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A Reverse Phase HPLC Method Development and Validation of 2,4,6 Trifluro Benzoic Acid and Its Impurities: A Key Raw Material Used in Preparation of Anti Migraine Drug Lasmiditan Hemisuccinate

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Abstract

A novel gradient reverse phase liquid chromatographic (RP-HPLC) method is developed and validated for the determination of purity of 2,4,6 Trifluro benzoic acid (TBA) in presence of its related impurities. TBA is very important key raw material used in the process development of Active Pharmaceutical Ingredient (API), Lasmiditan Hemisuccinate. The impurities formed during the synthesis of TBA will diminishes the pharmacological quality of the Lasmiditan Hemisuccinate. Hence, the stringent control of TBA and its related impurities were significantly important to achieve the niche quality of Lasmiditan Hemisuccinate. This HPLC method developed by using Zorbax SB-Aq, 5 µm, 4.6 x 250 mm column with mobile phase containing a gradient mixture of solvent A and B. The buffer is 0.1% triethyl amine solution is solvent A and acetonitrile, methanol, water in the ratio of 700:200:100 v/v/v solvent mixture is solvent B. The eluted compounds monitored at 205 nm. This method validated as per International Conference on Harmonization (ICH) guidelines with respect to specificity, limit of detection, limit of quantitation, precision, linearity, accuracy and system suitability.

Keywords: 2,4,6 Trifluro benzoic acid (TBA); Lasmiditan Hemisuccinate; HPLC-Development; Validation.

1. Introduction

2,4,6 trifluro benzoic acid (TBA) is an active moiety of anti-migraine drug Lasmiditan Hemisuccinate **(Figure 1)**. TBA impurities will affect the quality of the API. Hence, the separation of TBA and its related impurities at low level become significant and control of these impurities are necessary in Lasmiditan Hemisuccinate

Generally, TBA is prepared from 1,3,5 trifluro benzene. The route of synthesis of TBA is shown in (**Figure 2).** It involves reaction of 1,3,5-trichlorobenzene with potassium fluoride at high temperatures. Sequential displacement of chlorides by fluoride will furnish 1,3,5-trifluoro benzene. Further treatment of 1,3,5- trifluoro benzene with butyl lithium followed by quenching with dry ice provides the target. Based on the synthetic route, the plausible potential impurities shown in **Figure 3**. 1,3,5- trichloro benzene is the starting material and the unreacted starting material can react with butyl lithium and dry ice to produce 2,4,6-trichlorobenzoic acid. Incomplete fluorination may result in 2-chloro-4,6-difluoro benzoic acid, 4- chloro-2,6-difluoro benzoic acid and 2,4-dichloro-6-fluoro benzoic acid. Other possible impurities like difluoro and monofluoro controlled in an advanced intermediate. Hence, a validated analytical method is important to analyse the parent constituent, 2,4,6 trifluro benzoic acid (TBA) and it is to be developed either to determine only the parent constituent or only the impurity to separate and determine the parent compound in presence of its impurities [1]. Regulatory guidance in ICH Q1AR2 [2], Q3BR2 [3],

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Q6A [4] and FDA 21 CFR section 211[5] insists for the validated purity and assay methods.

Chromatographic and Spectroscopic techniques, either alone or in combination with other techniques were commonly practiced for the determination and characterization of impurities, which are produced either during formulation or upon aging of both API and formulations. HPLC has exploited for impurity profiling using a wide range of detectors, and stationary phases due to its sensitivity and cost effective separation. Few analytical methods found in literature for the determination of TBA in ground water by HPLC and environmental samples by LCMS [6], ion-chromatography-electrospray mass spectrometry [7]. A rapid ion-exchange method is developed to analyse the fluro benzoic acid of salt-rich reservoir [8] and nineteen fluro benzoic acid in saline water were identified with solid [9]. A sensitive UPLC method used to identify the fluorobenzoic acids in oil reservoir water [10] and solid-phase extraction and gas chromatography is used to identify the fluorobenzoic acid in tap water [11]. There is no specific literature is found, to identify the process related impurities in TBA which is a key raw material used in the process development of Lasmiditan Hemisuccinate. Hence, we have developed and validated a RP-LC method that can separate and quantitate TBA, its process related impurities.

2. Materials and Methods

2.1 Chemicals and Reagents

TBA (99.8%) and its five impurities, viz. Impurity-1 (97.5%), Impurity-2 (99.8%), Impurity-3 (97.8%), Impurity-4 (96.8%), Impurity-5 (98.2%) were obtained from our R & D division. Triethyl amine, orthophosphoric acid, methanol, acetonitrile, formic acid were obtained from Merck, India. All the solutions were prepared in Milli Q water (Millipore, USA).

2.1.1 HPLC Conditions

Agilent 1260/Infinity-II separation module (Agilent Corporation, USA) equipped with Ultra violet visible detector with EZ-Chrome elite software is used. For the analysis, Zorbax SB-Aq, 5 µm, 4.6 x 250 mm, Agilent, USA) column and a gradient mixture of solvent A and B were used as stationary and mobile phases, respectively. The solution –A is the buffer. The buffer used is 0.1% triethyl amine and the pH adjusted to 4.0 with diluted orthophosphoric acid. The solution-B is solvent mixture. The solvent mixture acetonitrile, methanol and water is prepared in the ratio of 700:200:100 v/v/v. The gradient program (T/%B) is set as 0/5, 5/5, 35/50, 45/90, 55/90 and 60/5, 65/5. An injection volume of 15 µL is used. The eluted compounds monitored at 205 nm. The column oven and auto sampler temperatures maintained at 40°C and 5°C.

2.1.2 Preparation of stock and standard solutions

A mixture of acetonitrile: methanol: water: formic acid (700:200:100:1.0 v/v/v/v) is used as diluent. 2 mg/ml stock solutions of TBA and 1 mg/mL of impurities were prepared in diluent for purity respectively. 0.15% of five impurities blend with respect to 2 mg/ml TBA is prepared in diluent.

3. Results and Discussions

3.1 Method Development and Optimization

HPLC method development for the separation of TBA and its five impurities initiated with the literature method [12]. In this method, TBA and Impurity-1 are co-eluting at same retention time. The TBA, Impurity-1, Impurity-2, Impurity-3, Impurity-4, Impurity-5 peak shapes were broad and tailing is more than 2.0. Hence, the effect of mobile phase pH studied viz., at pH 2.0, 4.5, 6.0 and pH 8.0. In changing the various pH conditions, at pH=4.0 the retention time separation is achieved with appropriate resolutions. However, in pH=4.0, the peak shapes were not improved. To improve the peak shapes, the different columns like Luna phenyl, Intersil ODS, Chromosil, Hypersil ODS etc., are tried

But in those columns the peak shapes and responses were not improved. Finally, the Zorbax SB-Aq (250 × 4.6 mm, 5 µ) column is tried and this column, separated all the impurities and the peak tailing reduced partially. To achieve the peak tailing less than 2.0 **(Figure-4),** the various diluents were studied like methanol, water, acetonitrile and methanol: water (50:50 v/v), acetonitrile: methanol (90:10 v/v), methanol: acetonitrile: water (70:20:10 v/v/v) were studied. The diluent acetonitrile: methanol: water: formic acid in the ratio of 700:200:100:1.0 v/v/v/v is improved the peak shapes of TBA and its impurities. But, the Impuriy-2, and Impurity-3 peaks were also closely eluting. Hence, the column temperature introduced as 40°C and with that temperature impurities resolution is improved. As the TBA and Impurity-1 is eluting early and Impurity-4 is eluting at longer retention time, the gradient programme modified to achieve the suitable retention times. The gradient program (T/%B) is set as 0/5, 5/5, 35/50, 45/90, 55/90 and 60/5, 65/5 which is found to be optimal as all the impurities of TBA are eluted at adequate retention times. The 205 nm wavelength selected as TBA and its five impurities are having maximum UV absorbance at this wavelength. Injection volume is 15 µL and 1 ml/min flow rate, 40°C temperature found to be ideal for a good chromatographic performance. The chromatographic

performance data presented in **Table 1**. A typical chromatogram of TBA spiked with 0.15 % of its five impurities shown in **Figure 5)**

3.2 Method Validation

The proposed method validated as per the ICH guideline. The critical parameters were like specificity, limit of detection, limit of quantitation, precision, linearity, accuracy, robustness and system suitability studied. Specificity of the developed LC method verified in the presence of its known impurities. All the known impurities are well resolved from one another and TBA peak, indicating the specificity of the proposed method to quantify TBA and its impurities. To establish the limit of detection and limit of quantitation**,** initially, 0.15% of TBA impurities blend with respect to 2 mg/ml TBA and injected. This solution further diluted to achieve the signal-to-noise (S/N) ratio of 3:1 and 10:1 for determining limit of detection (LOD) and limit of quantitation (LOQ), respectively. The S/N ratio is for TBA impurities were calculated using EZ-Chrome elite software. The S/N ratio of LOD and LOQ values found between 3 to 5 for LOD and 10 to 15 for LOQ. The precision of the method checked by injecting six individual preparations of TBA spiked with 0.15% of its five impurities (0.15% of impurities spiked with respect to 2 mg/ml TBA). % RSD of area for each impurity is calculated. LOQ precision also determined by injecting six individual preparations of TBA spiked at LOQ level of its five impurities. The intermediate precision of the method verified on two different days in the same laboratory using the specification level spiked TBA solutions. Columns with different packing lot particles used during this study and the results found to be in acceptance range. In all the precision study, % RSD is observed as less than 15%.

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. Linearity test solutions for impurities were prepared individually at six concentration levels in the range of LOQ to 200% of the specification level, viz. 0.15%. The peak area versus concentration data performed by least-squares linear regression analysis. The R^2 values found to 0.999.

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or as an accepted reference value and the value found. Standard addition and recovery experiments conducted to determine accuracy of the impurities of TBA for their quantification. The study carried out in at LOQ, 100% and 200% with respect to specification level, viz. 0.15%. The recoveries between 80% and 120%. The robustness is illustrated by getting the resolution between any two compounds to be greater than 2.0, when mobile phase flow rate (±0.2 ml/min), pH (±0.2), organic solvent ratio (±5%) and column temperature (±2 ◦C) were deliberately varied. The system suitability established by considering the 0.15% solution of TBA is prepared in diluent with respect to test concentration 2 mg/mL and six replicates injected into the chromatographic system. This solution used as system suitability solution. The % RSD for the six replicate injections of 0.10% of TBA is found as 1.8%. All the results of the validation parameters summarized in **Table-1.**

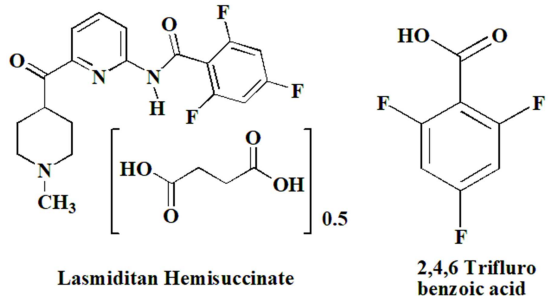

Fig. 1: Chemical structures of Lasmiditan Hemisuccinate and 2,4,6 Trifluro Benzoic Acid [TBA]

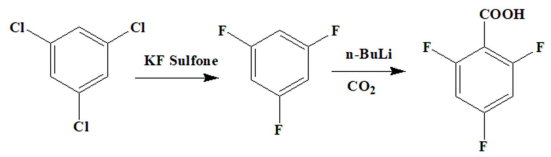

Fig. 2: Synthesis route for 2,4,6-Trifluoro Benzoic Acid [TBA]

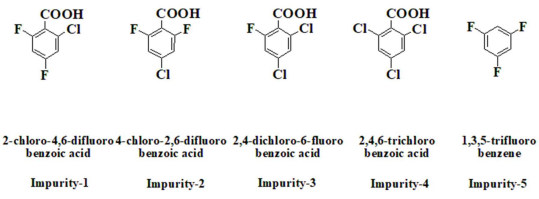

Fig. 3: Potential impurities in 2,4,6-Trifluoro Benzoic Acid [TBA]

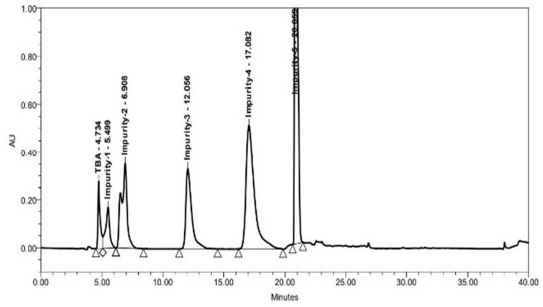

Fig. 4: Spiked impurities (Impurity-1, Impurity-2, Impurity-3, Impurity-4, Impurity-5) to TBA (peak tailing is more than 2.0)

(HPLC conditions: Mobile phase A: 10 mM KH_2PO_4 in water, pH=6.0 with triethyl amine; Mobile phase-B: acetonitrile; Column: Luna phenyl hexyl, 5 µm, 4.6 x 250 mm; Wave length: 205 nm; Diluent: acetonitrile; Gradient elution Time / %B: 0/5, 15/5, 20/50, 30/80, 35/80, 35.1/5, 40/5)

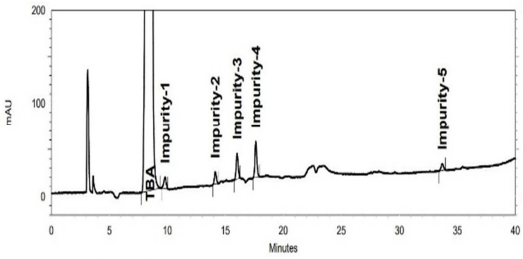

Fig. 5: Spiked 0.15% impurities (Impurity-1, Impurity-2, Impurity-3, Impurity-4, Impurity-5) to TBA

(*HPLC conditions: Mobile phase A: 0.1% triethyl amine in water, pH=4.0, with diluted ortho phosphoric acid; Mobile phase-B: acetonitrile: methanol: water (700:200:100 v/v/v); Column: Zorbax SB-Aq, 5 µm, 4.6 x 250 mm; Wave length: 205 nm; Diluent: acetonitrile: methanol: water: formic acid (700:200:100:1.0 v/v/v/v); Gradient elution Time / %B: 0/5, 5/5, 35/50, 45/90, 55/90, 60/5, 65/5)*

.

Table 1

Analytical method validation summary

S.No	Parameter	Acceptance criteria	Results
1	System suitability	% RSD of six replicate injections of 0.10% solution is Not more than 10.0	% RSD of TBA is 1.8
2	Selectivity	All the peaks to be separated from TBA and should not have any blank interference	Retention Times (minutes): TBA, 8.57, Impurity-1: 9.94, Impurity-2: 14.29, Impurity-3: 16.14, Impurity-4: 17.76
3	Limit of Detection (LOD)	S/N ratio not less than 3.0	TBA: 5.8, Impurity-1: 9.94, Impurity-2: 7.0, Impurity-3: 15.9, Impurity-4: 21.0, Impurity-5: 40.1
4	Limit of Quantification (LOQ)	S/N ratio not less than 10.0	TBA: 15, Impurity-1: 23.3, Impuirty-2: 21.0, Impurity-3: 46.9, Impurity-4: 65.0, Impurity-5: 11.4
5	LOQ precision (n=6)	% RSD for six replicates of LOQ solution is not more than 10	TBA: 3.54, Impurity-1: 2.26, Impurity-2: 3.20, Impurity-3: 2.49, Impurity-4: 1.99, Impurity-5: 1.10
6	Linearity (n=6)	Correlation co-efficient not less than 0.99	TBA: 0.999, Impurity-1: 0.998, Impurity-2: 0.997, Impurity-3: 1.000, Impurity-4: 0.997, Impuirty-5: 0.999
7	Accuracy	Recovery should be between 80% and 120%	LOQ level: Impurity-1: 87.6, Impurity-2: 101.0, Impurity-3: 97.3, Impurity-4: 101.4, Impurity-5: 105.3 100% level: Impurity-1: 84.25%, Impurity-2: 96.75%, Impurity-3: 83.75%, Impurity-4: 83.60, Impurity-5: 84.87 200 % level : Impurity-1: 90.74, Impurity-2: 84.78, Impurity-3: 81.49, Impurity-4: 83.65, Impurity-5: 85.07

4. Conclusions

A RP-HPLC method has been developed and validated for the determination of TBA and its impurities. This method is able to separate the TBA from its impurities and it can be used for checking the quality of TBA in presence of impurities.

5. Conflicts of interest

"There are no conflicts to declare".

6. Acknowledgments

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