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ABSTRACT

The objective of the present work was to explore the application of a hydrotropic solubilization phenomenon in the quantitative analysis of a model poorly water soluble (BCS Class II) drug. Other part of study was to develop and validate simple, easy, cost effective, eco-friendly unique spectrophotometric method of analysis for the novel poorly water soluble drug, Telmisartan, in pure and tablet dosage forms, utilizing the principle of single hydrotropic solubilization involving 20(M) Urea and 2(M) Sodium Benzoate. Solubility of Telmisartan in distilled water and each of the hydrotropes were determined and compared. The percentage label claims of Telmisartan in bulk and tablet forms were estimated by this novel spectrophotometric method. This method was validated for accuracy, linearity, precision, specificity and robustness as per recommendations in official guidelines. The study results revealed that the solubility of Telmisartan had been enhanced by more than 19 times in 20 (M) Urea and 11 times in 2(M) Sodium Benzoate respectively. The standard calibration curves were found obeying Beer-Lambert's principles as well as linearity at concentration range of 5 - 20 µg/ml (R^2 = 1 and % RSD < 2 both intra- and inter-day precision). Analysis of commercial tablets were found to contain drug content well within specified official label claim, (100.53% - 102.95%) of Telmisartan utilizing both the hydrotropes; while per cent recoveries in both the hydrotropes were found ranging from (100.13%-103.73%) - which was well within the specified official limit (90.0%-110%) of Pharmacoepoeal monograph of Telmisartan tablets (ref. USP 2011), even

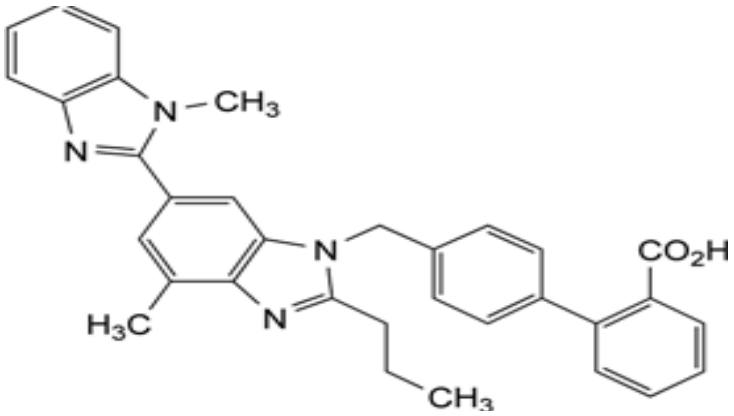
comparable to official HPLC method of analysis. %RSD and %CV were calculated and found < 2 with low standard error. LOD and LOQ were 0.575, 1.745 and 0.406, 1.231 in Urea and Sodium Benzoate respectively. In this assay method, there was no significant interference from any common pharmaceutical additives and diluents. The developed analytical method was proved as simple, specific, selective, reproducible, robust and validated for routine analysis of Telmisartan in pure and tablet dosage forms even without using costly and harmful organic solvents.

KEY WORDS: Angiotensin, ARB, BCS Class II, hydrotrope, LOD, LOQ, label claim.

INTRODUCTION

Telmisartan

Telmisartan is ***4'-[1,4'-dimethyl-2-propyl [2,6'-bi-benzimidazole]-1'-yl] methyl 1,1'-biphenyl 2-carboxylic acid*.** It is obtained as a white crystalline powder with melting point in the range of 221º C - 223º C. Telmisartan is practically insoluble in water; sparingly soluble in strong acid; soluble in strong bases.[1,2] Telmisartan prescribed for the treatment of essential hypertension is a new Angiotensin II receptor blocker that shows high affinity for the angiotensin II receptor type 1 (AT_1), with a binding affinity 3000 times greater for AT_1 than AT_2. It has the longest half-life of any ARB (24 hours) and the largest volume of distribution among ARBs (500 liters). It blocks the vasoconstrictor and aldosterone secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland.[2]



Chemical Structure of Telmisartan[1,2]

Hydrotropic Solubilization

Among the newly developed drug molecules, most of them are lipophilic in nature and poor solubility is one of the most difficult problems of these drugs for their analysis.[3,4] For spectrophotometric analysis the poorly water soluble drugs can be solubilized only in various organic solvents.[5,6] In order to avoid the usage of organic solvents, because of their toxicity, volatility and also high cost, attempts to develop alternative analytical methods have been explored so far. Lipophilic drugs having poor water solubility have encountered difficult problems during their spectrophotometric analysis in their formulation. Thus, it is necessary to enhance their aqueous solubility in order to facilitate their direct estimation in dosage forms or even dissolution testing. Among various methods available, HYDROTROPY is one of the advanced and most successful method,[5,7] in which aqueous solubility of poorly water soluble drugs is increased by co-dissolving with other highly water soluble inert substances. Such agents used to increase the solubility of poorly water soluble drug in aqueous medium are known as hydrotropic agent or HYDROTROPES like Sodium Benzoate, Niacinamide, Sodium Citrate, Sodium Acetate and Urea.[5,8-11] To preclude the use of organic solvents, hydrotropic solution may be a proper choice. Concentrated aqueous hydrotropic solutions of Sodium Benzoate, Sodium Salicylate, Urea, Nicotinamide, Sodium Citrate And Sodium Acetate have been observed to enhance the aqueous solubility of many poorly water-soluble drugs.[12,13,14] Maheswari et.al[4,6] have developed various analytical techniques using hydrotropic solubilization for analyzing many poorly water soluble drugs like Norfloxacin, Tinidazole, Ketoprofen etc.

Hydrotropy is the term originally put forward by Neuberg[15,16] to describe the increase in the solubility of a solute by the addition of fairly high concentrations of alkali metal salts of various organic acids. However, the term has been used in the literature to designate non-micelle-forming substances, either liquids or solids, organic or inorganic, capable of solubilizing insoluble compounds. The chemical structure of the conventional Neuberg hydrotropic salts (proto-type, sodium benzoate) consists generally of two essential parts, an anionic group and a hydrophobic aromatic ring or ring system. The anionic group is obviously involved in bringing about high aqueous solubility, which is a prerequisite for a hydrotropic substance. The type of anion or metal ion appeared to have a minor effect on the phenomenon.[16]

Advantages Of Hydrotropic Solubilization Technique [12,13,17,18]

1. Superior to other solubilization methods, such as miscibility, micellar solubilization, co-solvency and salting in, as the solvent character is independent of pH, highly selective and it does not need emulsification.
2. Simple mixing of the drug with the hydrotrope in water.
3. No chemical modification of hydrophobic drugs or preparation of emulsion system.
4. No use of any organic solvent

MATERIALS AND METHODS

Reagents and Chemicals

1. TELMISARTAN was obtained as free sample from M/s Auctus Pharma Ltd (Unit11).
2. Telmisartan 20 mg tablets of three different brands were purchased from different local retail pharmacies at Kolkata, India.
3. The following commercial tablets of Telmisartan 20 mg were taken for the study:

Drug product Information of Three Brands of Telmisartan Tablets (20mg)

SL NO.	BRAND NAME	BATCH NO.	MFG. DATE	EXP. DATE	PRICE/10 UNITS	MANUFACTURER
1	TELMA	18130466	Jul. 13	Jun. 16	Rs.75/15tab	Glenmarck Pharmaceuticals Ltd.
2	TAZLOC	48003034	Sep. 13	Aug. 15	Rs.42.50	USV Limited
3	TETAN	1301001501	Sep. 13	Aug. 15	Rs. 41.50	Alembic Pharmaceuticals Ltd.

All other chemicals and solvents used were of analytical grade. The reagents used were Methanol (AR), Ammonium Dihydrogen Phosphate (AR), HPLC grade water, Urea (GR), Sodium Benzoate (GR), Niacinamide (GR). Freshly prepared glass distilled water was used throughout the work.

Equipments and Instruments

1. UV Visible Spectrophotometer: Shimadzu UV-1560
2. Digital Balance: Mettler Toledo (ML204/A01)
3. High Performance Liquid Chromatography System : JASCO, JAPAN
4. HPLC Pump – JASCO, PU – 2080 Plus Intelligent HPLC Pump
5. HPLC Detector - JASCO, UV – 2075 Plus Intelligent UV/Vis Detector
6. HPLC Column - Column No. 044476; Hibar^{R} 250- 4,6; RP – 18e (5µm)
7. HPLC Software - Clarity Lite

Preliminary Solubility Study of Telmisartan in Hydrotropes

Solubility of Telmisartan was determined at 25±5°C. 10 mg of the drug was added to 50ml of each of Glass Distilled Water, 10(M) Urea solution, 20(M) Urea solution, 2(M) Niacinamide solution, 2(M) Sodium Benzoate solution respectively in separate stoppered conical flasks. The flasks were shaken mechanically for 48 hrs at 25±5°C, in a vibratory mechanical shaker. These solutions were next centrifuged for 5 mins at 2000 rpm. The supernatant of each conical flask was separately filtered through Whatman filter paper # 41. The individual filtrates were diluted suitably and analyzed spectrophotometrically against corresponding solvent blank. Enhancement ratio in solubility (Table 1) was also determined by following formula:

Enhancement ratio = Solubility of drug in hydrotropic solution / Solubility of drug in Distilled water. [19]

Preparation of Standard Calibration Curve of Telmisartan by Official HPLC Method

100 mg of Telmisartan pure drug was accurately weighed in a digital balance and transferred into a 100 ml volumetric flask. The drug substance was shaken to dissolve in Methanol of HPLC grade and the volume was made up to the mark with the Mobile Phase (Methanol : Buffer = 70:30). Then the solution was filtered through 0.45 µm membrane filter. From the resulting solution, further dilution was made in mobile phase to obtain a stock solution of 100 µg/ml. Aliquots from the above solution were taken and diluted further in mobile phase to have a set of final concentration e.g. 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 60µg/ml, 80µg/ml of Telmisartan respectively. Each of these drug solutions (20 µl) were injected into the column, and the peak area and retention time were recorded. Evaluation was performed with UV detector at 298 nm and a calibration curve (Fig. 2) was constructed for Telmisartan by plotting the peak area of drug (y - axis) against the concentration of drug (µg/ml) (x - axis).[1]

Preparation Of Standard Calibration Curve of Telmisartan by the Proposed Method

10mg of Telmisartan pure drug was accurately weighed and transferred into a 50ml volumetric flask. The drug was allowed to dissolve by shaking in 40ml of 20(M) Urea solution and the mixture was sonicated for 15mins. Then it was allowed to cool at room temperature and the volume was made up to the mark with glass distilled water to obtain a Telmisartan stock solution of concentration - 200µg/ml. The mixture was then kept at vibratory shaker incubator for 24 hrs. Aliquots from this solution were taken and diluted

further in glass distilled water to have a set of final concentration e.g. 4.2µg/ml, 8.4µg/ml, 10.5µg/ml, 14.7µg/ml of Telmisartan respectively. These solutions were scanned in the UV - range of 200-400nm against blank. The absorption maxima were found to be 315 nm against solvent blank (Fig. 3). A standard calibration curve (Fig. 4) was prepared for Telmisartan by plotting the absorbance of the drug (y- axis) at 315 nm against corresponding concentration of the drug (µg/ml) (x-axis).[19] The same procedure was repeated with 2(M) Sodium Benzoate. [20] (Fig. 5 & 6).

Analysis Of Telmisartan Commercial Tablets by the Official HPLC Method

Tablets of different manufacturer`s were procured from local market. Ten tablets from each of the three brands (TELMA 20, TAZLOC 20, TETAN 20) of Telmisartan 20mg tablets were separately weighed and finely powdered. The powdered tablets were passed through 60# sieve. An accurately weighed quantity of each lot of sieved powder equivalent to 100 mg of Telmisartan was transferred individually to 100 ml volumetric flasks. The mixtures were sonicated for 15mins and then allowed to cool at room temperature. The powdered drug product of each brand was allowed to mix & dissolve in Methanol (HPLC grade) and then the volume in each individual flask was adjusted up to the 100 ml mark with addition of mobile phase (Methanol : Buffer = 70:30). Then the respective solution of each brand from each individual flask was filtered through 0.45µm membrane filter. From each resulting filtrate, further dilution was made in mobile phase to obtain a stock solution of concentration - 100µg/ml. Aliquots from each respective solution were taken and diluted further in mobile phase to have a final set of concentration e.g. 20µg/ml, 40µg/ml, 60µg/ml of Telmisartan respectively. 20µl of each sample preparation of the respective three brands were injected into injector (column) of HPLC and the peak area and retention time were recorded. Evaluation was performed with UV detector at 298 nm. From the peak response of Telmisartan (TSN), the drug content in each individual sample of the respective brands was computed.[1]

Formula: $\%\ \text{of Sample} = (r_u/r_s) * (c_s/c_u)*100$

Where, r_u = peak response of TSN from the sample solution

r_s = peak response of TSN from the standard solution

c_s = Concentration of USP Telmisartan RS in the standard solution (mg/ml)

c_u = nominal concentration of TSN in the sample solution (mg/ml)

Analysis Of Telmisartan Commercial Tablets By The Proposed Method

Tablets of different manufacturer's were procured from local market. Ten tablets from each of the three brands (TELMA 20, TAZLOC 20, TETAN 20) of Telmisartan 20mg tablets were separately weighed and finely powdered. The powdered tablets were passed through 60# sieve. An accurately weighed quantity of each lot of sieved powder equivalent to 10mg of Telmisartan was transferred individually to 50ml volumetric flasks. To each of these flasks, 40ml of 20(M) Urea solution were added. The mixtures were sonicated for 15mins and then allowed to cool at room temperature. Then the volume in each individual flask was made up to 50 ml mark with glass distilled water to obtain a stock solution of concentration - 200µg/ml. The respective drug product solution of each brand was then kept at vibratory shaker incubator for 24hrs. Then the respective individual solution was filtered through Whatman filter paper # 41. From each of the individual filtrates, aliquot was taken and diluted further in glass distilled water to have a final set of concentration e.g. 8µg/ml, 12µg/ml, 16µg/ml of Telmisartan respectively. Finally, their absorbance was measured at 315nm against the respective reagent blank. The drug content of each branded preparation was calculated using the standard calibration curve.[19] The same procedure was repeated with 2(M) Sodium Benzoate.[20]

Recovery Study Of The Proposed Method

In order to check the accuracy, reproducibility and precision of the proposed method, recovery studies were conducted. Pre-analyzed tablet powder Brand I (TELMA) equivalent to 5 mg of Telmisartan was transferred in a 25 ml volumetric flask. In this flask, 2.5mg of pure drug substance, Telmisartan (corresponding spiked drug) was transferred. To this, 20 ml of 20(M) UREA solution was added and the flask was shaken about 5 min to solubilize the drug. Then the mixture was sonicated for 10mins and the volume was made up to the 25 ml mark with distilled water and filtered through Whatman filter paper # 41. The filtrate was diluted with distilled water appropriately and absorbance was noted at 315nm against corresponding reagent blank. Drug content was calculated and % recovery was estimated. Recovery studies using Brand II (TAZLOC) & Brand III (TETAN) were repeated in the same way. Drug contents were calculated and % recovery was estimated. The same procedure was carried out with 2(M) Sodium Benzoate. [21]

PROPOSED METHOD VALIDATION [19,22,23]

Accuracy

Accuracy of the developed method was confirmed by doing recovery study as per official guidelines. For the recovery studies of the proposed method, known amount of pure drug was added to the pre-analyzed powder sample and the mixture was analyzed for the drug content using the proposed method. The result of accuracy study was reported in [Table 5]. From the recovery study it was clear that the proposed method is *Accurate* for quantitative estimation of Telmisartan in tablet dosage form.

Precision

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated. Repeatability was performed for three times with commercial tablets of Telmisartan. The results of % relative standard deviation are given in [Table 6]. Intermediate Precision was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing the Telmisartan commercial tablets for three times using the concentration range of (8-16) µg/ml in the same day. Inter-day precision was determined by analyzing the same concentration range of solutions daily for three days and the results were recorded. The %RSD of intra-day and inter-day precision was determined and reported in [Table 7&8]. From the data obtained, the developed spectroscopic method was found to be *Precise and Accurate*.

Specificity

The specificity of the proposed method was checked for the interference of impurities in the analysis of a drug solution. It was determined by comparing the UV spectra obtained from 100 µg/ml solution of Telmisartan in pure and commercial samples in order to assess the level of interference from excipients and additives used in making commercial dosage forms of the drug. The method was found to be specific as there was no interference of impurities.

Linearity and Range

The linearity of an analytical procedure is its ability to obtain test results which are proportional to the concentration of analyte in the sample. In this study, three independent serially diluted volumes of stock solution of Telmisartan were prepared in each of the hydrotropes and each solution was analyzed in triplicate to obtain the linear calibration curve.

The Beer- Lambert's concentration range was found to be (5-20) µg/ml for Telmisartan in both hydrotropes. [Fig.4 & 6, Table 9]

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as **3.3** ***∂/S*** and **10** ***∂/S***, respectively as per ICH guidelines, where ***∂*** is the standard deviation of the response and ***S*** is the slope of the calibration plot. The LOD is the smallest concentration of the analyte that gives a measurable response. The LOQ is the smallest concentration of the analyte which gives response that can be quantified accurately. The result of accuracy study was reported in [Table 5]

Ruggedness

It was determined by carrying out the experiment on different instruments and by different operators. It was observed that there were no marked changes in the results, which demonstrated that the spectroscopic method developed, are rugged and robust.

RESULTS

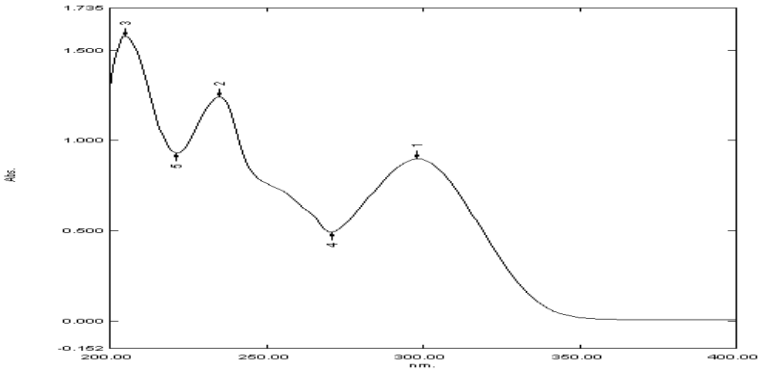


Fig. 1 U.V Spectra of Telmisartan in Methanol

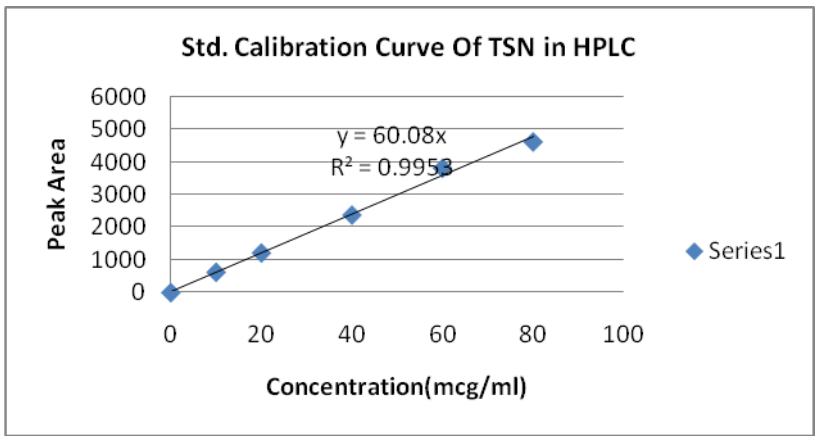


Fig. 2. Standard Calibration Curve of Telmisartan in Methanol Using HPLC

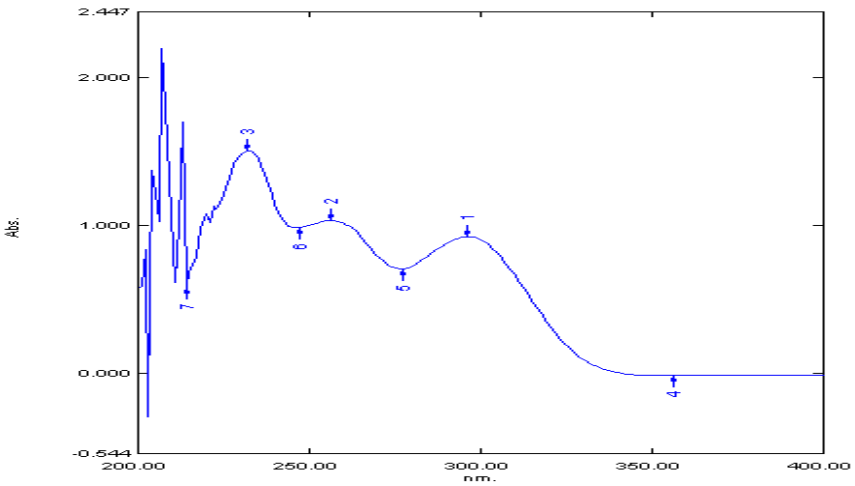


Fig. 3 U.V Spectra Of Telmisartan In 20(M)Urea

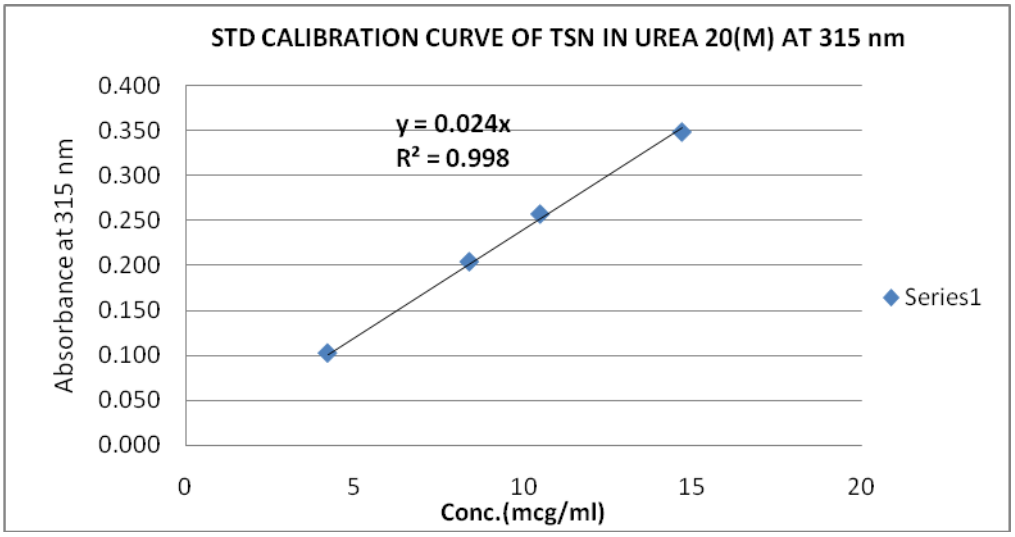


Fig. 4 Standard Calibration Curve of Telmisartan in 20(M)Urea at 315nm

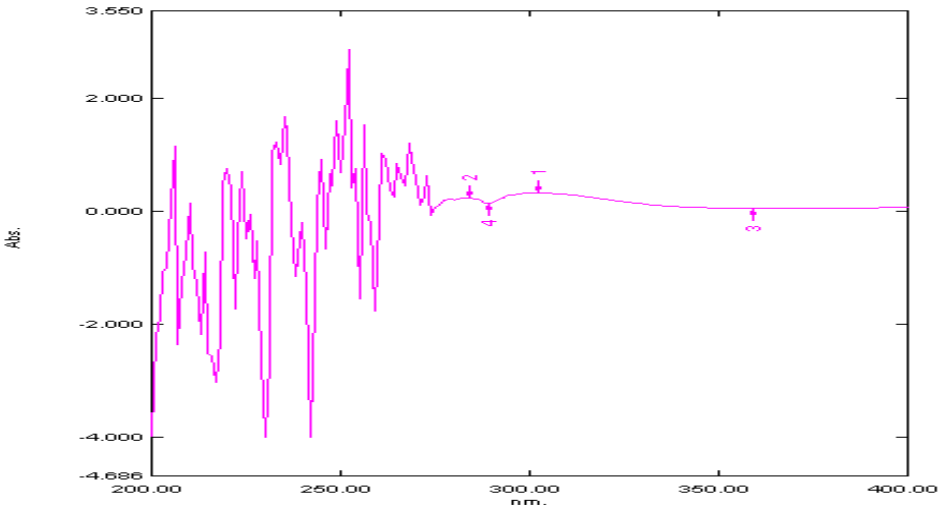


Fig. 5 U.V Spectra Of Telmisartan in 2(M) Sodium Benzoate

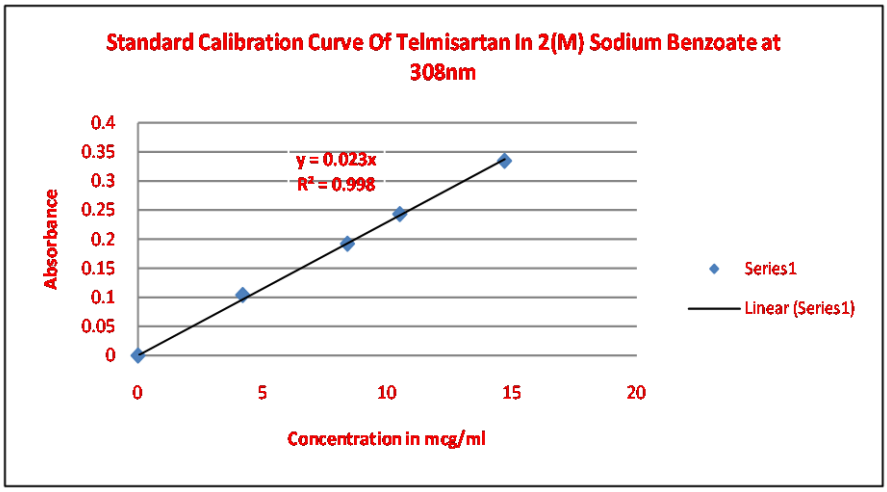


Fig. 6 Standard Calibration Curve of Telmisartan in 2(M) Sodium Benzoate at 308nm

Table 1: Solubility of Telmisartan In Different Hydrotropes

Drug	Solvent System*	Solubility (mg/ml)	Temperature (^{0}C)	Enhanced By
Telmisartan	DW	0.452	25±5°C	
Telmisartan	10(M)UR	5.309	25±5°C	11.74
Telmisartan	DW	0.452	25±5°C	_
Telmisartan	20(M) UR	8.856	25±5°C	19.59
Telmisartan	DW	0.452	25±5°C	_
Telmisartan	2(M)NIA	9.417	25±5°C	20.83
Telmisartan	DW	0.452	25±5°C	_
Telmisartan	2(M)SB	5.113	25±5°C	11.31

*DW- Distilled Water, UR- Urea, NIA- Niacinamide, SB- Sodium Benzoate

Table 2: Drug Content of Telmisartan brands using HPLC (as per official method in USP)

Brand	Label Claim (mg)	% Label Claim Estimated* (Mean ± S.D)	% Co-eff. Of Variation	Standard Error
TELMA	20	99.66 ± 1.36	1.37	0.79
TAZLOC	20	99.30 ± 1.38	1.39	0.80
TETAN	20	99.26 ± 1.25	1.26	0.72

*Average of six determinations

Table 3: Drug Content of Telmisartan brands with 20(M) Urea at 315nm

Brand	Label Claim (mg)	% Label Claim Estimated* (Mean ± S.D)	% Co-eff. Of Variation	Standard Error
TELMA	20	102.76 ± 0.66	0.65	0.38
TAZLOC	20	102.95 ± 0.95	0.93	0.55
TETAN	20	101.33 ± 1.46	1.45	0.85

*Average of six determinations

Table 4: Drug Content of Telmisartan brands with 2(M) Sodium Benzoate at 308nm

Brand	Label Claim (mg)	% Label Claim Estimated* (Mean ± S.D)	% Coeff. Of Variation	Standard Error
TELMA	20	100.68 ± 1.17	1.16	0.68
TAZLOC	20	100.53 ± 1.05	1.05	0.61
TETAN	20	101.38 ± 2.42	1.39	1.4

*Average of six determinations

Table 5: Recovery Studies For Commercial Tablets Of Telmisartan

Hydrotrope	Brand	Label Claim (mg)	Eqv. Amt taken for analysis (mg)	Spike amt of drug added (mg)	Derived amt of drug after analysis Estimated* (Mean±S.D)	% Recovery by analysis
20(M) UREA at 315nm	TELMA	20	5.02	2.5	7.77 ± 0.163	103.32
	TAZLOC	20	5.01	2.5	7.78 ± 0.107	103.60
	TETAN	20	5.09	2.5	7.59 ± 0.141	100.00
2(M) SODIUM BENZOATE at 308nm	TELMA	20	5.04	2.5	7.74 ± 0.178	102.65
	TAZLOC	20	5.01	2.5	7.79 ± 0.156	103.73
	TETAN	20	5.11	2.5	7.62 ± 0.053	100.13

*Average of six determinations

Table 6: Data For Repeatability For Commercial Tablets Of Telmisartan

	UREA AS HYDROTROPE						SODIUM BENZOATE AS HYDROTROPE					
Conc. (mcg/ml)	TAZLOC 20						TAZLOC 20					
	ABSORBANCE AT 315 nm			MEAN	SD	RSD%	ABSORBANCE AT 308 nm			MEAN	SD	RSD%
8	0.202	0.207	0.205	0.20	0.003	1.23	0.195	0.196	0.196	0.20	0.001	0.30
12	0.311	0.313	0.312	0.31	0.001	0.32	0.286	0.289	0.288	0.29	0.002	0.53
16	0.402	0.417	0.409	0.41	0.008	1.83	0.384	0.387	0.386	0.39	0.002	0.40
TELMA 20							TELMA 20					
	ABSORBANCE AT 315 nm			MEAN	SD	RSD%	ABSORBANCE AT 308 nm			MEAN	SD	RSD%
8	0.2	0.196	0.198	0.20	0.002	1.01	0.18	0.186	0.183	0.18	0.003	1.64
12	0.3	0.29	0.294	0.29	0.005	1.71	0.275	0.274	0.281	0.28	0.004	1.37
16	0.395	0.398	0.397	0.40	0.002	0.39	0.378	0.365	0.372	0.37	0.007	1.75
TETAN 20							TETAN 20					
	ABSORBANCE AT 315 nm			MEAN	SD	RSD%	ABSORBANCE AT 308 nm			MEAN	SD	RSD%
8	0.19	0.19	0.194	0.19	0.002	1.21	0.188	0.186	0.183	0.19	0.003	1.36
10	0.253	0.245	0.249	0.25	0.004	1.61	0.235	0.234	0.234	0.23	0.001	0.25
14	0.338	0.341	0.339	0.34	0.002	0.45	0.335	0.338	0.336	0.34	0.002	0.45

Table 7.1 : Data for Intra-day precision for Commercial Brands of Telmisartan tablets with 20(M)Urea as Hydrotrpe

Conc. (mcg/ml)	TAZLOC 20						TAZLOC 20					
	ABS. 2ND HR AT 315 nm			MEAN	SD	RSD%	ABS. 4TH HR. AT 315 nm			MEAN	SD	RSD%
8	0.201	0.204	0.204	0.20	0.002	0.85	0.201	0.208	0.205	0.20	0.004	1.72
12	0.32	0.31	0.31	0.31	0.006	1.84	0.309	0.315	0.312	0.31	0.003	0.96
16	0.401	0.41	0.409	0.41	0.005	1.21	0.405	0.412	0.409	0.41	0.004	0.86
TELMA 20							TELMA 20					
	ABS. 2ND HR AT 315 nm			MEAN	SD	RSD%	ABS. 4TH HR. AT 315 nm			MEAN	SD	RSD%
8	0.198	0.196	0.198	0.20	0.001	0.59	0.2	0.198	0.198	0.20	0.001	0.58
12	0.291	0.292	0.294	0.29	0.002	0.52	0.274	0.276	0.28	0.28	0.003	1.10
16	0.394	0.396	0.397	0.40	0.002	0.39	0.398	0.398	0.397	0.40	0.001	0.15
TETAN 20							TETAN 20					
	ABS. 2ND HR AT 315 nm			MEAN	SD	RSD%	ABS. 4TH HR. AT 315 nm			MEAN	SD	RSD%
8	0.188	0.189	0.194	0.19	0.003	1.69	0.197	0.195	0.198	0.20	0.002	0.78
10	0.252	0.243	0.249	0.25	0.005	1.85	0.279	0.28	0.286	0.28	0.004	1.34
14	0.336	0.343	0.339	0.34	0.004	1.03	0.391	0.397	0.397	0.40	0.003	0.88

	TAZLOC 20					
	ABS. 6TH HR. AT 315 nm			MEAN	SD	RSD%
8	0.188	0.189	0.194	0.19	0.003	1.69
12	0.252	0.243	0.249	0.25	0.005	1.85
16	0.336	0.343	0.339	0.34	0.004	1.03
	TELMA 20					
	ABS. 6TH HR. AT 315 nm			MEAN	SD	RSD%
8	0.189	0.19	0.194	0.19	0.003	1.39
12	0.252	0.247	0.249	0.25	0.003	1.01
16	0.332	0.342	0.339	0.34	0.005	1.52
	TETAN 20					
	ABS. 6TH HR. AT 315 nm			MEAN	SD	RSD%
8	0.19	0.189	0.194	0.19	0.003	1.39
10	0.255	0.248	0.249	0.25	0.004	1.51
14	0.334	0.342	0.339	0.34	0.004	1.19

Table 7.2 : Data for Intra-day precision for Commercial Brands of Telmisartan tablets with 2(M)Sodium Benzoate as Hydrotrope

Conc. (mcg/ml)	TAZLOC 20						TAZLOC 20					
	ABS. 2ND HR AT 308 nm			MEAN	SD	RSD%	ABS. 4TH HR. AT 308 nm			MEAN	SD	RSD%
8	0.193	0.199	0.196	0.20	0.003	1.53	0.19	0.196	0.196	0.19	0.003	1.79
12	0.283	0.29	0.288	0.29	0.004	1.26	0.287	0.289	0.288	0.29	0.001	0.35
16	0.38	0.385	0.386	0.38	0.003	0.84	0.384	0.387	0.386	0.39	0.002	0.40
TELMA 20							TELMA 20					
	ABS. 2ND HR AT 308 nm			MEAN	SD	RSD%	ABS. 4TH HR. AT 308 nm			MEAN	SD	RSD%
8	0.181	0.185	0.183	0.18	0.002	1.09	0.181	0.186	0.183	0.18	0.003	1.37
12	0.272	0.276	0.281	0.28	0.005	1.63	0.271	0.274	0.281	0.28	0.005	1.86
16	0.375	0.364	0.372	0.37	0.006	1.54	0.373	0.363	0.372	0.37	0.006	1.49
TETAN 20							TETAN 20					
	ABS. 2ND HR AT 308 nm			MEAN	SD	RSD%	ABS. 4TH HR. AT 308 nm			MEAN	SD	RSD%
8	0.182	0.186	0.183	0.18	0.002	1.13	0.185	0.186	0.183	0.18	0.002	0.83
10	0.23	0.232	0.234	0.23	0.002	0.86	0.233	0.236	0.234	0.23	0.002	0.65
14	0.332	0.336	0.336	0.33	0.002	0.69	0.33	0.335	0.336	0.33	0.003	0.96

Conc. (mcg/ml)	TAZLOC 20					
	ABS. 6TH HR. AT 308 nm			MEAN	SD	RSD%
8	0.19	0.197	0.196	0.19	0.004	1.95
12	0.285	0.288	0.288	0.29	0.002	0.60
16	0.381	0.388	0.386	0.39	0.004	0.94
	TELMA 20					
	ABS. 6TH HR. AT 308 nm			MEAN	SD	RSD%
8	0.183	0.185	0.183	0.18	0.001	0.63
12	0.273	0.273	0.281	0.28	0.005	1.68
16	0.374	0.365	0.372	0.37	0.005	1.28
	TETAN 20					
	ABS. 6TH HR. AT 308 nm			MEAN	SD	RSD%
8	0.181	0.187	0.183	0.18	0.003	1.66
10	0.231	0.235	0.234	0.23	0.002	0.89
14	0.333	0.337	0.336	0.34	0.002	0.62

Table 8.1 : Data for Inter-day precision for Commercial Brands Of Telmisartan tablets with 20(M) Urea

Conc. (mcg/ml)	TAZLOC 20						TAZLOC 20					
	ABS. 1ST DAY AT 315 nm			MEAN	SD	RSD%	ABS. 2ND DAY AT 315 nm			MEAN	SD	RSD%
8	0.203	0.206	0.204	0.20	0.002	0.75	0.202	0.207	0.205	0.20	0.003	**1.23**
12	0.31	0.311	0.31	0.31	0.001	0.20	0.311	0.313	0.312	0.31	0.001	**0.32**
16	0.401	0.41	0.409	0.41	0.005	1.21	0.402	0.41	0.409	0.41	0.004	**1.07**
TELMA 20							TELMA 20					
	ABS. 1ST DAY AT 315 nm			MEAN	SD	RSD%	ABS. 2ND DAY AT 315 nm			MEAN	SD	**RSD%**
8	0.2	0.196	0.198	0.20	0.002	1.01	0.2	0.196	0.198	0.20	0.002	**1.01**
12	0.292	0.29	0.294	0.29	0.002	0.68	0.271	0.272	0.28	0.27	0.005	**1.80**
16	0.395	0.398	0.397	0.40	0.002	0.39	0.395	0.398	0.397	0.40	0.002	**0.39**
TETAN 20							TETAN 20					
	ABS. 1ST DAY AT 315 nm			MEAN	SD	RSD%	ABS. 2ND DAY AT 315 nm			MEAN	SD	**RSD%**
8	0.188	0.19	0.194	0.19	0.003	1.60	0.188	0.19	0.194	0.19	0.003	**1.60**
10	0.253	0.245	0.249	0.25	0.004	1.61	0.253	0.245	0.249	0.25	0.004	**1.61**
14	0.338	0.341	0.339	0.34	0.002	0.45	0.338	0.341	0.339	0.34	0.002	**0.45**
Conc. (mcg/ml)	TAZLOC 20											
	ABS. 3RD DAY AT 315 nm			MEAN	SD	RSD%						
8	0.202	0.207	0.205	0.20	0.003	1.23						
12	0.311	0.313	0.312	0.31	0.001	0.32						
16	0.402	0.417	0.409	0.41	0.008	1.83						
	TELMA 20											
	ABS. 3RD DAY AT 315 nm			MEAN	SD	RSD%						
8	0.2	0.196	0.198	0.20	0.002	1.01						
12	0.276	0.28	0.286	0.28	0.005	1.79						
16	0.395	0.398	0.397	0.40	0.002	0.39						
	TETAN 20											
	ABS. 3RD DAY AT 315 nm			MEAN	SD	RSD%						
8	0.188	0.189	0.194	0.19	0.003	1.69						
10	0.253	0.245	0.249	0.25	0.004	1.61						
14	0.338	0.341	0.339	0.34	0.002	0.45						

Table 8.2 : Inter-day precision For Commercial Brands Of Telmisartan With 2(M) Sodium Benzoate

Conc. (mcg/ml)	TAZLOC 20						TAZLOC 20					
	ABS. 1ST DAY AT 308 nm			MEAN	SD	RSD%	ABS. 2ND DAY AT 308 nm			MEAN	SD	RSD%
8	0.194	0.199	0.196	0.20	0.003	1.28	0.193	0.196	0.196	0.20	0.002	0.89
12	0.285	0.29	0.288	0.29	0.003	0.87	0.285	0.289	0.288	0.29	0.002	0.72
16	0.381	0.385	0.386	0.38	0.003	0.69	0.386	0.387	0.386	0.39	0.001	0.15
TELMA 20							TELMA 20					
	ABS. 1ST DAY AT 308 nm			MEAN	SD	RSD%	ABS. 2ND DAY AT 308 nm			MEAN	SD	RSD%
8	0.182	0.187	0.183	0.18	0.003	1.44	0.184	0.186	0.183	0.18	0.002	0.83
12	0.273	0.274	0.281	0.28	0.004	1.58	0.271	0.274	0.281	0.28	0.005	1.86
16	0.376	0.364	0.372	0.37	0.006	1.65	0.375	0.363	0.372	0.37	0.006	1.69
TETAN 20							TETAN 20					
	ABS. 1ST DAY AT 308 nm			MEAN	SD	RSD%	ABS. 2ND DAY AT 308 nm			MEAN	SD	RSD%
8	0.184	0.186	0.183	0.18	0.002	0.83	0.187	0.186	0.183	0.19	0.002	1.12
10	0.232	0.234	0.234	0.23	0.001	0.49	0.232	0.236	0.234	0.23	0.002	0.85
14	0.331	0.336	0.336	0.33	0.003	0.86	0.33	0.337	0.336	0.33	0.004	1.13
Conc. (mcg/ml)	TAZLOC 20											
	ABS. 3RD DAY AT 308 nm			MEAN	SD	RSD%						
8	0.192	0.196	0.196	0.19	0.002	1.19						
12	0.283	0.289	0.288	0.29	0.003	1.12						
16	0.381	0.388	0.386	0.39	0.004	0.94						
	TELMA 20											
	ABS. 3RD DAY AT 308 nm			MEAN	SD	RSD%						
8	0.181	0.185	0.183	0.18	0.002	1.09						
12	0.272	0.275	0.281	0.28	0.005	1.66						
16	0.377	0.365	0.372	0.37	0.006	1.62						
	TETAN 20											
	ABS. 3RD DAY AT 308 nm			MEAN	SD	RSD%						
8	0.188	0.189	0.183	0.19	0.003	1.72						
10	0.234	0.238	0.234	0.24	0.002	0.98						
14	0.33	0.332	0.336	0.33	0.003	0.92						

Table 9 : Validation Parameters & Optical Characteristics of The Proposed Method

Parameters	20(M) Urea	2(M) Sodium Benzoate
Established λ_{max} (nm)	315nm	308nm
Beer's limit (µg/ml) (Linearity)	(5-20)	(5-20)
Correlation Coefficient (r^2)*	1	1
Regression Equation*	0.0235x+0.0055	0.0221x+0.01
Intercept*	0.0055	0.01
Slope*	0.0235	0.0221
LOD*(µg/ml)	0.575	0.406
LOQ*(µg/ml)	1.745	1.231
Intra-day* (CV)	0.15-1.85 %	0.35-1.95 %
Inter-day* (CV)	0.20-1.83 %	0.15-1.86 %
Robustness	Robust (% RSD < 2)	(% RSD < 2)
Ruggedness	Rugged	Rugged

*Mean of 6 determinations; CV= Coefficient of Variation; RSD= Relative standard deviation

Table 10: Comparison of drug content of Telmisartan brands using Official Method (as per USP) vs the Developed Method

LABEL CLAIM (mg)	BRAND	% LABEL CLAIM ESTIMATED			VARIANCE	MEAN	STD DEV
		HPLC	UREA	SOD BENZ	%		
20	TELMA	99.66	102.76	100.68	2.5	2.47	0.995
20	TAZLOC	99.3	102.95	100.53	3.45		
20	TETAN	99.26	101.33	101.38	1.46		

Table 11: Calculation of ANNOVA and VARIANCE between different brands of Telmisartan 20mg tablets

Brands	Sample Count	Sum	Avg. % Estimated	Variance Within Brand
TELMA	3	303.1	101.03	2.50
TAZLOC	3	302.78	100.92	3.45
TETAN	3	301.97	100.65	1.46

Source of Variation	SS	df= (N-1)	MS=SS/df	$F_{calculated}$	P- value	F_{CV}
Between Brands	0.22	2	0.11	**0.045**	0.95	**5.14**
Within Brands	14.81	6	2.46			
Total Variation	15.04	8				

The Critical Value of F

To find the critical value from an F distribution, the numerator (MSTR) and denominator (MSE) degrees of freedom, along with the significance level. F_{CV} has df1 and df2 degrees of freedom, where df1 is the numerator degrees of freedom equal to C-1 and df2 is the denominator degrees of freedom equal to N-C. [C = no of brands (3) & N = Total nos. of analytical methods applied (3 concentration ranges of each of three brands)] In our example, df1 = (3 – 1) = 2 and df2 = (3*3 – 3) = 6. Hence we need to find F $CV_{2,6}$ corresponding to **α** = 5%. [Using the F tables in reference text, it was observed that F $CV_{2,6}$ = 5.143253]

Decision Rule

We reject the null hypothesis if: F (observed value) > F_{CV} (critical value).

We accept the null hypothesis if: F (observed value) < F_{CV} (critical value).

Final decision

In this case, F (observed value) is 0.46 and F_{CV} (critical value) is 5.14. Hence we accept the Hypothesis that there is significant similarity between the analytical methods applied with P value <1.

DISCUSSION

Based on the solubility enhancement, stability and spectral characteristics of the drug Telmisartan; 20(M) Urea and 2(M) Sodium Benzoate were selected as hydrotropic agents. The results of solubility study of Telmisartan in distilled water and hydrotropic agents showed that the solubility enhancement were found to be more than 19 fold in Urea and 11 fold in Sodium Benzoate [Table 1]. After solubilizing the Telmisartan in selected hydrotropic agents, it was scanned in spectrum mode and the working wavelength for the estimation, were found to be 315nm and 308nm for Urea and Sodium Benzoate respectively. Spectra of Telmisartan with Urea and Sodium Benzoate are shown in Figure 3 and Figure 5 respectively. Calibration curve of Telmisartan were plotted between concentrations versus absorbance with Urea [Fig. 4] and with Sodium Benzoate [Fig. 6]. Apart from these hydrotropes, the drug was also scanned with Methanol and a calibration curve was prepared following the Official Pharmacoepial method using HPLC [Fig. 1 &2]. [Table 2] shows the results of analysis of Telmisartan in commercial formulations by the Official Pharmacoepial Method using HPLC. [Table 3 & 4] shows the results of analysis of Telmisartan in commercial formulations using the developed method. As evident from [Table 3], Assay of

Drug content was estimated using the proposed method which had been ranged as percent label claim from 101.33 ± 1.46 to 102.95 ± 0.95 and from [Table 4], it was found that ranged from 101.38 ± 2.42 to 100.68 ± 1.17. Since the % label claims are within specified limit (90.0% to 110%) of official Pharmacopoeal monograph of Telmisartan tablets (ref. USP 2011), the proposed method can be described as accurate and gets further validated statistically by low values of standard deviation, % coefficient of variation and standard error. The result of recovery studies (presented in Table 5) indicates that % recovery estimated with Urea ranged from (100% to 103.60%) and that with Sodium Benzoate ranged from (100.13% to 103.73%) by use of proposed method. Since the percent recovery values are within specified limit (90.0% to 110%) of official Pharmacopoeal monograph of Telmisartan tablets (ref. USP 2011), this indicates the accuracy of the proposed method. Values of standard deviation, % coefficient of variation and standard error are satisfactorily low and confirm further the accuracy, reproducibility, precision of the proposed method. Results for repeatability and precision (intra and inter day) were presented in [Table 6-8]. [Table 9] shows the optical characteristics of the proposed method. [Table10] presents a comparative study for the analysis of commercial brands of Telmisartan using the Official Pharmacopoeal Method with that of the developed method. [Table 11&12] represents the results for the Annova Single Factor and Variance between the commercial brands of Telmisartan comparing the official method of analysis with that of the developed method which concludes that the methods are significant. From this study, it is obvious that there was no interference of Urea and Sodium Benzoate in the estimation of Telmisartan (λ-max–298 nm). Urea and Sodium Benzoate does not interfere above 250 nm and 300 nm respectively. Just like Telmisartan as model drug (poorly water soluble or practically insoluble), a large number of poorly water-soluble drugs having λ-max above 300 nm may be tried for estimation by the proposed method provided that their preliminary solubility studies are conducted to observe the enhancement effect on solubility in order to preclude the use of organic solvents.

CONCLUSION

Thus, it may be concluded that the proposed method of analysis of Telmisartan in tablet dosage form using Urea and Sodium Benzoate as the hydrotropic solubilizing agents is new, simple, cost-effective, environmentally friendly, safe, accurate and reproducible. As seen from recovery studies, Urea and the commonly used tablet excipients did not interfere in Spectrophotometric estimation at 315 nm. Sodium Benzoate also did not interfere in Spectrophotometric estimation at 308nm. Decided advantage is that organic solvents are

precluded but not at the expense of accuracy. The proposed method is worth adopting in Pharmacopoea. By proper choice of hydrotropic agents, the use of organic solvents in analysis may be discouraged to a large extent. The proposed method shall prove equally effective to analyze Telmisartan in the corresponding drug sample and may prove to be of great importance in pharmaceutical analysis. There is a good scope for other poorly water soluble drugs which may be tried to get solubilized by suitable hydrotropic agents to carry out their spectrophotometric analysis excluding the use of costlier and unsafe organic solvents.

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