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Original Article

RP-HPLC METHOD FOR ESTIMATION OF TIAPRIDE RELATED SUBSTANCE IN TABLET FORMULATION

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ABSTRACT

Objective: To develop a simple, precise, accurate related substance, reverse phase high-performance liquid chomatographic (RP-HPLC) method for the quantitative estimation of impurities which are present in dosage form of Tiapride Hydrochloride.

Methods: The chomatographic separation was achieved with Inertsil C8 (250 × 4.6) mm, 5µ column with mobile phase containing a gradient mixture of 0.05 mM aqueous Potassium di hydrogen phosphate (KH_2PO_4) solution buffer: (with octanesulphonate and final pH of buffer was adjusted to 2.7 with Orthophosphoric acid): Acetonitrile: Methanol (800:150:50 v/v), flow rate of 1.5 ml/min and a detection wavelength of 240 nm.

Results: The method exhibited linearity between range 0.125 to 1200 µg/ml, shows well resolved degradation products from Tiapride hydrochloride tablet with 0.063 µg/ml of LOD (limit of detection) and 0.125 µg/ml of LOQ (limit of Quantification). Forced degradation studies proved that the method is specific for Tiapride Hydrochloride and N-oxide Tiapride reported in European pharmacopeia and British pharmacopeia is one of the degradation impurity confirmed by liquid chromatography mass spectrometry (LC-MS) analysis.

Conclusion: An accurate, precise, linear, robust and specific related substance RP-HPLC method was developed and validated for the quantitative estimation of impurities presented in pharmaceutical dosage form of Tiapride Hydrochloride as per ICH guidelines. The method is stability indicating used for separation of degradation products and can be used for the identification of process related impurity.

Keywords: HPLC, LC-MS, Forced degradation, ICH guidelines, Tiapride Hydrochloride.

INTRODUCTION

Tiapride (N-2–Diethylaminoethyl)-2-methoxy-5 methylsulfonyl benzamide) hydrochloride is an atypical neuroleptic agent and used for the treatment of schizophrenia [1]. It is a selective dopamine D2-receptor antagonist with little propensity for causing catalepsy and sedation. It is likely that the small quantity of these un reacted intermediates may left over during the variety of reaction steps and finally decrease the yield and quality of the finished products. Monitoring of reactions as well as the purity and yield is very important to assess the viability of the processes used for the commercial production of Tiapride. Thus, there is a great need for the development of analytical methods for separation and determination of related substances for process development and quality control of Tiapride [2-4].

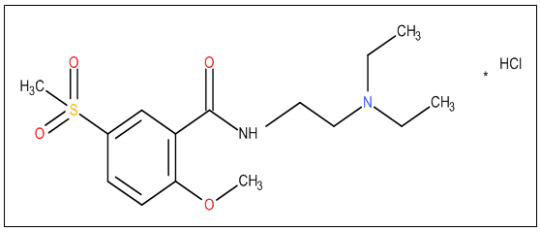

Fig. 1: Structure of tiapride hydrochloride

Literature search revealed that Liquid Chomatography with different detection systems such as spectrofluorimetric [5], UV spectrometric [6], photodiode array, mass spectrometric was used for quantitative determination of Tiapride in biological fluids. Several other approaches like cell based assay, immune assay, electrophoretic [7], polarimetric and high performance thin layer chomatography (HPTLC)[8],gas Chomatography (GC)[9],hydrophilic interaction liquid chomatography-tandem mass spectrometry (HILC)[10] have been tried to analyse tiapride in various matrices. Japan Pharmacopeia (JP)[11] adopted isocratic method for impurity profiling A-E of Tiapride API in HPLC, similarly capillary zone electrophoresis (CZE) [12] and differential pulse anodic voltametric determination [13] method for separation of Tiapride hydrochloride and related impurities in pharmaceutical formulations as well as in human urine and plasma [14] also reported. European and British pharmacopeia has reported related substance reverse phase high performance liquid chomatographic method for active pharmaceutical ingredient of Tiapride Hydrochloride [15, 16]. But no single method is available for formulation and thus it is a challenging task for developing related substance method to detect minute amount of impurities in presence of pharmaceutical excipients using HPLC.

Forced degradation studies help to identify reactions that cause degradation of pharmaceutical products. They are part of development and integral component of validating analytical methods that indicate stability and detect impurities, impurities formed during manufacturing; storage or use and their properties are different from the desired product.

Any significant degradation product should be evaluated for characterization and quantification for its potential hazards [17]. The British Pharmacopeia and United State Pharmacopeia have also mentioned limits of impurities present in the APIs and formulations [18]. The regulatory authorities have also developed guidelines regarding stress testing on drug substances and drug products to detect the degradation products and degradation pathways. It also helps in establishing intrinsic stability of the drug substance as well as in developing stability indicating assay method [19-22].

The present paper describes a reverse phase high-performance liquid chomatographic (RP-HPLC) method for separation and determination of Tiapride on related impurity by using Inertsil C8 (250 × 4.6) mm, 5µ column. Mobile phase used in the method was the mixture of Buffer: Acetonitrile: Methanol (800:150:50 v/v) where the phosphate buffer 0.05 M with pH 2.7 was used. The method was validated and found to be suitable for the quality assessment of Tiapride in pharmaceutical formulations.

MATERIALS AND METHODS

Chemical and reagents

All Reagent were of analytical grade acetonitrile (HPLC Grade) was purchased from Finar Ltd. Methanol (HPLC Grade), Hydrochloric acid were purchased from Merck Specialities Pvt. Ltd., Worli, Mumbai. Purified water of Nano pure Diamond water purification system. Potssiumdihydrogen, Sodium hydroxide (AR Grade), orthophosphate (AR Grade), Hydrogen Peroxide (AR Grade) were purchased from Rankem Ltd. Sodium octane sulphonate (AR Grade), Orthophosphoric acid (HPLC Grade) were purchased from Spectrochem Ltd. Working Standard of Tiapride Hydrochloride was purchased from Easybuyer (Hong Kong) Ltd and marketed formulation Tiapride hydrochloride Tablet was purchased from INTAS Pharmaceutical LTD. Ahmedabad.

Instrumentation

Liquid chromatography

The high performance liquid chromatography (HPLC) System consist of Agilent Model of 1100/1200 series with Diode Array Detector and quaternary pump with Chomeleon software. pH measurement was carried out by PHAN (Lab India LTD) having resolution of±0.01 pH and accuracy of±0.01pH was used. pH meter was equipped with a combined glass–calomel electrode calibrated using standard buffer solutions of pH 4.0, 7.0 and 9.2.

Computer system was using Chomeleon Software before mobile phase delivered in to system. Mobile phase consists of Buffer: Acetonitrile: Methanol (800:150:50) where the phosphate buffer 0.05 M with pH 2.7 was filtered through 0.45 µ nylon filter and degassed by sonication for 15 min. HPLC analysis was carried out on a Inertsil C8 (250 × 4.6 mm), 5µ manufactured by GL Science at 40 °C at a flow rate of 1.5 ml/min and the injection volume was 10 µl. The detection was performed at 240 nm.

Liquid chomatography mass spectroscopy (LC/MS)

The liquid chromatography mass spectroscopy (LC/MS) system consist of Agilent model of 6400 series of triple quadrupole LC/MS with Trap control software. Mobile phase consist of gradient programme with mobile phase A (0.1 % Formic Acid) and mobile phase B (Acetonitrile HPLC Grade) with pH 2.7 were filtered through 0.45 µ nylon filter and degassed by sonication for 15 min. LCMS analysis was carried out on Inertsil C8 (250 × 4.6 mm), 5µ manufactured by GL Science at 40 °C at a flow rate of 1.5 ml/min, the injection volume was 10 µl. The detection was performed at 240 nm. Mass detection was done by quadrupole mass analyser.

Force degradation studies

Tiapride Hydrochloride was subjected to different stress conditions such as hydrolysis, oxidation, dry heat and photolysis. A stock solution of 1000 µg/ml of the drug was prepared in the mobile phase. This was used further for all the stress studies. Tiapride Hydrochloride was subjected to hydrolytic decomposition under acidic condition using 8 ml of 5 M HCl for 24 hs at 80 °C, under basic condition using 8 ml of 5 M NaOH for 24 Hs at 80 °C with reflux, and under neutral condition with water at 80 °C for 24 h. For oxidative stress, the drug was treated with 30% H_2O_2 to reflux at 80 °C for 24 h. For thermal degradation, the solid drug and its 1% solution form in water were heated in a stability chamber at a temperature of 105 °C/75% relative humidity (RH) for 48 h. For photolytic degradation, the solid drug and its 1% solution form in water was exposed to the sample to light providing an overall illumination of not less than 72 h.

Sample preparation

Accurately weighed eight intact tablets (each tablet containing Tiapride Hydrochloride API equivalent to 25 mg) containing 200 mgTiapride Hydrochloride API and transferred into a200 ml volumetric flask, then 150 ml of diluent was added and sonicated for 15 min to disperse the tablets while mixing and resulting solution filter though 0.45 µ nylon filter before analysis.

Validation

Preparation of solution

Stock solution of Tiapride hydrochloride was prepared in mobile phase as diluent to study accuracy, precision, linearity, limit of detection and quantification. The specified concentration of Tiapride was taken as 1000 µg/ml.

Accuracy

The accuracy of the method was determined by performing the recovery studies from previously analysed tablet sample by standard addition method at four different levels (LOQ, 50,100,120 %). Accuracy is performed at range of LOQ Level to 120 % level for Tiapride Hydrochloride.

Precision

Accurately weighed 8 intact tablets and transferred into 200 ml volumetric flask.150 ml of diluent was added and sonicated for 15 min to disperse the tablets, resulting solution was allowed to cool at ambient temperature and volume was made up with diluent, mixed well. It was filtered through 0.45 µ nylon filter.

Robustness

Robustness was performed by carrying out deliberate changes in flow rate (±0.2 ml/min), mobile phase ratio (±5 ml) and pH of the mobile phase (±0.2 units), temperature (±0.2 units), variation of extraction Time (±5).

RESULTS AND DISCUSSION

Stress studies and method development

Tiapride is a very stable substance, but it can be degraded under certain conditions. Several analytical methods were reported for determination of Tiapride API including pharmacopeia but no method has been reported for the dosage form. Moon Y *et al.*, has developed HILIC/MS/MS for determination of Tiapride in human plasma. Similarly Metwally *et al.*, described stability indicating method for Tiapride in pure form, pharmaceutical preparation and in human plasma. In order, to develop an efficient and simple RP-HPLC method for the analysis of drug in tablet dosage forms, preliminary tests were conducted to select optimum and adequate chomatographic conditions. Initially Potassium di hydrogen phosphate, sodium per chlorate, ammonium acetate was evaluated for system suitability parameters and overall chomatographic performance. In the sequential trials Potassium di hydrogen phosphate was found to be suitable for effective separation of parent peak and impurities.

The pH had an effect on the retention times of the Tiapride and its related compounds. Resolutions and peak symmetry are found good at pH 2.7. C18, Phenyl and C8 column has been tried but in phenyl column impurities were merging with the main peak and in C18 column good separation was observed but poor peak shape formed.

Then again try with Inertsil C8 (250 × 4.6 mm), 5 µm with [acetonitrile: methanol: buffer] 150:52:797 v/v/v and mixture of 5.44 gm Potassium di hydrogen phosphate (KH_2P0_4) and 0.08 gm of sodium octanesulphonate with 1.5 ml/min, column temperature 40 °C, in order to get good resolution between Tiapride and its impurities and g*et al.* l symmetrical peaks. Thus method was finally optimised with mixture of 5.44 gm Potassium di hydrogen phosphate (KH_2P0_4) and 0.08 gm of sodium octanesulphonate, pH adjusted to 2.7 with ortho phosphoric acid and using the ratio of buffer: acetonitrile: methanol 800:150:50 v/v/v.

The force degradation study was carried out in different stress conditions. Results obtained concluded that no peaks were detected at the retention time of Tiapride peak from the blank and placebo preparation. Tiapride Hydrochloride tablet was degraded in oxidative, alkali and in acid hydrolytic conditions, all degradation products were well separated from Tiapride peak, no degradation observed in water stress condition and thermal conditions. Tiapride peak was homogenous and spectrally pure in all degradation sample preparation of related substance (table 1).

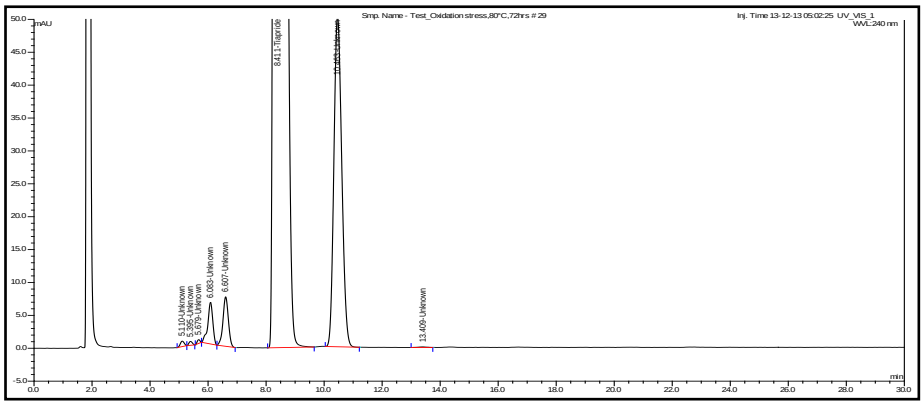

Fig. 2: HPLC Chomatogram of test in oxidative condition (30 % H_2O_2/80 °C, 72 H)

Table 1: Force degradation study of tiapride hydrochloride tablet

Condition	Related substance		Peak purity Match factor	% Assay of tiapride	% Degradation
	% Single impurity	% Total impurity			
Without Degradation	0.046	0.066	997.711	99.9	0
Hydrolytic Degradation (Acid) 5M HCl/80 °C/72 Hs	4.322	5.379	998.201	94.1	5.8
Hydrolytic Degradation (Base) 5M NaOH/80 °C/24 Hs	5.293	6.766	998.334	92.9	7.00
Oxidative Degradation (80 °C/30 %H_2O_2/72 Hs)	6.097	7.323	998.426	91.6	7.00
Thermal Degradation (105 °C)	0.058	0.148	997.803	99.2	7.7
Hydrolytic Degradation (Water)80 °C/72 Hs	0.046	0.093	997.864	99.4	ND
Photo Degradation (UV Uncontrolled)	0.049	0.068	997.938	99.5	ND
Photo Degradation (UV controlled)	0.045	0.064	997.803	99.6	ND

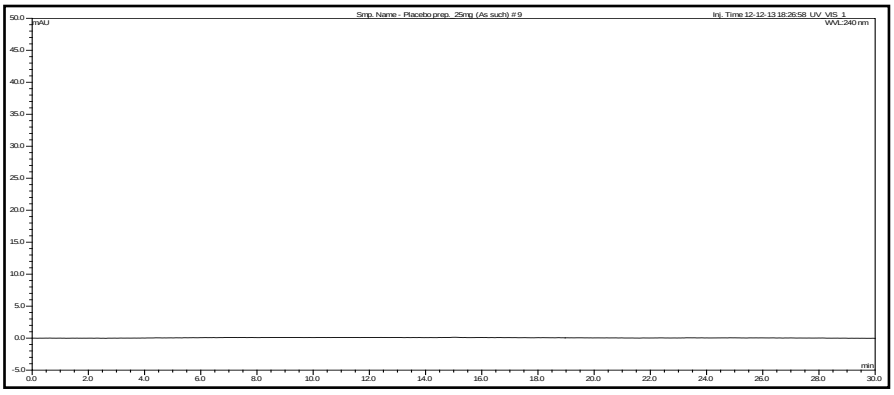

Fig. 3: HPLC Chomatogram of placebo

Validation

Robustness

It was observed that deliberate changes made in the method with regards to the flow rate, mobile phase composition and the pH of the mobile phase did not cause any significant changes in the resolution, accuracy and precision indicating that the method is robust.

Linearity

The response of the drug was found to be linear over the concentration range from 0.125µg/ml to 1200 µg/ml. The regression equation was observed to be Y = 16.056x–10.569.

Specificity

The method was found to be specific as absence of any interference at retention times of peak of interest, and it was evaluated by observing the chromatogram of blank samples and samples of Tiapride. The elution peak of unknown impurity was separated from Tiapride.

Limits of detection (LOD) and quantification (LOQ)

Limit of detection, LOD was found to be 0.063µg/ml and the limit of quantification, LOQ was found to be 0.125µg/ml using % test concentration method.

Accuracy

Recovery studies were performed from 0.125 µg/ml to 1200 µg/ml for Tiapride.

The method showed % recovery in the range of 99.3% to 108.0 % which suggest that the method is accurate. Recovery data is given in table 2.

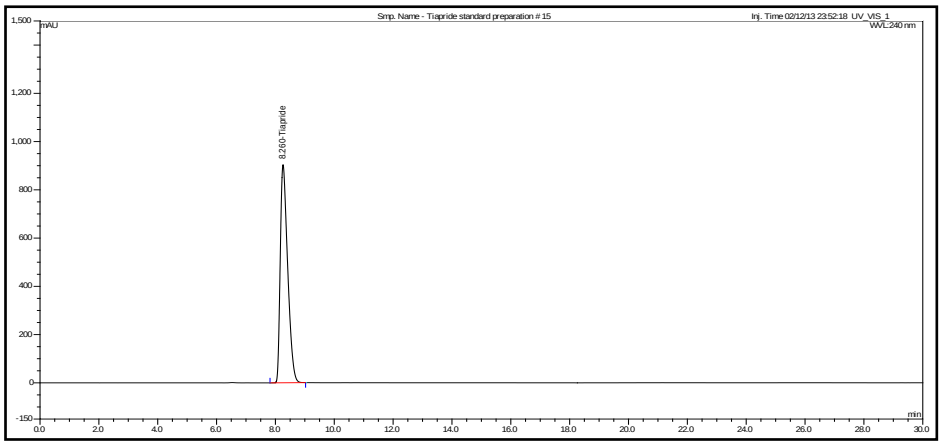

Fig. 4: **HPLC chromatogram of standard tiapride HCl (1000 µg/ml)**

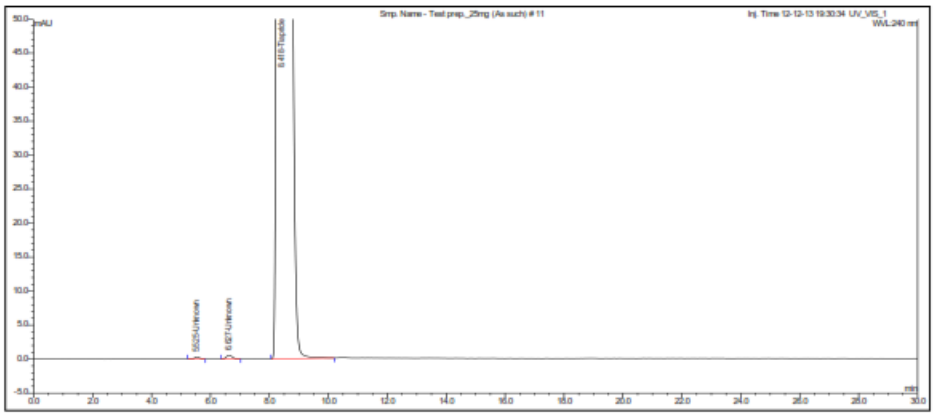

Fig. 5: HPLC chromatogram of tiapride HCl tablet (1000 µg/ml)

Table 2: Results of recovery studies

Accuracy level	Concentration of tiapride (µg/ml)	Area	Recovered amount of tiapride (µg/ml)	%Recovery
LOQ	0.125	2.17248	0.135	108.0
		2.06807	0.129	103.2
		2.15334	0.134	107.2
			Mean	106.1
			% RSD	2.42
50 %	499.99	8038.138	500.611	100.1
		8017.607	499.332	99.9
		8010.751	498.292	99.8
			Mean	99.9
			% RSD	0.15
100%	999.991	15953.16	993.555	99.4
		15968.48	994.508	99.5
		15962.62	994.144	99.4
			Mean	99.4
			% RSD	0.06
120 %	1199.989	19142.09	1192.159	99.3
		19158.39	1193.175	99.4
		19257.15	1199.325	99.9
			Mean	99.2
			% RSD	0.32

Precision

Absolute difference in individual impurity and total impurity of test preparation was found to be less than 0.05 and 0.010 respectively. Method precision data is represented in table 3.

Characterization of Impurity by LC/MS study of degraded samples

10 µg/ml of Tiapride N Oxide impurity solution was injected in liquid chromatography system and retention time (RT) was obtained at 11.884 min (fig. 6). Overlay chromatogram of the reference standard of Tiapride N oxide impurity and degradation sample was shown in fig. 7.

Mass spectrum of Tiapride N-Oxide is shown in fig. 8. The molecular weight of Tiapride N-oxide is 344 and $[M+H]^+$ion peak obtained at m/z 345.08. LC/MS analysis shows peak obtained at RT 9.09 min with molecular weight 344 exactly match with Tiapride N-Oxide confirmed that this molecular mass was of Tiapride N-Oxide.

N-oxide impurity was generated during all degradation conditions. Thus, Tiapride N-Oxide is a degradation impurity which is reported impurity in European pharmacopeia, Japan pharmacopeia, and British pharmacopeia.

Table 3: Results of precision studies

	%Single Impurity		% Total impurities	
No	**Method precision**	**Intermediate precision**	**Method precision**	**Intermediate precision**
1	0.045	0.048	0.064	0.066
2	0.045	0.047	0.065	0.065
3	0.045	0.049	0.064	0.067
4	0.045	0.049	0.065	0.067
5	0.046	0.046	0.064	0.064
6	0.045	0.047	0.065	0.065
Mean	0.045	0.048	0.065	0.066
% RSD	1.14	2.54	0.85	1.84
Absolute difference in mean		0.003	Absolute difference in mean	0.001

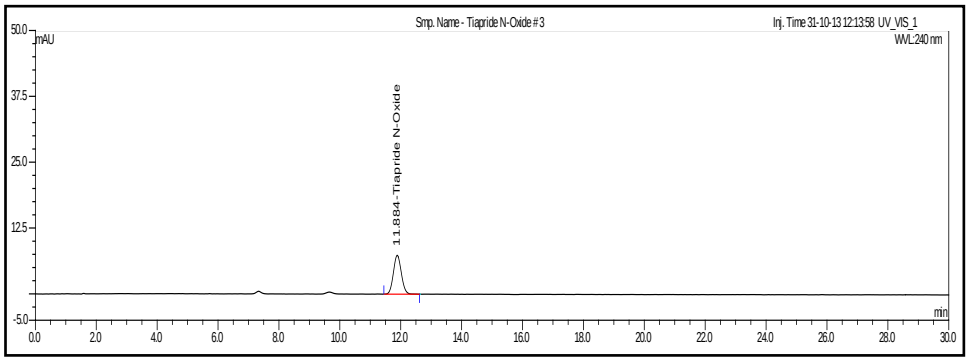

Fig. 6: Chomatogram of reference standard of n oxide impurity

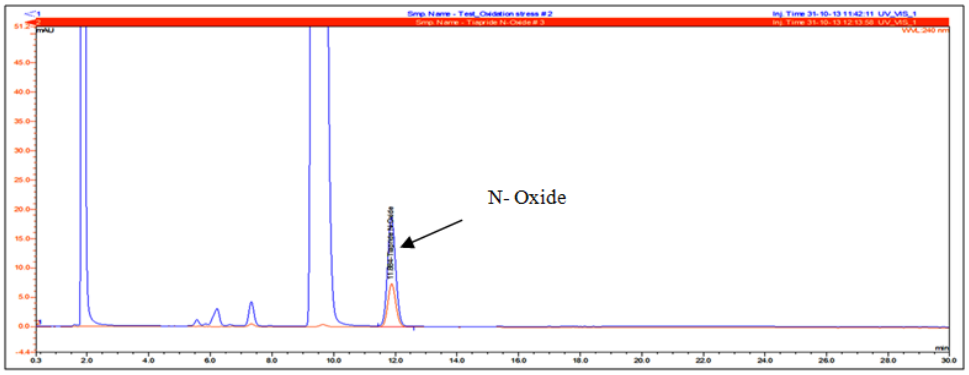

Fig. 7: Overlay HPLC chomatogram of standard impurity N oxide and oxidative degradation sample

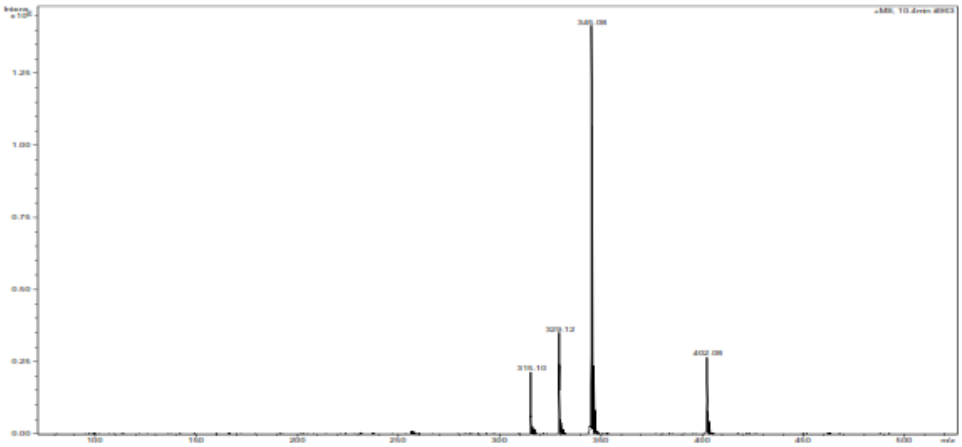

Fig. 8: Mass spectrum of tiapride n oxide

CONCLUSION

European Pharmacopeia has reported related substance reverse phase high performance liquid chomatographic method for the active pharmaceutical ingredient of Tiapride Hydrochloride. This method is not yet applied for formulations. In current project validated method is applied for the formulation and validation was carried out as per ICH (Q3) guideline, which confirms that method is specific, precise, robust and accurate for the developed formulation analysis. This method can also work as stability indicating method as it can separate degradation products peak from each other as well as from drug peak. The method can also be used as in identification of process impurity testing. The impurity Tiapride N-Oxide which is reported in European Pharmacopeia, British Pharmacopeia and Japan Pharmacopeia is confirmed as a degradation impurity by using Liquid chromatography mass spectrometry (LC-MS) analysis.

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CONFLICT OF INTERESTS

Declared None

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