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VALIDATED HPLC METHOD FOR DETERMINITION OF N-METHYL-O-PHENYLENEDIAMINE DIHYDROCHLORIDE IN TELMISARTAN DRUG SUBSTANCES

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ABSTRACT

A reproducible RP-HPLC method was developed and validated with the aim of quantifying the levels of genotoxic impurity in telmisartan drug substances. A limit level concentration of 18.75 µg/g for N-methyl-O-phenylenediamine dihydrochloride was calculated by applying the concept of threshold of toxicological concern. The impurity was separated on Kromasil C18 150 x 4.6 mm, 5 µm analytical column with a mobile phase consisting of the buffer pH 3.0 and acetonitrile by using the gradient program at a flow rate 1.0 mL/min. The effluent was monitored by UV detection at 230 nm with column temperature maintained at 30 °C and the injection volume 20µL. The developed method was validated as per ICH guidelines in terms of specificity, limit of detection, limit of quantification, linearity, precision, accuracy and robustness. The LOD and LOQ value were found to be 2.53 µg/g and 7.52 µg/g and accuracy results were well in the range 90.68 to 110.21 %. The linearity curve showed the correlation coefficient of 0.9998 and method very sensitive. The validated method was applied for identification and quantification of impurity in different batches of the API.

KEYWORDS: RP-HPLC, API, CH guidelines, Impurity, TTC, Validation.

INTRODUCTION

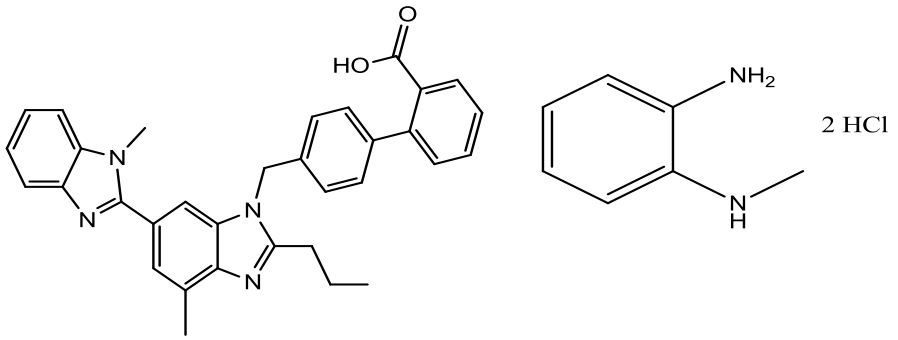
Telmisartan is orally active, potent, non-peptide, long-lasting, noncompetitive drug belongs to a class of antihypertensive agents called angiotensin II receptor blockers.[1] Its molecular formula $C_{33}H_{30}N_4O_2$, the formula weight is 514.62 and chemically described as 4'-[[4-methyl-6-(1-methyl-2-benzimidazolyl)-2-propyl-1-benzimidazolyl]-methyl]-2-biphenylcarboxylic acid.[2] The drug is used to treatment of hypertension and protects the damage of kidneys from diabetes.[3] The drug is available in 20, 40 and 80 mg tablet dosage form either individually or combination with other drugs, some of the brands include Telista, Sartel, Tellzy, Cresar-R, Arbitel AV, Tazloc-AM, Telmikind-AMH, Telma, Telmaxx.[3,4]

N-methyl-O-phenylenediamine dihydrochloride is the most important raw material used in the synthesis of telmisartan drug substances.[5] This is identified as a genotoxic impurity in according to the guidelines.[6, 7] The evaluation limits for genotoxic impurity in the drug was calculated based on TTC and the maximum daily dose of drug i.e. 80 mg. A maximum daily exposure target of genotoxic impurities is 1.5 µg per day per person.[6]

$$\text{Evaluation Limits (µg/g)} = \frac{1.5\mu g/day}{dailydose(g/day)} = \frac{1.5}{0.080} = 18.75\mu g/g$$

Hence 18.75µg/g is set as the evaluation limit of potentiality genotoxic impurities in telmisartan drug.

The chemical structures of telmisartan and N-methyl-O-phenylenediamine dihydrochloride are presented in fig. 1.[5]



(a) (b)

Fig. 1: Chemical structure of (a) Telmisartan and (b) N-methyl-O-phenylenediamine dihydrochloride

The literature survey revealed that some spectrophotometric methods,[8-9] HPLC methods,[10-14] and LC/MS/MS were developed for the determination of telmisartan in different combinations of drugs and biological matrices.[15-19] A sensitive method for the analysis of N-methyl-O-phenylenediamine dihydrochloride genotoxic impurity was not available. Hence the aim of this study was to develop a specific and sensitive analytical method, which separate the impurity from the drug substances, but also could quantify the impurity at the trace level.

EXPERIMENTAL SECTION

Chemical and Reagents

HPLC grade of water, orthophosphoric acid, methanol, and acetonitrile were purchased from Rankem, Mumbai India. AR grade of 1-pentane sulphonate and Potassium dihydrogen phosphate monohydrate was purchased from Merck, Mumbai India. All pure drug substances and impurities are used for research purpose were procured in-house Macleods pharmaceutical LTD.

Instrumentation

The HPLC system consisted of Shimadzu model LC 2010 $C_{HT,}$ UV and PDA detector. The output signals were monitored and integrated using LC solution software. Sartorius analytical balance and Pico^{+} pH meter were used.

Chromatographic Conditions

The chromatographic separation was achieved on a gradient method using Kromasil C18 (150 x 4.6 mm, 5µm) column. The mobile phase was consisting of buffer pH 3.0 as mobile phase A and the acetonitrile as mobile phase B. The flow rate of mobile phase was 1.0 mL/min. The run time for was kept 40 min. The HPLC gradient program set as, time (min) / % mobile phase B: 0.01/10, 15/10, 20/80, 30/80, 31/10 and 40/10. The column temperature was maintained at 30°C and the detection was monitored at 230 nm. The injection volume was 20 µL and acetonitrile was used as diluent.

Preparation of Buffer pH 3.0: Dissolved 3.8 g 1-pentane sulphonate and 2.00 g potassium dihydrogen phosphate and in 1000 ml of water. pH is adjusted to 3.0 with orthophosphoric acid and filter the buffer through 0.45µ filter paper.

Preparation of standard and sample solutions

A stock solution of N-methyl-O-phenylenediamine dihydrochloride 38 µg/mL was prepared in diluent. An appropriate dilution was made from the stock to get the standard solution of 0.38 µg/mL of the genotoxic impurity. The sample solution (20 mg/mL) was prepared by weighing 400 mg of drug substances and transferred to 20 mL volumetric flask.

RESULTS AND DISCUSSIONS

Method Development

TLM is non-polar molecule hence strongly retained in reverse-phase HPLC columns and polar mobile phase. The method was developed by considering the main parameters like the selection of wavelength, HPLC column, mobile phase, column oven temperature, flow rate, injection volume and diluent. The solubility was checked for telmisartan, N-methyl-O-phenylenediamine dihydrochloride, and other impurities in water, methanol, acetonitrile and the combination of water: methanol, water: acetonitrile in different ratios. All compounds had a good solubility in 5% orthophoshoric acid in methanol than others diluent. Hence, 5% orthophoshoric acid in methanol was selected as diluent.

The impurity standard solution was prepared and injected into the HPLC system with PDA detector and a spectrum was obtained. The maximum absorption wavelength of the solution had shown about 230.4 nm, hence 230 nm was selected for the quantification of this impurity in the telmisartan drug substances.

The selection of HPLC column carried out by conducted trials on various packaging material of ODS, C8 and C18 in different length, internal diameter, particle size and pore size manufactured by different industries. After performing trials the decisive separation was accomplished on Kromasil C18 (150 x 4.6mm) 5µm HPLC column.

The selection of mobile phase was carried out on isocratic condition by prepared water as mobile phase A

and acetonitrile as mobile phase B in the ratio 50:50 v/v and the standard solution of impurity was injected. The result was observed that the analyte peak was not eluted within 60 minutes. The trial was continued by applied gradient condition with same mobile phase result was observed that peak shape of analyte was not proper with noisy baseline and the pharmacopeial impurities were co-eluted along with main peak. After performing many trials with experimental data the chromatographic separation was finalized by the following gradient program (Time/% mobile phase B) was fixed as 0.01/10, 15/10, 20/80, 30/80, 31/10 and 40/10 by using buffer pH 3.0 and acetonitrile were used as Mobile Phase A and B.

The column temperature was selected by taking many trials with different column oven temperature (20°C to 55°C) in 5°C steps. The analyte was well separated and the reproducible result was obtained at 30°C.

The flow rate of the mobile phase was optimized from 0.5-1.5 mL/min for separation of analyte peak from blank and impurities peaks. It was found from the experiments that 1.0 ml/min flow rate was ideal for the successful elution of the compound.

The standard solution was injected from 10µL to 100µL injection volume into HPLC system. Based on the response and shape of the peak 20 µL injection volume was selected.

METHOD VALIDATION

The developed HPLC method has been validated for genotoxic impurity determination in the telmisartan sample according to ICH guideline.[20] The individual parameter of system suitability, specificity, limit of detection, limit of quantification, linearity, precision, accuracy, solution stability and robustness was experimentally evaluated by injecting standard and sample solution.

System Suitability

According to USP, system suitability test is an integral part of liquid chromatographic methods to verify that the system is adequate for the analysis.[21] The standard solution was prepared and 20 µL of six replicates was injected into HPLC system. The obtained peak was calculated for the theoretical plates, tailing factor and % RSD of six replicate areas. The result was found to comply with USP requirements and indicated that the chromatographic system is adequate for the intended analysis. The results are presented in table 1 and overlay chromatograms of replicate standard injection are presented in Fig. 2.

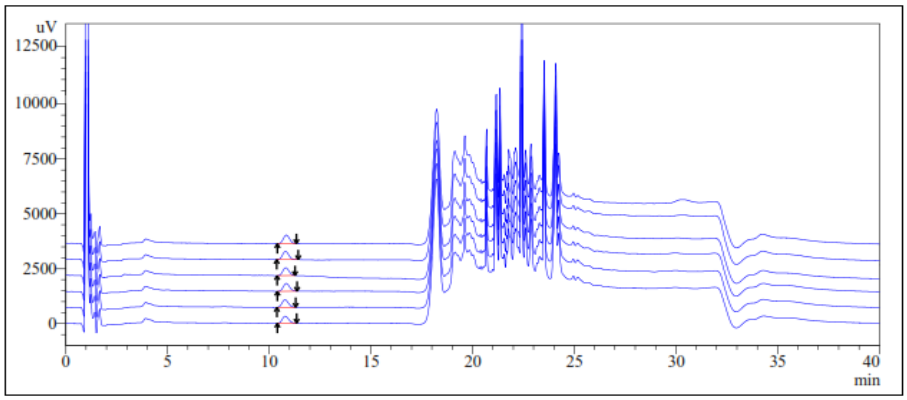


Fig. 2: Overlay chromatograms of replicate standard injections

Table 1: Validation Results

Parameters	Acceptance Criteria			Results
System Suitability	The theoretical plates in standard solution NLT 2000			3456
	The tailing factor in standard solution NMT 2.0			1.12
	The % RSD in standard solution NMT 5.0 %			0.58
Specificity	No interference	Blank	Retention time	No Peak
		Telmisartan impurity A		19.02 min
		Telmisartan impurity B		20.98 min
		Telmisartan impurity E		20.23 min
		Telmisartan impurity F		20.84 min
		Telmisartan methyl ester		22.15 min
		N-methyl-O-phenylenediamine dihydrochloride		10.73 min
Limit of Detection and Limit of Quantitation	LOD concentration (µg/g)			2.53
	S/N ratio LOD should be 3:1			3.52
	LOQ concentration (µg/g)			7.52
	S/N ratio LOD should be 10:1			13.28

	% RSD at LOQ level should NMT 10.0 %	2.54
Linearity	Slope (Record Results)	452.06
	Intercept (Record Results)	80.93
	Correlation Coefficient (NLT 0.990)	0.9998
	Residual sum of square (Record Results)	20884.204
Precision	% RSD of repeatability study NMT 10.0 %	0.59 %
	% RSD of Intermediate precision study NMT 10.0 %	0.48 %
Solution stability	Absolute difference in impurity should be not more than 15 % of evaluation limit	Complies
Robustness	Deliberate changes in the developed condition should be not impact on system suitability	Complies

Specificity

For demonstrating the specificity of the method blank, USP listed known / process impurities, N-methyl-O-phenylenediamine dihydrochloride standard, telmisartan sample were prepared individually at specification limit in the diluent and the solution of telmisartan spiked with N-methyl-O-phenylenediamine dihydrochloride evaluation limit and injected into developed chromatographic condition. No chromatographic interference (Fig. 3) from any of the blank, impurities and sample peak was found at the retention time of N-methyl-O-phenylenediamine dihydrochloride. These results (Table 1) confirm the specificity of the method without any interfering peak around the retention time of N-methyl-O-phenylenediamine dihydrochloride; also the base line did not show any significant noise around the peak.

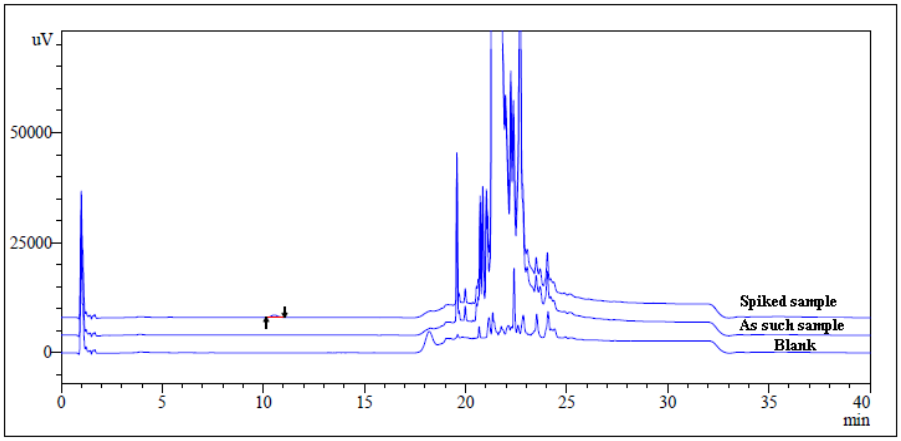


Fig. 3: Overlay chromatogram of blank, sample and spiked sample

Limit of detection and Limit of quantitation

The LOD and LOQ for N-methyl-O-phenylenediamine dihydrochloride were estimated through the signal-to-noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solutions having known concentrations. LOD of the impurity is defined as the lowest concentration that can be detected. LOD was found to be 2.53µg/g (Fig. 4). LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy. LOQ was found to be 7.52µg/g (Fig. 4). The low values of LOD and LOQ indicate the adequate sensitivity of the method. The precision study was also carried out at LOQ level by injecting six individual preparations and calculating the % RSD of the area. The results are present in table 1.

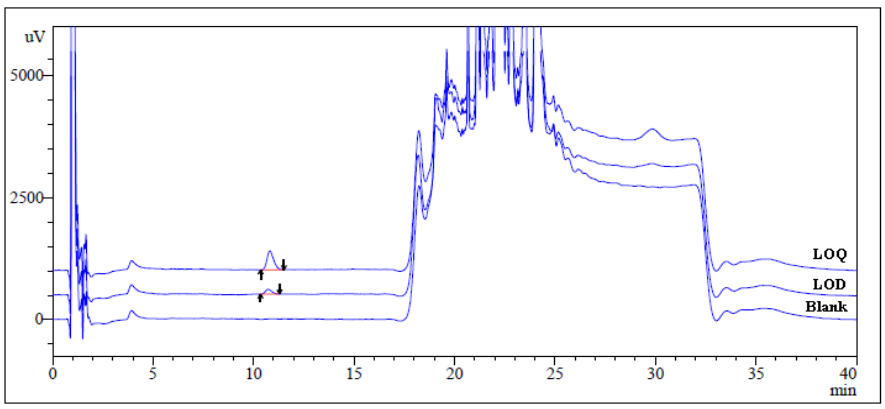


Fig. 4: Chromatogram of Limit of detection and Limit of quantitation

Linearity

To establish the linearity of the developed method, calibration solution were prepared by diluting the impurity stock solution to obtain solutions at LOQ, 50%, 80%, 100%, 120% and 150% of the evaluation limits. Each solution was injected and area of responses was recorded at 230 nm. The graph of peak area vs concentration in µg/g was plotted (Fig. 5). The slope, intercept, correlation coefficient of the regression line and residual sum of square were calculated. The correlation coefficient obtained was greater than 0.990. The result shows that an excellent correlation existed between the peak area and the concentration of the impurity over the entire concentration. The results are summarized in table 1.

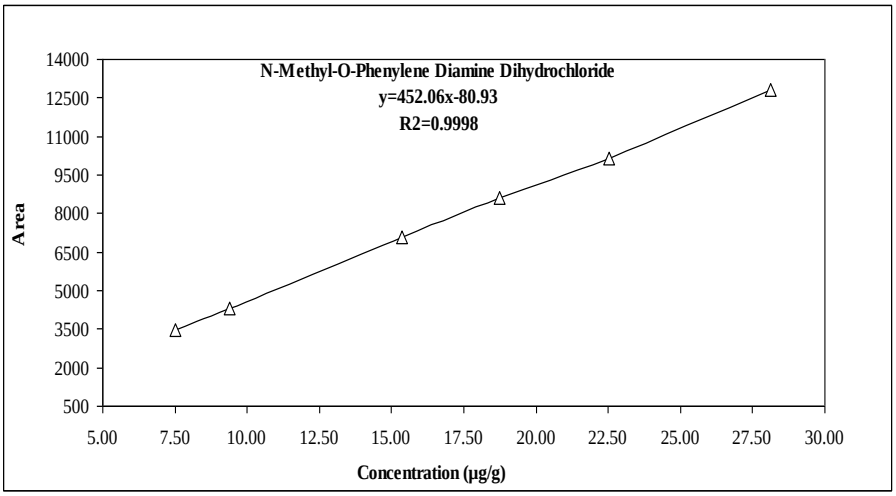


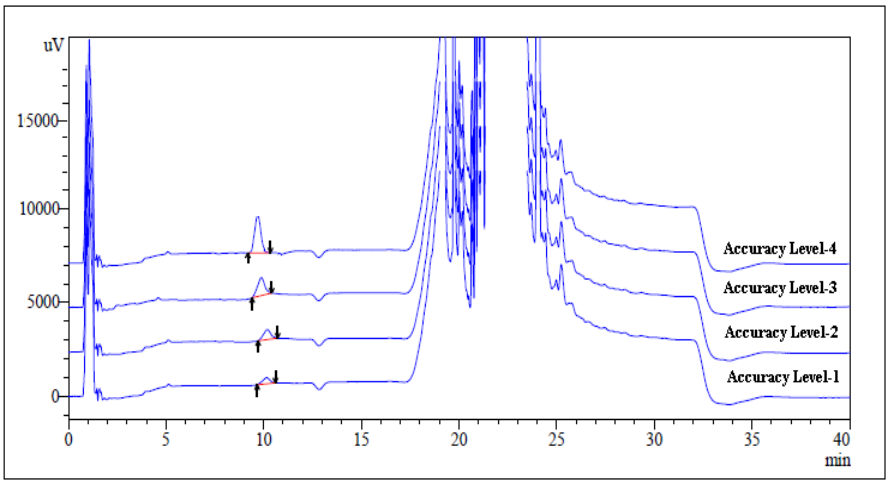
Fig. 5: Linearity of N-methyl-O-phenylenediamine dihydrochloride

Precision

Precision was determined through repeatability and intermediate precision, Precision of the method was checked by injecting six individual preparation of telmisartan spiked with the impurity at evaluation limit. The percentage RSD of the content of impurity was calculated. Intermediate precision of the method was evaluated by injecting six individual preparation of the spiked sample at evaluation limit on a different day in the same laboratory. The % RSD for the content of N-methyl-O-phenylenediamine dihydrochloride impurity was very low, confirmed that the high precision of the method. The results are present in table 1.

Accuracy

The accuracy of the method was determined by analyzing the drug substances spiked with impurity. A known amount of impurity was spiked to the telmisartan sample at different concentration levels of LOQ, 50%, 100% and 150% of the evaluation limit. Each concentration level was prepared in triplicate. The percentage recovery of impurity in the drug substances was calculated. The recovery of the N-methyl-O-phenylenediamine dihydrochloride in telmisartan ranged from 90.68 to 110.21% which is well within acceptance criteria 80% to 120%. The results are summarized in table 2 and it was observed that the method was accurate within a determined range. Overlay chromatograms of accuracy are presented in fig. 6.



. **Fig. 6: Overlay chromatograms of accuracy (at LOQ, 50 %, 100 %, and 150 %)**

Table 2: Accuracy at different spiking concentration

Level	Amount in sample	Amount added (µg/g)	Amount found (µg/g)	Recovery (%)
	nil	7.425	7.201	96.98
	nil	7.856	7.124	90.68
	nil	7.654	7.34	95.90
50 %	nil	9.502	9.384	98.76
	nil	9.512	9.632	101.26
	nil	9.508	9.532	100.25
100 %	nil	19.007	20.644	108.61
	nil	19.014	20.956	110.21
	nil	19.022	20.394	107.21
150 %	nil	28.508	29.154	102.27
	nil	28.523	29.235	102.50
	nil	28.532	29.568	103.63

Solution stability

The solution stability was established by spiking N-methyl-O-phenylenediamine dihydrochloride impurity in telmisartan sample. The prepared solution was stored at room temperature for 24 h. The content of impurity was determined at 4 h interval for 24 h. The result was observed that no significant change in the content of the impurity.

Robustness

To determine the robustness of the method, the experimental conditions were deliberately altered and the system suitability result was evaluated. To study the effect of flow rate, it was changed by 0.1 units from 1.0 mL/min to 0.9 mL/min and 1.1 mL/min. The effect of column temperature was studied by changed 5°C units from 30 °C to 25 °C and 35 °C. The results were found that the deliberate changes in the method, i.e. flow rate of mobile phase and column oven temperature have no impact on system suitability.

Batch analysis

The three production batches of telmisartan drug substance were analyzed in the validated method for determination of N-methyl-O-phenylenediamine dihydrochloride and found the impurity was not detected in all three batches. Overlay chromatograms of three production batches are presented in fig. 7.

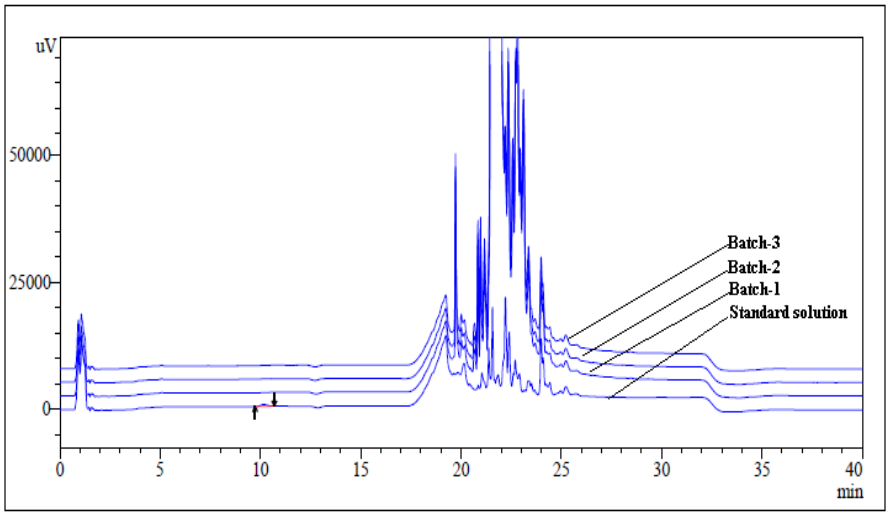


Fig. 7: Overlap chromatogram of three production batches of telmisartan sample with standard solution

CONCLUSION

The present study represents a gradient HPLC method developed for the trace level quantitative determination of genotoxic N-methyl-O-phenylenediamine dihydrochloride in telmisartan is linear, precise, accurate, rugged and robust. Satisfactory results were obtained from validation of the method according to ICH guideline. This method exhibited an excellent performance in terms of sensitivity and specificity with no sample matrix and impurity interference observed. This method can be used for routine analysis of the trace level quantitative determination of N-methyl-O-phenylenediamine dihydrochloride in telmisartan drug substances.

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