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Note

Simultaneous determination of maleic acid and timolol by high-performance liquid chromatography

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Recently, pharmaceutical and biochemical studies (*in vitro* and *in vivo*) concerning the actions and properties of various formulations containing timolol maleate, a β-adrenergic blocking agent, were undertaken in our laboratories. These studies generated the need for a rapid, accurate, selective and precise simultaneous assay procedure for both timolol and maleic acid. High-performance liquid chromatography (HPLC) has been employed successfully in the analysis of many pharmaceuticals due to its ability to produce efficient, relatively rapid separations for a large variety of compounds. HPLC was thus the technique of choice for a simultaneous determination of maleic acid and timolol.

Little has been published on the HPLC of maleic acid even though the maleate salt is a commonly encountered form for many marketed pharmaceuticals. Demian *et al.*[1] used reversed-phase HPLC to determine the products of the reaction of maleic anhydride with alcohols while other authors[2,3] evaluated reversed-phase HPLC for the determination of maleic acid in various matrices. Ion-exchange HPLC has been employed to separate maleic acid from organic acids in sugar beet extracts[4].

A variety of reversed-phase HPLC methods have been reported, however, for timolol and related β-adrenergic blocking agents. Octadecyl silane columns were used to develop screening tests for pharmaceuticals[5,6] and were also applied in pharmacokinetic studies of β-blocking agents[7]. A C_2-bonded silica column assay was used for the detection of nine β-blocking agents in bulk material and tablet formulations[8]. To date, however, no method for the combined HPLC of maleic acid and any β-adrenergic blocking agent has been reported.

In this paper a gradient elution, detector wavelength programmed, reversed-phase HPLC method is presented which permits the concurrent determination of maleic acid and timolol. The method is shown to be selective and precise, linear over the range of concentrations studied and accurate when checked with samples of known concentrations. This method has been applied in the determination of maleic acid and timolol from samples resulting from *in vivo* and *in vitro* evaluations of pharmaceutical formulations containing timolol maleate.

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EXPERIMENTAL

The liquid chromatographic system employed consisted of a Hewlett-Packard (Avondale, PA, U.S.A.) HP1084B binary gradient liquid chromatograph equipped with a Hewlett-Packard HP79841A autoinjector and a Hewlett-Packard HP79875A programmable variable-wavelength UV absorbance detector. All chromatograms were obtained under the conditions in Table I and were recorded and integrated with a Hewlett-Packard HP79850B LC terminal. The compounds of interest were separated on a 150 mm × 4.6 mm I.D. Zorbax (DuPont, Wilmington, DE, U.S.A.) C_8-bonded silica column (6-μm particle size).

TABLE I

HPLC CONDITIONS FOR THE SIMULTANEOUS DETERMINATION OF MALEIC ACID AND TIMOLOL

Column	Zorbax C_8-bonded silica (150 mm × 4.6 mm I.D., 6-μm particle size) (DuPont, Wilmington, DE, U.S.A.).
Mobile phase	Component A: 0.04 *N* orthophosphoric acid (aq.), 0.75 g/l sodium hexane sulfonate added. Component B: acetonitrile.
Gradient	Step gradient from 100% A to 25% B in A at 5 min.
Temperature	Ambient.
Flow-rate	0.8 ml/min
Detection	UV absorbance (235 nm changed to 294 nm at 5 min)
Injection volume	20 μl
Signal attenuation	2^6 (0.064 a.u.f.s.)
Sample concentration	Approximately 0.05 mg/ml as timolol base

All solvents used for chromatography were HPLC grade and were vacuum degassed prior to use. Reagent grade sulfuric acid was used.

Timolol maleate[9], the hydrogen maleate salt of 5-(−)-1-(*tert.*-butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxyl]-2-propanol, was used as the reference standard for both maleic acid and timolol and was obtained from the reference standard supply at this laboratory. A stock solution was prepared by dissolving approximately 70 mg of timolol maleate in 100.0 ml of 0.1 *N* sulfuric acid. Standard solutions were prepared by diluting the stock solution with 0.1 *N* sulfuric acid to the desired concentrations. Based on the formulae of maleic acid, timolol and timolol maleate, the concentrations of maleic acid and timolol in each standard were respectively calculated as 26.8% and 73.2% of the timolol maleate concentration.

Chromatographic parameters were calculated based on the guidelines listed in the USP XX[10].

RESULTS AND DISCUSSION

The development of HPLC conditions which permitted the determination of maleic acid and timolol in a single chromatogram presented the problem of simultaneously determining two molecules with vastly different molecular weights, solubilities and UV absorbances as well as the challenge of chromatographing an acid (maleic acid) and a base (timolol) concurrently.

Octadecyl-bonded silica was the obvious choice for preliminary investigative work since C_{18}-bonded silicas have been used in the HPLC of both timolol and maleic acid. The choice of a mobile phase, however, was not obvious since ideal chromatographic conditions for the respective compounds were inherently different. For example, the hydrophilic nature of timolol necessitated the addition of an ion-pairing reagent (sodium hexane sulfonate) to the mobile phase in order to achieve an acceptable peak shape. Addition of 25% (or more) organic modifier to the aqueous mobile-phase was also needed to attain a reasonable timolol retention time. On the other hand, no ion-pairing reagent or organic modifier was needed for the HPLC of maleic acid on C_{18}-bonded silica; in fact, the addition of too much of either of these caused this compound to elute with the void volume. A mobile phase pH $\leqslant$ 1.9, however, was dictated by the pK values of maleic acid (6.93 and 1.92) in order to ensure the existence of the majority of the compound as one ionic form.

Simultaneous chromatography of maleic acid and timolol on C_{18}-bonded silica with a mobile phase consisting of various mixtures of 0.04 N orthophosphoric acid (0.75 g/l hexane sulfonic acid added) and acetonitrile unfortunately was not successful. Maleic acid showed no affinity for the C_{18}-bonded silica stationary phase under these conditions and eluted in the void volume even with 0% organic modifier in the mobile phase. A slightly more polar stationary phase (C_8-bonded silica) was then attempted even though a longer retention time for timolol was excepted.

Octyl-bonded silica and maleic acid did interact sufficiently to produce an acceptable retention time and, by using a 15.0-cm long C_8 column instead of a standard 25.0-cm column, the timolol retention time was kept to a reasonable length. The use of a 15.0-cm column also permitted a less drastic increase in organic modifier concentration in the mobile phase in order to produce the same retention time for timolol thereby minimizing equilibration time from the end of the gradient program back to initial conditions. Fig. 1 is a graphical depiction of the gradient-detector program which, when administered with a 15.0-cm C_8-bonded silica column, provided the best

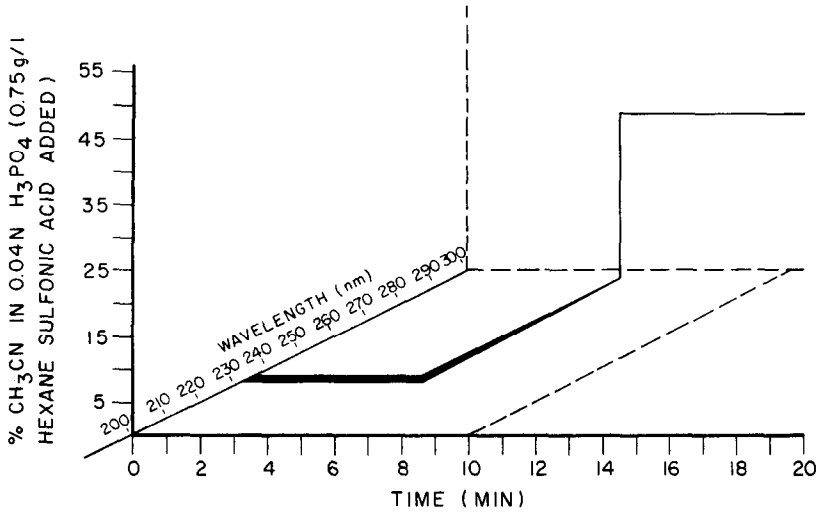


Fig. 1. Graphical depiction of the gradient program used to develop chromatograms of maleic acid and timolol from single injections of samples containing both components.

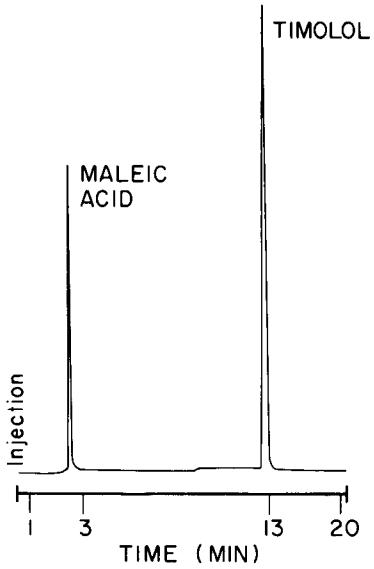


Fig. 2. Chromatogram of maleic acid and timolol obtained under the conditions in Table I. The peaks represent 327 ng maleic acid and 894 ng timolol, respectively.

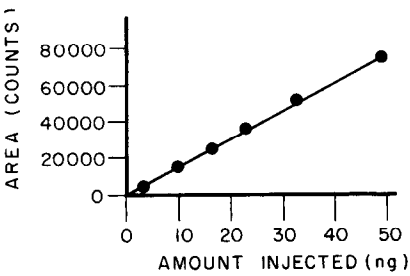
chromatography for both compounds in question. This figure shows the instantaneous change from 0 to 25% acetonitrile in the mobile phase at 5.0 min as well as the change in detector wavelength (235 to 294 nm) which also occurred at 5.0 min. Experimentation with even less non-polar stationary phases (C_3- and C_1-bonded silicas) indicated that too little increase in maleic acid retention resulted to compensate for the drastic and intolerable increase in timolol retention time. Fig. 2 shows a chromatogram of maleic acid and timolol obtained under the conditions in Table I. Table II lists the significant chromatographic parameters for each component of this chromatogram.

In order to determine the sensitivity and linearity of the proposed method for each of the compounds in question, calibration curves were constructed using a timolol maleate solution. Figs. 3 and 4 show the curves and linear regression analysis results obtained for maleic acid and timolol in the concentration range of most frequent study. In both cases, a correlation coefficient (R) of >0.999 indicated a linear relationship between detector response and amount injected. Extension of these cal-

TABLE II

CHROMATOGRAPHIC PARAMETERS FOR MALEIC ACID AND TIMOLOL BASED ON THE HPLC CONDITIONS LISTED IN TABLE I

	Maleic acid	*Timolol*
Retention time (t_R)	2.68 min	12.67 min
Capacity factor (k')	0.4	5.5
Tailing factor – 5% from base ($T_{5\%}$)	1.4	1.1
Efficiency (number of plates, N)	6,000	30,000



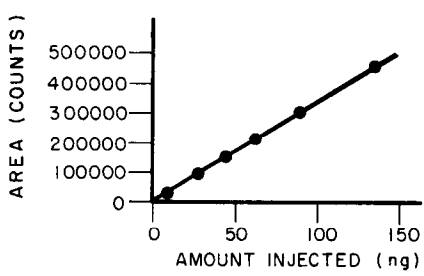


Fig. 3. Calibration curve for maleic acid obtained under the conditions in Table I. $y = (1518.8) - 196.16$, $R = 0.9994$.

Fig. 4. Calibration curve for timolol obtained under the conditions in Table I. $y = (3358.0) - 2652.2$, $R = 0.9998$.

ibration curves to more dilute sample solutions showed that linearity was maintained to a detection limit of *ca.* 500 pg injected for both analytes.

Accuracy and selectivity of the assay procedure were also determined. Testing with control samples of known concentrations indicated that the HPLC determination as described provided results accurate to >98% of the true value. The procedure was found to be selective based on the types of interferents encountered in the *in vivo* and *in vitro* studies. Neither the maleic acid nor the timolol peak was altered by co-elution of other sample components found in the *in vitro* and/or *in vivo* samples.

CONCLUSION

Maleic acid and timolol were determined concurrently in a gradient elution, detector wavelength-programmed HPLC method. Octyl-bonded silica columns of intermediate (15.0 cm) length proved to be more suitable for this determination than 25 cm C_1-, C_3- or C_{18}-bonded silicas. The method was selective, precise, accurate and reliable enough to be used in the assay of samples from *in vitro* and *in vivo* evaluations of pharmaceutical formulations containing timolol meleate. Reversed-phase HPLC was shown to be of use in the analysis of pharmaceutical products of vastly different physical and chemical properties and, as such, should continue to be the method of choice for the analysis of the complex mixtures resulting from the pursuit of new and improved pharmaceutical formulations.

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