Short Research Article

PREPARATION OF ANALYTICAL STANDARD OF BISOPROLOL IMPURITY A

ABSTRACT

Aims: Research of the convenient method for obtaining (RS)-1-(4-Hydroxymethyl-phenoxy)-3-isopropylaminopropan-2-ol, known as the Impurity A of Bisoprolol, of high purity as close as 100%.
Study design: Impurity A may be formed as a by-product in the processes used for commercial synthesis of bisoprolol fumarate. Impurity A may be also formed as a result of degradation (hydrolysis) of active substance. This compound is available as the reference standard, but the offered purity is between 95% and 97%, what suggest that its purification to the pharmaceutical quality is demanding. The most common method of purification of chemical standards for pharmacy is preparative chromatography and is commonly used for obtaining the reference standards of high purity, but it is unattainable in many cases, so there is a need for simple, convenient and repeatable laboratory procedures elaboration.
Place of Study: ICN Polfa Rzeszów S.A., Poland, Synthesis Laboratory
Methodology: The synthesis of Bisoprolol Impurity A was performed starting from p-hydroxybenzyl alcohol and subsequent reactions with epichlorohydrin and isopropylamine, whereas purification process consisted particularly of obtaining and isolation of fumarate salt of Impurity A, its crystallization and basification.
Results: The analytical standard of Bisoprolol Impurity A of a purity of 95.5% was obtained with convenient chemical process without need of any advanced methodology. The structure was elucidated with IR, NMR and EA methods and the purity was determined by HPLC technique.
Conclusion: The method of obtaining the analytical standard of Impurity A of purity as close as 100% is described in this paper.

Keywords: Bisoprolol fumarate; Impurity A reference standard; Convenient purification

1. INTRODUCTION

Active pharmaceutical ingredients (API) and the drug products should fulfil the regional registration requirements. In the European Union such the requirements are common and as regards the acceptable content of impurities (relative substances), the guidelines Q3A(R2**)** [1] and Q3B(R2) [2] for active substances and drug products respectively were adapted. Relative substances in drug substances and drug products, according to the mentioned guidelines, are divided into: degradation products, unreacted raw materials, intermediates and process impurities originated from raw materials, and finally by-products. Additionally, relative substances in drugs, drug substances and also excipients are divided into specified (characterised by chromatographic factors as retention time or retardation factor) and unspecified. The specified impurities can be subsequently divided into identified and unidentified [1, 2]. Following the rules, the identified impurity content can be

determined with the analytical method and converted on the known amount of reference standard, i.e. specified impurity or other substance used as a reference. The reference standard for determination of the impurity can be both pure chemical compound or a mixture of known percentage composition. The content of the chemical compound used as the reference standard in pharmacy should be as close as 100%.

The basic purification methods in chemical art, as repeatable crystallization, rectification or extraction, are not sufficiently effective in many cases and obtaining chemical substance of high quality may not be possible, and more advanced techniques may be required.

The most effective method of purification in chemistry is chromatography, used to separate an individual compound from the mixture, but the disadvantage of advanced chromatographic techniques is that the special and expensive equipment is required. The column chromatography (flash chromatography) is frequently used for purification [3, 4, 5], but the modern chromatographic methods as preparative HPLC [6, 7, 8, 9, 10, 11] and preparative TLC are also suitable for separation of the reference quality material [12]. Less used methods as simulated moving bed (SMB) could be the costless alternative [3, 13] to the chromatographic techniques.

Reference standards of impurities (related substances) for drugs analysis both pharmacopoeial and non pharmacopoeial are widely available on the market, but the methods of synthesis and purification are not described in a great majority. The convenient methods of purification [14] of the reference standards are cost effective alternative, in comparison to the chromatographic techniques described above, but they are rather sparsely used.

Bisoprolol fumarate is a β1-selective adrenoreceptor blocking agent marketed as the racemate, where the S-isomer is responsible for majority of the β1-blocking activity. The major impurity of this active substance is a racemic compound (RS)-1-(4-hydroxymethyl-phenoxy)-3-isopropylaminopropan-2-ol, known as specified Impurity A according to European Pharmacopoeia (EP).

Bisoprolol Impurity A is a by-product which may be formed in the most common synthesis processes of bisoprolol fumarate, *i.e*. according to Jonas [15, 16] (see **Figure 1**) and according to O'Neill [17] methods (see **Figure 2**).

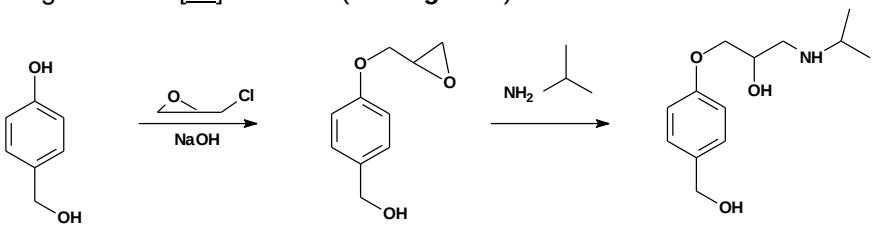


Figure 1. Scheme of possible formation of impurity A in the synthesis of bisoprolol according to Jonas.

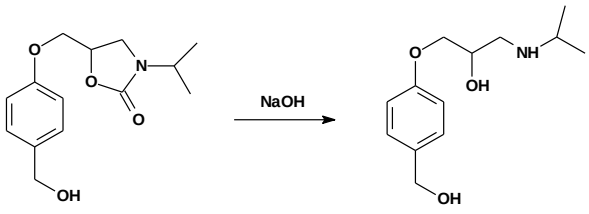


Figure 2. Scheme of possible formation of impurity A in the synthesis of bisoprolol according to O'Neill

Impurity A is also a degradation product of bisoprolol hydrolysis (see **Figure 3**).

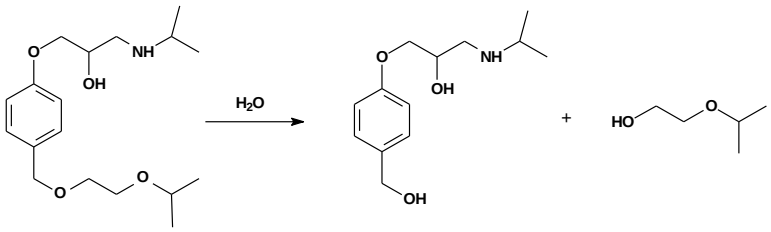


Figure 3. Scheme of possible formation of impurity A in the hydrolysis of bisoprolol

Probably the most inconvenient impurity derived from the process and degradation of bisoprolol is 4-[((2RS)-2-hydroxy-3-(isopropylamino)propyl)oxy]benzaldehyde (see **Figure 4**), known as Impurity L according to EP. This impurity removal from API is very difficult with simple methods, that is why it is often removed *via* formation of chemical derivatives. For example, impurity L may be simply hydrogenated with sodium borohydride [18], but this process is the next possible source of Impurity A (see **Figure 4**).

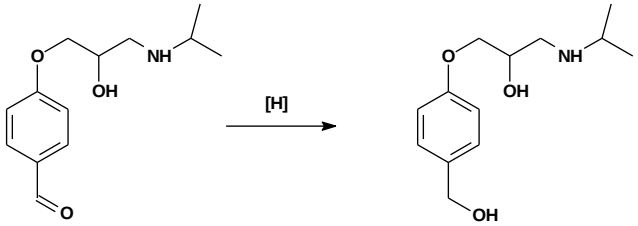


Figure 4. Scheme of possible formation of Impurity A in hydrogenation of Impurity L

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1. Synthesis procedure of crude impurity A

24.4 g of p-hydroxybenzyl alcohol, 13.6 g of potassium carbonate and 37 mL of epichlorohydrin was boiled for 5 hrs. The suspension was chilled and filtered. The filtrate was distilled under vacuum to obtain 32.0 g of yellow liquid. The product was reacted with 64.5 mL of isopropyloamine for 3 days, under room temperature. After evaporation of excess reagent, the product in the amount of 40.6 g was dissolved in 120 mL of hot ethyl acetate and decolorized with 1.0 g charcoal activated. After crystallization the deposit was filtered and dried. 14.0 g of almost white solid was obtained.

2.2. Purification of Impurity A

The crude product was dissolved in the mixture of 70.0 mL of water and 3.7 g of fumaric acid. The solution was then mixed with charcoal activated, filtered and subsequently basified with sodium hydroxide. The precipitate was filtered and dried, next crystalized in 38 mL of acetone (filtered after dissolving). The product was dissolved in the mixture of 30 mL of acetone, 30 mL of isopropanol and 1.35 g of fumaric acid. After filtration the mixture was chilled and the precipitate filtered. Subsequently the solid product was neutralized with sodium hydroxide in water. The product was filtered, washed with water and methylene chloride. 3.37 g of the product was obtained.

2.3. HPLC procedure for purity determination

The procedure applied for determination of purity of Bisoprolol Impurity A was adapted from EP monograph for bisoprolol fumarate:

Stationary phase:	Column Nucleosil 100-5 C18, 5 µm, 4.6 x 250 mm		
Mobile phase A: Mobile phase B:	Phosphoric acid 10 g/L Phosphoric acid 10 g/L in acetonitrile		
Gradient elution:	Time, in min	Mobile phase A, in % (v/v)	Mobile phase B, in % (v/v)
	0→4	95	5
	4→8	95→80	5→20
	8→15	80	20
	15→34	80→20	20→80
	34→36	20	80
	36→40	20→95	80→5
Flow rate:	1.0 ml/min		
Detector:	UV 225 nm		
Temperature:	20 ± 2°C		
Sample volume:	10 µl		

3. RESULTS AND DISCUSSION

The synthesis and purification of Impurity A was performed according to the route presented on **Figure 5**. Although the pathway of Impurity A formation in Jonas synthesis process was suggested by Khan, and in his work the presence of this impurity in Bisoprolol was confirmed with MS analysis [16], the synthesis of this compound is not described in art, as well as the way of its purification.

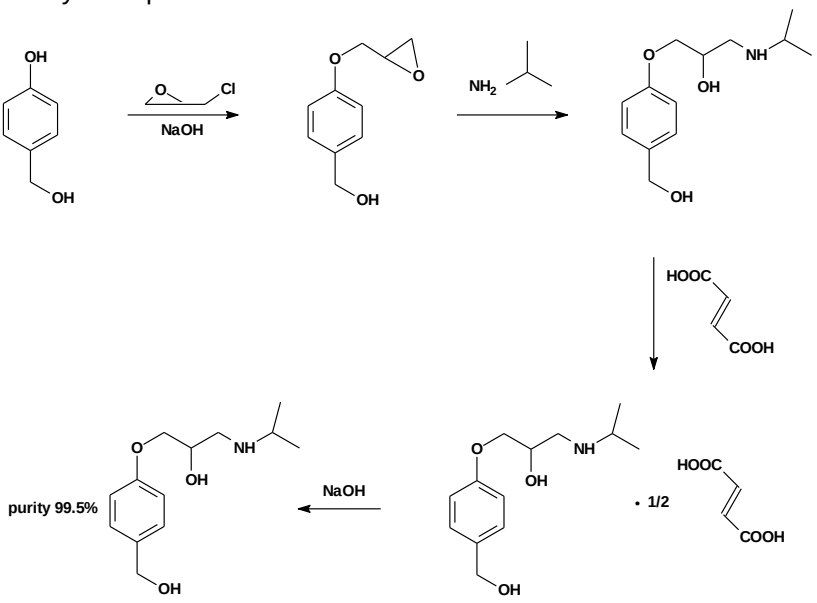


Figure 5. Scheme of synthesis and purification of Bisoprolol Impurity A

The synthesis was performed starting from p-hydroxybenzyl alcohol and excess epichlorohydrin in basic environment. The obtained epoxide was then reacted with excess

isopropylamine. Impurity A thus synthesised was initially purified from coloured impurities thorough dissolving in ethyl acetate and treating with activated charcoal.

The purification method of Impurity A consisted firstly of formation of a salt with fumaric acid, which was soluble in water in opposite to unreacted traces of p-hydroxybenzyl alcohol. The second step of purification was basification and here residual reagents epichlorohydrin and isopropylamine were removed as soluble in filtrate. The obtained product was then dissolved in warm acetone, filtered (at this step all possible process inorganic impurities were removed) and finally crystallized. The last step of purification was obtaining afresh fumarate salt, but instead of water – in a mixture of organic solvents (equal volume of acetone and isopropanol), which was next crystalized to dispense with organic by-products. The last step was again basification and final washing.

The structure of the compound was elucidated by EA (see **Table 1**), NMR (see **Table 2**), MS (see **Table 3**) techniques and Infrared spectroscopy (wavenumbers in cm^{-1}: 3334, 3285, 3103, 3047, 2952, 2926, 2831, 1617, 1584, 1519, 1481, 1257, 1083, 1033, 834, 638).

Table 1. Elemental analysis of Impurity A

Element	Detected, %	S.D.	% Rel. S.D.	Variance	Calculated, %
Carbon	66.09	6.32E-03	9.57E-03	4.00E-05	65.25
Hydrogen	8.79	5.54E-02	0.6300	3.07E-03	8.84
Nitrogen	5.40	5.28E-02	0.9781	2.78E-03	5.85
Oxygen	19.11	0.0682	0.3568	4.65E-03	20.06

Table 2. ^{1}H NMR analysis of Impurity A (50 mg in 1 mL)

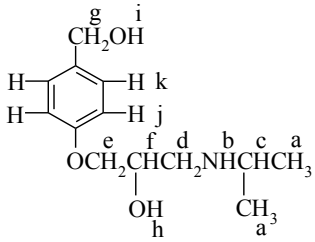
Group	Chemical shift, ppm	Multiplicity	Integration
			
a	0.877, 0.955	doublet	6H
b	1.20 ÷ 2.40	broad	1H
c + d	2.426 ÷ 2.807	multiplet	3H
e + f	3.826	singlet	3H
g	4.369	singlet	2H
h + i	4.953	singlet	2H
j	6.783, 6.891	doublet	2H
k	7.135, 7.243	doublet	2H

Table 3. MS analysis of Impurity A (fragmentation)

Mass	Attribution
239	$[M]^+$
224	$[M]^+ - [CH_3]$
195	$[M]^+ - [CH_3, C_2H_5]$
153	$[M]^+ - [CH_3, C_2H_5, C_2H_4N]$
109	$[M]^+ - [CH_3, C_2H_5, C_2H_4N, CH_2OH, CH]$
93	$[M]^+ - [CH_3, C_2H_5, C_2H_4N, CH_2OH, CH, O]$

The purity of Bisoprolol Impurity A was determined with HPLC method according to procedure contained in EP monograph for Bisoprolol fumarate.

4. CONCLUSION

The possible pathway of formation of (RS)-1-(4-hydroxymethyl-phenoxy)-3-isopropylaminopropan-2-ol (Impurity A) in the Jonas synthesis of bisoprolol is known [16], but the process of obtaining the reference standard of this substance, especially of the purity as close as 100%, is not yet described. Moreover, the fumarate salt of Bisoprolol Impurity A is not mentioned anywhere, even though in the context of purification of Impurity A.

The proposed process of synthesis and purification of Bisoprolol Impurity A reference standard to the purity of 99.5% is efficient and cost-effective in comparison to the chromatographic techniques *e.g.* preparative TLC or preparative HPLC, it is also less laborious than SMB method. The crude compound may be purified to the purity of not less than 99.5% using simple, convenient and useful method.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

API – Active Pharmaceutical Ingredient
HPLC – High Performance Liquid Chromatography
EP – European Pharmacopoiea
EA – Elemental Analysis
NMR – Nuclear Magnetic Resonance
MS – Mass Spectroscopy
Rel. S.D. – Relative Standard Deviation
SD – Standard Deviation
SMB – Simulated Moving Bed
TLC – Thin Layer Chromatography