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RESEARCH ARTICLE

Simple and Reliable Gas Chromatography Method for the Determination of 3-Quinuclidinol Content in Solifenacin Succinate Drug Substance

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Abstract: A simple and reliable gas chromatography method has been developed and validated for the determination of 3-quinuclidinol content in solifenacin succinate drug substance, using dimethyl-sulfoxide (DMSO) as internal standard (IS). Efficient chromatographic separation was achieved on DB-Wax, 15 m long with 0.53 mm i.d., 1.0 µm particle diameter column consists with 100% polyethylene glycol as stationary phase including carrier gas as Helium. The analyte and internal standard were extracted in chloroform and monitored by flame ionization detector. The performance of the method was assessed by evaluating specificity, precision (repeatability, reproducibility), linearity, robustness and accuracy. No interference of organic solvents used in the synthesis was observed. The proposed method has a potential application to drug substances which may contains 3-quinuclidinol. The limits of detection (LOD) and limits of quantification (LOQ) established for 3-quinuclidinol, 40 µg g^{-1} and 120 µg g^{-1} respectively. The correlation coefficient value of linearity experiment is 0.9997. The average recovery for 3-quinuclidinol is 100.8%. The results proves that the validated method was suitable for determining 3-quinuclidinol content and method can be successfully applied for the routine analysis of solifenacin succinate drug substance.

Keywords: Gas chromatography, Solifenacin succinate, 3-Quinuclidinol, Extraction, Validation

Introduction

Chemically solifenacin succinate is butanedioic acid, compounded with (1S)-(3R)-1-azabicyclo[2.2.2]oct-3-yl 3,4-dihydro-1-phenyl-2(1*H*)iso-quinolinecarboxylate (1:1) having white to pale-yellowish-white crystal or crystalline powder with an empirical formula of $C_{23}H_{26}N_2O_2 \bullet C_4H_6O_4$ and a molecular weight of 480.55. Solifenacin succinate is a competitive M_3 selective muscarinic receptor antagonist used in the treatment of an overactive bladder (OAB), such as urinary incontinence, urinary urgency and urinary frequency.

This can help to reduce the episodes of urinary incontinence or reduce the feeling or urgency that bladder spasms can use[1-3]. Solifenacin succinate was discovered and developed by Yamanouchi Pharmaceuticals Company limited. It is available 5 mg and 10 mg tablets for oral administration and appears world-wide under the brand name of Vesicare. In the synthesis process of solifenacin succinate, 3-quinuclidinol was used as intermediate as it is highly valuable for several muscarine-active compounds are described. This residual organic intermediate can come through the manufacturing process of the drug substance, the criteria for acceptance is based on pharmaceutical studies or known safety data[4]. The toxicological properties of 3-quinuclidinol have not been fully investigated. The levels of 3-quinuclidinol in solifenacin succinate drug substance needs to be monitored and controlled with appropiate methods due to quality and importance of the drug. In the available literature many of analytical procedures have been reported for the estimation of solifenacin and its related substances[5-12]. Very few analytical methods have been identified for the determination of 3-quinuclidinol. In 2002, Petr Bednar and *et al*, developed for the determination of 3-quinuclidinol and related quaternary derivatives spiked into a sample of pond water by capillary electrophoresis with mass spectroscopy (CE/MS)[13].

The aim of this study was to develop a simple and sensitive gas chromatography (GC) method with flame ionization detector for determination of UV inactive 3-quinuclidinol content in solifenacin succinate drug substance with better separation and sufficiently low levels of detection. To the best of our knowledge no report has been published on the analysis of 3-quinuclidinol in solifenacin succinate drug substance.

Experimental

The standard, samples of solifenacin succinate drug substance and 3-quinuclidinol were procured from APL Research Centre-II (A division of Aurobindo Pharma Ltd., Hyderabad). Analytical reagent (AR grade) dimethylsulfoxide, methanol, ethanol, ethyl acetate, benzene, toluene, triethylamine and tetradecane obtained from sigma-aldrich limited. HPLC grade chloroform and sodium hydroxide pellets procured from E Merck India. Highly purified water obtained from Millipore purification system. High purity gases of helium obtained form Matheson K-Air India Pvt. Limited and hydrogen, zero air, nitrogen gases are obtained from Sri Balaji gases & Chemicals India Pvt. Limited.

Gas chromatography

Two different makes of gas chromatograph systems (Agilent 6890N network GC system equipped GERSTEL Multi Purpose Sampler (MPS2) with data handling system HPCHEM station / Shimadzu GC-2010 Gas Chromatograph equipped CTC Analytics COMBI PAL sampler with data handling system GC solutions) with flame ionization detector was used. High purity helium gas was used as carrier gas. The analysis was carried on DB-Wax, 15 m long with 0.53 mm i.d., 1.0 µm particle diameter column consists with 100% polyethylene glycol as stationary phase (Agilent J&W GC columns).

The capillary injector temperature: 200 °C and flame ionization detector temperature: 260 °C.

Column pressure programme: 40 kpa (15 min) $\xrightarrow{10\ \text{kpa/min}}$ 100 kpa (54 min).

Column oven temperature programme: 80 °C (2 min) $\xrightarrow{10\ °\text{C/min}}$ 170 °C (10 min) $\xrightarrow{10\ °\text{C/min}}$ 230 °C (48 min).

Flow gases at detector for ignition: Hydrogen – 50 mL/min; Zero air – 500 mL/min and Nitrogen – 40 mL/min. The injection volume of standard and sample was 2.0 µL and

introduced with 1:5 split ratio. The run time was 75 min. The retention times of the dimethylsulfoxide and 3-quinuclidinol are about 5.5 and 10.4 minutes respectively. Retention time (RT) and relative retention time (RRT) should be confirmed from standard solution. Relative standard deviation for the ratio of peak area of 3-quinuclidinol to the peak area of internal standard (dimethylsulfoxide) for six injections of the standard solution is not more than 5.0%.

Standard and sample solutions

Preparation of 6N sodium hydroxide solution

About 24 g of sodium hydroxide pellets was dissolved in 100 mL of water.

Preparation of internal standard solution

About 0.067 g of dimethylsulfoxide accurately weighed and transferred into a 10 mL clean, dry volumetric flask containing about 5 mL of chloroform, mixed and made up to volume with chloroform. 2.0 mL of this solution was diluted to 200 mL with chloroform.

Preparation of blank solution

Into a clean, dry separating funnel, 3 mL of 6 N sodium hydroxide solution and 2 mL of internal standard solution was added and shaken vigorously for about 1 min. The two phases were allowed to separate and collected the lower layer (chloroform).

Preparation of standard stock solution

About 0.0161 g of 3-quinuclidinol accurately weighed and transferred into a 25 mL clean, dry volumetric flask containing about 15 mL of internal standard solution, mixed and made up to volume with internal standard solution. Dilute 1.0 mL of this solution to 25 mL with internal standard solution.

Preparation of standard solution

2 mL of standard stock solution was transferred into a clean, dry separating funnel, containing 3 mL of 6 N sodium hydroxide solution and shaken vigorously for about 1 min. The two phases were allowed to separate and collected the lower layer (chloroform).

Sample solution

About 0.05 g of sample was accurately weighed and transferred into a clean, dry separating funnel, 3 mL of 6 N sodium hydroxide solution was added and shaken well the sample. 2 mL of internal standard solution was added immediately and shaken vigorously for about 1 min. The two phases were allowed to separate and collected the lower layer (chloroform).

Results and Discussion

Method development and optimization

The objective of this work is, to determine low level concentrations of 3-quinuclidinol in solifenacin succinate drug substance by using gas chromatography (GC) system. In the synthesis process of solifenacin succinate, 3-quinuclidinol was used as intermediate. As there is no chromophore present in 3-quinuclidinol, there was no possibility for UV or fluorescence detection and no suitable groups are present for derivatization. Method development was initiated with, solifenacin succinate, 3-quinuclidinol solubility, miscibility and extraction studies, based on that extraction method was chosen with 6 N sodium hydroxide solution and chloroform solvents. Preliminary experiment was carried out by

using DB-CAM, 30 m long with 0.53 mm i.d., 1.0 µm particle diameter column consisted with deactivated polyethylene glycol as stationary phase and carrier gas was helium, with constant column pressure 50 kpa and column oven temperature 120 °C (2 min) 10 °C/min 180 °C (12 min). In this trial 3-quinuclidinol and dimethylsulfoxide (internal standard) were separated from each other. In sample analysis 3-quinuclidinol peak interfering with unknown peak, eluted at about 10.4 minutes, which was initially not found. As long as time increases, 3-quinuclidinol peak area decreasing, simultaneously unknown peak area increasing, whereas this observation was not found in standard. After that many trials were performed to resolve this problem, by changing different columns like DB-waxetr, DB-FFAP and DB-624, using carrier gas as helium. In all above trials, tailing of analyte peaks was observed. Satisfactory separtation was achieved on DB-Wax, 15 m long with 0.53 mm i.d., 1.0 µm particle diameter column consisted with 100% polyethylene glycol stationary phase (Make:Agilent J&W GC columns), using carrier gas as helium in constant column pressure mode and varying column oven temperature, analytes were separated from each other with good shape. In sample analysis, extraction with 1.0N NaOH and chloroform, 3-quinuclidinol, internal standard (dimethylsulfoxide) peaks are not interfering with unknown peak, which is eluted about 67 minutes and unable to identify by gas chromatography with mass spectroscopy (GC/MS) and also poor recovery results were observed. To overcome this problem again several trials were made using chloroform and by changing NaOH concentration ranging from 1.0 N to 6.0 N by increment of every 1.0 N interval. Extraction with 2.0 N NaOH, accuracy results were improved. After that by increasing the NaOH concentration upto 6.0 N, 100% accuracy results were achieved.

Finally, satisfactory separation with better peak shapes were achieved, on chromatographic conditions which have been mentioned in gas chromatography (GC), was used for validation study to evaluate its performance characteristics.

Method validation

In order to determine the content of 3-quinuclidinol in solifenacin succinate drug substance, the method was validated as per the ICH guidelines[14], individually in terms of specificity, limit of detection, limit of quantification, linearity, accuracy, robustness and precision (system precision, method precision and ruggedness).

Specificity

Specificity is the ability of the method to measure the analyte response in presence of all residual solvents (ethanol, ethyl acetate, benzene, toluene, triethylamine), which are used in the synthesis process of solifenacin succinate drug substance. For specificity determination, blank, all residual solvents of solifenacin including 3-quinuclidinol, chloroform and dimethylsulfoxide solutions were prepared individually as per methodology and injected into GC to confirm the retention times. After that solutions of solifenacin succinate drug substance, solifenacin succinate drug substance spiked with 3-quinuclidinol (considered as spiked sample), solifenacin succinate drug substance spiked with all residual solvents which are used in the synthesis process of solifenacin succinate including 3-quinuclidinol (considered as all spiked sample) were prepared as per methodology and injected into GC to confirm any co-elution with analyte peaks from respective blank, any of residual solvent peak. An overlay chromatogram of blank, solifenacin succinate drug substance, spiked sample and all spiked sample chromatogram is shown Figure 1.

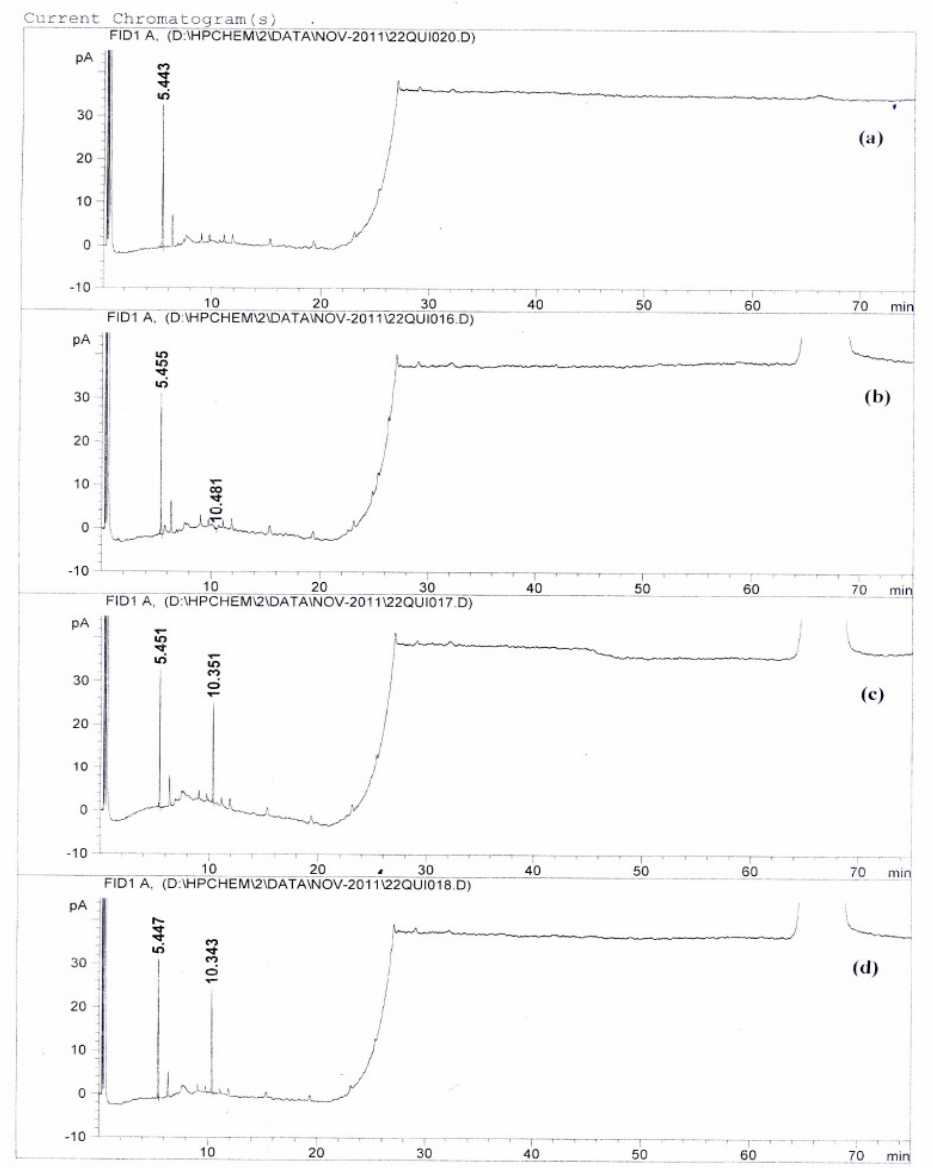

Figure 1. A typical representative overlay chromatograms of (a) Blank, (b) Solifenacin succinate drug substance, (c) Spiked sample and (d) All spiked sample

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

Standard solution of 3-quinuclidinol, concentration at 1019 µg g^{-1} was injected into gas chromatograph. Based on signal to noise (S/N) ratio method, LOD and LOQ concentrations were predicted by using standard solution concentration (C) and standard solution S/N ratio value, with the formula [3.3 x C / (S/N)] for LOD and 10 x C / (S/N)] for LOQ. For evaluation of LOD and LOQ, solutions were prepared at predicted concentration levels and precised by analyzing six times. The achieved precised values are shown in Table 1.

Table 1. Statistical data of linearity, LOD/LOQ for 3-quinuclidinol

Statistical parameters	3-Quinuclidinol
Correlation coefficient	0.9997
Intercept	-0.0112
Residual standard on deviation response	0.0100
Slope	0.0009
Concentration range, µg g^{-1}	120 – 1500
Limit of detection, µg g^{-1a}	40
Limit of quantification, µg g^{-1a}	120
Precision for Limit Of Detection, % RSD	4.0
Precision for Limit Of Quantification, % RSD	3.4

a : Precised LOD and LOQ values

Linearity

The linearity was determined by preparing solutions of 3-quinuclidinol, concentration from 120 (LOQ) - 1500 µg g^{-1} and injected into GC. A plot of peak ratio versus concentration was drawn. The data subjected to statistical analysis using a linear-regression model. The statistical parameters slope, intercept, residual standard on deviation response and correlation coefficient values are calculated and shown in Table 1.

Accuracy

Accuracy experiment was performed using standard addition technique. The recoveries were determined by spiking 3-quinuclidinol at four levels [120 (LOQ level), 500, 1000 and 1500 µg g^{-1}] into solifenacin succinate drug substance. These samples were prepared and analyzed in triplicate. The calculated recovery values for 3-quinuclidinol ranged from 99.1% - 102.4% and average recovery of four levels (twelve determinations) was 100.8%. The completely validated accuracy results are shown in Table 2.

Table 2. Accuracy data of 3-quinuclidinol

Identification	3-Quinuclidinol			
	LOQ Level, µg g^{-1}	500 µg g^{-1}, Level	1000 µg g^{-1}, Level	1500 µg g^{-1}, Level
*Added, µg g^{-1}	121	531	1019	1515
*Found, µg g^{-1}	121	544	1036	1502
Recovery, %	100.0	102.4	101.7	99.1
* % RSD	2.2	1.2	0.6	0.8

**Average of 3 replicates*

Precision

The precision was the study of the method using repeatability and reproducibility (ruggedness). The performance of the method was evaluated with replicate injections of standard and sample solutions. Standard solution was analyzed six times for checking the performance of the gas chromatography system under the chromatographic conditions on the day tested (System precision). The relative standard deviation for 3-quinuclidinol is 2.9%. Repeatability and reproducibility of the method was studied by analyzing six sample solutions separately. Repeatability was the intra-day variation (Method precision), demonstrated by preparing six sample solutions individually using a single batch of solifenacin succinate drug substance spiked with 3-quinuclidinol at a known concentration level (about 1000 µg g^{-1}) as per methodology and content was determined. The relative

standard deviation for the content of 3-quinuclidinol is 1.2%. The intermediate precision was the inter-day variation (Ruggedness), was defined as the degree of reproducibility obtained by following the same procedure as mentioned for method precision experiment. The analysis of the same sample (which is used in the method precision) under a variety of conditions using different Lot No. column and carrier gas, with different analyst on different day by preparing new standards and the content was determined. The relative standard deviation for the content of 3-quinuclidinol is 0.9%. The completely validated precision (System precision, Method precision and Ruggedness) results are shown in Table 3.

Table 3. Statistical data of precision for 3-quinuclidinol

Injection ID	System precision Ratio of area counts [3-Quinuclidinol / Dimethylsulfoxide]	Method precision 3-Quinuclidinol content, µg g^{-1}	Ruggedness 3-Quinuclidinol Content, µg g^{-1}
1	0.8956	1072	1094
2	0.8818	1065	1091
3	0.8722	1067	1079
4	0.8408	1092	1089
5	0.8562	1063	1081
6	0.8291	1054	1067
Average	0.8626	1069	1084
SD	0.0253	12.8	10.0
% RSD	2.9	1.2	0.9

Robustness

To assess the robustness of the method, experimental conditions were deliberately altered. The study was carried out with respect to flow pressure variation of carrier gas initial pressure and ramp pressure ±10% and column oven temperature initial temperature and ramp temperatures ± 2 °C as follows.

Conditions: In each robustness condition remaining gas chromatography conditions are same as per test method.

i) Column pressure programme / (Flow -10%) : 36 kpa (15 min) 9 kpa/min 100 kpa (54 min).

ii) Column pressure programme /(Flow +10%): 44 kpa (15 min) 11 kpa/min 100 kpa (54 min).

iii) Column oven temp. / (Temp. -2 °C) : 78 °C (2 min) 8 °C/min 170 °C (10 min) 8 °C/min 230 °C (48 min).

iv) Column oven temp. / (Temp. +2 °C) : 82 °C (2min) 12 °C/min 170 °C (10 min) 12 °C/min 230 °C (48 min).

Test method conditions

Column pressure programme: 40 kpa (15 min) 10 kpa/min 100 kpa (54 min).

Column oven temperature programme : 80 °C (2 min) 10 °C/min 170 °C (10 min) 10 °C/min 230 °C (48 min).

In each robustness condition, solutions of blank, standard and solifenacin succinate drug substance spiked with 3-quinuclidinol were prepared as per methodology and injected into GC to confirm the retention times. There is no much variation in the relative retention time

(RRT) of 3-quinuclidinol obtained at different deliberately varied robustness conditions from the developed methodology. Hence the test method is robust for all varied conditions. The completely robustness results are shown in Table 4.

Table 4. Robustness data of 3-quinuclidinol

Robustness condition	Variation	Dimethylsulfoxide		3-Quinuclidinol	
		RT, min.	RRT	RT, min.	RRT
Methodology (As per test method)	-	5.246	1.00	10.110	1.93
Flow pressure variation - Initial pressure and Ramp	-10% & -10%/min	5.491	1.00	10.346	1.88
	+10% & +10%/min	4.991	1.00	9.832	1.97
Temperature variation - Initial oven and Ramps	-2°C & -2°C/min	5.749	1.00	11.661	2.03
	+2°C & + 2 °C/min	4.786	1.00	8.968	1.87

Conclusion

A simple and reliable gas chromatography method was developed and validated for the determination of 3-quinuclidinol in solifenacin succinate drug substance. The results of various validation parameters demonstrated that the method is specific, sensitive, linear, precise, robust and accurate. Hence the proposed method is simple and user friendly, for the determination of 3-quinuclidinol content in solifenacin succinate drug substance.

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