Forced degradation of timolol maleate on high temperature for verification of HPLC method for related substances in Timolol eye drop 0.5%

Aleksandra Veleska Stojanovska1; Tijana Serafimovska2; Marija Darkovska Serafimovska1

1Faculty of Medical sciences, University "Goce Delcev", Krste Misirkov No.10-A, 2000 Stip, North Macedonia
2Faculty of Pharmacy, University "Ss. Cyril and Methodius", Mother Tereza 47, 1000 Skopje, North Macedonia

INTRODUCTION

Timolol is a potent β-adrenergic blocker, useful in treatment of ocular hypertension or open-angle glaucoma. Many chromatographic analysis methods have been applied for the determination of pharmaceutical compounds containing heterocyclic rings (as timolol maleate), but the most commonly applied chromatographic technique is HPLC.

MATERIAL AND METHODS:

Chemicals and Regents
Mobile phase was consisting of mixture of 0.02M sodium octane-sulfonate and methanol in ratio 42,5:57,5; adjusted to pH 3.0 using glacial acetic acid.

Apparatus: Liquid chromatography was performed with stainless steel column (30cm x 4.6mm) packed with end-capped octadecyl silyl silica gel for chromatography (10 μm) (μBondapakTM C18 was used) on HPLC system (Agilent Technologies Infinity II 1260, DAD HS 1260: Serial No. DEAEK06706).

Chromatographic Conditions:
An isocratic method elution was used with flow rate of 1.5 mL per minute on ambient column temperature. UV detection was on wavelength of 295 nm. Injection volume was 20 μL of each solution and run time was 4 times of the retention time of the principal peak of timolol maleate.

RESULTS:

Verification of the method

Linearity was performed using timolol maleate standard solutions in concentration range from 0.005 mg/ml (limit of quantification-LOQ) to 0.03 mg/ml or 25%-150% from the reference limit for impurities. The response was linear and the coefficient of correlation was greater than 0.999.
The range (R2=0.9999) is shown on Figure 1. Linearity of the method is shown in Table 1.

Limit of Detection (LOD) and (LOQ) were determined by evaluation peak areas of timolol maleate at lower concentrations. Acceptance criteria were signal to noise ratio about 3:1 for LOD and about 10:1 for LOQ. According to results LOD for timolol was 0,05 μg/ml, and LOQ for timolol was 0,15 μg/ml.

Range (%)	Concentration levels (mg/ml)	Peak Area (mAU*s)	Mean Peak Area (n=3)
LOQ*	0.0001	3.5899	3.71137
	0.0001	3.59118	
	0.0001	3.76928	
	0.0001	3.89849	
	0.0001	3.70801	
25%	0.005	93.1	92.25
	0.005	92.48	
	0.005	91.68	
	0.005	91.63	
	0.005	92.34	
50%	0.01001	181.38	181.05
	0.01001	180.92	
	0.01001	181.31	
	0.01001	181.06	
	0.01001	180.6	
75%	0.01501	276.95	276.89
	0.01501	277.1	
	0.01501	276.71	
	0.01501	276.74	
	0.01501	276.96	
100%	0.02002	363.06	363.19
	0.02002	363.78	
	0.02002	362.97	
	0.02002	363.09	
	0.02002	363.04	
150%	0.03003	546.71	546.52
	0.03003	547.19	
	0.03003	545.92	
	0.03003	546.13	
	0.03003	546.65	

Table 1. Linearity of the method

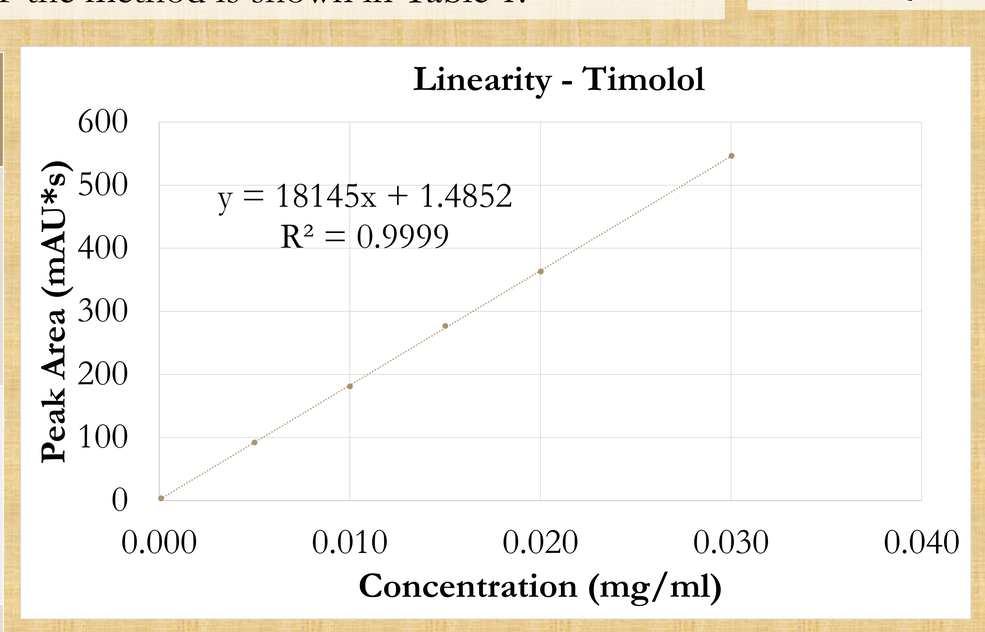
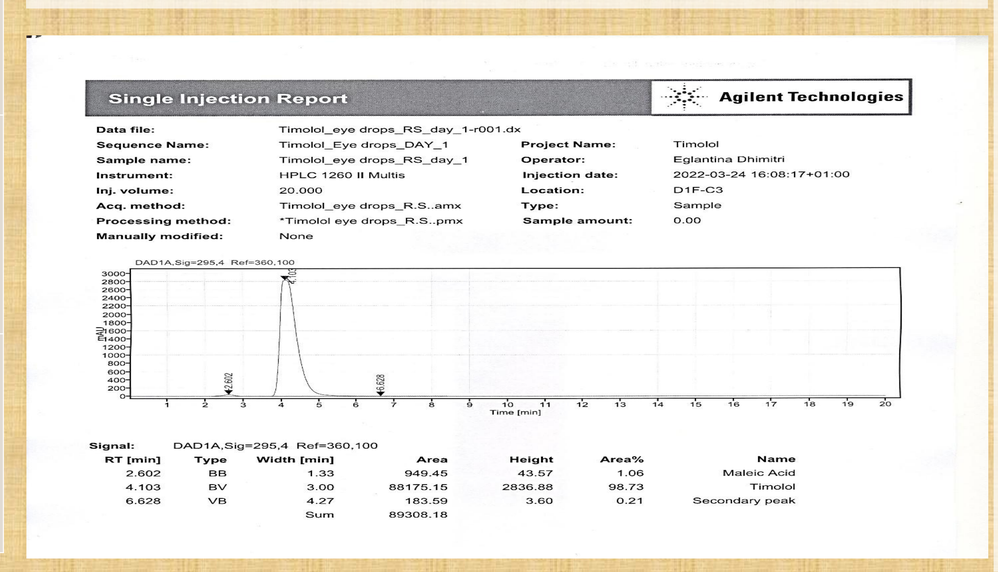


Figure 1. Linearity and the response R2=0.9999.

The parameter specificity, demonstrate good separation of all peaks derived from active substance (timolol maleate, secondary peak because of force degradation and maleic acid). Specificity is shown in Figure 2.


Single Injection Report — Agilent Technologies

Data file:	Timolol_eye drops_RS_day_1-r001.dx		
Sequence Name:	Timolol_Eye drops_DAY_1	Project Name:	Timolol
Sample name:	Timolol_eye drops_RS_day_1	Operator:	Eglantina Dhimitri
Instrument:	HPLC 1260 II Multis	Injection date:	2022-03-24 16:08:17+01:00
Inj. volume:	20.000	Location:	D1F-C3
Acq. method:	Timolol_eye drops_R.S..amx	Type:	Sample
Processing method:	*Timolol eye drops_R.S..pmx	Sample amount:	0.00
Manually modified:	None		

Signal: DAD1A,Sig=295,4 Ref=360,100

RT [min]	Type	Width [min]	Area	Height	Area%	Name
2.602	BB	1.33	949.45	43.57	1.06	Maleic Acid
4.103	BV	3.00	88175.15	2836.88	98.73	Timolol
6.628	VB	4.27	183.59	3.60	0.21	Secondary peak
		Sum	89308.18			

Figure 2. Specificity of the method and secondary peak.

The precision and reproducibility of the proposed method were evaluated by performing replicate analysis of the standard solution timolol maleate in concentration of 0,02mg/ml (reference limit for impurities), to determine intraday and inter-day variability [within day (n = 6) and between days (n = 6)].

Relative standard deviations were calculated to obtain the precision of the method. The results of precision, and reproducibility of the method demonstrate a good precision (RSD =1,05%).

CONCLUSION

Forced degradation was done only by exposing the API and DP on high temperature at 80°C for 48 hours to obtain degradation products quickly and to verify the method for determination of related substances listed in the British pharmacopoeia.

For forced degradation studies the chosen stress condition (temperature of 80°C for 48 hours) leads us to only one secondary peak at RRT 1,6 due to exposition of the product at higher temperature.