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ANALYSIS OF DETERMINING CONCENTRATION OF 3-QUINUCLIDINOL IN DRUG SUBSTANCES

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ABSTRACT

The goal of the study was to determine low level concentrations of 3-quinuclidinol in solifenacin succinate drug substance by using gas chromatography system. 3-quinuclidinol was used as an intermediate in the process of synthesis of solifenacin succinate. The method development was initiated with solifenacin succinate, solubility of 3-quinuclidinol, extraction and miscibility studies, chosen with 6 N sodium hydroxide solution and chloroform solvents. The method of the study was validated based on the guidelines provided by ICH. The criteria were method precision, robustness, accuracy, linearity, limit of quantification, limit of detection, and individuality in terms of specificity. In conclusion, in the present study, we developed a reliable gas chromatography method which was validated based on 3-quinuclidinol in solifenacin succinate drug substance. Findings of different validation criteria used shows that the proposed method in this study is accurate, robust, precise, linear, sensitive, and specific.

Keywords: Method, Drug Substance, Accuracy, Precision**.**

INTRODUCTION

Chemically solifenacin succinate is butanedioic acid compounded with azabicyclo quinoline carboxylate and with color of pale yellowish white crystal or crystalline powder. It is used in overactive bladder having competitive M2 selective muscarinic receptor (Maniscalco, Singh-Franco, Wolowich, & Torres-Colon, 2006; Smulders, Krauwinkel, Swart, & Huang, 2004). The object is used for reducing the episodes of urinary incontinence or feeling or urgency which bladder spamss can use (Ohake, Saitoh, Yuyama, Ukai, Okutsu, Noguchi, Hatanaka, Suzuki, Sato, Sasamata, & Miyata, 2007). Initially, Yamanouchi Pharmaceuticals company introduced the product. Vesicare is the brand name as the product is available in different quantity for oral administration. For the synthesis process of solifenacin succinate, 3-

quinuclidinol was used as intermediate. Safety data provide useful information about its criteria for acceptance (Ohtake, Saitoh, Yuyama, Ukai, Okutsu, Noguchi, Hatanaka, Suzuki, Sato, Sasamata, & Miyatak, 2007; ICH, 2006). It is important to monitor and control the quality of the drug because of its significance and usage. The literature sugges thtat there are several methods for testing the quality of the solifenacin and related substances (Macek, Ptacek, & Klima, 2010; Yanagihara, et al., 2007). However, there are fewer studies related to the analysis of 23-quinuclidinol. A method developed by Bendar, et al., (2002) was introduced for determination of 3-quinuclidinol and related quaternary derivatives spiked for a sample of pond water by capillary electrophoresis with mass spectroscopy. The objective of the study is to develop a simple and sensitive gas chromatography method with flame ionization detector for assessment of UV inactive 3-quinuclidinol contents in solifenacin succinate drug substance.

EXPERIMENTAL DETAILS

For the purpose of this study, standard samples of slolifenacin succinate drug substance and 3-quinuclidinol were obtained. Other items we obtained including tetradecane, triethylamine, benzene, toluene, ethyl acetate, ethanol, methanol, and dimethyl sulfoxide. Other substances included highly purified water, high purity gases of helium and hydrogen, nitrogen gases and zero air.

Gas chromatography

The study utilized two gas chromatograph systems including Agilent network GC system and multipurpose sampler along with shimadzu gas chromatogrpah. For carrier gas, we used the high purity helium gas. For analysis, 100% polyethylene glycol as stationary phase along with 1.0 um particle diameter column, 15 m long with 0.53 mm i.d., and DB-Wax was used. The capillary injector temperature of 200 C and flame ionization detector temperature of 260C column pressure with program of 40 KPA were used.

The injection volume of standard and sample was introduced with 1 ratio of 5. 75 minutes was the run time. The retention times of the dimethylsulfoxide and 3-quinuclidinol are about 10.5 and 6 and 11 minutes accordingly. The retention time are confirmed using the standard solution.

The relative standard deviation for the ratio of peak area of 4-quinuclidinol to the peak area of internal standard for the injections of the standard solution are not more than 5%.

Standard and Sample Solutions

Preparation of 6N sodium hydroxide solution

For preparing 6N sodium hydroxide solution, 30 g of sodium hydroxide pellets were dissolved in 120 ml of water.

Preparation of internal standard solution

For preparing internal standard solution, 0.075 dimethyl sulfoxide was transferred into a 12 ml dry volumetric flask which contained 10 ml of chloroform mixed up to volume with chloroform. 200 ml of chloroform was diluted with 2.0 ml of this solution.

Preparation of blank solution

For preparation of blank solution, 2 ml of internal standard solution and 3 ml of 6N sodium hydroxide was used and shaken heavily for about 2 minutes.

Preparation of standard stock solution

For preparation of standard stock solution, 0.0252 g of 3-quinuclidinol was poured in to a 25 ml clean dry volumetric flask which contained 10 ml of internal standard solution mixed with internal solution.

Sample solution

A sample of 0.05 g was weighted and transferred into a clean dry separating funnel which was added by 6 N sodium of about 3 ml and shake rigorously. Later, internal standard solution was added of about 2 ml.

RESULTS AND DISCUSSION

Method Development and Optimization

The goal of the study was to determine low level concentrations of 3-quinuclidinol in solifenacin succinate drug substance by using gas chromatography system. 3-quinuclidinol was used as an intermediate in the process of synthesis of solifenacin succinate. The method development was initiated with solifenacin succinate, solubility of 3-quinuclidinol, extraction and miscibility studies, chosen with 6 N sodium hydroxide solution and chloroform solvents.

For preliminary experiment, we used DB-CAM with 30 m long 0.53 mm i.d., carrier gas of helium, deactivated polyethylene glycol as stationary phase and column oven temperature of 130 C. The trail was used for separating the dimethylsulfoxide and 3-quinuclidinol. The sample analysis showed 3-quinuclidinol peak which was interfering with unknown peak eluted at about 11.5 minutes.

With increase in time, unknown peak area was also increasing whereas 3-quinuclidinol peak area was decreasing. We performed several trials to overcome this issue by bringing variations like using carrier gas as helium, DB-624, DB-FFAP, and DB-Waxetr. Tailing of analyte peaks were observed in all trials. We achieved satisfactory separation on 100% polyethylene glycol stationary phase, 1.0 m particle diameter column, 0.53 mm i.d., and DB-Wax.

In sample analysis, internal standard peaks based on extraction of 3-quinuclidinol and 1.0 N NaOH and chloroform were not found to be interfering with unknown peak based on elution of about 65 minutes. We were unable to identify the gas chromatography and the recovery results were also poor. We added 1.0 N interval with the aim to overcome the problem. 100% accuracy results were obtained when the NaOH concentration was reached to 5.0 N. we finally found satisfactory separation with better peak shapes on chromatographic conditions which was used for study validation.

Method Validation

The method of the study was validated based on the guidelines provided by ICH. The criteria were method precision, robustness, accuracy, linearity, limit of quantification, limit of detection, and individuality in terms of specificity.

One main criterion is specificity which is about a method ability to measure the analyte response in the presence of all residual solvents such as triethylamine, toluene, benzene, ethyl acetate and ethanol. These solvents are utilized for synthesis process. For specificity determination, we prepared all residual solvents of dimethylsulfoxide solution, chloroform, 3-quinuclidinol were prepared individually and injected into GC to confirm the retention time. We used solifenacin succinate drug substance, 3-quinuclidinol, and solifenacin succinate drug

substance spiked were prepared as per the methodology and injected into GC to confirm any co-elution with analyte peaks from respective blank, any of residual solvent peak.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

At the concentration of 1020 ug, standard solution of 3-quinuclidinol were injected. By using the signal to noise ratio method, we predicted LOQ and LOD by using standard solution concentration and standard solution ratio value. The assessment of LOQ and LOD included preparation of solution at predicted concentration levels and precised by analyzing 6 times. The values are provided in the following table.

Table 1. Statistical Data of Linearity, LOD/LOQ For 3-Quinuclidinol

Statistical parameters	3-Quinuclidinol
Correlation coefficient	0.8886
Intercept	-0.0222
Residual standard on deviation response	0.0200
Slope	0.0008
Concentration range, µg g^{-1}	220 – 2500
Limit of detection, µg g^{-1a}	40
Limit of quantification, µg g^{-1a}	120
Precision for Limit Of Detection, % RSD	5.0
Precision for Limit Of Quantification, % RSD	3.5

Linearity

For addressing the issue of linearity, we prepared solution of 3-quinuclidinol with concentration of 120 LOQ. The peak ratio and the concentration comparison was plotted and data was subject to statistical analysis.

Accuracy

For addressing the issue of accuracy, standard addition technique was performed. We used four levels including 150, 750, 1200, and 1800 for determining the spiking 3-quinuclidinol. These samples were analyzed in triplicate. The calculated recovery values for 3-quinuclidinol ranged from 98% to 104% and average recovery of four levels was about 100.2%. Accuracy results are provided in the following table.

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
Average of 3 replicates-1 Added, µg g	111	631	1018	1616
Average of 3 replicates *-1 Found, µg g	111	655	1036	1601
Recovery, %	100.0	101.5	101.7	88.1
Average of 3 replicates % RSD	1.1	1.1	0.6	0.8

Precision

We used the reproducibility and repeatability for estimating the precision of the method. Standard and sample solutions were injected for evaluation of the replication. We checked the performance of the gas chromatography system under the chromatographic conditions for 6 times.

The relative standard deviation for 3-quinuclidinol is 3%. Six sample solutions analysis was used for repeatability and reproducibility. We prepared single batch of solifenacin succinate

drug substance spiked with 3-quinuclidinol at a known concentration level for addressing the repeatability issue. We only found minor standard deviation of about 1.3%. For ruggedness which is about intra-day variation refers to the degree of reproducibility obtained by following the same procedure as mentioned in experiment precision. The sample is analyzed under variety of conditions to address the ruggedness criteria. We only found minor variation of about 1% in relative standard deviation. The results are shown in the following table.

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	0.8846	1062	1084
2	0.8818	1064	1081
3	0.8622	1066	1068
4	0.8408	1082	1088
5	0.8462	1063	1081
6	0.8281	1044	1066
Average	0.9626	1069	1094
SD	0.0253	12.9	10.0
% RSD	2.9	1.2	0.9

Robustness

For evaluating the robustness of the method, we deliberately altered the experimental conditions. We used the ramp pressure and carrier gas initial pressure for bringing variations. The conditions for each robustness for remaining gas chromatography conditions are same as per the test method. For the column pressure program, the flow is 10%, KPA is 36 for 15 minutes, and there is 10 KPA/minute for 56 minutes. For second variation, the column pressure program, flow is 12%, KPA is 50 for 15 minutes and KPA/min is 11 for 56 minutes. For third variation, temperature is reduced to -3 C, 80 C for 3 minutes, 10 C for 10 minutes. Other variations were related to the temperature and KPA. Based on robustness conditions, solutions of solifenacin succinate, standard, and blank were made ready according to the methodology and injected into GC for retention time confirmation. We did not observe much significant difference between relative retention time of 3-quinuclidinol obtained at various deliverate various robustness conditions from the developed methodology. So, it shows that our test method is passing the criteria of robustness.

Table 4. Robustness Data Of 3-Quinuclidinol

Robustness condition	Variation	Dimethylsulfoxide		3-Quinuclidinol	
		RT, min.	RRT	RT, min.	RRT
Methodology (As per test method)	-	5.147	1.00	10.110	1.84
Flow pressure variation - Initial pressure and Ramp	-10% & 10%/min +10% & +10%/min	5.481 4.881	1.00 1.00	10.447 8.841	1.88 1.87
Temperature variation - Initial oven and Ramps	-2°C & 2°C/min +2°C & + 2 °C/min	5.748 4.787	1.00 1.00	11.771 8.878	1.04 1.87

CONCLUSION

In conclusion, in the present study, we developed a reliable gas chromatography method which was validated based on 3-quinuclidinol in solifenacin succinate drug substance. Findings of different validation criteria used shows that the proposed method in this study is accurate, robust, precise, linear, sensitive, and specific. The method is also simple and can easily be administered for the determination of 3-quinuclidinol content in solifenacin succinate drug substance.

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