

Method development and validation for the determination of residual solvents in quinabut API by using gas chromatography. Message 2

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Abstract

Aim. The aim of study was to develop and validate a simple, precise and accurate method using gas chromatography for analysis of residual solvents – acetone and 2-propanol – in quinabut API.

Materials and methods. All experiments were performed on a gas chromatographic system equipped with FID detector (Shimadzu GC System) using the DB-624 (30 m × 0.32 mm ID, 3.0 μm film thickness) column as stationary phase. Nitrogen was used as carrier gas with flow rate 7.5 mL/min. Split ratio was 1:5, injector temperature was 140 °C, detector temperature was 250 °C, oven temperature was programmed from 40 °C (2 min) to 50 °C at 1 °C/min and then increased at a rate of 15 °C/min up to 215 °C; and maintained for 2 min. All solutions were prepared using water as diluent.

Results. This proposed method is assessed for separation of residual solvent from quinabut with quantification. The obtained results are compared with the corresponding specified limits of ICH standard guidelines. The method validation was done by evaluating specificity, limit of detection (LOD) and limit of quantitation (LOQ), linearity, accuracy, repeatability, ruggedness, system suitability and method precision of residual solvents as indicated in the ICH harmonized tripartite guideline. The separation between acetone and 2-propanol peaks is 2.07. Hence method was found to be specific. The linear relationship evaluated across range of 15 to 180% for acetone and 2-propanol of ICH specified limit of residual solvents. The graphs of theoretical concentration *versus* obtained concentration are linear and the regression coefficients ‘R’ for residual solvents were more than 0.9968. The values of LOD and LOQ were much less than the lower limit of the concentration range and cannot affect the accuracy of the test. The technique was characterized by high intra-laboratory accuracy at concentrations close to the nominal acetone and 2-propanol concentration. All solutions were stable in water for at least 1 hour when stored at room temperature.

Conclusion. A simple, specific, accurate, precise and rugged gas chromatography method was developed and validated for the quantification of residual solvents present in quinabut API through an understanding of the synthetic process, nature of solvents and nature of stationary phases of columns. The residual solvents acetone and 2-propanol were determined.

Keywords

Gas Chromatography, Quinabut, Residual Solvents, Validation

Introduction

Organic solvents are routinely applied during synthesis of drug substances, excipients, or during drug product formulation (Nasanov 2003; Feuba 2004; Simon and Botting 2004; Warner and Mitchel 2004; Balabanova 2010; Levin and Laufer 2012). They are not desirable in the final product, mainly because of their toxicity, influence on the quality of crystals of the drug substance, and their odor or taste, which can be unpleasant for patients. To remove them, various manufacturing processes or techniques are in use. Even after such processes, some solvents still remain, albeit in small quantities. These small quantities of organic solvents are commonly known as organic volatile impurities or residual solvents. Different manufacturers produce the same pharmaceutical products using different organic solvents. Therefore, analysis of residual solvents becomes a challenging analytical task in pharmaceutical analysis and control. Unknown residual solvents are frequently detected during routine quality control testing. An error may occur while using existing official methods for their determination. Hence, we need to develop a rapid, sensitive method which identifies, and quantitate all residual solvents in pharmaceuticals.

Inadequate attention has been paid during pharmaceutical investigations. Gas chromatography (GC) is a technique where the liquid or solid sample is set in a closed vessel until the volatile components reach equilibrium between the sample and the gas volume above i.e. the so called "headspace". An aliquot of the headspace is sampled and introduced into gas chromatographic column for analysis. Regulatory agencies and pharmacopoeias suggest gas chromatography as the most suitable technique for residual solvent testing for active substances and formulations soluble in water. Residual solvent specification limits, set in accordance with the toxicity of solvents, vary from a few ppm to thousands of ppm. GC determination of residual solvents is nowadays a mature technique (Hymer 2003; Jacobsa et al. 2006; Grodowska and Parczewski 2010). Direct injection of analytes evaporated through equilibration between liquid (or solid) phase and gas phase into a GC system minimized the contamination of the GC system and the deterioration of the GC column. In addition, the automation of equilibrium and injection procedure reduced analysis time and improved reproducibility in the injection procedure.

Quinabut (Fig. 1) (Sodium 4-(3-methyl-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)butanoate) – the original newly synthesized (Kovalenko et al. 2012 [Patent of Ukraine №97586]; Stepanyuk et al. 2013 [Patent of Ukraine №81634]).

The manufacturing of active pharmaceutical ingredients (API) under GMP (good manufacturing practice) conditions requires adequate control of the quality of the different ingredients involved in the synthesis. Organic residual solvents must therefore be controlled, and their purity determined, before any GMP synthesis. The acceptable limits for these substances are given in ICH

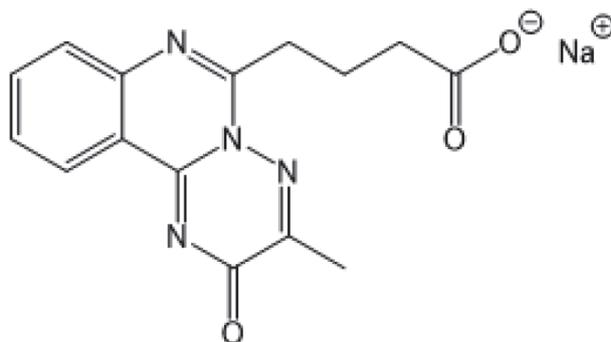


Figure 1. Chemical structure of quinabut.

guidelines (Guideline for Residual solvents, Q3C (ICH Q3C 2006; ICH Q2 1995).

Acetone and 2-propanol (IPA) are used as solvents in quinabut manufacturing steps and not removed consistently. Acetone the same as IPA belongs to 3 class of residual solvents and may be regarded as less toxic and of lower risk to human health.

The purpose of this research study was to develop an accurate and precise gas chromatography method for analysis of residual solvents – acetone and 2-propanol – in quinabut API.

Materials and methods

Chemicals and reagents

Quinabut API (purity 99.9%) was synthesized and characterized in-house. Used chemicals – acetone and 2-propanol – were obtained from Sigma – Aldrich, USA. Water Milli Q has been used as a diluent and was obtained from in-house Milli Q water instrumentation.

Instrumentation and chromatographic conditions

A gas chromatograph (Shimadzu GC System ser. 2014) with autosampler AOC-20 series was used to load the sample. An analytical balance (ME204 from Mettler Toledo) and auto pipette (100–1000 μ L from Eppendorf) were used.

For gas chromatographic analysis, a DB-624 fused silica capillary column from Agilent (G43 phase: 6% cyanopropyl phenyl, 94% polydimethylsiloxane) (30 m length, ID 0.32 mm, and 3 μ m film thickness) was used. The temperature of the injection port was maintained at 140 °C at a split ratio of 1:5, with nitrogen as a carrier gas (flow rate of 7.5 mL/min). The temperature of the detector was set at 250 °C. Oven temperature was programmed from 40 °C (2 min) to 50 °C at 1 °C/min and then increased at a rate of 15 °C/min up to 215 °C; and maintained for 2 min. A volume of 1 ml solutions was injected into the GC injection port.

The chromatographic system was considered suitable if relative standard deviation of the peak area for 5 injections not more than 10.0%.

Standard solutions and sample preparation

A common standard stock solution in water containing all the known residual solvents of quinabut API (i.e., acetone and IPA) was prepared in such a way that it had a final concentration of 5000 ppm for acetone and 5000 ppm for IPA.

Spiked model solutions for specificity, linearity, accuracy and precision were prepared using 1.0 g of quinabut API, 500 mg of IPA and 500 mg acetone in 50 ml of water. Further, 5 ml of this solution was diluted to 10 ml with diluent.

To obtain quinabut API sample solution 1.0 g of quinabut API was accurately weighed in a 20 ml volumetric flask containing 5 ml of diluent (water) and diluted up to the mark.

Final concentrations of all dilutions were achieved using auto pipette. All vials were immediately equipped with septum, metallic cap and crimped properly.

Validation of the developed method

The method validation was done by evaluating specificity, limit of detection (LOD) and limit of quantitation (LOQ), linearity, accuracy, repeatability, robustness, system suitability and method precision of residual solvents as indicated in the ICH harmonized tripartite guideline (Guideline for Residual solvents, Q3C (ICH Q3C 2006; ICH Q2 1995).

Specificity of the analytical method was performed by injecting both solvents IPA (5000 ppm) and acetone (5000 ppm), individually and blank (i.e. water) under the same chromatographic condition. System suitability of method was performed by injecting five replicates of standard solution. The system suitability was confirmed by resolution and % RSD.

For the instrumental method, LOD was determined as the lowest amount to detect and LOQ was the lowest amount to quantify by the detector. The LOD and LOQ were calculated by statistical methods (determined based on the standard deviation of the response and the slope).

Detector response linearity, accuracy and precision were assessed by investigation of nine model solutions of IPA and acetone prepared over the range 15–180% of specification limit (750 to 9000 ppm) in diluent.

Method reproducibility was determined by measuring repeatability and intermediate precision of peak area for IPA and acetone. The repeatability of method was determined by analyzing six replicate injections containing quinabut API spiking with IPA (5000 ppm) and acetone (5000 ppm). The inter-day precision was determined by performing injections of six freshly prepared spiked model solutions on different day and analyst.

Robustness of the method was assessed by deliberately altering the experimental conditions such as carrier gas flow rate (± 0.75 mL/min), column temperature program (± 2 °C), and injector port temperature (± 15 °C) by keeping all the other chromatographic conditions constant as described above.

Table 1. Parameters of specificity.

Peak name	Retention time, min	The number of theoretical plates	Peak symmetry
Acetone	3.83	21047	1.19
2-propanol	4.06	23016	1.11

Results and discussion

Method development

Quinabut will be used in solid dosage form; the residual solvents in it should be quantified as per ICH guideline. According to ICH guideline Q3C (R6) on impurities: guideline for residual solvents it is considered that amounts of IPA and acetone of 50 mg per day or less (corresponding to 5000 ppm or 0.5%) would be acceptable without justification. Thus, proposed method was assessed for separation of residual solvent from quinabut API with quantification. The obtained results were compared with the corresponding specified limits of ICH standard guidelines.

Method validation

System suitability

Using the Shimadzu GC system suitability software, resolution between acetone and IPA was calculated. The criterion for system suitability was that the resolution between these residual solvents should not be less than 1.5 and it was found well above the minimum passing limit.

Also %RSD for acetone and IPA areas in five replicates of standard solution injections should not be more than 10%. Results indicate an acceptable level of precision for the analytical system (1.56% for acetone and 2.43% for IPA).

Specificity

The quinabut API sample was spiked with acetone and 2-propanol individually and each sample was chromatographed to examine interference, if any, of the residual solvent peaks with each other.

The specificity parameters are shown in Table 1. The diluent and API do not show interference at the retention time of any residual solvents.

The separation between acetone and 2-propanol peaks was 2.07. Hence method was found to be specific.

The retention time for standards of acetone and 2-propanol was found to be 3.83 and 4.06 min, respectively. Chromatograms of blank, standard, specificity are shown in Figs 2–4.

Linearity and range

The linearity of the method was determined by making injections of acetone and IPA model spiked solutions over the range 15–180% of specification limit. Three replicates were performed at each level. The calibration curves were obtained with the average of peak area ratios of three replicates. The linearity data are shown in Tables 2, 3.

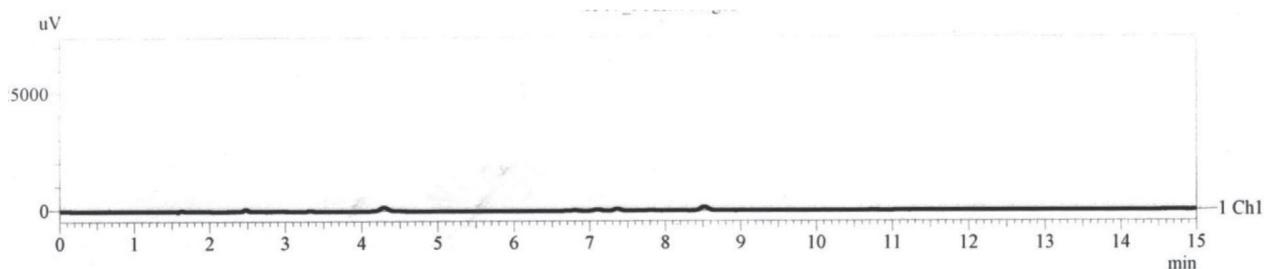


Figure 2. Typical chromatogram of blank (water).

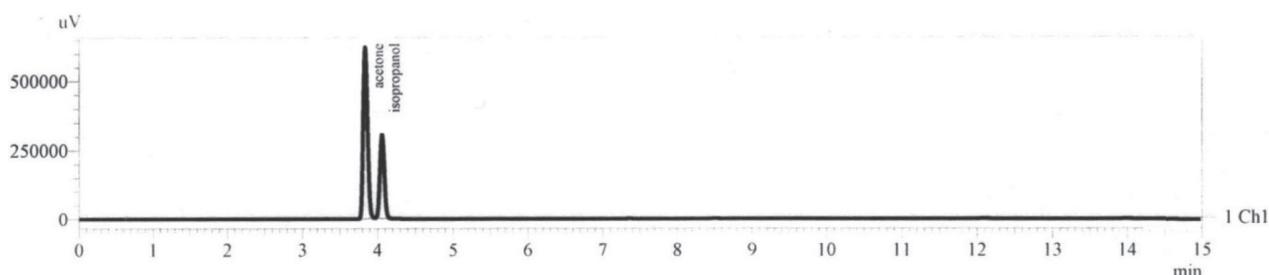


Figure 3. Typical chromatogram of acetone and IPA standard solution.

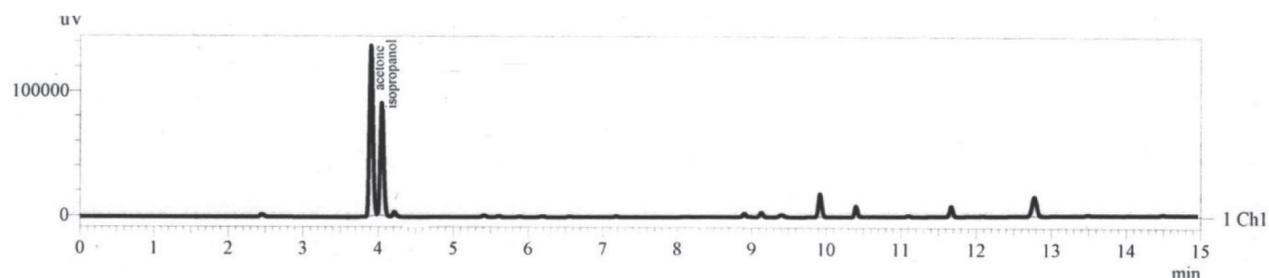


Figure 4. Chromatogram of specificity (spiked solution of quinabut API, acetone and 2-propanol).

Table 2. Metrological characteristics of linear dependence are calculated by method of least squares for model solutions of acetone.

Parameter	Value	Critical values (15–180) %, g = 9	Conclusion
b	0.992379111	–	–
s _b	0.010586652	–	–
a	0.005766911	1) ≤ 1.895*s _a , 2) if not executed 1), than ≤ 1.92	Valid
s _a	0.014219746	–	–
s _r	0.017029	≤ 1.58	Valid
r	0.999823281	≥ 0.996865	Valid

Table 3. Metrological characteristics of linear dependence are calculated by method of least squares for model solutions of IPA.

Parameter	Value	Critical values (15–180) %, g = 9	Conclusion
b	1.00493247	–	–
s _b	0.026492986	–	–
a	-0.015926285	1) ≤ 1.895*s _a , 2) if not executed 1), than ≤ 1.92	Valid
s _a	0.035311827	–	–
s _r	0.042609153	≤ 1.58	Valid
r	0.998858991	≥ 0.996865	Valid

Thus, it can be concluded that the linearity of the method is confirmed over the entire concentration range (10–180%) for acetone and for 2-propyl alcohol.

Limit of detection (LOD) / Limit of quantitation (LOQ)

The LODs and LOQs of residual solvents in quinabut API were determined based on the standard deviation of the response and the slope.

$$\text{LOD (acetone)} = 3.3 \times s_a / b = 0.047\%;$$

$$\text{LOQ (acetone)} = 10 \times s_a / b = 0.143\%;$$

$$\text{LOD (IPA)} = 3.3 \times s_a / b = 0.0116\%;$$

$$\text{LOQ (IPA)} = 10 \times s_a / b = 0.351\%.$$

The values of LOD and LOQ are much less than the lower limit of the concentration range and cannot affect the accuracy of the test. The LOQ values are well below the ICH specification limit of the residual solvents.

Accuracy and precision

Accuracy, closeness of measured values and its actual or standard value, was determined by injecting known amount of residual solvent at placebo levels from 15% to 180% of standard solution. The recovery data of investigated residual solvents are listed in Tables 4–7. These values are well within the prescribed limits; hence method is precise for determination of residual solvents in quinabut API.

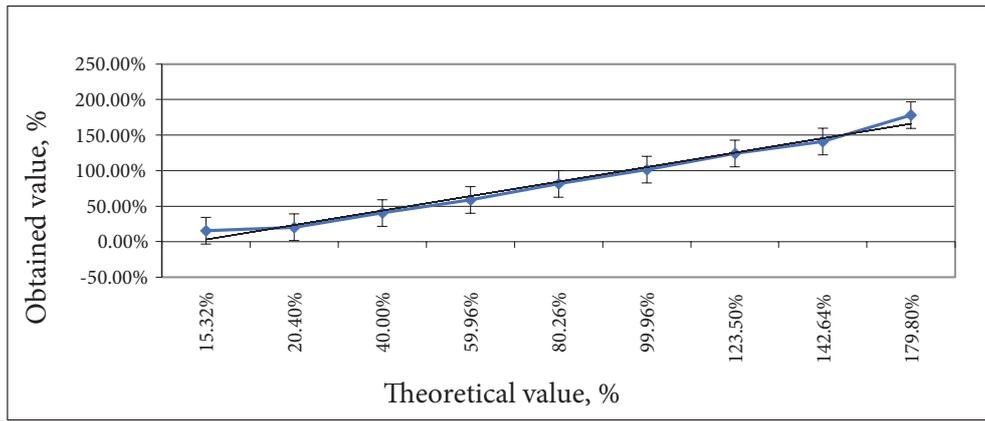


Figure 5. Linearity of acetone solutions.

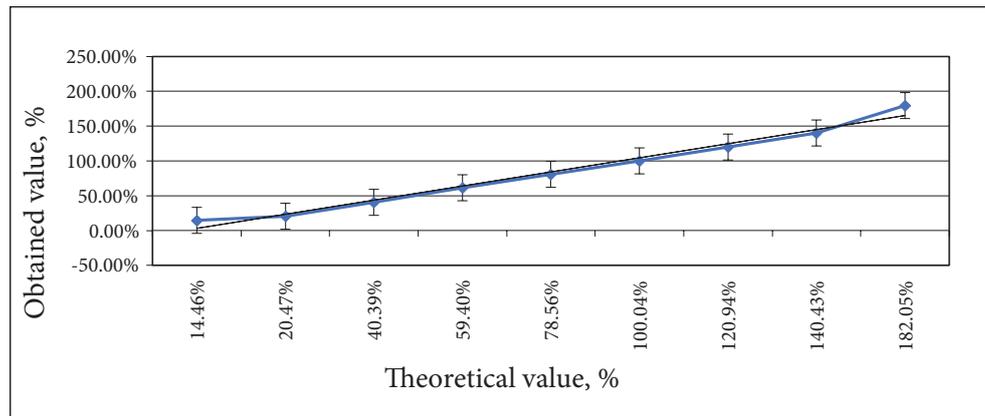


Figure 6. Linearity of IPA solutions.

Table 4. The results of the analysis of model mixtures of quinabut API with acetone content.

Number of model solution	Weight, mg	Theoretical value, %	The peak area of acetone	Obtained value, %	Found in % to theoretical value
lin15	76.6	15.32	360513	15.46	100.89
lin20	102	20.40	476635	20.43	100.17
lin40	200	40.00	940261	40.31	100.78
lin60	299.8	59.96	1371118	58.78	98.04
lin80	401.3	80.26	1897507	81.35	101.36
lin100	499.8	99.96	2366792	101.47	101.51
lin120	617.5	123.50	2896049	124.16	100.54
lin140	713.2	142.64	3289593	141.03	98.87
lin180	899	179.80	4156937	178.22	99.12
\bar{Z} , %				100.14	
Relative standard deviation SD_s , %				1.20	
Relative confidence interval $\Delta_z = t(95\%, g-1) \times SD_z = 1.860 \times s_z$				2.23%	
Δ_{AS}				3.00%	
Systematic error $\delta = \bar{Z} - 100 $				0.14%	
The criterion for the insignificance of a systematic error:				Valid	
1) $\delta \leq \Delta_z / 3 = 0.74$					
2) if not executed 1), than $\delta \leq 0.96$				Valid	
Conclusion				Method is correct	

$\Delta_z = 2.23\% < \max \Delta_{AS} = 3.0\%$, the technique is characterized by sufficient precision over the entire concentration range. If $n = 9$, then the technique has no significant systematic error as the magnitude $\delta\% = 0.14\% \leq \frac{\Delta_z}{\sqrt{n}} = 0.74\%$ (satisfies the criterion 1) and is not significantly different

Table 5. The results of the analysis of model mixtures of quinabut API with IPA content.

Number of model solution	Weight, mg	Theoretical value, %	The peak area of 2-propanol	Obtained value, %	Found in % to theoretical value
lin15	72.9	14.58	178085	14.46	99.21
lin20	102.2	20.44	252073	20.47	100.17
lin40	202.3	40.46	497321	40.39	99.84
lin60	307.1	61.42	731300	59.40	96.71
lin80	403.6	80.72	967239	78.56	97.33
lin100	500.2	100.04	1231646	100.04	100.00
lin120	599.5	119.90	1488921	120.94	100.86
lin140	700.7	140.14	1728933	140.43	100.21
lin180	897.9	179.58	2241279	182.05	101.37
\bar{Z} , %				99.52	
Relative standard deviation SD_s , %				1.56	
Relative confidence interval $\Delta_z = t(95\%, g-1) \times SD_z = 1.860 \times s_z$				2.90%	
Δ_{AS}				3.00%	
Systematic error $\delta = \bar{Z} - 100 $				0.48%	
The criterion for the insignificance of a systematic error:				Valid	
1) $\delta \leq \Delta_z / 3 = 0.96$					
2) if not executed 1), than $\delta \leq 0.96$				Valid	
Conclusion				Method is correct	

from zero, the criterion of practical insignificance $\delta\% = 0.14\% \leq 0.96\%$ (satisfies the criterion 2) (Table 4).

$\Delta_z = 2.90\% < \Delta_{AS} = 3.0\%$, the technique is characterized by sufficient precision over the entire concentration range. If $n = 9$, then the technique has no significant systematic

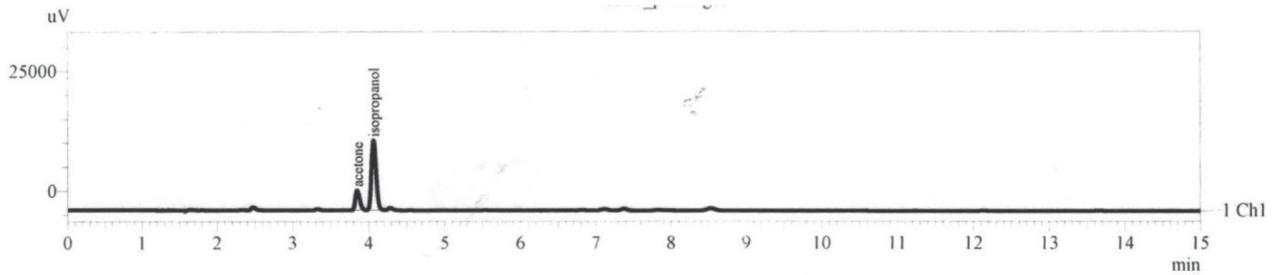


Figure 7. Chromatogram of quinabut API sample.

Table 6. Inter-day data of model mixtures of quinabut API with acetone content.

Solution number	Value Zi, %	
	Analyst 1	Analyst 2
Solution 1	101.51	100.66
Solution 2	101.74	91.85
Solution 3	101.64	100.51
Solution 4	102.21	97.89
Solution 5	95.94	94.16
\bar{Z}	100.61	97.01
$Z_{intra}, \%$	98.81	
$S_z, \%$	2.62	3.91
$SD_{intra} \bar{Z}, \%$	3.66	
$\Delta_{intra}, \%$	2.89	

error as the magnitude $\delta\% = 0.48\% \leq \frac{\Delta_z}{\bar{z}} = 0.96\%$ (satisfies the criterion 1) and is not significantly different from zero, the criterion of practical insignificance $\delta\% = 0.48\% \leq 0.96\%$ (satisfies the criterion 2) (Table 5).

Δ_{intra} shall not exceed the maximum permissible uncertainty of the method (Table 6), ie:

$$\Delta_{intra} = t[95\%, (n * m - 1)] * RSD_{intra} / \sqrt{5} = 1.761 * RSD_{intra} / \sqrt{5} = 0.79 * RSD_{intra} \leq \max \Delta_{AS}$$

$$\Delta_{intra} = 2.89\% \leq \max \Delta_{AS} = 3.20\%.$$

Thus, the technique was characterized by high intra-laboratory accuracy over the entire IPA concentration range, close to the nominal concentration.

$$\Delta_{intra} = t[95\%, (n * m - 1)] * RSD_{intra} / \sqrt{5} = 1.761 * RSD_{intra} / \sqrt{5} = 0.79 * RSD_{intra} \leq \max \Delta_{AS}$$

$$\Delta_{intra} = 1.27\% \leq \max \Delta_{AS} = 3.20\%.$$

Robustness

To test the robustness of the gas chromatographic method, the stability of solutions over time, the influence of the subjective factor (various analysts) were investigated. The method for determining the amount of residual solvents does not specify the time after which the peak area should be measured, so its stability was checked over time. Measurements of the areas of the peaks of the residual solvents were performed with a time interval of 15 min for 1 h for the model solution (A) and the standard solution (A₀) (Tables 8, 9).

Table 8. The stability of acetone solutions over time.

	t, min					Average	RSD _t , %	Δ_t , %	max δ , %
	0	15	30	45	60				
A ₀	2379374	2383254	2387936	2374158	2364781	2381181	0.0037	0.01	1.024
A	2366797	2366794	2377154	2361725	2364696	2367433	0.0025	0.01	

Table 7. Inter-day data of model mixtures of quinabut API with IPA content.

Solution number	Value Zi, %	
	Analyst 1	Analyst 2
Solution 1	99.92	100.68
Solution 2	97.12	100.00
Solution 3	97.88	100.39
Solution 4	103.11	100.09
Solution 5	100.20	100.53
\bar{Z}	99.65	100.34
$Z_{intra}, \%$	99.99	
$S_z, \%$	2.34	0.29
$SD_{intra} \bar{Z}, \%$	1.61	
$\Delta_{intra}, \%$	1.27	

Thus, the technique was characterized by high intra-laboratory accuracy over the entire acetone concentration range, close to the nominal concentration.

Δ_{intra} shall not exceed the maximum permissible uncertainty of the method (Table 7), i.e.:

This confirms that the method is accurate and precise for determination of residual solvent in quinabut API in routine analysis.

$$\max \delta = \max \Delta_{AS} * 0.32 = 3.2 * 0.32 = 1.024.$$

$$\Delta_t = t(95\%, (n_t - 1)) * RSD_t = 2.13 * RSD_t < \max \delta = 1.024,$$

the technique is stable over time.

From the results above, it is apparent that acetone and IPA model and standard solutions are stable in water for at least 1 hour when stored at room temperature.

The column temperature program, carrier gas flow and injection port temperature within $\pm 10\%$ did not significantly affect the test results. The results were

Table 9. The stability of 2-propanol solutions over time.

	t, min					Average	RSD _t , %	Δ_t , %	max δ , %
	0	15	30	45	60				
A ₀	1231646	1254793	1232487	1237983	1247215	1239227	0.0080	0.02	1.024
A	1234567	1235476	1236123	1237825	1244674	1237733	0.0033	0.01	

found to be within the assay variability limits during the entire process.

Application

The developed method was applied to the research and quantification of IPA and acetone in quinabut API sample.

The residual solvent content is calculated by the formula:

$$\text{Residual solvent, ppm} = \frac{A_{\text{samp}}}{A_{\text{std}}} \times \frac{C_{\text{std}}}{W_{\text{samp}}} \times 10^6,$$

where A_{samp} – peak area of residual solvent in the sample solution; A_{std} – peak area of residual solvent in the standard solution; C_{std} – concentration of residual solvent in the standard solution, mg/ml; W_{samp} – weight of the sample, mg.

The obtained results for acetone and 2-propanol in the quinabut API sample levels was respectively 47.7 ppm and

354.9 ppm; which are widely inferior to the amount specified by the ICH guidelines (5000 ppm).

Conclusion

A simple, rapid and highly selective gas chromatography method was developed and validated for the quantification of residual solvents present in quinabut API through an understanding of the synthetic process, nature of solvents and nature of stationary phases of columns. The developed method is specific, accurate, precise and rugged as per ICH guidelines. The result of this validation shows that residual solvents (acetone and 2-propanol) can be analyzed in quinabut API in the range of 1–9000 ppm according to the method described in this article with reliability for further analytical studies.

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