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“LC-MS Method Development and Quantification by LC-MS in Marketed Formulations”.

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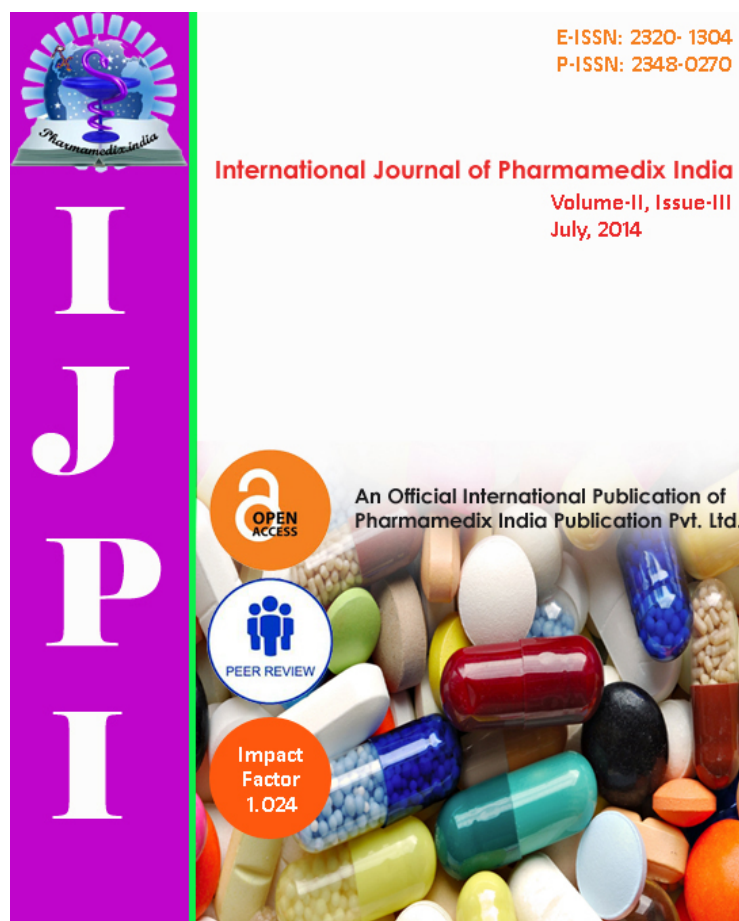
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Abstract:

A simple, sensitive method has been developed for the quantitative estimation of Telmisartan (TELM) in the pharmaceutical formulations. Chromatographic separation was achieved on Merck chromolith RP18 column (25mmX4.6mm). The flow rate was 0.5 ml/min and the injection volume was 5µL. An isocratic programme was used as ACN: water (0.1% formic acid, v/v); in the ratio, 55:45). The retention time of Telmisartan was found to be 4.12 min. Calibration plots were linear and correlation coefficient (R^2) 0.997. The proposed method was validated by testing its linearity, recovery and LOD/LOQ values and it was successfully employed for the determination of Telmisartan in pharmaceutical tablet formulations.

Keyword: LCMS, Acetonitrile, Telmisartan, formic acid.

Introduction

Telmisartan (TELM) chemically described as 4[(1,4-dimethyl-2-propyl(2,6-bis-1H-benzimidazol-1-yl)methyl)[1,1-biphenyl]-2-carboxylic acid is a potent, long-lasting, nonpeptide antagonist of the angiotensin II (AT1) receptor that is indicated for the treatment of essential hypertension. It selectively and insurmountably inhibits stimulation of the AT1 receptor by angiotensin II without affecting other receptor systems involved in cardiovascular regulation. In clinical studies, TELM shows comparable antihypertensive activity to other major antihypertensive classes, such as angiotensin converting enzyme (ACE) inhibitors, beta-blockers and calcium antagonists¹⁻².

Telmisartan is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Generally, angiotensin II receptor blockers (ARBs) such as telmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to a reduction in arterial blood pressure. Recent studies suggest that telmisartan may also have PPAR-gamma agonistic properties that could potentially confer beneficial metabolic effects. The long elimination half-life of telmisartan ensures the drug provides effective reductions in blood pressure (BP) across the entire 24-hour dosage interval. Extensive evidence from well designed clinical trials and the clinical practice setting indicates that telmisartan, either as

monotherapy or in combination with other antihypertensive agents, provides long-term antihypertensive efficacy and is well tolerated in a broad spectrum of hypertensive patients, including the elderly and those with coexisting type 2 diabetes mellitus, metabolic syndrome and/or renal impairment. Notably, BP control is sustained throughout the 24-hour dosage interval, including during the last 6 hours of this period. The consistent antihypertensive efficacy during the entire 24-hour dosage interval and sustained BP-lowering effect in the long term, combined with its favourable tolerability profile, mean that telmisartan is a valuable first-line treatment option for the management of essential hypertension.³⁻⁶ Telmisartan is estimated by LC-MS⁷ LC-tandem MS⁸⁻⁹

Experimental:

Materials and Method

A rapid and sensitive liquid chromatography-tandem mass spectrometry method has been developed for the identification and quantification of Telmisartan in the formulation Telmikin-40mg.

Reagents and Materials

MS grade Methanol, MS grade ACN, Millipore water, analytical grade formic acid and Standard drug Telmisartan and Borosil glassware were used.

Stock Solution(1000ppm)- Stock solution was prepared by accurately weighing 10 mg of standard drug, and dissolving in a 10 ml of methanol, in a volumetric flask.

Sample Preparation- Samples of Telmisartan were prepared by taking 0.01 ml of stock solution in 10ml volumetric flask and making up the volume with methanol to obtain 1ppm, i.e. 1000ppb (ng/ml) of standard solution. Further solutions of a concentration range, 2500, 1000, 500, 250, 100, 50, 10 and 5 ppb were prepared similarly from the stock.

NOTE- All standard solutions were filtered through a 0.1µm PTFE disposal filter (Whatman) and injected directly. The stock and working solutions were stored at +4°C.

METHOD- An isocratic programme was used as, ACN : water (0.1% formic acid, v/v); in the ratio, 55:45. The flow rate was monitored at 0.3mL/min and the injection volume was 5µL and column temperature was maintained at 30°C. A 6410B triple quad LC/MS system from Agilent was used for the detection, which is a hybrid triple quadrupole mass spectrometer equipped with Turbo V sources. The analyses were performed using an electrospray ionization (ESI) source in positive and negative modes. Nitrogen was used in all cases. The dwell time was set at 100ms. (Telmisartan in positive mode). Agilent Mass Hunter software (version B.04.00) was used for data acquisition and processing.

METHOD PARAMETERS

The operating conditions were as follows:

Scan range -100–600 amu, **Gas Temperature**

325°C, **Nebulizer** -60psi, **Gas flow**- 12 L/min

Capillary voltage- 4000V.

INSTRUMENT DETAILS

LC-MS USED- Agilent 1260 liquid chromatography system (Agilent, USA)- Equipped with a quaternary solvent delivery system, an autosampler and a column compartment was used.

SOFTWARE USED- Agilent Technologies MASS HUNTER

LC-MS PARAMETERS

Mobile phase A- water (0.1% formic acid,v/v)

B- Acetonitrile

Column used- Merck chromolith RP18column (25mmX4.6mm) protected by a chromolith guard column of the same company

Oven temp.- 30°C,**Injection volume**- 5

µl,**Flow rate**- 0.5 ml/min,**Flow condition**-

Isocratic program : **Solvent ratio A**- 45%,**Solvent ratio B** -55%

Optimization of MS parameter

The precursor and product ion of reference compounds were selected by infusing individual standard solutions into the mass spectrometer using HPLC system. In the MS1 scan mass spectra, most analytes formed protonated molecular ions $[M+H]^+$. So these predominant precursor ions of analyte were chosen for MS/MS analysis.

Several fragment ions of the analytes were observed in the product ion spectra and the predominant fragment ions were selected in MRM acquisition for quantification. Then fragmentor and CE were optimized in order to obtain the maximum sensitivity of analysis. Both negative and positive ion modes were conducted, which showed that the positive ion mode was more sensitive for Telmisartan.

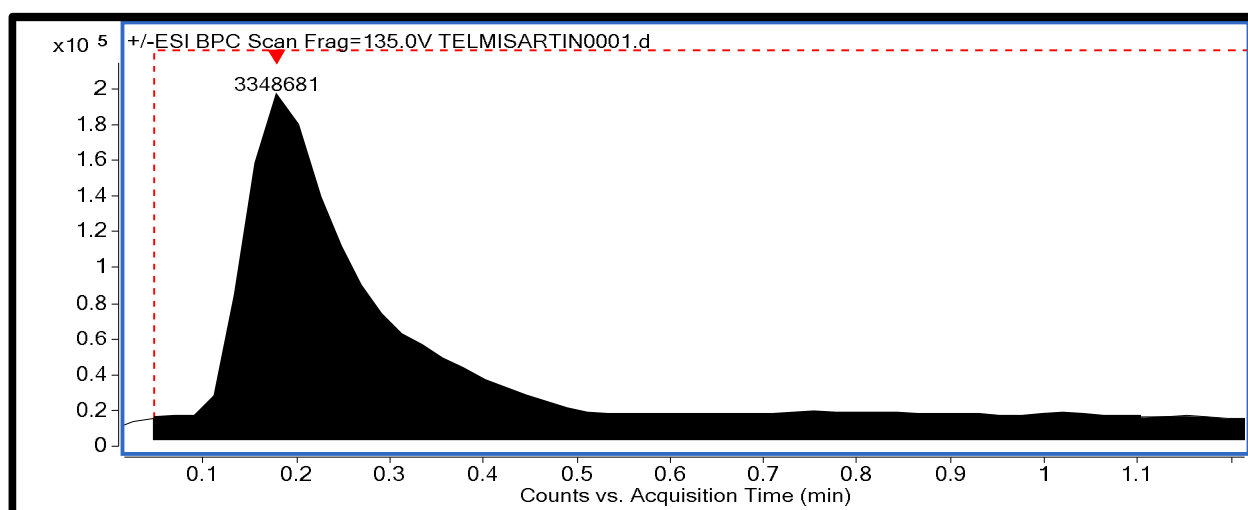


Fig.(a) MS2 scan of Telmisartan

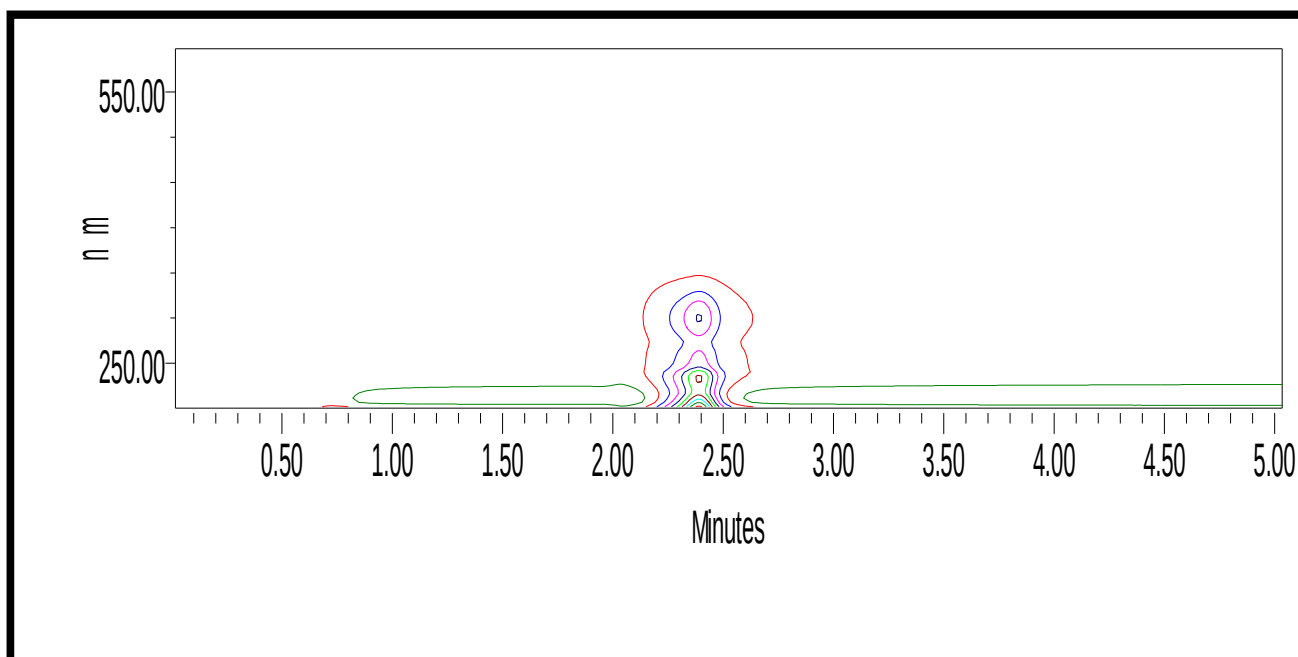


Fig.(b) Contour plot of Telmisartan and inside chromatograph and UV spectrum of Telmisartan

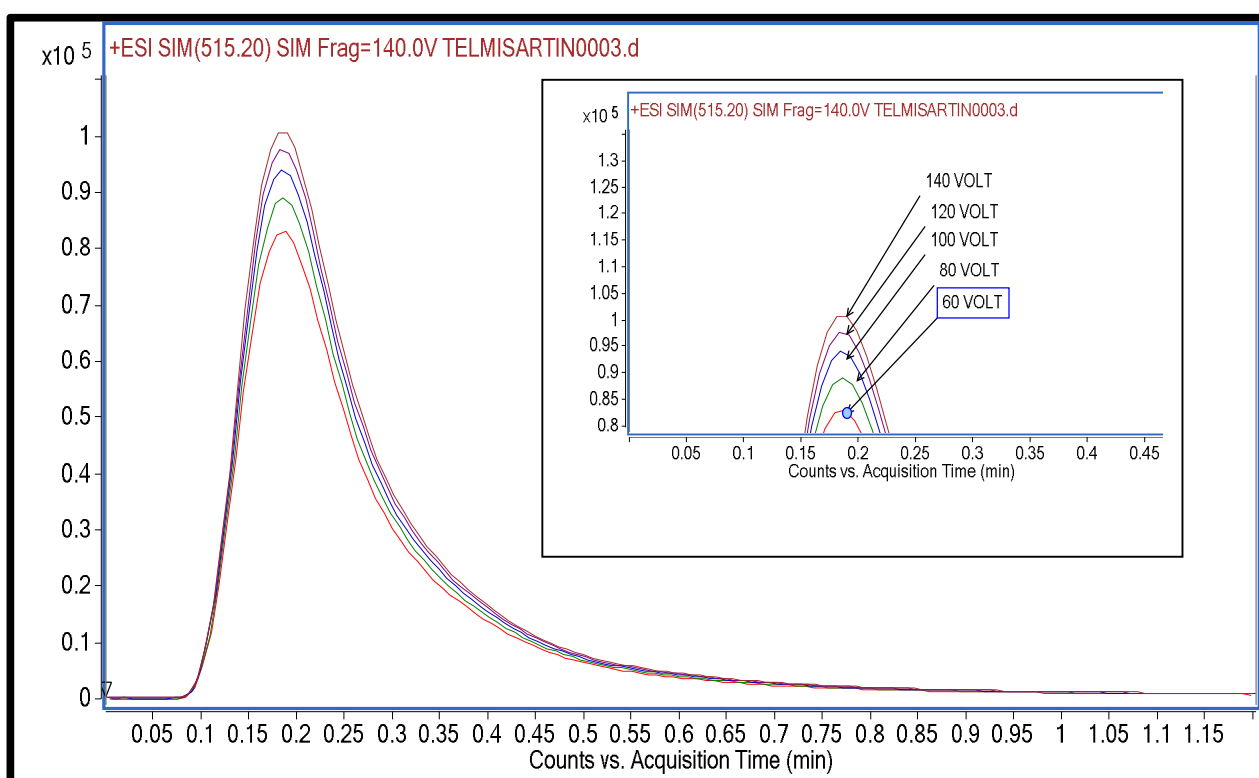


Fig. (c) Optimization of Telmisartan from fragmentor voltage 60-140V.

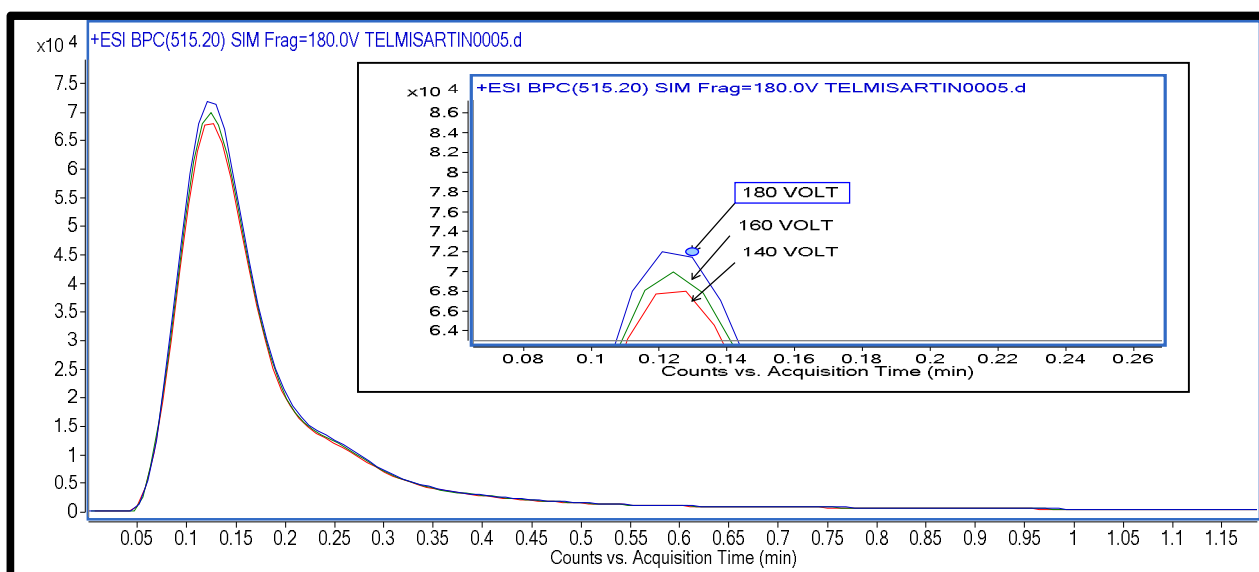


Fig.(d) Optimization of Telmisartan from fragmentor voltage 140-180V.

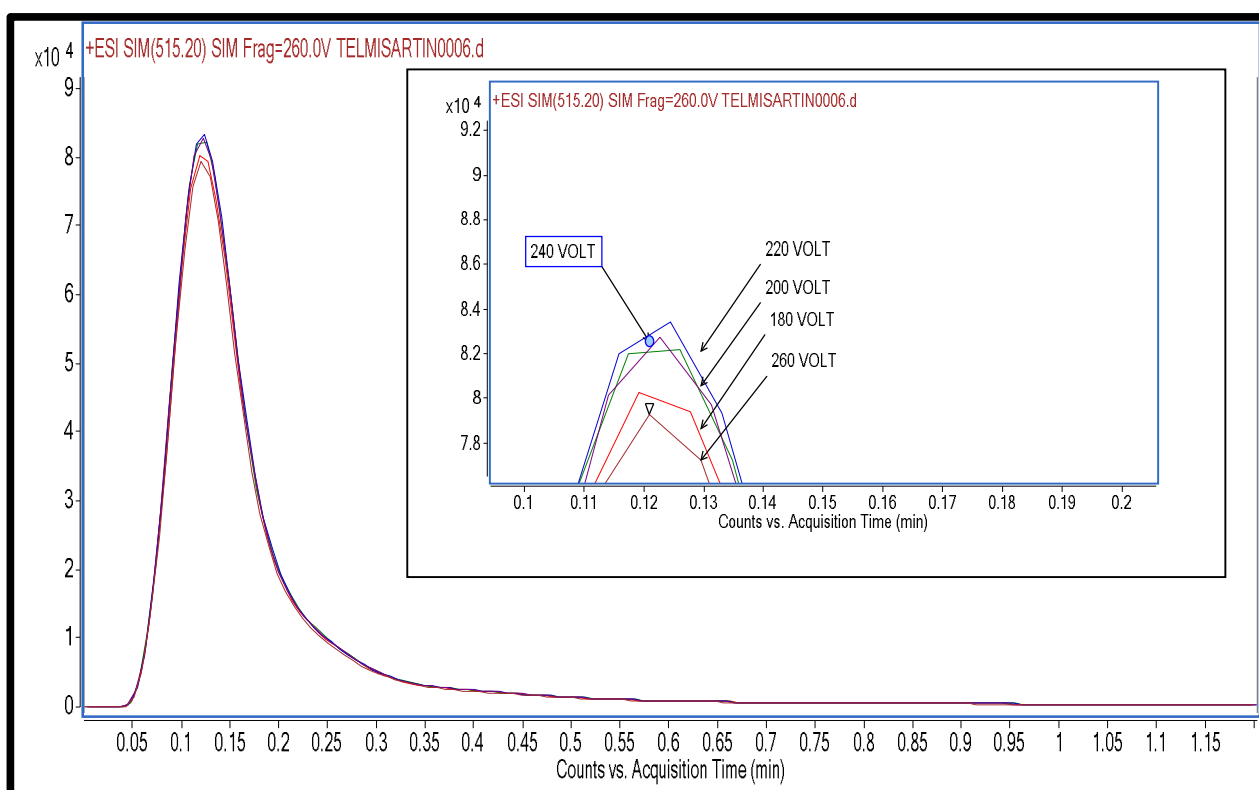


Fig.(e) Optimization of Telmisartan from fragmentor voltage 180-260V.

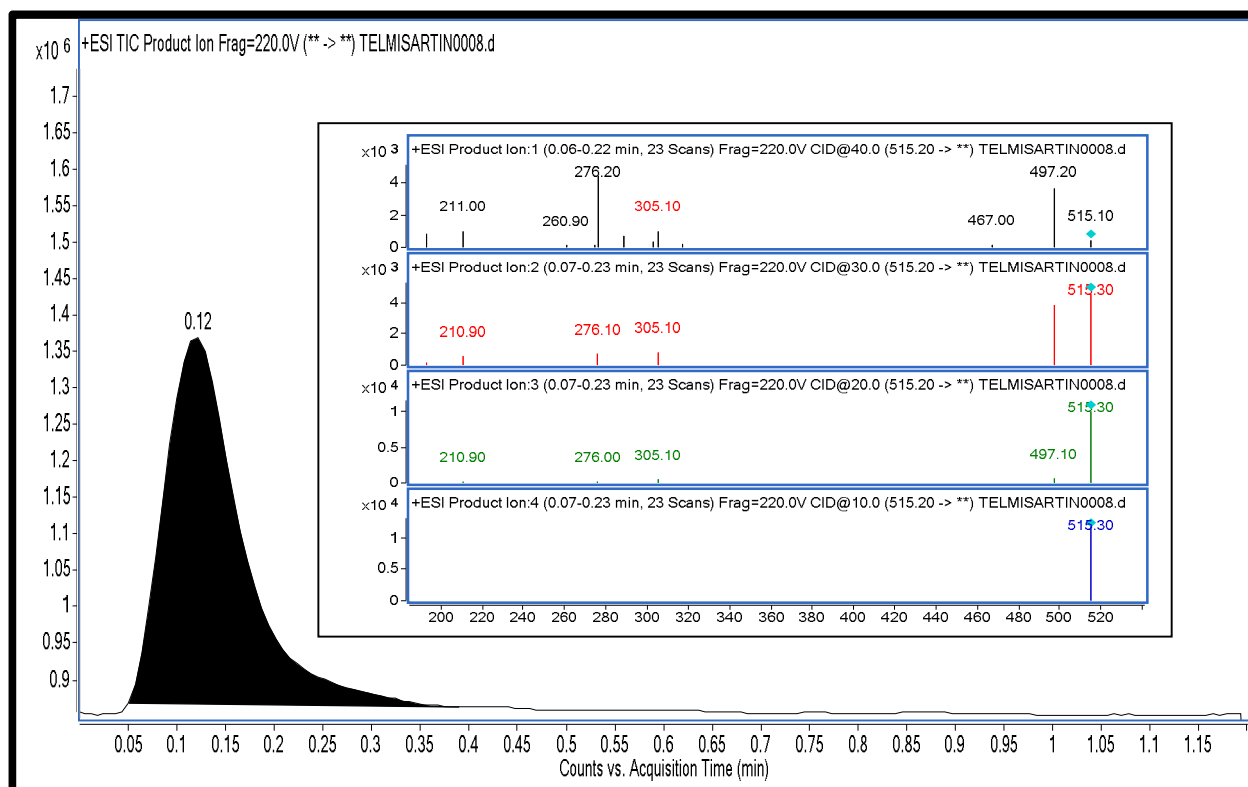


Fig.(f)Product ion of Telmisartan from 10-40eV.

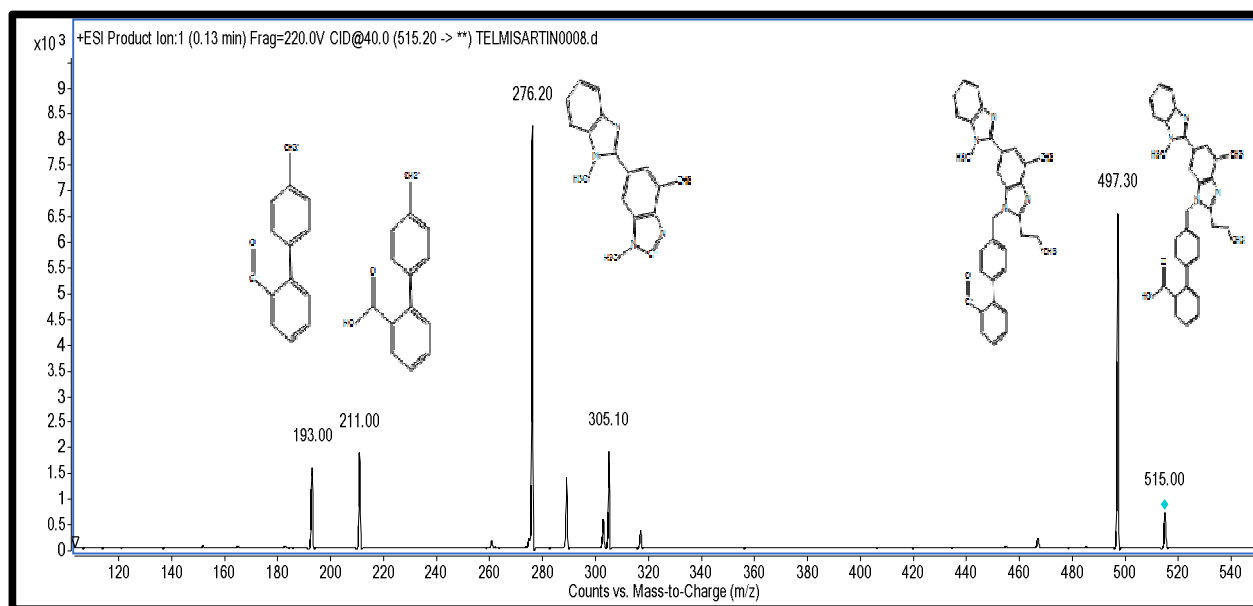


Fig.(g)Fragmentation pattern of Telmisartan

Based on the results, the precursor/product ion pair of transition mass of m/z 515.1 $\rightarrow$ 497.2, taken as quantifier and 515.1 $\rightarrow$ 276.2, taken as qualifier for Telmisartan.

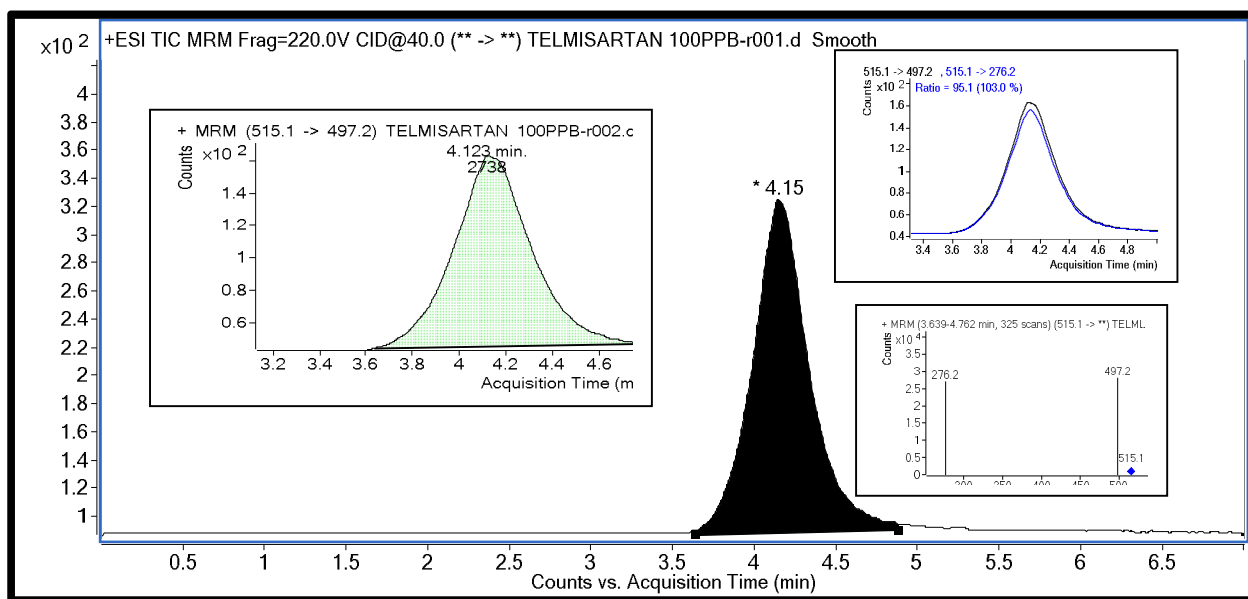


Fig.(h) Chromatogram showing qualifier andquantifier

MRM were performed with 100 ms dwell time. Peak areas obtained from the MRM of standards were utilized for quantification. Sample solution of different conc. were injected directly, separated and detected under the optimize condition.

During MRM method development, fragmentor voltage for maximum abundance of Telmisartan [M+H] and collision energy for the generation of product ion, were optimized to get good signal of fragmented ions. Maximum resolution was obtained at fragmentor voltage of 220 V.

Identification of Telmisartan was done on the basis of retention time and comparison of presence of peak in the MRM in sample and standards. It can be seen from Fig. .(h) that the retention time of the investigated compound was 4.15 min.

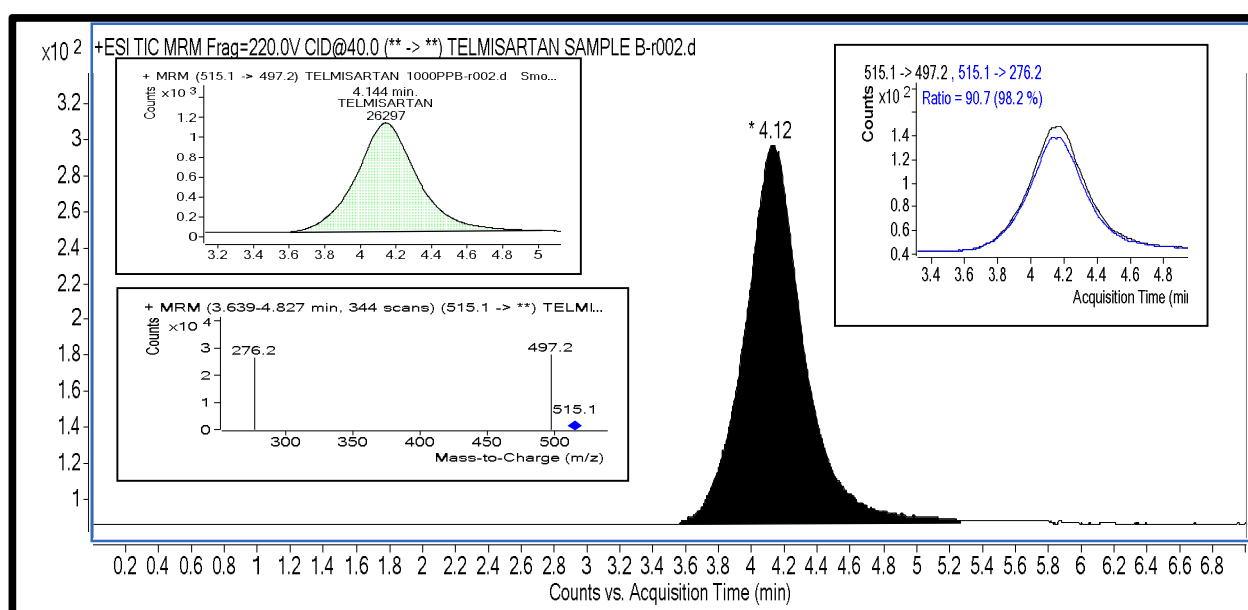


Fig.(i): MRM of Telmisartan sample

Stock solution of standard compound

A stock solution of Telmisartan (1mg/ml) was prepared in volumetric flasks in mobile phase. Standard working solutions were then obtained by mixing and making appropriate dilutions of stock solutions using mobile phase. The concentration utilized for the preparation of five point calibration curve ranged between 5-2500 ng/ml for Telmisartan. All standard solutions were filtered through a 0.1µm PTFE disposal filter (Whatman) and injected directly. The stock and working solution were stored at +4 °C.

Sample preparation of formulation (Telmikind) for quantitative analysis by LC-ESI-MS/MS

A stock solution of formulation Telmikind (1mg/ml) was prepared in volumetric flasks in mobile phase. Then different working solutions were prepared and analysed through LC-ESI-MS.

Preparation of calibration curves and LC-ESI-MS-MS analysis

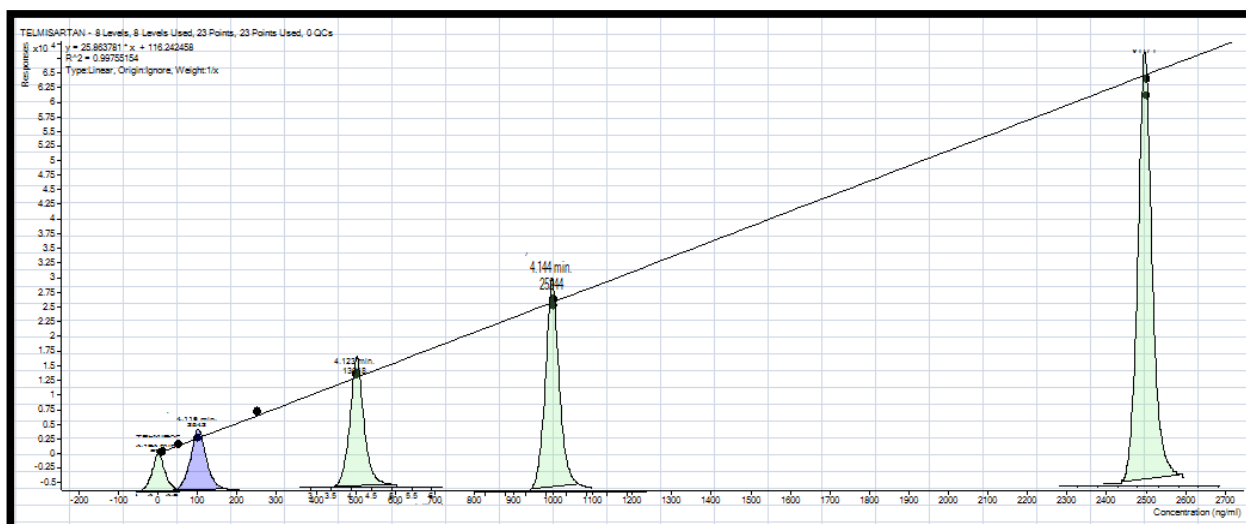


Fig (j) Chromatogram showing increasing conc. Of Telmisartan standard

Before making calibration curve three blank injections were run to check the noise level of the system. The calibration equation of Telmisartan was obtained by plotting LC-MS peak area (y) versus the concentration (x, mg/ml) of calibrators as $y = 25.863781x + 116.242$ ($R^2 = 0.997$). The equation showed very good linearity over the range.

Investigated compounds identities were confirmed by comparison of fragmentation pattern with that of standard. Samples were analyzed using the optimum LC-MS–MS conditions showed protonated molecular ion $[M+H]^+$ of Telmisartan at retention time (4.23 min) indicating presence. Precursor ion scan experiment of Telmisartan confirmed the origin of daughter ions from the same molecular ion thereby confirming presence in the sample. All the quantification, were performed in triplicate

Results and Discussion:

The attempt was made to develop an alternative and economical method for simultaneous estimation of Telmisartan by LCMS. Quantification were carried out on freshly prepared standard stock solution of Telmisartan.

Table.(i) . Linearity of standard curves, quantification/detection limits, Qualifier, quantifier ions for Telmisartan

Name of Compounds and [M+ H]	Retention time (min)	Regression equation	R ²	Linear range (ng/ml)	LOQ (ng/ml)	LOD (ng/ml)	Qualifier\ Quantifier	Fragmentor voltage and Collision energy
Telmisartan	4.123	y=25.86378 1x +116.242	0.997	5-2500	16.5	2.92	497.2\ 276.2	220;40

Conclusion

A simple, specific, linear, and accurate LCMS method has been developed and Quantified for quantitative determination of Telmisartan in tablet formulation. The method is very simple and specific . it's suitable for routine quality control analysis work.

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