RESEARCH ARTICLE

In silico toxicity assessment and trace level quantification of two genotoxic impurities in silodosin using capillary gas chromatography

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Abstract

A capillary gas chromatographic method using flame ionization detection was developed and validated for the trace quantification of 2-bromoethanol (2-BE) and 2-bromoethylmethanesulfonate (2-BEM) in silodosin, used in the treatment of benign prostatic hyperplasia. Chromatographic separation was performed in spilt mode using nitrogen as carrier gas on a column containing crosslinked polyethylene glycol (30 m × 0.32 mm, 0.25 μm) stationary phase modified with nitroterephthalic acid. A simple matrix precipitation strategy was implemented to eliminate the sample overload and the matrix interference problems. The developed method was linear and accurate in the concentration range of 24–3000 ppm for 2-BE and 24–300 ppm for 2-BEM with $r^2 > 0.999$ and percent recoveries greater than 90% for both the analytes. The developed method was precise for both the analytes with RSD(%) of not more than 4.5%. In silico genotoxicity and carcinogenicity potential of 2-BEM were assessed using ICH M7 principles. The developed method can be applied in the quality control laboratories of pharmaceutical industries for trace level quantification of 2-BE and 2-BEM in silodosin.

Keywords Gas chromatography, Matrix precipitation, Genotoxic impurity, In silico toxicity, Method validation

Introduction

In the era of the modern pharmaceutical world, global regulatory agencies and manufacturers across the globe are focusing on the identification, assessment, and control of genotoxic impurities in drug substances (Giordani et al. 2011). Impurities originating from drug substances yield no therapeutic benefit to the patient but have the potential to cause risk to the patient. Some of the trace level impurities present in the drug substance may cause deleterious changes in the genetic material of the cells. Therefore, the levels of such potential genotoxic impurities (PGIs) present in the drug substances are to be assessed and controlled for the patient safety. The International Council on Harmonization (ICH) covers both the safety and quality frameworks for establishing acceptable limits that assures negligible risk to patients (ICH 2017). The ICH M7 recommended limits for daily intake of PGIs are 120, 20, 10, and < 1.5 μg/day, for < 1 month , > 1–12 months, > 1–10 years, and > 10 years to lifetime, respectively. Based on the maximum daily dose (MDD) of the drug substance, a limit for the quantitation of mutagenic impurities is established.

Silodosin is a third-generation selective α_{1A}-adrenoceptor antagonist indicated for the treatment of benign prostatic

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hyperplasia (BPH). Silodosin is effective in relieving BPH symptoms and also in the treatment of conditions like difficulty in urination, urinating urgency and high frequency of urination (Wada et al. 2011). The key starting materials used in the synthesis of silodosin are shown in Fig. 1. In the synthesis of compound 3, 2-bromoethanol (2-BE) is used to protect the alcohol functional group during the conversion of compound 1 to compound 3 (Guorong 2018). Further to form compound 4, the alcohol functional group in compound 3 is protected with a good leaving group, methane sulfonate (using methane sulfonyl chloride). During this conversion, the residual 2-BE (added for the synthesis compound 3) reacts with methane sulfonyl chloride (added for the synthesis compound 4) and generate 2-bromoethylmethane sulfonate (2-BEM). The mechanism formation of such sulfonate esters from the alcoholic solvents has already been reported in the literature (Elder and Snodin 2009; Teasdale et al. 2009, 2010). The reaction kinetics is relatively slow for the formation of sulfonate esters (Teasdale et al. 2009); however, trace levels of these compounds might be generated following this mechanism. As per the structural alerts classification (Muller et al. 2006; Snodin 2010), 2-BE is a well-known alkylating agent used to synthesize the intermediate (compound 3) of silodosin key starting material. On the other hand, 2-BEM is the byproduct of the reaction involved in the synthesis of other key starting material (compound 4) of silodosin (Fig. 1). The unreacted 2-BE and 2-BEM can be present in silodosin as impurities.

The genetic toxicology information of 2-BE, being a well-known alkylating agent, is well established and described in the national toxicology testing program (NTP) (National Toxicology Program USA 2021). Based on the salmonella test results (Zeiger et al. 1988a), 2-BE is positive to the salmonella mutagenicity test, and hence in silico toxicity studies were not performed. However, there were no published reports on the toxicity of 2-BEM in the literature. Hence, in silico toxicity assessment was carried out for 2-BEM according to the ICH M7 principles by using an expert knowledge-based (DEREK) and statistical-based (SARAH) approaches.

In the trace level quantification of genotoxic impurities in a drug substance, a high concentration of drug substance sample is required in the analysis. If the analysis is performed on a gas chromatography (GC) instrument with an auto-liquid sampler, it can create multiple problems due to accumulation of drug substance in the injection port liner as well as in the column. We have to substantially decrease the sample matrix load in order to reduce its interference. One method that is used to extract the impurities of interest present in the drug substance involves sonication followed by filtration of the undissolved drug substance. However, this method has a risk of not extracting the impurities of interest completely due to their entrapment in the crystal lattice leading to false-negative results. Other methods involving sample pretreatment techniques such as liquid–liquid extraction (LLE) (Zheng et al. 2009) and solid-phase extraction (SPE) (Szekely et al. 2012) may be used for the sample cleanup. However, LLE and SPE involve multiple steps and pre-concentration of the extract is required before the analysis, which might result in poor recoveries and reproducibility of the method. Hence, the matrix precipitation strategy (Yang et al. 2015) was followed to
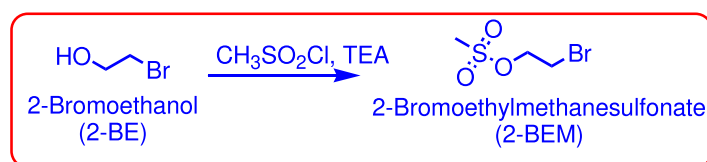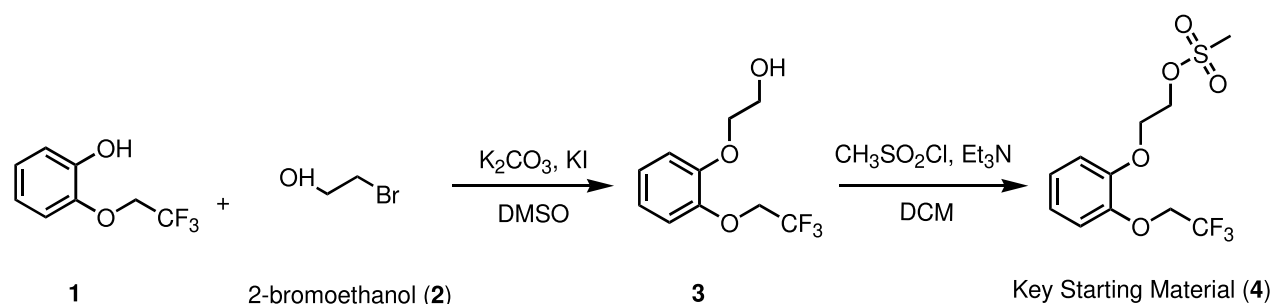
Fig. 1 Synthetic scheme of Silodosin (Guorong 2018)

substantially reduce the matrix interference problem by dissolving the silodosin in ethanol and diluting it with di-isopropyl ether (DIPE). During the process of dilution, silodosin gets precipitated.

In the present study, a GC coupled with flame ionization detector (GC-FID) was selected for the analysis as both the analytes (2-BE and 2-BEM) are volatile in nature. There are some reported methods for the analysis of silodosin (Zhao et al. 2009; Priyanka and Shrivastav 2018; Yin et al. 2018) and related substances (Shaik et al. 2014; Raman et al. 2011) in the literature. However, there are no reported methods for trace level quantification of 2-BE and 2-BEM in silodosin. The existing methods are not sensitive for the trace level quantification of the two PGIs in silodosin. This study aims to perform the in silico toxicity for 2-BEM followed by development and validation of GC-FID method for trace level quantification of 2-BE and 2-BEM impurities in silodosin using simple sample pre-treatment to reduce the matrix interference.

Experimental

Chemicals and materials

Silodosin (HPLC purity > 99%), and 2-BEM standard (GC purity > 99%) were provided by Herrlich Pharma Ltd., (Hyderabad, India) as gift samples. 2-BE (GC purity > 99%), HPLC grade acetonitrile, di-isopropyl ether (DIPE), ethanol, methanol, dichloromethane, tetrahydrofuran, chloroform, acetone, pyridine, acetic acid, ethyl acetate, n-heptane, N,N-dimethylformamide (DMF), N,N-dimethyl sulfoxide (DMSO), and trimethylamine were procured from Merck (Mumbai, India). Ultra-high pure nitrogen, hydrogen and helium gases and zero-air were procured from Siddi Vinayaka Industrial Gases Private Limited (Hyderabad, India). Polyvinylidene difluoride (PVDF) syringe filter was procured from Merck Millipore (Millipore® MA, USA).

Chromatographic conditions

An Agilent 7890B GC unit, equipped with a split/splitless auto-injector and flame ionization detector (FID), was used in the study. Analytes were separated on a ZB-FFAP column (30 m × 0.32 mm, film thickness 0.25 μm). The initial oven program started at 50 °C and held isothermal for 3 min, then ramped at 7 °C min^{-1} to 220 °C and held isothermal at 220 °C for 3 min. The injection volume was 1.0 μL, with a split ratio of 1:5. Nitrogen was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. Detector and injector temperatures were operated at 250 °C and 220 °C, respectively. Ethanol was used as injector wash solvent and diluent. DIPE was used as precipitation solvent in the sample pre-treatment. The final optimized method conditions used in quantification of both the analytes (2-BE and 2-BEM) are presented in Additional

file 1: Table S1. During the optimization of sample pre-treatment studies for the precipitation of silodosin, the filtrate was analyzed using HPLC on Poroshell 120 EC-C18 column (50 × 4.6 mm, 2.7 μm).

Sample pre-treatment to reduce matrix interference

Silodosin sample solution was prepared by dissolving 200 mg in 2.0 mL of ethanol by sonication. Then 2.0 mL of DIPE was added to precipitate the silodosin matrix. Before injection, all sample solutions were filtered using 0.45 μm PVDF filter to remove the precipitated matrix components.

Softwares and in silico tools used in the study

Chemstation (Version B.04.03, Agilent) software was used for controlling the GC instrument and the integration of the data. According to ICH M7 guidelines, knowledge- and statistical-based in silico models were used for the prediction of toxicity of potential mutagenic impurities. DEREK Nexus (Version 6.0.0, LHASA Limited, Leeds UK) and SARAH Nexus (Version 3.0.0, LHASA Limited, Leeds UK) were used for the in silico toxicity prediction. The validation calculations were performed with Microsoft Excel (2013 version office package).

Preparation of standard and sample solutions

A primary stock solution containing 2-BE and 2-BEM at 1.0 mg mL^{-1} was prepared using DIPE as the diluent. The primary stock solution was diluted with a 1:1 ratio of ethanol and DIPE to produce a secondary stock solution containing 50 μg mL^{-1} of 2-BE and 5 μg mL^{-1} of 2-BEM. The primary stock solution was further diluted with a 1:1 ratio of ethanol and DIPE to yield calibration curve standard solutions ranging from 1.2 to 150.0 μg mL^{-1} (equivalent to 24–3000 ppm with respect to 50 mg mL^{-1} of silodosin) for 2-BE and 1.2–15.0 μg mL^{-1} (equivalent to 24–300 ppm with respect to 50 mg mL^{-1} of silodosin) for 2-BEM. For system suitability studies, secondary stock solution containing 50 μg mL^{-1} of 2-BE and 5 μg mL^{-1} of 2-BEM was used. For accuracy studies, spiking solutions at LOQ, 50%, 100%, 150% and 300% levels were prepared by diluting the primary stock with DIPE. The above spiking solutions were added to silodosin sample solutions containing 100 mg mL^{-1} concentration of the drug substance.

For LOD and LOQ determination, different concentrations of 2-BE and 2-BEM standard solutions were prepared in the range of 0.2–2.0 μg mL^{-1} (equivalent to 4.8–48 ppm with respect to 50 mg mL^{-1} of silodosin) from the primary stock solution and secondary stock solution.

Panchakarla *et al. Journal of Analytical Science and Technology*   (2023) 14:15

Page 4 of 10

For repeatability and intermediate precision studies, six individual spiked samples were used. Silodosin sample solutions were prepared at 100 mg mL^{-1} in ethanol and then added with primary stock solution to yield spiked solution containing 50 µg mL^{-1} of 2-BE and 5 µg mL^{-1} of 2-BEM, respectively. Secondary stock solution and repeatability solutions were used for solution stability and robustness experiments.

Test sample solutions of silodosin were prepared at 100 mg mL^{-1} in ethanol and diluted with DIPE to yield 50 mg mL^{-1}. The sample and standard solutions were filtered using a 0.45 µm polyvinylidene difluoride (PVDF) syringe filter (Millipore® MA, USA).

Method validation and application
The developed method was validated according to the regulatory guidelines (ICH 2022; USFDA 2021). Validation parameters such as sensitivity, selectivity, accuracy, precision, linearity, robustness, and solution stability were studied.

System suitability
System suitability of the method was assessed by injecting the secondary stock solution containing 50 µg mL^{-1} of 2-BE and 5 µg mL^{-1} of 2-BEM and assessed the RSD(%).

Specificity
To assess the specificity of the method, the chromatograms of diluent (solvent mixture containing 1:1 ratio of ethanol and DIPE), individual injections of process residual solvents (methanol, dichloromethane, isopropanol, acetone, acetonitrile, chloroform, *n*-heptane, ethyl acetate, tetrahydrofuran, pyridine, acetic acid, DMF and DMSO), silodosin sample solution, 2-BE, 2-BEM, secondary stock solution and resolution solution were compared to check the interference at the retention times of the analytes. Process residual solvents were prepared at a concentration of 1 µg mL^{-1} in DIPE. Specificity of the method was established by injecting the solutions of individual process residual solvents and the resolution solution. Resolution between the closely eluting peaks with 2-BE and 2-BEM was determined.

Sensitivity
The sensitivity of a method is defined by its LOD and LOQ for each of the analytes being analyzed using the method. The LOD is the lowest analyte concentration detected but not necessarily quantified, while LOQ is the lowest analyte concentration that can be quantified by the method. The LOD and LOQ of the 2-BE and 2-BEM were determined by injecting the solutions containing 2-BE and 2-BEM in the range 0.2–2.0 µg mL^{-1} (as

described in section "Preparation of standard and sample solutions") to obtain a signal-to-noise (S/N) ratio greater than or equal to 3:1 and 10:1, respectively.

Linearity
Five-point calibration curves at concentrations of 1.2, 25.0, 50.0, 75.0 and 150.0 µg mL^{-1} (equivalent to 24, 500, 1000, 1500 and 3000 ppm with respect to 50 mg mL^{-1} of silodosin) for 2-BE and at concentrations of 1.2, 2.5, 5.0, 7.5 and 15.0 µg mL^{-1} (equivalent to 24, 50, 100, 150, and 300 ppm with respect to 50 mg mL^{-1} of silodosin) for 2-BEM were constructed by plotting peak area of the analyte (2-BE or 2-BEM) versus the concentration of the analyte. Linearity of the calibration curves was assessed based on the statistical parameters obtained from the least-square regression analysis of the calibration data.

Precision
The levels of 2-BE and 2-BEM were found to be below the detection limit in the three batches of silodosin analyzed using the developed method. Therefore, the method precision (repeatability) and intermediate precision (reproducibility) studies were performed using the six individual solutions of silodosin spiked with 2-BE and 2-BEM at the specification level (i.e., 1000 ppm of 2-BE and 100 ppm of 2-BEM). Six replicate spiked samples of 2-BE and 2-BEM were injected to determine the RSD(%) values. In the precision studies, RSD(%) values of 2-BE and 2-BEM responses from the six replicates should be less than 10% to indicate that the developed method is precise.

Accuracy
The accuracy of the method was evaluated by comparing the percentage recovery values and RSD(%) values for 2-BE and 2-BEM. For this study, the responses obtained from un-spiked and spiked samples in triplicate determination at four different levels ranging from LOQ, 50%, 100%, 150% and 300% of the specification limit of 1000 ppm for 2-BE and 100 ppm for 2-BEM were compared. Percentage recovery values should be within 100 ± 20%, while the RSD(%) values should be less than 10% to establish that the method is accurate.

Robustness
The robustness of the developed method was assessed by making deliberate changes to the column flow rate (± 10% to the set value of 1.0 mL min^{-1}) and initial oven temperature (± 10% to the set value of 50 °C). System suitability and mean percentage recovery of the spiked samples were determined by changing the method parameters described above. RSD(%) values obtained from system suitability should be ≤ 10%, and the mean

Panchakarla *et al. Journal of Analytical Science and Technology*   (2023) 14:15

Page 5 of 10

percentage recovery should be within $100 \pm 20\%$ for 2-BE and 2-BEM for the method to be robust.

Solution Stability

Short-term solution stability studies were performed using a secondary stock solution containing 1000 ppm of 2-BE and 100 ppm of 2-BEM and the spiked resolution sample, prepared as part of method precision study. Stability studies were performed on the samples up to 48 h at ambient laboratory temperature (25 ± 5 °C) and refrigerated conditions (2–8 °C). The percent recoveries for 2-BE and 2-BEM were calculated against the freshly prepared solutions.

Results and discussion

In silico prediction of mutagenicity and carcinogenicity of 2-BEM

Bacterial mutation (Ames) test for 2-BE in salmonella bacteria was reported in the literature and the results from the investigations indicated that 2-BE was positive to bacterial mutation/Ames test (Zeiger et al. 1988b). Based on the reported results on genotoxicity, 2-BE was classified as ICH M7 Class-1 genotoxic impurity. Till date, there was no genetic toxicological data reported for 2-BEM in the literature. Hence, in silico toxicity studies were performed using knowledge-based (DEREK Nexus) and statistical-based (SARAH Nexus) tools using ICH M7 principles. The DEREK Nexus prediction for 2-BEM is "Plausible". The structure of 2-BEM has alkyl halide and alkyl sulfonate structural alerts, both corresponds to alkylating agents and matching with example alert 027 from DEREK data base. In vitro mutagenicity in bacterium and in vitro mammalian chromosome damage is plausible based on the alert structure 027 from DEREK knowledge base 2018 1.1. Statistical-based (SARAH) prediction was performed using Sarah model 2.0, and 2-BEM was predicted to be positive with 51% confidence for the mutagenicity in vitro (i.e., Ames test positive). The supporting hypothesis contains similar examples from the training set. Summary of DEREK and SARAH Nexus results for 2-BEM are presented in Table 1 and Fig. 2. Based on the predictions from the DEREK and SARAH, 2-BEM was classified as ICH M7 class-3 impurity. Limit for 2-BEM was calculated as per ICH M7 principles using the threshold of toxicological concern (TTC) and the MDD of silodosin. Using TTC of 1.5 µg/day and MDD of 8 mg/day the 2-BEM should be controlled at limit of 187.5 ppm. However, in the current study stringent limit of 100 ppm for the quantification of 2-BEM in silodosin drug substance was selected to have better control approach.

### Optimization of matrix precipitation conditions

In the trace level quantification of impurities present in drug substances, to reduce sample load and matrix interference, precipitation of the sample matrix can be performed in reverse precipitation mode or normal precipitation mode. In this study, normal precipitation mode was selected as it was well suited for the direct GC analysis. Polar solvents like methanol, ethanol, and acetonitrile were evaluated as potential solvents for solubilizing silodosin. Ethanol was selected as solvent due to the higher solubility (100 mg mL^{-1}) of silodosin in ethanol compared to other solvents. Matrix precipitation conditions were evaluated by diluting 100 mg mL^{-1} of silodosin with 0.5 mL, 1.0 mL, 1.5 mL and 2.0 mL of DIPE and MTBE as precipitating solvents. The resultant solution was filtered using a 0.45 µm PVDF filter, and the extent of precipitation was determined by quantifying the concentration of silodosin in the filtrate against the external standard by using HPLC–UV method. The content of silodosin was found to be 1.45 mg mL^{-1} in the filtrate and the percentage of precipitation was found to be more than 97.1% with 2.0 mL of DIPE. DIPE was selected as the final precipitation solvent due to the close elution of MTBE with 2-BE and merging of the peaks. Moreover, the precipitation rate was higher in DIPE compared to MTBE. Hence, DIPE (2 mL) was selected for the effective precipitation of the sample matrix.

Table 1 In silico toxicity prediction results for 2-BE and 2-BEM using Derek and Sarah Nexus software's

Impurity	DEREK prediction	SARAH prediction
2-Bromoethanol (2-BE)	Not performed as 2-BE is already reported to be genotoxic based on salmonella mutagenicity tests (National Toxicology Program USA 2021)	
2-Bromoethylmethanesulfonate (2-BEM)	Chromosome damage in vitro in mammal is "PLAUSIBLE"[a] Alert matched to 27 Alkylating agent Mutagenicity in vitro in mammal is "PLAUSIBLE"	The compound is predicted to be "POSITIVE"[b] with 51% confidence for the "Mutagenicity in vitro" Hypotheses analysis was found to be "POSITIVE" with structure ID# H-680 and H-654

[a] As per DEREK prediction outcome definition, "PLAUSIBLE" indicate that the weight of evidence supports the proposition

[b] As per SARAH prediction outcome definition, "POSITIVE" indicate that the query structure is predicted to be positive in a bacterial reverse mutation assay (Ames test)
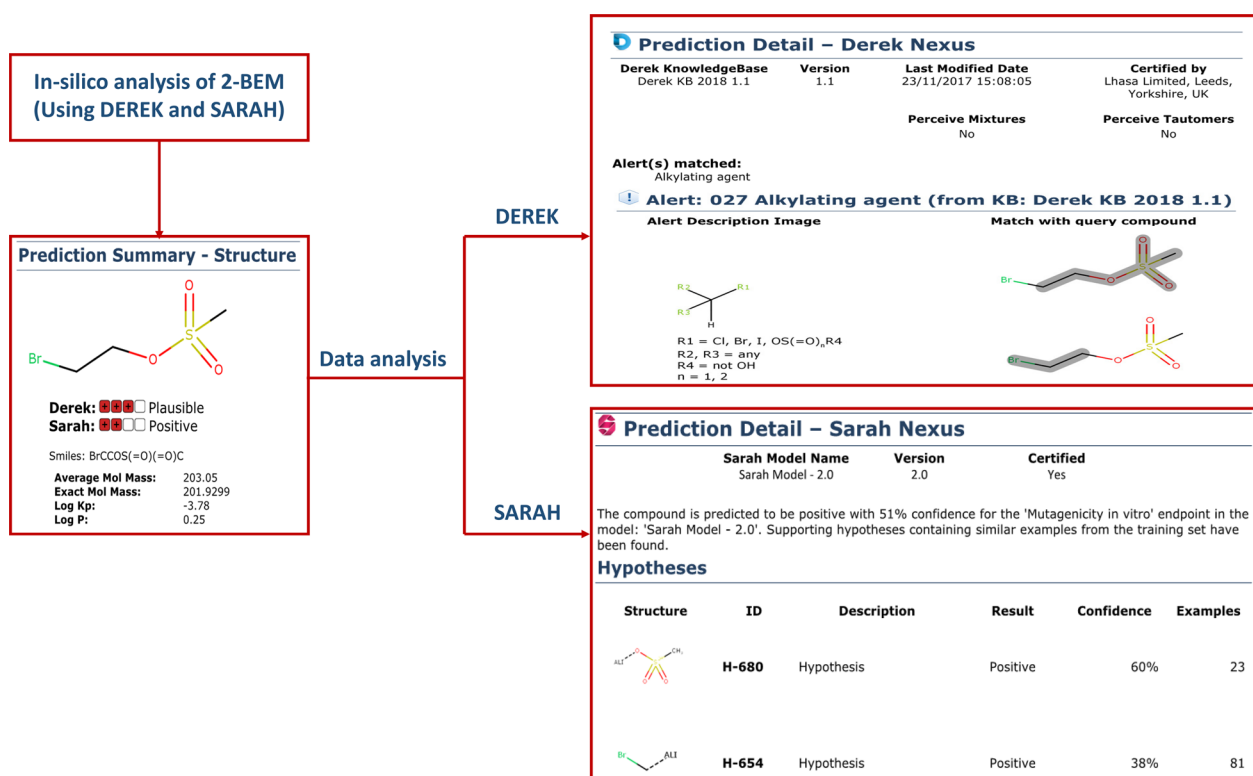
Fig. 2 In silico toxicity results for 2-BEM

Method development and optimization of GC conditions

The initial phase of any method development for the determination of GTIs in any drug substance is the selection of the right analytical technique based on the nature of the analytes and target specification limit of the GTIs (Liu et al. 2010). Based on the maximum daily dose (MDD) of silodosin, the specification limits for 2-BE and 2-BEM were found to be 9512.5 and 187.5 ppm, respectively. However, in the intermediate specification for compound 4 (Fig. 1) the specification limits for 2-BE and 2-BEM are set at 1000 and 100 ppm, respectively. Hence, the same limits were applied to silodosin.

Based on the volatile nature of 2-BE and 2-BEM, GC coupled with FID was chosen as the suitable analytical instrument for their quantification in silodosin. However, due to the low volatility of the silodosin and high sample concentration, it is necessary to remove it from the samples before GC analysis. Hence, matrix precipitation was carried out using the procedure as defined in section "Optimization of matrix precipitation conditions".

In the initial method development trials, residual solvents quantification in silodosin was performed using DB-624 (30 m × 0.32 mm, film thickness 1.8 μm) column with static headspace sampler. Good resolution (USP Resolution; Rs > 1.5) was achieved between the known solvents and 2-BE peaks, but 2-BEM peak was not detected with the headspace sampler due to its less volatility. Hence, the auto-liquid injector was opted for this study. The peak shape for 2-BEM was distorted and had a tailing factor of 3.5 on the DB-624 column, making it inappropriate for trace level quantification. Different stationary phases such as DB-WAX (30 m × 0.32 mm, film thickness 0.5 μm), ZB-FFAP (30 m × 0.32 mm, film thickness 0.25 μm), DB-1701 (30 m × 0.32 mm, film thickness 1.0 μm), and DB-1 (30 m × 0.32 mm, film thickness 1.0 μm) were screened. Good resolution (Rs > 3.0) between the target analytes and the matrix peaks and the residual solvent peaks was achieved on DB-WAX and ZB-FFAP columns. However, better peak shape (Tailing factor < 2.0) was achieved only on the ZB-FFAP column. Other experimental variables such as column flow rate, injector temperature and temperature program were studied, and the final optimized conditions are summarized in Additional file 1: Table S1. A representative overlaid chromatogram of blank, standard solution, and sample solution is shown in Fig. 3.

Method validation

The developed method was validated as per the regulatory guidelines (ICH 2022; USFDA 2021) and the results are summarized in Table 2. As shown in Figs. 3
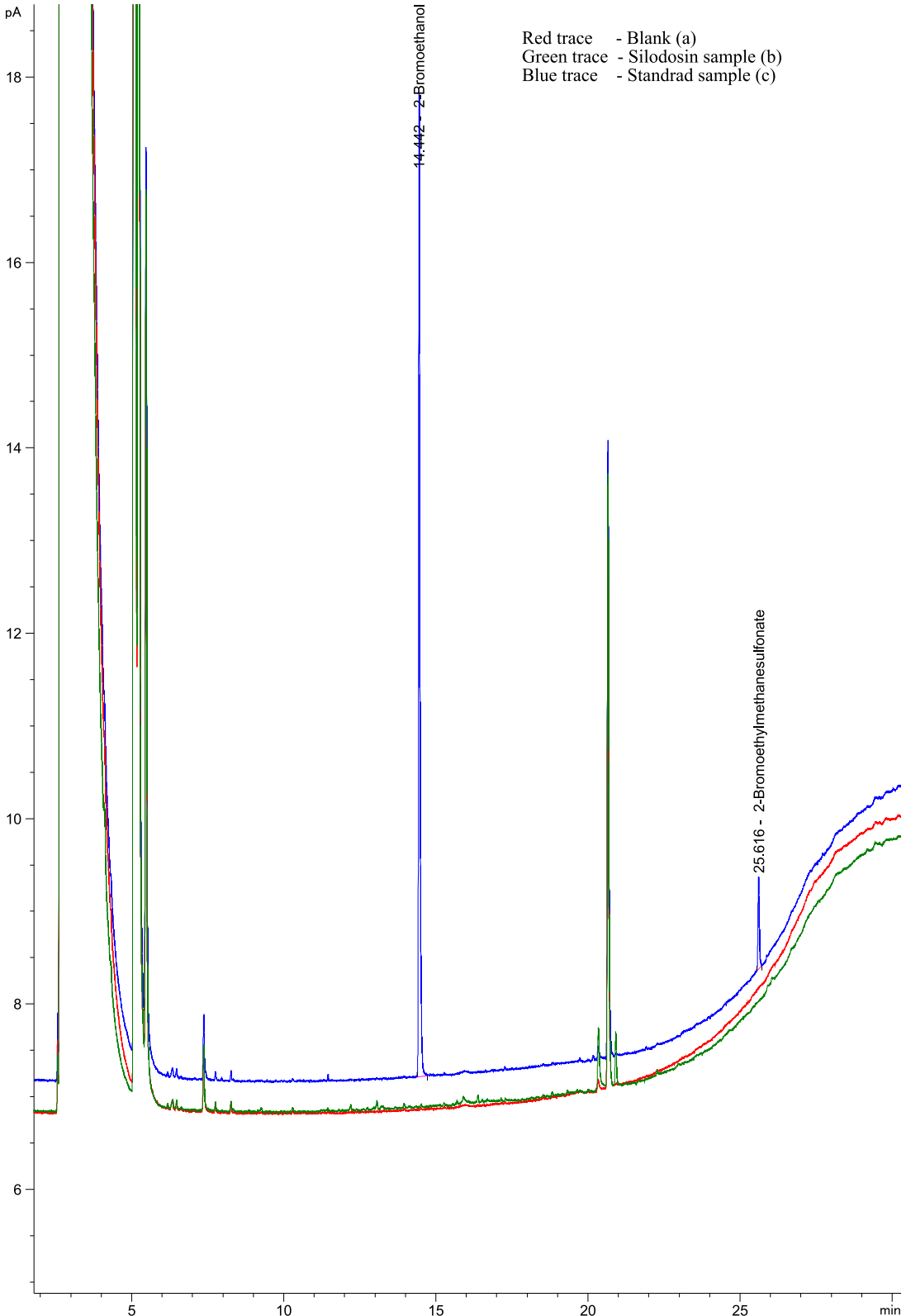
Fig. 3 Overlaid chromatogram of **a** Blank, **b** sample solution, and **c** standard solution

**Table 2** Method validation data summary

Test parameter	Typical acceptance criteria	2-Bromoethanol (2-BE)	2-Bromoethylmethanesulfonate (2-BEM)
System suitability	RSD (%) for peak area response ($n = 6$)	1.27%	0.28%
	Cumulative RSD (%) for peak area response (all injections)	1.10%	3.15%
System suitability (for Intermediate precision)	RSD (%) for peak area response ($n = 6$)	0.36%	1.44%
	Cumulative RSD (%) for peak area response (all injections)	0.83%	1.43%
Specificity	Blank interference and USP Resolution	No blank interference, 2-BE and 2-BEM are well resolved from residual solvent and matrix peaks	
Sensitivity	Concentration	LOD—8.1 ppm LOQ—24.3 ppm	LOD—8.0 ppm LOQ—24.1 ppm
	S/N for LOD solution should be > 3:1	4:1	3:1
	S/N for LOQ solution should be > 10:1	13:1	11:1
	RSD (%) for six replicate injections of LOQ solution should be $\leq$ 15.0%	2.61%	1.09%
Linearity	Range	24.1–3013.5 ppm	25.1–301.4 ppm
	Calibration Equation	$y = 0.7513 x - 0.0538$	$y = 0.8364 x - 0.1050$
	r^2	0.9999	0.9999
	Residual plots	Random scatter	Random scatter
Accuracy	Average recovery ($n = 3$) from the spiked samples performed at 5 levels should be between 80 and 100%; RSD (%) should be $\leq$ 10.0%	LOQ—95.8%; 4.75% 50—95.9%; 2.16% 100—99.8%; 3.60% 150—94.5%; 1.01% 300—101.1%; 0.29%	LOQ—93.5%; 1.27% 50—90.6%; 4.50% 100—104.4%; 0.67% 150—95.7%; 0.40% 300—96.7%; 0.43%
Precision	RSD (%) for six preparations at 100% spike level should be $\leq$ 10.0%	1.39%	2.73%
Intermediate Precision	RSD (%) for six preparations at 100% spike level should be $\leq$ 10.0%	1.33%	2.35%
Robustness 0.9 mL min^{-1} Flow	RSD (%) for peak area response ($n = 6$) %Recovery ($n = 3$) for 100% spiked solution	0.39% 97.6%	1.70% 93.5%
Robustness 1.1 mL min^{-1} Flow	RSD (%) for peak area response ($n = 6$) %Recovery ($n = 3$) for 100% spiked solution	1.04% 91.3%	0.95% 96.9%
Robustness 45° Oven Temp	RSD (%) for peak area response ($n = 6$) %Recovery ($n = 3$) for 100% spiked solution	0.47% 93.7%	1.35% 94.9%
Robustness 55° Oven Temp	RSD (%) for peak area response ($n = 6$) %Recovery ($n = 3$) for 100% spiked solution	1.02% 95.9%	0.71% 95.3%

and 4, the specificity study demonstrates that 2-BE and 2-BEM eluted at 14.44 mins and 25.62 mins, respectively. Both the target analytes were well resolved from process residual solvent peaks and the blank artifact peaks. Methanol, dichloromethane, isopropanol, acetone, acetonitrile, chloroform peaks are eluting on the shoulder of DIPE, and ethanol peaks before 5.0 min. Whereas *n*-heptane, ethyl acetate, tetrahydrofuran, pyridine, acetic acid, DMSO, and DMF eluted at the retention time of 8.28, 9.73, 11.35, 13.55, 15.83, 20.90 and 20.90 min, respectively. The LOD and LOQ values for 2-BE and 2-BEM were calculated from the S/N data.

The LOD value was found to be 0.42 µg mL^{-1} (8 ppm) for 2-BE and 0.28 µg mL^{-1} (6 ppm) for 2-BEM, whereas LOQ values were found to be 1.20 µg mL^{-1} (24 ppm) for both 2-BE and 2-BEM. The RSD(%) values of LOQ precision were found to be less than 2.6%. The LOQ results for 2-BE and 2-BEM are summarized in Additional file 1: Table S2.

Linear regression analysis of each analyte demonstrated high r^2 (> 0.999) and low % *y*-intercept (< 10.0%). The random scatter of residual plots of the calibration curve data of both the analytes indicate that the methods are linear for both the analytes over
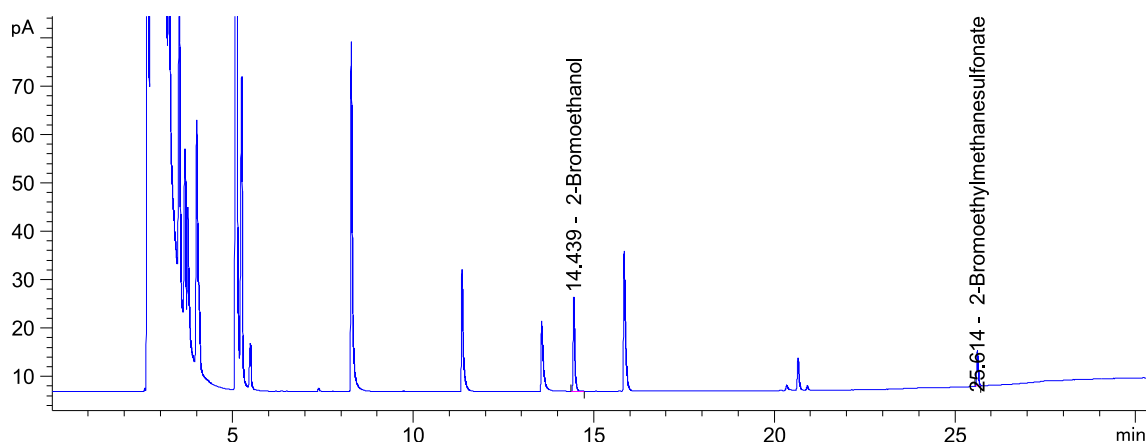
Fig. 4 Chromatogram of Silodosin spiked with residual solvents, 2-BE, and 2-BEM

their corresponding calibration ranges. The RSD(%) values from method precision and intermediate precision studies were found to be less than 5.0% for both analytes. The experimental results from the precision study are summarized in Additional file 1: Table S3 and Table S4, respectively. The recoveries of 2-BE and 2-BEM from the accuracy experiments were found to be in the range of 91.7–112.8%. RSD(%) of 2-BE and 2-BEM from the triplicate preparations at LOQ, 50%, 100%, 150% and 300% levels were found to be less than 6.82%. The percentage recovery values, and the RSD(%) values were within the acceptance criteria of 80–120% and less than 10.0%, respectively. Robustness results have shown that change in the flow rate and the initial oven temperature had no significant impact on the resolution or the peak shape. However, the retention times of the analytes changed with the change in flow rate and the initial oven temperature. In the stability studies, the maximum percentage deviation was found to be less than 10.0% for 2-BE and 2-BEM in the secondary stock and spiked solutions; indicating solutions are stable for 48 h when stored at ambient laboratory conditions (25 ± 5 °C) and refrigerated conditions. The experimental results from accuracy and robustness study are summarized in Additional file 1: Table S5–S8.

Conclusion

A simple and sensitive GC-FID method was developed and validated for the quantification of two genotoxic impurities, 2-BE and 2-BEM, in silodosin using a simple matrix precipitation method to overcome the sample overload and matrix interference problems. Toxicity studies revealed that 2-BEM is genotoxic and classified as a class 3 impurity as per ICH. The developed method was specific, sensitive, accurate, and precise for the quantification of the 2-BE and 2-BEM in the silodosin.

The developed method can be implemented in the quality control laboratory for routine analysis and can be adapted for analysis of 2-BE and 2-BEM present in other drug substances with minimal tweaking of the sample preparation.

Abbreviations

2-BE	2-Bromoethanol
2-BEM	2-Bromoethylmethane sulfonate
BPH	Benign prostatic hyperplasia
DIPE	Di-isopropyl ether
DMF	*N,N*-Dimethylformamide
DMSO	*N,N*-Dimethyl sulfoxide
FID	Flame ionization detector
GC	Gas chromatography
PGI	Potential genotoxic impurity
ICH	International council for harmonization
LLE	Liquid–liquid extraction
HPLC	High-performance liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MDD	Maximum daily dose
MTBE	Methyl tert-butyl ether
NTP	National toxicology testing program
PVDF	Polyvinylidene difluoride
RSD	Relative standard deviation
SPE	Solid-phase extraction
S/N	Signal-to-noise ratio
TTC	Threshold of toxicological concern
USP	United states pharmacopeia
UV	Ultraviolet

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-023-00378-1.

Additional file 1: Table S1. Gas chromatographic conditions. **Table S2.** Summary of LOQ precision for 2-BE and 2-BEM. **Table S3.** Method precision results. **Table S4.** Intermediate precision results. **Table S5.** Accuracy data for 2-BE. **Table S6.** Accuracy data for 2-BEM. **Table S7.** Summary of robustness results for 2-BE. **Table S8.** Summary of robustness results for 2-BEM

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The author declares no competing interests.

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