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Keywords :	Tetra-n-butylammonium bromide; TBAB; Teneligliptin; GC-MS; Validation

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ABSTRACT

Teneligliptin (DPP-4 inhibitor) is a potential drug to control type 2 diabetes. During the synthesis of Teneligliptin, the tetra butylammonium bromide (TBAB) is used as a phase transfer catalyst, but, it cannot be completely controlled to reach the drug. Hence it is essential to estimate the TBAB, but the existing methods are very limited and particularly no appreciable effective GC method exist. As a consequence, we involved in the development of a sensitive and rapid GC-MS method for the determination of TBAB in drug. The GC-MS analysis was performed on Agilent 5977B GC/MSD equipped with DB-1 capillary column (60 m × 0.32 mm x 0.25 µm) and helium carrier gas. The detection was performed at the *m/z* of 100, 142, and 185 using selective ion monitoring (SIM) mode. The concentration range of 200-1500 ppm with the correlation coefficient of 0.9995 was determined for the linearity curve. The detection (LOD) and quantitation limits (LOQ) of TBAB were calculated as 3.3 µg/ml and 10.0 µg/ml, respectively, which are corresponding to 66 µg/g and 200 µg/g of TBAB with respect to the sample weight. The accuracy of the proposed method was determined by recovery studies and was found to be 88 to 102%. RSD area of the six replicates injection of TBAB standard was <5%. The proposed method showed excellent linearity, accuracy, precision, specificity, LOD, LOQ, and system suitability according to ICH guidelines. In addition, commercial batches were also analyzed to prove this method's suitability.

Keywords: Tetra-n-butylammonium bromide; TBAB; Teneligliptin; GC-MS; Validation.

INTRODUCTION

In people with type 2 diabetes, the Teneligliptin, a DPP-4 inhibitor is used in conjunction with a proper diet and exercise program to control blood sugar levels (Kishimoto, 2013; Sharma *et al.*, 2016). Teneligliptin increases the level of natural substances such as incretins that help to control blood sugar by releasing insulin, especially after a meal. Drug substances are generally obtained by a series of manufacturing steps and through these steps, either from the raw materials or from the process (intermediate stages and/or degradation products) the impurities will be incorporated with the drug substances. Further, through the manufacturing process, residual solvents and catalysts may also be present in the pharmaceuticals as impurities, which are subject to evaluation and in fact to be controlled as much as possible because they do not contribute to the therapeutic activity (United States Pharmacopoeia USP, 2016). But, in reality, they cannot be completely removed by practical manufacturing techniques (International Conference on Harmonization ICH, 2011). The use of TBAB as a phase transfer catalyst (PTC) in Teneligliptin synthesis leads to the presence of TBAB as a residual impurity in the final drug substance. Numerous efforts are being made during the synthetic process to remove the potentially toxic organic ionic impurity; however, hitherto it is not been completely eradicated from the final drug. As per the current governing authorities' guidance on drugs, it is extremely important to control the level of impurities in the formulations of drug substances and drug products below the threshold, based on dosage.

There are no considerable number of reports on the estimation of TBAB in drug either by GC or HPLC in the literature. Generally, the estimation of amines is difficult due to their interaction with the stationary phase of both GC and LC. Particularly, while lowering the molecular weight, the interaction with the stationary phase will be higher. In addition, the non-

availability of chromophores in low molecular weight amines making them difficult to be detected. However, there are few reports available with low molecular weight amines using HPLC associated with the derivatization techniques (Meseguer-Lloret *et al.*, 2004; Hao *et al.*, 2004; Herraez-Hernandez *et al.*, 2006). As we know that the derivatization techniques are laborious and less sensitive towards pharmaceutical products, it is no longer recommended for the trace level estimation. On the other hand, a recent report on the direct estimation of TBAB in Daclatasvir dihydrochloride API by LC-MS/MS seems to be specific but the LOQ (310 ppm) was in higher range (Reddy *et al.*, 2019).

In GC, mostly the derivatization reports are available due to the sorption on stationary phase along with other difficulties such as basic, volatile and polar nature of the amines. In order to overcome the above issues derivatization techniques were adopted for the estimation of some aliphatic amines (Jerome *et al.*, 2008; Chang *et al.*, 2005; Zhao *et al.*, 2003). But they were not more sensitive and performed/applicable on effluents rather than pharmaceuticals. Instead of derivatization, another method that involved pyrolysis of TBAB to produce n-butyl bromide and *tert*-butylamine for the estimation of TBAB by GC separation. Even if the method was also laborious, not suitable for measuring TBAB in trace level according to the impurity control strategy; their quantification with linearity basis was in the range of 0.0485 - 0.2416 g/ml (Lopez *et al.*, 1988).

Alternatively, the low molecular weight aliphatic amines such as amylamine, trimethylamine, tert-butylamine and piperazine were separated from the pharmaceuticals (Hajos *et al.*, 2002; Jagota *et al.*, 1996; Tan *et al.*, 1995; Hall *et al.*, 1995), whereas certain low molecular weight amine, ammonium, hydroxylamine and ethanolamine were estimated from the effluents (Christian *et al.*, 2020; Fernando *et al.*, 2022), saline water (Fernanda *et al.*, 2017) and

natural gas (Maryam *et al.*, 2021; Kadnar, 1999) using ion chromatography (IC) on appropriate column through either suppressed or non-suppressed conductivity detection (Kumagai *et al.*, 1996; Krol, 1992). An appreciable effort has had been carried out on the direct determination of TBAB in Levetiracetam using IC with cation exchange non-suppressed conductivity detection and reported as a sensitive method (Subramanian *et al.*, 2009).

In fact, there are considerable number of reports on the estimation of low molecular weight amines using IC including one direct method of estimation of TBAB in Levetiracetam, whereas, to our knowledge best, no GC or LC method has had been demonstrated for the direct estimation of TBAB in trace level in pharmaceuticals except the one with tandem mass LC with low sensitivity. Keeping all the above facts in mind, we were involved in the development of a suitable, selective, and sensitive direct method to determine the TBAB in Teneligliptin drug.

MATERIALS AND METHODS

Materials

Analytical grade reagents and solvents were used for the research work. TBAB was procured from Sigma-Aldrich Chemicals Pvt. Ltd., India.

Optimized GC-MS conditions

To ensure the GC system's suitability in providing a valid peak shape and acceptable recovery, a range of chromatographic parameters were optimized during the method development process. The parameters screened in the method optimization include the column temperature (80 – 150 °C), flow rate (1.0 – 1.5 ml with constant flow), injector temperature (IT +10 °C), and capillary GC columns (HP-5, DB-1701, and DB-1 with varying film thickness. In terms of retention time at lower temperatures, the HP-5 (30 m x 0.32 mm x 1 µm) and the DB-

1701 (30 m length x 0.32 mm x 1 m film thickness) columns performed well. The stationary phases, however, showed greater bleed and less uniform baseline across the temperature range tested. On the DB-1 capillary column (60 m × 0.32 mm x 0.25 µm) with helium as carrier gas, we achieved an acceptable level of selectivity, sensitivity, resolution and chromatographic separation with a stable baseline. Hence, Agilent 5977B GC/MSD, USA was used for the analysis (GC paired with a quadrupole mass spectrometer) using DB-1. Injection volume was chosen to be 1µl with a split inlet of 10:1. GC oven temperatures were set at 120 °C and held for 1 minute before being ramped to 280 °C at 15 °C/minute. It took 15 minutes to reach the final temperature. GC-MS interface, ion source and injection temperatures were 260, 250, and 230 °C, respectively. Helium (carrier gas) was used at a flow rate of 2 ml/minute and 70 eV was used to ionize the gas. We collected three mass spectra (*m/z*): 100, 142, and 185 using selective ion monitoring (SIM) mode. The analyses were performed using GC-MS solution software, Version 2.50. Based on the mass spectral library of National Institute of Standard Technology (NIST), the compounds were identified.

Standard preparation

The standard stock solution was prepared by diluting 250 mg of TBAB in 100 ml of acetonitrile (CH_3CN). Further, 1ml of the above solution was diluted to 50 ml.

Sample preparation

A 500 mg of sample was diluted to 10 ml using CH_3CN.

RESULTS AND DISCUSSION

Method development

The attempt to separate TBAB using a DB-5 column (5% phenyl: 95% dimethylpolysiloxane) was unsuccessful because of the irregular peak shape, whereas the replacement of DB-5 column by DB-1 (100% dimethylpolysiloxane) ended up with sharp peaks. By injecting 1µl of solution, the effects on separation and determination of injection volume ratios were studied. The split ratio was fixed at 10:1 according to the detector response. The separation of TBAB was investigated as a function of column temperature (120 °C was preferred as the initial column temperature) and the injections were performed as follows: blank (1 injection), standard solution (6 injections), blank (1 injection), sample solution (2 injections), and standard solution-bracketing (1 injection). Interference caused by the diluent was also corrected necessarily.

Method validation

Method validation was executed according to the validation of analytical methods outlined in the ICH guidelines[25,26]. To determine the suitability of GC-MS, a standard solution of TBAB of 1000 ppm was injected and the data is provided in **Table 1**.

For the six preparations of standard solution, there should not be a difference greater than 15% in the relative standard deviation (RSD) area of TBAB peak. The TBAB content in Teneligliptin was estimated using the system and the % RSD was found to be in the acceptance criteria.

Specificity

The solvents used to manufacture Teneligliptin were injected as part of the specificity study. From the study, it is observed that the analyte components did not interfere with each other.

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

Calibration curves (CC) were used according to the ICH guidelines for the determination of LOD and LOQ values and are outlined in the **Table 2** for better perception. TBAB was found to possess LOD and LOQ values of 66 and 200 ppm, respectively. Based on six LOQ preparations, the RSD peak area of TBAB is 2.02.

Linearity

In order to achieve a stable base line, the system and column were conditioned. The injections were performed as specified and observations were recorded in **Table 3**. The correlation coefficient was 0.9995 and linearity was tested between 50 and 150%.

RSD of the area of each solvent peak determined as a result of the six preparations of linearity levels 1 and 6 did not exceed the acceptance limit 15%. The results clearly indicate that the proposed method meets the acceptance criteria as evidenced by the correlation coefficient (>0.99) and the %RSD of each solvent peak.

Precision and accuracy

Six replicate preparations were injected with the standard solution containing 1000 ppm of TBAB to determine the method's precision. TBAB showed a RSD of 2.4 on six replicates, within the permissible limits as indicated in **Table 4**. Analysis of the peak areas of the analyte confirms the precision of this method with low RSD. The TBAB sample was spiked at QL levels, 100% and 150% for accuracy testing and the data are reproduced in Table 5. All accuracy levels of TBAB should have a recovery rate between 80 and 120 according to the acceptance criteria. The average recovery percentage was well within the permissible range.

As an outcome of the successful method validation, a few of the significant recommended parameters are shown in Table 6 as standard test procedure of the estimation of TBAB content by GC-MS.

Mass spectral analysis

In GC-MS, the TBAB appears at 12.35 minutes (**Figs. 1-3**), and the presence of TBAB ($C_{16}H_{36}NBr$, *m/z* 322) is confirmed by its mass spectra (**Fig. 4**) through the major fragments of TBAB at *m/z* 185, 142, 100 and 57.

Batch analysis report

Three batches of Tenelegliptins were analysed according to the above developed and validated method to find the practical application in industries. As an outcome of the batch analyses, the results are found to be below the detection limit, which ensures the absence of TBAB in the tested batches above 65 ppm (LOD is 66 ppm). The complete batch analysis reports of the three different batches are systematically summarized with appropriate chromatograms and provided as a Supplementary material.

CONCLUSIONS

Determination of low-molecular weight amines are challenging to get a better reproducibility in GC due to the aminic (basic) nature of the analyte, which make strong binding with free silanol groups in the GC columns. As an outcome of this interaction, precision gets collapsed and exhibits generally poor detectability. However, we attempted to directly estimate the presence of TBAB (molecular weight: 322.86) in Teneligliptin and thus the developed method resulted with better peak shape, high reproducibility and good accuracy as well. The developed method was validated as per ICH guidelines.

In light of the experimental and validation results, the method appears to be simple, specific, rapid, robust, linear, precise and accurate. Hence, we recommend this method for the quantitative determination of TBAB in Teneligliptin. This GC-MS method may be useful for determining the presence of TBAB in gliptins and other drug substances as well. Over and above, this method can be readily implemented in any pharmaceutical analytical labs since it does not require any derivatizing agents.

Author contribution

The manuscript was compiled by the contributions of all authors. The final version of the manuscript has been approved by all authors.

Conflict of interest (If any)

The authors declare no conflicts of interest.

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None to declare.

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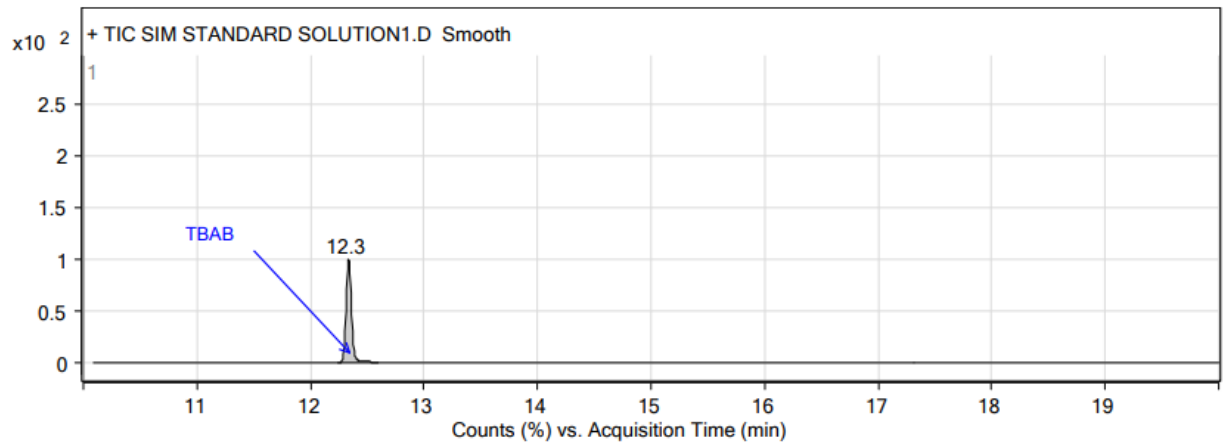

Figure 1. GC-MS chromatogram of TBAB standard (1000 ppm)

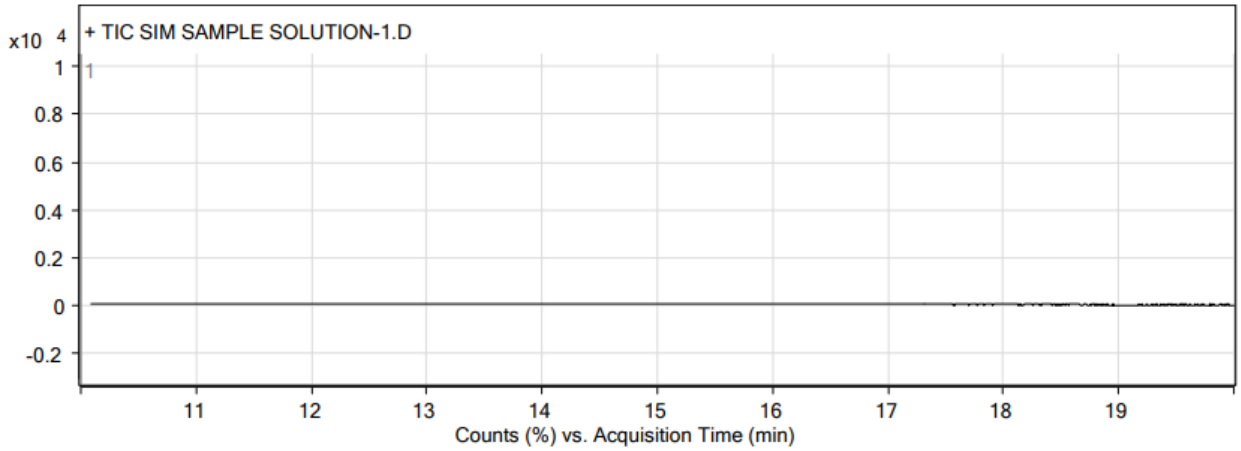

Figure 2. GC-MS chromatogram of Teneligliptin

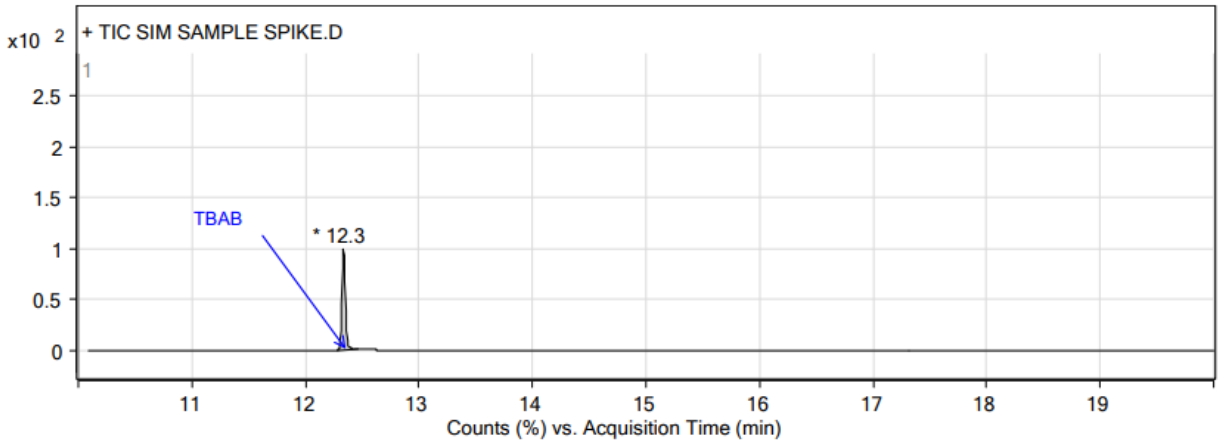

Figure 3. GC-MS chromatogram of Teneligliptin spiked with TBAB

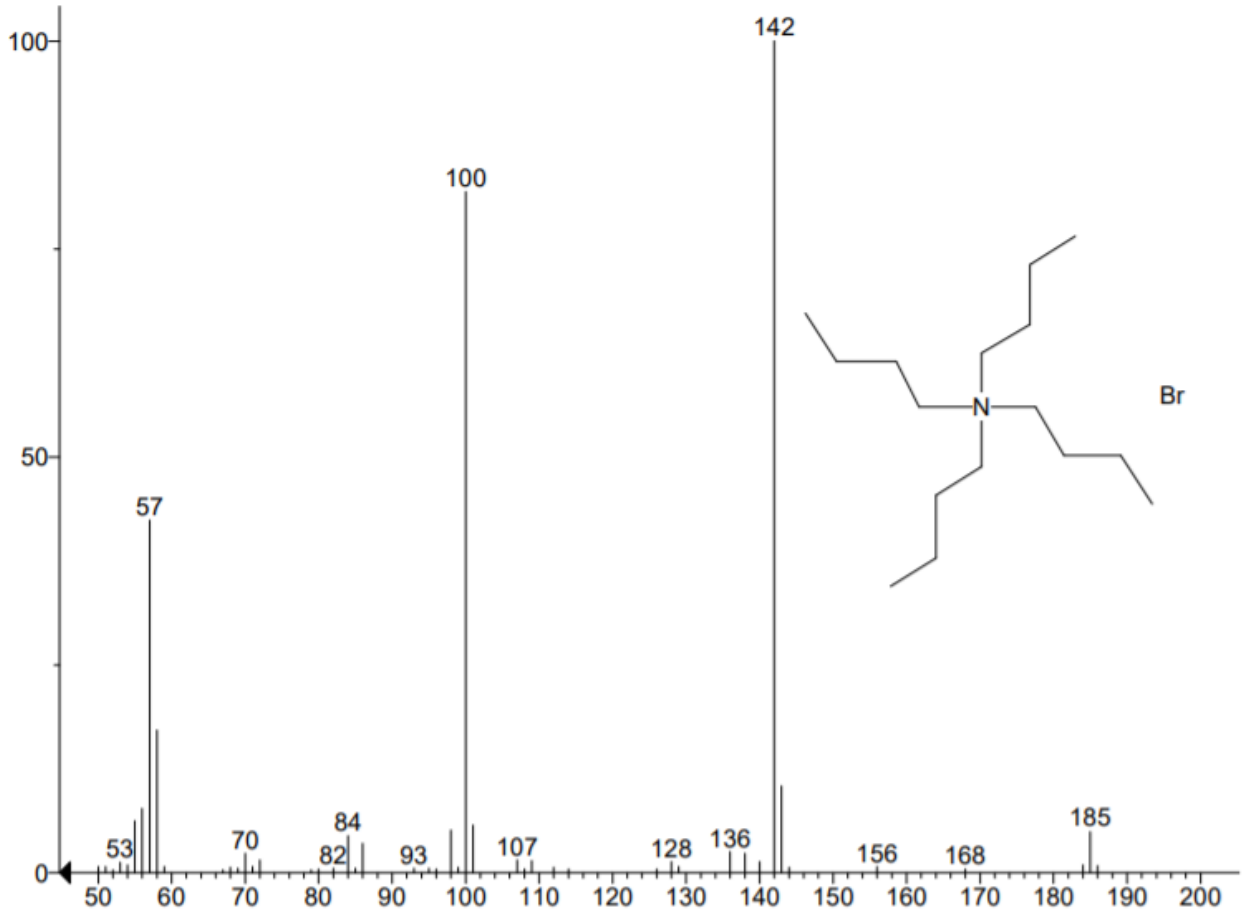

Figure 4. Mass pattern of TBAB (Ionization potential: 70 eV; Scan range (*m/z*): 29-400 Da).

Table 1. System suitability data for standard samples

Preparation	Response of TBAB
Std-1	20565556
Std-2	21566985
Std-3	22871496
Std-4	21566986
Std-5	22587968
Std-6	20698756
Average	21642958
S.D.	945114.35
% RSD	**4.37**

Table 2. Precision at LOQ Level

Concentration of TBAB (200 ppm)	**Response of TBAB**
LOQ-1	457808
LOQ-2	459632
LOQ-3	445698
LOQ-4	436985
LOQ-5	445687
LOQ-6	441256
Average	447844
S.D.	9042.30
% RSD	**2.02**

Table 3. Linearity of TBAB

Linearity level	Concentration (ppm)	Area of injection-1	Area of injection-2	Average Response
LOQ Level	200	419632	407808	413720
50% Level	500	8515876	9025636	8770756
100% level	1000	19566985	20565556	20066271
120% Level	1250	26587126	25987456	26287291
150% Level	1500	33065986	32556986	32811486
		Correlation coefficient		**0.9995**
		y-intercept		-4135609.14
		%y-intercept		-20.61
		Slope		24500.58
		Residual sum of square		**0.9995**

Table 4. System precision analysis

Preparation	TBAB (ppm)
1	1005
2	1007
3	1010
4	1058
5	1057
6	1025
Mean	1027
SD	24.65
%RSD	**2.40**

Table 5. Accuracy of TBAB

Level	% Recovery
QL level	88.19
100%	99.83
150%	101.77

Table 6. Method recommendations for TBAB

Substance	LOD (ppm)	LOQ (ppm)	Retention time (minutes)
TBAB	66	200	12.3