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Stability indicating HPTLC determination of timolol maleate as bulk drug and in pharmaceutical preparations

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Abstract

Timolol was the first β blocker to be used as an anti-glaucoma agent and to date remains as the standard because none of the newer β blockers were found to be more effective. The high performance thin layer chromatographic method of analysis of timolol maleate is reported. The mobile phase selected was ethyl acetate–methanol–isopropyl alcohol–ammonia (25%) (80:20:2:1, v/v/v/v). The calibration curve of the drug was linear in the range of 100–600 ng. The spectrodensitometric analysis was carried out at 294 nm. The mean ($\pm$RSD) values of slope, correlation coefficient and intercept were 2487.5 ($\pm$0.9), 0.996 ($\pm$0.081) and 90463 ($\pm$1.1), respectively. The system precision and the method precision were excellent with an RSD of 2.8 and 1.004, respectively. The limits of detection and quantitation were 10 and 40 ng, respectively. The mean percent recovery was found to be 98.6. Timolol maleate was degraded by exposing the drug to heat, acid and base. The degraded products were found to be well separated from the pure drug with significantly different R_f values suggesting a stability indicating analysis method for quantification of timolol maleate in pharmaceutical preparations and as bulk drug. The method was utilized to analyze timolol maleate from conventional eye drops and novel sustained release solid polymeric ocular inserts and oral preparations. The reported method is simple, selective, precise, accurate, time saving and economic as compared to reported HPLC methods.

Keywords: Timolol maleate; HPTLC; Degradation

1. Introduction

Timolol maleate (Fig. 1) is a nonspecific β-adrenergic blocker. It was the first β-blocker to be used as an antiglaucoma agent. None of the newer β-blockers were found to be more effective than timolol. Timolol is effective for treating hypertension, arrhythmias and angina pectoris[1,2]. It is also useful for the secondary prevention of myocardial infarction [3].

Various GLC [4,5] and LC methods from plasma [6,7] and from pharmaceuticals [8–10] have been reported for the determination of timolol. As versus LC methods, HPTLC is most suited

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for a small as well as large number of samples. Mobile phases having pH 8 and above can be employed. Unlike GC methods sample preparation is simple. Suspensions, dirty or turbid samples can be directly applied.

High performance thin layer chromatography (HPTLC) is a sophisticated instrumental technique which utilizes full potential of thin layer chromatographic method. Technically it is simple to learn and operate. It facilitates automated application and scanning in situ. It offers extreme flexibility for various steps: stationary phase, mobile phase, developing technique, detection (pre and post chromatographic determination). Unlike HPLC, consumption of mobile phase per sample basis is quite low. This saves cost per analysis and analysis time as well. HPTLC facilitates repeated detection (scanning) of the chromatogram with the same or different parameters. HPTLC technique is most suited for impurity profile of drug substances and content uniformity test as per compendial specifications. Simultaneous assay of several components in a multicomponent formulation is possible.

This paper describes a simple, accurate, precise and specific HPTLC method for determination of timolol maleate as bulk drug and from pharmaceutical dosage forms.

2. Experimental

2.1. Chemicals and reagents

Timolol maleate U.S.P. grade (99.7% pure) was supplied by Centaur Chemicals (Mumbai, India) as a gift sample. Analytical grade solvents and reagents were purchased from Ranbaxy Fine Chemicals (New Delhi, India).

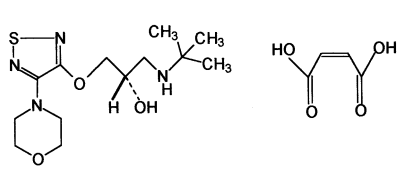

Fig. 1. Structure of timolol maleate.

2.2. Preparation of standard solution

A stock solution of Timolol maleate (1 mg/ml, equivalent to timolol base) was prepared in distilled water. A standard solution of 100 μg/ml was used for the analysis.

2.3. Instrumentation

The samples were spotted on HPTLC aluminium plates (10 × 10 cm) precoated with Silica gel 60 F 254 (layer thickness 0.2 mm) (E. Merck). Prewashing was carried out by placing the plates in Camag twin trough chamber for 60 min. The chamber was closed by a lid having a slit. This provided prewashing by continuous mode for some time. After washing plates were kept in hot air oven (105°C) for a sufficient time (at least 30 min) to ensure complete removal of washing solvent. Spotting was done using Camag Linomat IV model. The samples were spotted in the form of narrow bands of length 3 mm, 15 mm from the bottom edge, 10 mm from margin, 5 mm apart at a constant rate of 15 s/μl using a nitrogen aspirator. The migration distance was 8 cm with a migration time of 15 min. The separation was observed under the short wavelength (254 nm) ultraviolet lamp. Densitometric analysis of the separated components was carried out using Camag TLC scanner II in the absorbance/reflectance mode at 294 nm. The slit dimensions were 4 × 0.3 mm and sensitivity was kept at auto mode. Scanning speed was 1 mm/s. Integration of the chromatogram was carried out using the Camag TLC scanner/integrator system.

2.4. Selection of mobile phase

Various solvent systems reported for TLC analysis of timolol maleate were tried. A reported TLC system can be adapted for HPTLC by carrying out modifications in the solvent strength and selectivity [11]. The selection of mobile phase was therefore attempted by modification of TLC systems reported for identification and purity testing of timolol from bulk and pharmaceuticals. The solvent systems consisting of ethyl acetate, methanol, isopropyl alcohol and 25% ammonia in

various ratios were tried to get dense and compact spots with sufficient separation in R_f values of timolol and its degradation products.

2.5. Calibration curve of timolol maleate

Appropriate volumes of standard solutions (100 μg/ml) were spotted to obtain timolol in the concentration range of 100–1000 ng ($n = 3$). Calibration curve was plotted using area under curve versus concentration (ng).

2.6. Validation of the assay

2.6.1. System precision

Six spots of each were applied from a single standard solution (100 μg/ml) to get the concentration of 600 and 100 ng.

2.6.2. Method precision

Method precision study was carried out by applying the spots (600 and 100 ng each) from six different solutions (100 μg/ml each). The rest of the analysis was carried out using the proposed method.

2.6.3. Analysis of timolol from formulations

Marketed eye drops, novel sustained release solid polymeric ocular inserts and novel sustained release multiparticulate matrix of timolol for oral use of timolol maleate were analyzed by the proposed method. The sample preparation was done by extracting the drug in distilled water and suitably diluted solutions were spotted after filtration through nylon membrane filter (13 mm, 0.22 μ).

2.6.4. Recovery study

Recovery study was carried out by adding 30% (90 ng) and 80% (240 ng) of standard drug to the preanalysed sample of 300 ng. The resulting mixture was analyzed by the proposed method.

2.6.5. Degradation of timolol maleate

The degradation of timolol maleate solution (0.5 mg/ml) was carried out using 0.2 M HCl and 0.2 M NaOH. The solutions were refluxed for 3 h. 1 μl solution was spotted on the plates and analyzed by HPTLC. The drug was also degraded by dry heat in solid state at 190°C for 10 min.

3. Results and discussion

An R_f value of 0.51 for timolol was obtained using the selected mobile phase of ethyl acetate–methanol–isopropyl alcohol–ammonia (25%) (16:4:0.4:0.2, v/v/v/v). Standard curves of timolol ($n = 3$) were prepared over a concentration range of 100–1000 ng. Linearity was obtained in the range 100–600 ng. The mean values ($\pm$RSD) of correlation coefficient, slope and intercept were 0.996 ($\pm$0.081), 2487.5 ($\pm$0.9) and 90463 ($\pm$1.1), respectively. The limit of detection (LOD) was determined on the basis of signal to noise ratio. Mean of 15 noise peak areas and their absolute standard deviation values were determined. LOD was the amount of applied sample producing a peak area that is equal to the sum of mean blank area and three times standard deviation. Limit of quantitation (LOQ) was the amount of applied sample producing a peak area that is equal to the sum of mean blank area and ten times its standard deviation. LOD and LOQ for timolol maleate were 10 and 40 ng (RSD 2.1), respectively. The lowest concentration of timolol that was accurately detected and integrated by the instrument used was 40 ng. Six applications of the same concentration from a single (system precision) as well as six different solutions (method precision) were observed. Relative standard deviations at this level (40 ng) for system precision and method precision were 2.6 and 3.1, respectively.

Accuracy and precision studies were carried out at two levels (100 and 600 ng). The method was found to be highly accurate and precise over the linearity range as evident by the figures depicted in Table 1 (RSD < 3).

Stability in sample solution: Solutions of two different concentrations (100 and 600 ng) were prepared from stock sample solution and stored at room temperature for 24 hours and 240, 120, 60, 30 and 10 min and then applied on the same chromatoplate, after development the chromatogram was evaluated (Table 2) for additional spots if any. There was no indication of compound instability in the sample solution.

Stability on the sorbent layer prior to development (spot stability): The time the sample is left to stand on the sorbent prior to chromatographic

Table 1
Precision of the assay

Sample no.	Area under the chromatographic peak			
	System precision		Method precision	
	100 ng	600 ng	100 ng	600 ng
1	342 416	1 345 891	330 892	1 434 070
2	338 532	1 417 802	331 946	1 407 735
3	335 591	1 464 427	334 552	1 422 313
4	340 068	1 457 933	343 845	1 392 939
5	341 625	1 455 973	337 892	1 417 677
6	334 798	1 415 635	340 528	1 432 325
RSD	0.9	2.9	1.5	1.0

Table 2
Stability of timolol in sample solution

Actual (ng)	Mean	Range	RSD
100	99.3	97.1–101.8	1.8
600	596.8	578.4–612.3	2.2

Table 3
Assay of timolol from pharmaceutical formulations

Sample no.	Drug content (%)[a]		
	E[b]	S1[c]	S2[d]
1	97.90	99.23	98.56
2	97.02	100.25	101.89
3	98.23	101.56	97.56
Average drug content	97.71	100.34	99.33

[a] S1 and S2 formulated in-house using common excipients.
[b] Marketed eye drops.
[c] Sustained release ocular inserts.
[d] Sustained release multiparticulate oral matrix formulations.

development can influence the stability of separated spots and are required to be investigated for validation [12]. Two-dimensional chromatography using same solvent system was used to find out any decomposition occurring during spotting and development. In case, if decomposition occurs during development, peak(s) of decomposition product(s) shall be obtained for the analyte both in the first and second direction of the run. No decomposition was observed during spotting and development.

Timolol content from marketed eye drops (E), sustained release ocular inserts developed inhouse (S1) and sustained release multiparticulate oral matrix formulations developed inhouse (S2) was determined (Table 3). The drug content figures (%) in Table 3 assure the extraction efficiency of the method from pharmaceutical formulations. None of the excipients — hydroxypropylmethylcellulose, hydroxypropylcellulose, Eudragit RSPO, Eudragit RLPO (methacrylates), ethylcellulose, triacetin, glycerin, castor oil, polyethylene glycol 400, diethylphthalate — interfered during timolol analysis.

The recovery of timolol from formulations was determined by comparing peak areas obtained from formulations to which had been added timolol (90 or 240 ng) with the peak areas obtained from preanalysed formulations (300 ng). The results are shown in Table 4. The mean percent recovery was found to be 98.6. This study confirmed the reproducibility of the method.

The degraded products of timolol maleate obtained by various treatments using acid, base and dry heat resulted in products with various R_f values lower than the drug (with one exception). The R_f of degraded products were found to be 0.1, 0.2 and 0.64 which are quite different from that of the drug (0.51). Fig. 2 shows the separation of the degradation products obtained by the various methods. The lower R_f values of some

Table 4
Recovery studies using preanalysed sample of 300 ng

Sample[a]	Label claim	% Recovery ($\pm$SD)
E[b]	0.25% w/v	98.29 ($\pm$0.60)
S1[c]	125 μg/insert	99.56 ($\pm$1.20)
S2[d]	15 mg/capsule	97.88 ($\pm$0.87)
Average recovery		98.6

[a] S1 and S2 formulated in-house using common excipients.
[b] Marketed eye drops.
[c] Sustained release ocular inserts.
[d] Sustained release multiparticulate oral matrix formulations.

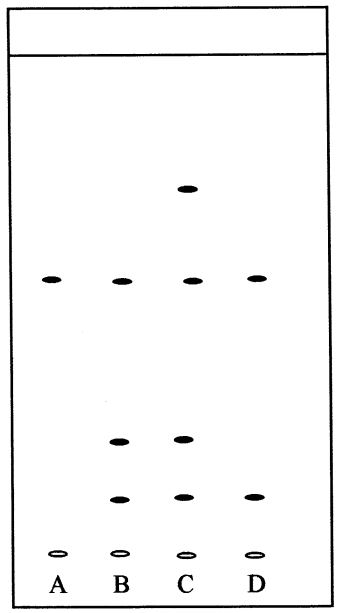

Fig. 2. Separation of degradates of timolol by HPTLC. A, timolol maleate; B, acid degraded; C, base degraded; D, heat degraded.

degraded components indicated that they were less polar than the analyte itself. The potential degradation products of timolol could be isotimolol or 4-hydroxy-3-morpholino-1,2,5-thiadiazol as reported by D.J. Mazzo et al. [9].

The developed analytical method could be considered as a stability indicating method for analysis of timolol maleate. A single spot at an R_f value of 0.51 was observed in the samples from various pharmaceutical formulations tested. It could therefore be suggested that no degradation of timolol maleate occurred in these formulations.

4. Conclusions

The proposed method is simple, rapid, accurate and precise. It could be used as a stability indicating assay method for analysis of timolol maleate as the bulk drug and from pharmaceutical dosage forms. It could also be extended to study the degradation kinetics of timolol maleate and for its estimation in plasma and other biological fluids.

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