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RP-UPLC method development and validation for the quantitative determination of potential impurities of trimipramine maleate

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ABSTRACT

Objective: The objective of the study was to develop and evaluate the reverse phase ultra performance liquid chromatography (RP-UPLC) method for the quantitative determination of potential impurities of trimipramine maleate active pharmaceutical ingredient. **Method:** The method uses a waters acquity BEH RP_{18} column (100 × 2.1mm, 1.7µm) with mobile phase A consisted, 100 mM dipotassium hydrogen phosphate and 10 mM potassium dihydrogen phosphate, pH adjusted to 8.0 and mobile phase B consisted mixture of acetonitrile and methanol (80:20) with a gradient programme. The column temperature was maintained at 40 ˚C and the detection was carried out at 220 nm. **Results and Discussions:** Efficient and reproducible chromatographic separation was achieved on BEH RP_{18} stationary phase in gradient elution profile. The newly developed UPLC method was validated according to ICH guidelines considering four impurities to demonstrate precision, linearity, accuracy and robustness of the method. The developed UPLC method was found to be rapid (10.5 min run time), accurate and sensitive. The correlation coefficient values are greater than 0.99 for trimipramine maleate and its four impurities. Detection limit and quantitation limit was 0.003% and 0.009% respectively, indicating the high sensitivity of the newly developed method. Accuracy of the method was established based on the recovery obtained between 93.8% and 106.2% for all impurities. The result of robustness study also indicates that the method is robust and is unaffected by small variation in chromatographic conditions. **Conclusion:** The proposed UPLC method provides reliable, reproducible, accurate and sensitive for the quantification of trimipramine maleate related substances.

KEYWORDS: Trimipramine maleate; Impurities; RP-UPLC; Validation.

INTRODUCTION:

Trimipramine is a dibenzazepine derivative tricyclic antidepressant, which is widely used for the treatment of a variety of depressive states and other psychiatric disorders. It has antidepressant, anxiolytic, antipsychotic and sedative effects[1]. Trimipramine is primary use in medicine for the treatment of major depressive disorder, especially where sedation is required due to its prominent sedative effects[2]. Trimipramine also has some weak antipsychotic effects which are less pronounced than with the phenothiazine antipsychotic perazine[3]. Trimipramine is the only effective drug against insomnia known so far that does not alter the normal sleep architecture[4,5]. The anxiolytic action produces restoration of normal sleep patterns and a subjective improvement in the patients. The antidepressant action produces mood elevation, usually within 7 to 10 days[6] . Trimipramine is metabolised in the liver to its major metabolite desmethyltrimipramine. Trimipramine is excreted in the urine, mainly in the form of its metabolites, and has been shown to have a mean elimination half life of 24 hours after a 50mg oral dose[7]. Trimipramine has been shown to be extensively bound to plasma proteins (average of 94.9%) and it has been suggested that it undergoes high first-pass hepatic clearance[8,9].

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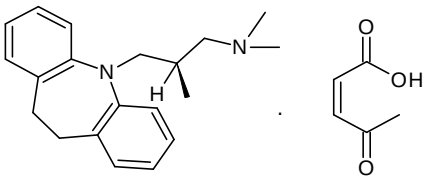
Trimipramine is a chiral compound with an asymmetric center at the side chain and the two enantiomers are different from each other in terms of physiological and behavioural effects[10] . Chemical structure of trimipramine maleate is given in figure 1.



Molecular weight = 410.51
Molecular formula = $C_{20}H_{26}N_2.C_4H_4O_4$

Fig. 1: Structure of Trimipramine maleate

Several analytical methods have been reported to determine trimipramine in bulk drug, formulation and in biological matrices. These methods include spectrophotometry[11,12], high performance thin layer chromatography (HPTLC)[12], high performance liquid chromatography (HPLC) [13-15] and liquid chromatography tandem mass spectrometry (LC/MS) [16-18]. Trimipramine maleate is an official drug in USP, EP, BP, and IP. Extensive literature survey reveals that no UPLC methods have been reported for the analysis of trimipramine maleate drug substance. Hence it was felt necessary to develop an accurate, rapid and sensitive UPLC method for the determination of trimipramine maleate impurities.

UPLC is relatively new method developed in the liquid chromatography. It represents a significant decrease in separation time without loss of its efficiency and resolution. Objective of the current study was to develop a UPLC–UV method for the quantitative determination of impurities in trimipramine maleate and check the suitability of the method as per ICH guidelines. Pharmacopeia methods for trimipramine maleate are isocratic and time consuming. The newly developed UPLC method separates all impurities with short run time.

MATERIALS AND METHODS:

Reagents and Chemicals

Samples of trimipramine maleate and standards of Imp-1, Imp-2, Imp-3 and Imp-4 (Table 1) were received from Deepta laboratories, Mysore, India. HPLC grade methanol and acetonitrile was purchased from Rankem, Mumbai, India. Deionized water was prepared using a Milli-Q plus water purification system from Millipore (Bedford, MA, USA). Analytical reagent grade of dipotassium hydrogen orthophosphate, potassium hydrogen phosphate, diammonium hydrogen orthophosphate, ammonium hydrogen phosphate, ammonium acetate, sodium hydroxide, hydrogen peroxide and hydrochloric acid were purchased from Merck India Limited (Mumbai, India).

Instruments

The LC method development and validation were done using Agilent 1290 Infinity UPLC equipped with PDA detector (Agilent technologies, CA, USA). The data were collected and the peak purity of the trimipramine peak was checked using chemstation software.

Chromatographic conditions

The chromatographic separations were achieved on Waters Acquity BEH RP_{18} column (100 mm length × 2.1 mm ID with 1.7µm particle size, Waters corporation, MA, USA). Mobile phase A consisted, 100 mM dipotassium hydrogen phosphate and 10 mM potassium dihydrogen phosphate, pH adjusted to 8.0 and mobile phase B consisted mixture of acetonitrile and methanol (80:20) with a gradient programme (T_{min}A:B) $T_0$85:15, $T_{4.5}$50:50, $T_{7.5}$20:80, $T_{10.0}$20:80, $T_{10.5}$85:15 with a post-run time of 2.5 min. The column temperature was maintained at 40 ˚C and the detection was carried out at 220 nm. The flow rate was set to 0.5 mL/min. The test concentration was about 200 µg/mL and the injection volume was 2µL. A degassed mixture of mobile phase A and mobile phase B (1:1) was used as diluent for standard and sample preparations.

Preparation of stock solutions for method validation

A test preparation of 200 µg/mL of trimipramine maleate API sample was prepared by dissolving in diluent. A stock solution of impurities was prepared by dissolving 5 mg each of Imp-1, Imp-2, Imp-3, Imp-4 and 5 mg of trimipramine maleate in diluent and made up to 50 mL with diluent. Transferred 5 mL of each individual stock solution into a 100 mL volumetric flask and made up to volume with diluent. From this stock solution, standard solution of 0.40 µg/mL of each impurity and 0.40 µg/mL of trimipramine maleate was prepared.

RESULTS AND DISCUSSION:

Method development

The determination of the suitability of a HPLC method is based upon the level of development. However, at a minimum HPLC method should provide baseline separation of starting materials, desired products, known impurities, and expected by-products. The chromatographic conditions should also be chemically compatible with the analytes. The main objective of the UPLC method development for trimipramine was to achieve efficient separation of impurities and a short run time method.

Selection of wavelength

The optimum wavelength of detection is the wavelength that gives the highest sensitivity for the significant related substances and minimizes the difference in response factors between those of the active pharmaceutical ingredient and the related substances. Trimipramine and its impurities give good detector response at 220 nm, therefore the final absorption wavelength for detection was chosen at 220 nm.

Mobile phase selection

In reverse phase chromatography, the mobile phase consists of an aqueous buffer and a non-UV active water miscible organic solvent. Choosing a right buffer and pH is very critical for method development. Buffers are recommended to control the pH stability of the mobile phase. Buffers like ammonium acetate, dipotassium hydrogen orthophosphate, potassium hydrogen phosphate, diammonium hydrogen orthophosphate, ammonium hydrogen phosphate, and its combination were studied for the trimipramine maleate UPLC method development. Trimipramine is basic in nature with a pKa value of 9.4[19]. At sufficiently low pH, basic analytes are in ionised form and will elute more quickly but with improved peak shape. Conversely, at higher pH basic compounds will be more retained. Peak splitting may be observed if the pH of the mobile phase is similar to the pKa of the compound and the analyte elutes as both a charged and uncharged species. Therefore we have adjusted the pH of the buffer is 8 to get a better peak shape for all the experiments. Acetonitrile and methanol has chosen as organic modifiers. The principle difference in the behavior of acetonitrile and methanol is that where acetonitrile forms a thick multi-molecular adsorbed layer on the surface of reverse phase adsorbent (C_1-C_{18} and phenyl phases), while methanol is adsorbed only in monomolecular fashion[14] . This brings a principal difference in the analyte retention mechanism in these two hydro-organic systems.

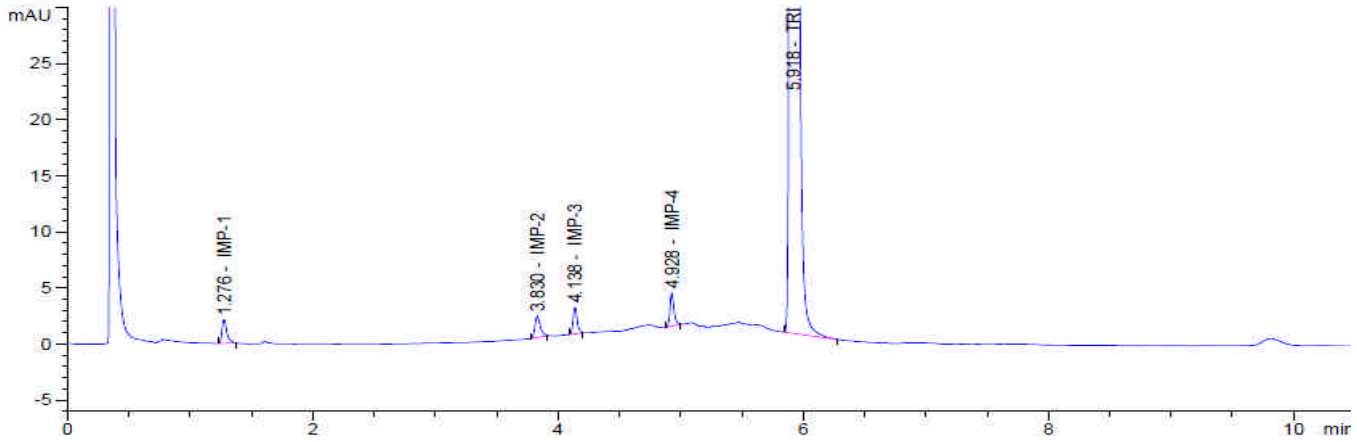


Fig. 2: Chromatogram of Trimipramine maleate spiked with impurities.

Column selection

The heart of a HPLC system is the column. Changing a column will have the greatest effect on the resolution of analytes during method development. Silica-based packing materials dominate in applications for RP separations in the pharmaceutical industry. The vast majority of RP LC separations take place on column that contain C_{18} bonded stationary phases due to their stability, retentivity and reproducibility. In addition, these hydrophobic ligands provide the desired separation most of the time. However screening several different types of stationary phases during method development for a particular separation is often useful because different columns usually have different selectivity for components in a sample. Three different types of columns were preferred for the method development of trimipramine maleate ie BEH (Ethylene Bridged Hybrid) C_{18}, BEH RP_{18} and HSS (High Strength Silica) C_{18} columns. All columns used were fully end capped.

Several experiments were conducted to get a baseline resolution between trimipramine maleate and impurities. The resolution between

Table 1: Potential impurities of Trimipramine

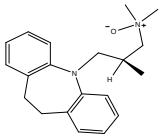
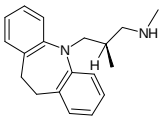
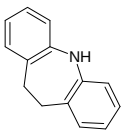
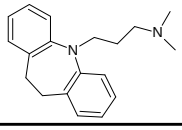
Sl.No.	Structure	Mol. Wt.	IUPAC name	Code Origin
1		310.44	(2RS)-3-(10,11-Dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,N,2-trimethylpropan-1-amine N-oxide	IMP-1 Process
2		280.41	(2RS)-3-(10,11-Dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,2-dimethylpropan-1-amine	IMP-2 Process
3		195.26	10,11-Dihydro-5H-dibenzo[b,f]azepine	IMP-3 Process
4		280.41	(2RS)-3-(10,11-Dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,N-dimethylpropan-1-amine	IMP-4 Process

Table 2: Method validation summary report

Parameter	Imp-1	Imp-2	Imp-3	Imp-4	Trimipramine
System suitability					
RT	1.28	3.83	4.14	4.93	5.92
RRT	0.22	0.65	0.70	0.83	1.00
Rs	-	37.13	4.83	13.57	17.49
N	6897	41120	81831	118540	175641
T	1.22	1.35	1.18	1.06	1.09
Linearity					
r	0.9985	0.9946	0.9993	0.9969	0.9978
Slope	18.28	18.10	16.31	16.62	18.15
Intercept	0.0931	-0.1322	0.1683	0.0632	0.0587
Detection limit (%)	0.003	0.003	0.003	0.003	0.003
Quantitation limit (%)	0.009	0.009	0.009	0.009	0.009
Precision (QL)					
% RSD (n 6)	3.1	4.0	2.5	2.5	3.5
Repeatability (intra day)					
% RSD (n 6)	1.6	1.2	0.7	1.6	0.4
Intermediate precision					
(inter day)					
% RSD (n 12)	1.5	2.0	3.1	1.8	0.3
Accuracy at QL level (n 3)					
Amount added (%)	0.0090	0.0090	0.0090	0.0090	-
Amount recovered (%)	0.0084	0.0095	0.0084	0.0088	
% Recovery	93.3	105.6	93.3	97.8	
Accuracy at 80% level (n 3)					
Amount added (%)	0.16	0.16	0.16	0.16	-
Amount recovered (%)	0.1509	0.1699	0.1539	0.1559	
% Recovery	93.8	106.2	96.2	97.4	
Accuracy at 100% level (n 3)					
Amount added (%)	0.20	0.20	0.20	0.20	-
Amount recovered (%)	0.1921	0.2022	0.2056	0.2006	
% Recovery	96.1	101.1	102.8	100.3	
Accuracy at 120% level (n 3)					
Amount added (%)	0.24	0.24	0.24	0.24	-
Amount recovered (%)	0.2335	0.2315	0.2435	0.2475	
% Recovery	97.3	96.5	101.5	103.1	

n, number of determinations; RT, retention time; RRT, relative retention time; Rs , USP resolution; N, number of theoretical plates; T, USP tailing factor; r, correlation coefficient.

Imp-2 and Imp-3 were poor when different UPLC columns viz; C_{18}, RP_{18} and HSS C_{18} were used in different mobile phases containing phosphate and acetate buffers along with acetonitrile with pH 8.0. Use of methanol as an organic modifier shown significance improvement in resolution between Imp-2 and Imp-3 however poor peak shape resulted. Use of RP_{18} column with a 100 mm length × 2.1mm ID column and 1.7µm particle size, use of mixture of 100 mM dipotassium hydrogen phosphate and 10 mM potassium dihydrogen phosphate, pH adjusted to 8.0 as mobile phase A and acetonitrile–methanol mixture (80:20) as mobile phase-B was significant in achieving the desired resolution of trimipramine maleate and its impurities. After several trials for gradient profile, chromatographic conditions were finalized as described under section chromatographic conditions.

METHOD VALIDATION:

The newly developed method was validated for sensitivity, linearity, precision and accuracy, robustness and system suitability according to ICH guidelines[20]. Validation study was carried out for Imp-1, Imp-2, Imp-3 and Imp-4. The system suitability and selectivity were checked by injecting 200 µg/mL of trimipramine maleate solution containing 0.4µg/mL of all impurities monitored throughout the validation. Method validation results are summarized in Table 2.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection and limit of quantitation were determined for trimipramine maleate and for each of the related substances as per ICH $Q2R_1$ guideline. The LOD and LOQ for Imp-1, Imp-2, Imp-3, Imp-4 and Imp-5 and trimipramine maleate were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively by injecting a series of diluted solutions with known concentration. The limit of detection and the limit of quantitation for Imp-1, Imp-2, Imp-3, Imp-4 and trimipramine maleate were about 0.003% and 0.009% of analyte concentration i.e. 200 µg/mL respectively. Precision study was also carried at the LOQ level by injecting six individual preparations of all impurities and the relative standard deviation for LOQ concentration for all impurities were below 5%.

Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample. A linearity test solution for related substance method was prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 150% of the permitted maximum level of the impurity (i.e. LOQ, 0.04 µg/mL ,0.10 µg/mL, 0.20 µg/mL 0.32 µg/mL, 0.40 µg/mL and, 0.60 µg/mL) was subjected to linear regression analysis with the least squares method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The residuals and sum of the residual squares were calculated from the predicted responses. The correlation coefficient obtained was greater than 0.99 for all impurities. The result showed an excellent correlation between the peak and concentration of all impurities. The range of the method was from LOQ to 0.60 µg/mL of the analyte concentration (200 µg /mL).

Precision

Precision of the method was studied for method precision and intermediate precision. Method precision was checked by injecting six individual preparations of (200 µg/mL)Trimipramine maleate spiked with 0.4µg/mL of each impurity. In the intermediate precision study, the similar procedure of method precision was carried out by a different day. % RSD of areas of each impurity was within 5.0, confirming good precision at low level of the developed analytical method.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was evaluated in triplicate at LOQ, 80% level (0.32 µg/mL), 100% level (0.40 µg/mL) and 150% level (0.60µg/mL). The percentage recovery of all impurities in drug substance has been calculated. Chromatogram of Trimipramine maleate spiked with four impurities was depicted in Figure 2.

Robustness

To determine the robustness of the method, experimental conditions were deliberately changed and the resolution between closely eluting peaks, Imp-2 and Imp-3 were evaluated. Close observation of analysis results of deliberately changed chromatographic conditions viz; flow rate (0.5±0.05 mL/min), mobile phase composition (± 2% acetonitrile and methanol), pH 8.0±0.1 and column temperature (40±5 °C) shown that resolution between Imp-1 and Imp-2 was greater than 4.0 and no significant change in relative retention time for all impurities in spiked sample illustrating the robustness of the method.

Solution stability and mobile phase stability

The solution stability of trimipramine maleate and its related impurities was carried out by leaving both spiked and unspiked sample solutions in tightly capped HPLC vials for 72 h in an auto sampler. Content of each impurity was determined against freshly prepared standard solution. No significant changes were observed in the content of any of the impurities. The solution stability and mobile phase stability experiment data confirms that the sample solutions and mobile phase used during related substance determination were stable for at least 72 hour.

CONCLUSION:

The developed UPLC method provides reliable, reproducible, accurate and sensitive for the quantification of trimipramine maleate related substances. This newly developed method has been validated as per regulatory requirements and has shown acceptable precision, accuracy and adequate sensitivity. This method can be used for the

routine analysis of trimipramine maleate active pharmaceutical ingredient related substances.

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