Optimization and Validation of GC Method for Determination of Methanol as Organic Volatile Impurity in Atenolol Bulk Drug

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Abstract Methanol is used during synthesis of atenolol and therefore present as organic impurity in bulk drug. Gas chromatography with headspace is widely used for the determination of residual solvents in API's. The presented study is performed to develop and validate gas chromatography method for determination of methanol in atenolol API. The developed method was validated in terms of specificity, linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ). The developed method was utilized for the investigation of methanol content in bulk drug. The presented method can be utilized for methanol determination in atenolol in pharmaceutical industries.

Keywords: *atenolol, organic volatile impurity (OVI), active pharmaceutical ingredient, ß-Adreno receptor antagonists*

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1. Introduction

Cardiovascular diseases are the major cause of deaths worldwide, even surpassing deaths due to cancer. Among these, hypertension is central to the pathogenesis of coronary artery disease (angina, myocardial infarction), heart failure, cerebral (stroke), and peripheral vascular diseases. Pharmacological treatment of hypertension includes mainly the use of six drug classes: diuretics, *ß*-adrenergic blocking agents, calcium antagonist, angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists, and *α*-adrenergic blockers [1].
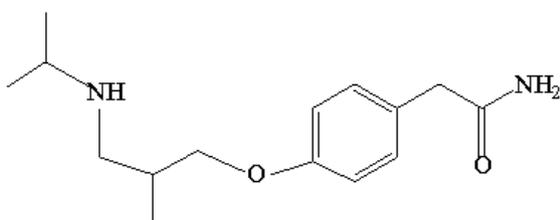

Figure 1. The chemical structure of atenolol

ß-Adreno receptor antagonists are a group of compounds that competitively inhibit, the effects of catecholamines at b-adrenergic receptors. These agents are widely used in clinical medicine for the treatment of various conditions. Racemic atenolol is one of the top five best-selling drugs in the world today, for the treatment of hypertension, angina and also in the treatment of post-myocardial infarction, yet the S isomer has found to avoid the occasional side effect of a lowered heart rate encountered with race mate [2].

Atenolol (RS)-2-{4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl} ace amide (ATE) is a selective *ß1* receptor antagonist, a drug belonging to the group of beta blockers (sometimes written *ß*-blockers), a class of drugs used primarily in cardiovascular diseases. Introduced in 1976, atenolol was developed as a replacement for propranolol in the treatment of hypertension [3]. This is also used to treat myocardial infarction (heart attack), arrhythmias (rhythm disorders), angina (chest pains), and disorders arising from decreased circulation and vascular constriction, including migraine [4].

For pharmacopeial purposes, residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. The residual solvents are not completely removed by practical manufacturing techniques. Appropriate selection of the solvent for the synthesis of a drug substance or an excipient may enhance the yield, or determine characteristics such as crystal form, purity, and solubility. Therefore, the solvent may sometimes be a critical element in the synthetic process [5].

Analytical chemists have to analyze a variety of complex samples often originating in different matrices to answer questions about the quality and quantity of

different analytes. [6] The need for a rapid and reliable method for the determination of residual solvents has become significant due to the toxicity of residual solvents in drug substances and drug products. The determination of residual solvents in drug substances, excipients or drug products is known to be one of the most difficult and demanding analytical tasks in the pharmaceutical industry. Furthermore, determination of the polar residual solvents in pharmaceutical preparations is still an analytical challenge mainly because these compounds are quite difficult to remove from water or polar solvents [7].
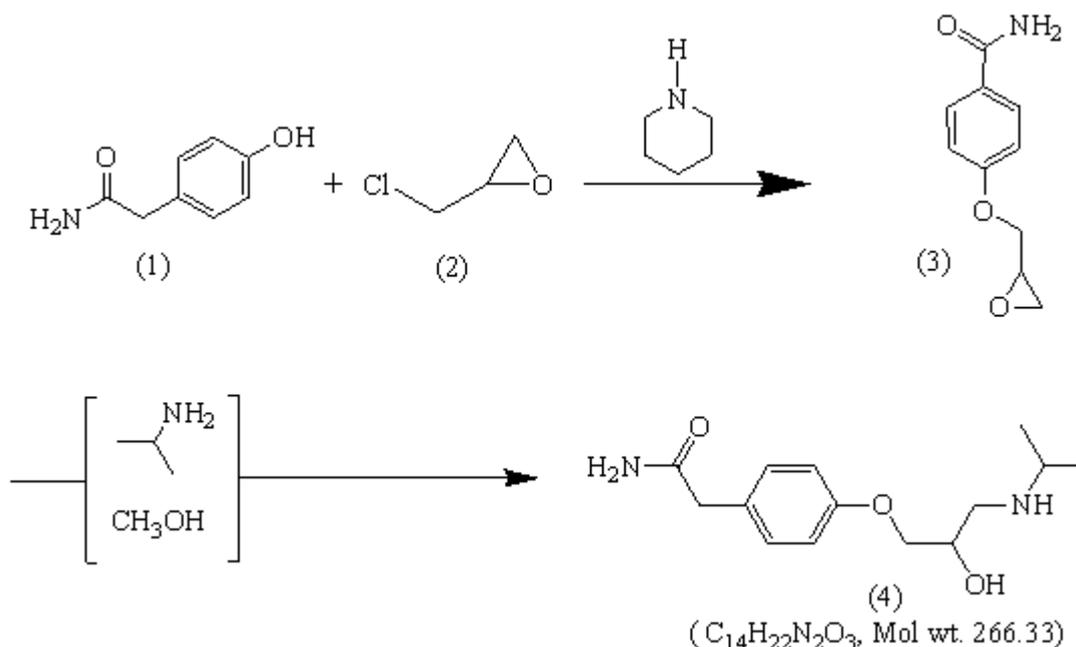

Figure 2. Scheme for the synthesis of atenolol showing usage of methanol

Atenolol is synthetized started from commercial available 4-hydroxyphenylacetamide (1) and epichlorohydrine (2) in 15 fold molar excess, (1) with piper dine as a catalyst (see Figure 2). The reaction mixture is stirred 4-6 hours at 95-100°C, then allowed to cool at room temperature and left standing overnight. The precipitate is collected by filteration and thoroughly washed with methanol to remove unreacted epichlorohydrine. The product, 1-(4-carmoylmethylphenoxy) 2,3-epoxy-propane (3), may be recrystallized from methanol. The recrystallized compound (3) is reacted with isopropyl amine (24 fold molar excess), in methanol as solvent, at the boiling point, for 5-30 minutes. The solvent and excess of isopropyl amine are distilled off, and the crude atenolol recrystallized from water [8].

Here in this paper rapid GC method for the estimation of methanol in atenolol is discussed. As per USP methanol is classified under class 2 solvent, may be limited nongenotoxic animal carcinogens or possible causative agent of other irreversible toxicity, such as neurotoxicity or teratogenicity [5]. The maximum concentration prescribed is 3000 ppm [5].

2. Material and Method

2.1. Material

Pure atenolol was provided by IPCA laboratories, Ratlam and methanol were purchased from Fluka Chemical Co., Inc. (Milwaukee, WI, USA).

2.2. Chromatographic Conditions

Gas chromatograph Perkin Elmer Authosystem XL with headspace HS – 40 XL or equivalent is utilized with Turbochrom Navigator software. Capillary column CP-SIL-8CB (25 × 0.53 mm) ID, 5 µm from Restek Corporations was used. GC system is equipped with Flame ionization detector. Oven temperature is presented under Table 1, injector and detector temperature are 200 and 250 °C respectively. Nitrogen was used as carrier gas in pressure of 13 psi through headspace. Split flow was 33 ml/min. Head space conditions are shown under Table 2.

Table 1. Oven Temperature of the Proposed GC Method

Temperature (°C)	Hold (min)	Rate (°C/min)
45	4.0	10
200	2.5	00

Table 2. Conditions for Head Space

Sample temperature	110°C
Needle temperature	130°C
Transfer line temperature	140°C
Thermo stating time	43 min.
Pressurizing time	3.0 min
Injection time	0.2 min
Withdrawal time	0.1 min
GC cycle time	26 min

2.3. Optimization of Chromatographic Conditions

Method was optimized by varying some parameters as presented under Table 3, while other parameters such as carrier gas, detector temperature and withdrawal time kept constant. It was found that condition 10th was most suitable condition for our purpose and hence chosen for proceeding study. Figure 3 represents different chromatograms obtained during optimization cycle.

Table 3. Optimization Studies Performed in the Proposed Method

| Parameters	Over all Conditions									
	GC Condition									
	1	2	3	4	5	6	7	8	9	10
Oven Initial Temperature (°C)	40	45	42	43	43	44	45	45	45	45
Final Temperature (°C)	150	175	175	180	190	195	198	199	199	200
Split flow/Column flow rate	10 ml/min	28 ml/min	29 ml/min	30 ml/min	32 ml/min	33 ml/min	33 ml/min	33 ml/min	33 ml/min	33 ml/min
Head Space Condition										
Sample Temperature (°C)	85	105	109	109	110	110	105	110	110	110
Needle Temperature (°C)	120	128	128	128	130	130	130	130	130	130
Transfer Line temperature (°C)	130	135	137	137	139	140	140	140	140	140
Thermo stating time/Equilibrium time (min)	20	36	38	38	38	40	41	42	43	43
Pressurizing Time (min)	1	2	3	3	3	3	3	3	3	3
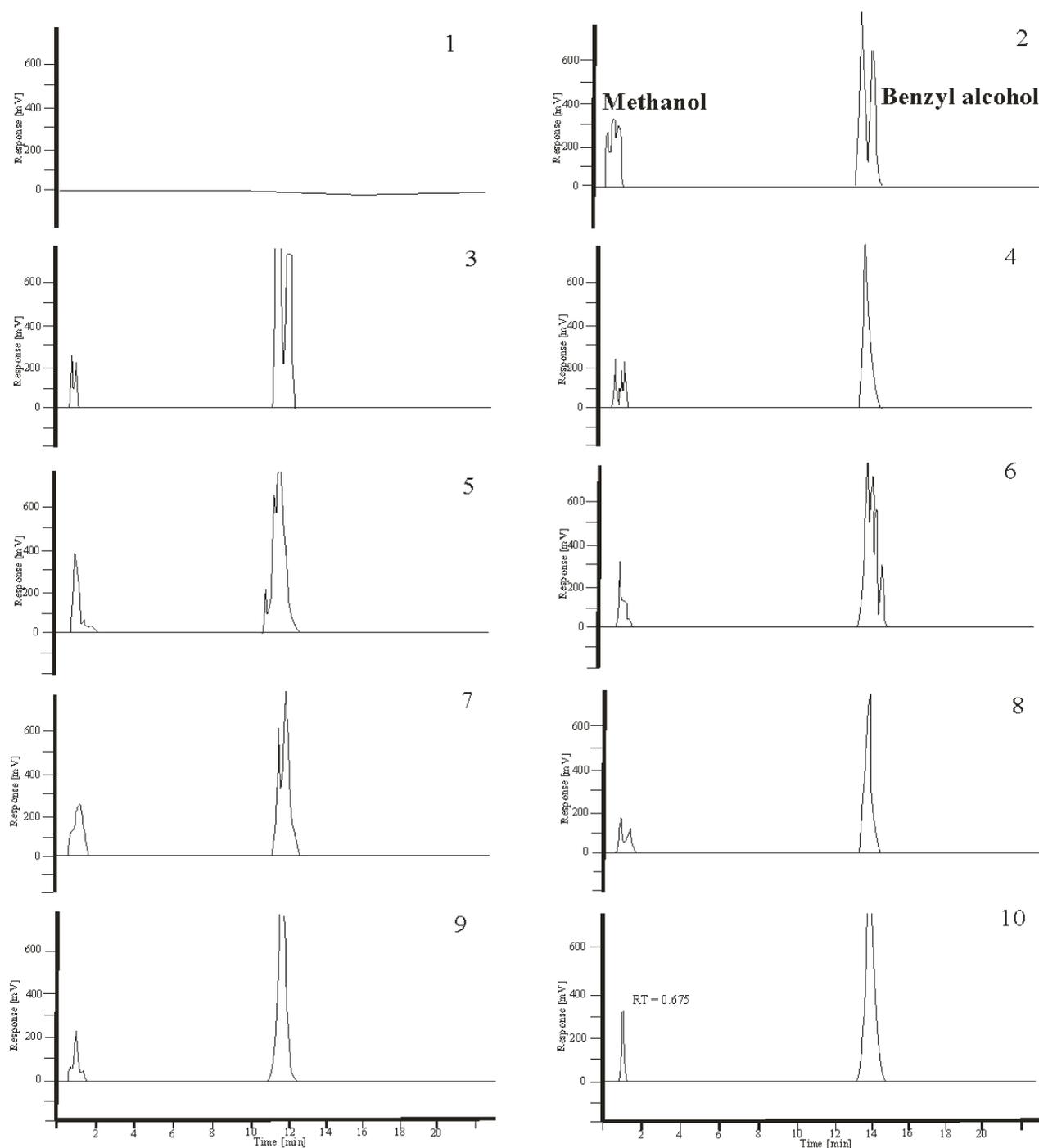
Figure 3. Chromatograms obtained during optimization studies as described under Table 3

3. Preparation of Solutions Used in Method

Blank preparation: 5 ml of benzyl alcohol accurately measured and sealed properly in dry HS 40 vials.

Standard stock solution: Accurately known concentration of 3000 ppm of methanol was prepared in AR grade benzyl alcohol.

Standard preparation: From stock solution 10 ml of solution accurately measured and transferred to 100 ml standard volumetric flask and dilute to the mark with benzyl alcohol.

4. Validation

Proposed method was validated as per ICH guidelines [8].

Blank sample produces no interference near to the RT of the drug shows method is specific.

To evaluate linearity Stock solution was diluted to produce 50, 100, 500, 2000, 3000 and 5000 µg/ml solution of methanol. Calibration curve was prepared by plotting graph between area and concentration (Figure 4). R^2 value equal to 1 proves linearity of the proposed method.
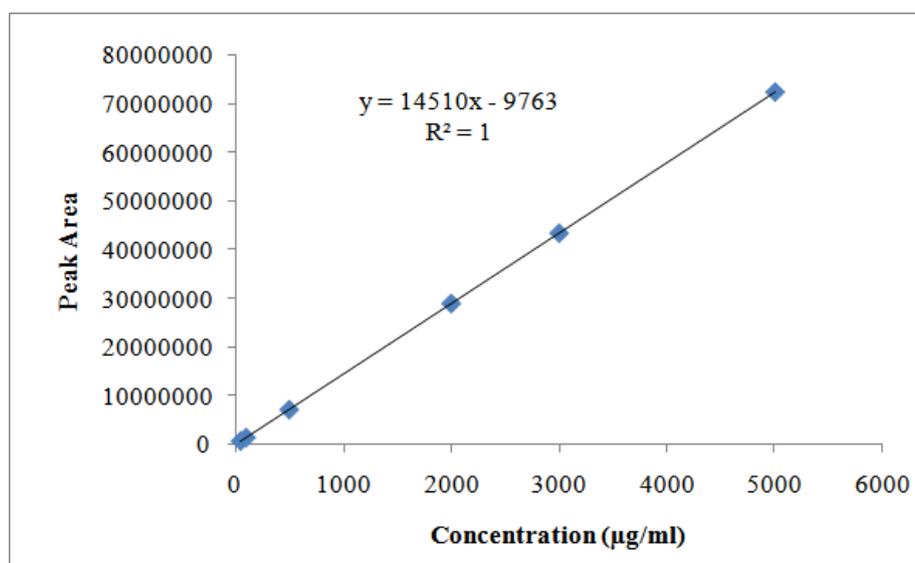

Figure 4. Calibration curve of methanol

Dilution of 100 µg/ml was prepared from stock solution and diluted upto the mark in benzyl alcohol. Same solution was injected six times and relative standard deviation of area found was less than 1. For intermediate precision same operation is repeated for three days and again RSD observed less than 1 proves method to be sufficiently precise.

To establish method accuracy blank sample was spiked with known quantity of methanol at three concentration

levels (100, 1000 and 4000 µg/ml) solution and injected three times. Table 4 presents recovery studies of the proposed method.

The LOD and LOQ [10] of methanol in atenolol were determined based on signal-to-noise ratio of 3 : 1. The LOQs of residual solvents were determined based on signal to noise ratio 10: 1. Six replicates were performed at each level. LOD and LOQ were found to be 1.25 and 5 ppm respectively.

Table 4. Recovery Studies for the Proposed Method

Conc. MeOH	Concentration found	% Recovery	Mean±SD
100	90	90	90.66 ± 3.05
	94	94	
	88	88	
1000	990	99	97.6 ± 1.51
	978	97.8	
	960	96	
4000	3965	99.125	98.14 ± 0.94
	3922	98.05	
	3890	97.25	

5. Determination of Atenolol in API

Acurately weighed 500 mg of Atenolol API is transferred to dry HS 40 vials in dublicate and 5 ml of benzyl alcohol added in each vial and sealed properly. Samples were then sonicated for 5 minutes.

Result in ppm was calculated by using formula:

$$\frac{AT}{AS} \times \frac{WS}{100} \times \frac{10}{100} \times \frac{5}{WT} \times 100000$$

Where,

AT = peak area response of methanol obtained from test solution

AS = Mean peak area response of methanol obtained from standard solution

WS = weight of methanol in standard solution in mg
WT = weight of test sample in mg
The methanol content found to be 2.39 ppm. Since the methanol content found is between LOD and LOQ, it has to be reported as <5.0 ppm.

System suitability was checked from relative standard deviation (RSD) of peak responses from six replicate injections of the standard preparation found to be 2 % shows suitability of the equipment for the proposed method.

6. Conclusion

In this way method for the determination of methanol present as OVI in atenolol is now developed. Method was validated in terms of Specificity, linearity, precision, accuracy, LOD and LOQ. The methanol content found in the atenolol API under investigation found to be < 5. ppm which is quite less than maximum limit prescribed in USP (3000 ppm). In this way the presented study can be used for determination of methanol as OVI in atenolol bulk in pharmaceutical industries.

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