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Short communication

Simultaneous determination of dorzolamide HCL and timolol maleate in eye drops by two different spectroscopic methods

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Abstract

Two-component mixtures of dorzolamide hydrochloride and timolol maleate were assayed by first derivative and ratio derivative spectrophotometric methods. The first method, derivative spectrophotometry, by the zero-crossing measurements, was used due to the drugs closely overlapping absorption spectra. Linear calibration graphs of first derivative values at 250.3 nm for dorzolamide hydrochloride and 315.8 nm for timolol maleate. The second method, is based on ratio first derivative spectrophotometry, the amplitudes in the first derivative of the ratio spectra at 242.9 and at 223.5 nm were selected to determine dorzolamide and timolol maleate in the binary mixture. Calibration graphs were established for 8.0–30.0 μg ml^{-1} for dorzolamide hydrochloride and 3.0–24.6 μg ml^{-1} for timolol maleate in binary mixture. Good linearity, precision and selectivity were found, and the proposed methods were applied successfully to the pharmaceutical dosage from containing the above-mentioned drug combination without any interference by the excipients. Vierordt's method was also developed for a comparison method.

Keywords: Dorzolamide hydrochloride; Timolol maleate; Zero-crossing first derivative spectrophotometry; Derivative ratio spectra spectrophotometry; Vierordt's method; Pharmaceutical formulations

1. Introduction

Dorzolamide hydrochloride, [(−)-(*SS*)-4-ethylamino-5,6-dihydro-6-methyl-7,7-dioxide-4H-thieno(2,3-b)thio-pyran-2-sulfonamide, is recently approved for the treatment of glaucoma. Timolol maleate, (*S*)-1-[(1,1-dimethyl)amino]-3-[[4-(4-morpholinyl9-1,2,5-thiadiazol-3-yl]oxy]-2-propanol, is a nonspecific β-adrenergic blocker. Timolol maleate 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. The two drugs can be combined in eye drops for the treatment of antiglaucoma agent. Their chemical structures are shown in Scheme 1.

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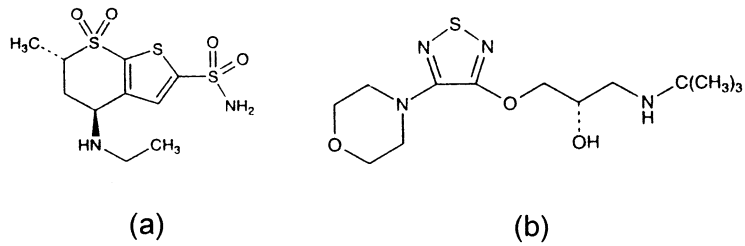

Scheme 1. Chemical structures of (a) dorzolamide hydrochloride, (b) timolol maleate.

In the literature, very few methods appeared in the literature for the determination of dorzolamide individually based on high performance liquid chromatographic assay with ultraviolet detection in human serum and urine [1–3] and capillary electrophoresis [4]. Several analytical procedures have been reported on the determination of timolol maleate, including GLC [5] and high performance liquid chromatography from plasma [6,7], HPTLC [8] and from pharmaceuticals [9–11].

More recently, dorzolamide hydrochloride has been marketed in combination with timolol maleate in eye drops. The dorzolamide hydrochloride is not yet offical in any pharmacopoeia either alone or in combinations with other drugs. To the best of my knowledge, no spectrophotometric methods have been described for the simultaneous determination of both drugs in eye drops. Therefore, it was desirable to develop a simple and fast procedure that could be applied in quality control laboratories for the determination of both drugs in the presence of each other. This paper reports a simply, and rapidly method for the simultaneous quantitation of the two drugs in eye drops by first derivative spectrophotometry, and ratio first derivative spectrophotometry providing accurate and precise results. The proposed methods do not need prior separation of dorzolamide hydrochloride and timolol maleate before analysis.

2. Experimental

2.1. Instrument

Spectrophotometric analysis was carried out on a Shimadzu 1601 double beam spectrophotometer with a fixed slit width (2 nm) connected to an IBM-PC computer loaded with a HP 1100 printer was used for all the absorbance signals and treatment of data.

2.2. Chemicals used

Dorzolamide hydrochloride and timolol maleate were kindly donated by MSD Pharm. Ind. Methanol was of analytical grade (Merck Chem.Ind.).

2.3. Pharmaceutical preparation

A commercial pharmaceutical preparation (COSOPT® eye drops MSD Pharm. Ind., Turkey, containing 20.0 mg of dorzolamide, 5.00 mg of timolol, 0.00075% benzalkonium chloride and water q.s per drop) was assayed.

2.4. Standard solutions and calibration curves

Stock solutions of 1 mg ml^{-1} of dorzolamide hydrochloride and timolol maleate were prepared in methanol. These solutions of dorzolamide hyrochloride and timolol maleate containing concentration ranges 8.0–30.0 and 3.0–24.6 μg ml^{-1} were prepared in methanol, respectively. Dorzolamide hydrochloride and timolol maleate is stable in solution form [15].

Fresh stock standard solutions were prepared every day.

2.5. Assay procedure for dosage form

1 ml of each commercial eye drops was trans-

ferred into a volumetric flask and diluted to volume with methanol.

3. Results and discussion

3.1. 'Zero-crossing' derivative spectrophotometry

The stability of working solutions of dorzolamide hydrochloride and timolol maleate and commercial sample solutions were cheched for 4 h and the UV–vis absorption spectra of all sample solutions were found to be stable for this period of time.

Fig. 1A shows the absorption spectra of dorzolamide hydrochloride and timolol maleate. As it can be seen, timolol maleate could be directly determined in presence of dorzolamide hydrochloride because its absorption spectrum exhibits a spectral zone where the other component does not absorb. This fact makes it extremely difficult to determine dorzolamide hydrochloride in the presence of timolol maleate by conventional UV spectrophotometry. This problem has been solved satisfactorily by derivative spectrophotometry. When derivative UV spectra were recorded, sharp bands of large amplitudes of dorzolamide hydrochloride and timolol maleate were produced which may offer more selective identification and specific simultaneous determination of these drugs. Fig. 1B shows the first derivative spectra of both drugs. The zero-crossing method is the most common procedure for the preparation of analytical calibration. Dorzolamide hydrochloride was determined by measurement of its first derivative amplitude at the zero-crossing of timolol maleate (at 315.8 nm ($^1D_{315.8}$)[1]). While timolol maleate was determined by measurement of its first derivative amplitude at the zero-crossing of dorzolamide hydrochloride (at 250.3 nm ($^1D_{250.3}$)). From among these wavelengths, these values were selected as optima to determine dorzolamide hydrochloride and timolol maleate in their mixtures.

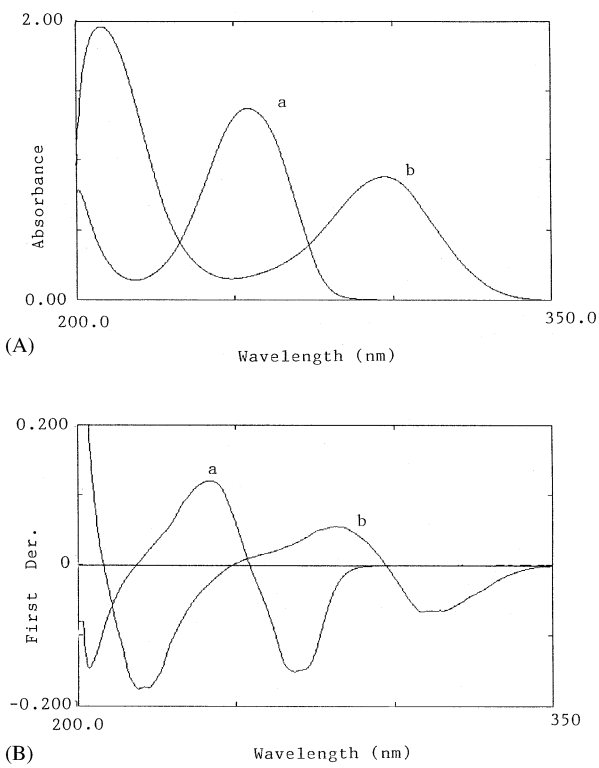

Fig. 1. (A) Zero-order (a) 24.0 μg ml^{-1} dorzolamide hydrochloride, (b) 18.0 μg ml^{-1} timolol maleate in methanol. (B) First derivative spectra (a) 24.0 μg ml^{-1} dorzolamide hydrochloride, (b) 18.0 μg ml^{-1} timolol maleate in methanol.

3.2. Ratio first derivative spectrophotometry

Fig. 1A shows the absorption spectra of the solutions of the compounds in methanol, which overlap sufficiently to demonstrate the resolving power of the proposed method.

Fig. 2 shows the ratio spectra of different amounts of dorzolamide hydrochloride standards (spectra divided by the standard spectrum of a 10.0 μg ml^{-1} solution of timolol maleate) and their first derivatives. As can be seen, the height of the maximum at 242.9 nm ($^1DD_{242.9}$)[2] in the derivative spectra corresponds to the dorzolamide hydrochloride present in the solution, so it can be

[1] order derivative$^{\text{Derivative}}_{\text{wavelength measure}}$.

[2] order derivative$^{\text{Derivative Divided}}_{\text{wavelength measure}}$.

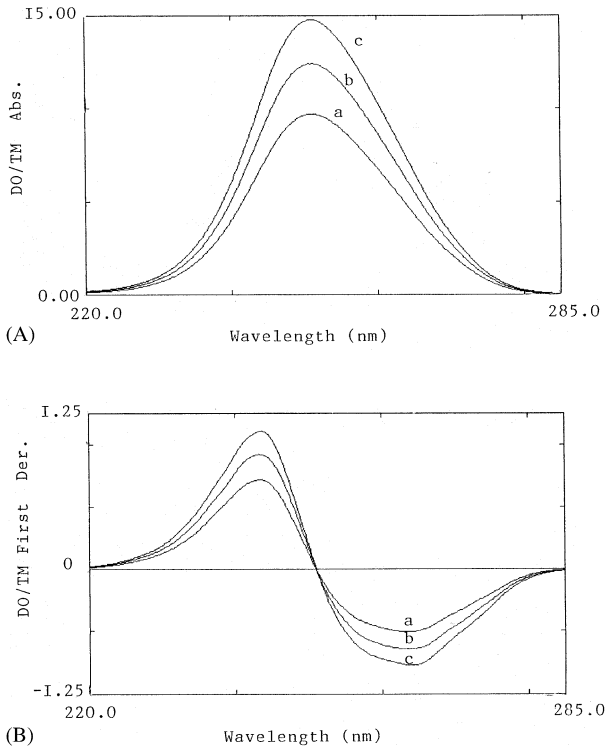

Fig. 2. (A) Ratio spectra of the dorzolamide hydrochloride (a) 8.0 μg ml^{-1}; (b) 19.0 μg ml^{-1}; and (c) 30.0 μg ml^{-1}, where 10.0 μg ml^{-1} timolol maleate used as divisor in methanol. (B) First ratio derivative spectra of the dorzolamide hydrochloride (a) 8.0 μg ml^{-1}; (b) 19.0 μg ml^{-1}; and (c) 30.0 μg ml^{-1}, where 10.0 μg ml^{-1} timolol maleate used as divisor in methanol.

used for its quantitative determination. Likewise, Fig. 3 shows the ratio spectra of different timolol maleate standards (spectra divided by the spectrum of a 20.0 μg ml^{-1} dorzolamide hydrochloride solution) as well as the corresponding first derivative spectra, on the basis of which the drug can be quantified by measuring at 223.5 nm ($^1DD_{223.5}$), corresponding to a minimum wavelength.

The influence of $\Delta\lambda$ for obtaining the first derivative of the ratio spectra was tested to obtain the optimum wavelength interval; $\Delta\lambda = 6$ nm was considered as suitable. These spectra were smothed with 16 experimental points due to the high noise of the signals obtained [12]. For selecting the standard solution as divisor, its concentration was modified.

3.3. Vierordt's method

Absorptivity A_1^1 (1%, 1 cm) values for each compound in the binary mixtures at zero-order spectra were calculated by using the absorbances measured at the appropriate wavelengths in methanol. Similarly the absorbances of the mixed sample solutions were measured and then the concentration of each compound was calculated from the following simultaneous equations:

$$A_1 = \alpha_1 C_{do} + \alpha_1 C_{tm} \qquad A_2 = \alpha_2 C_{do} + \alpha_2 C_{tm}$$

where C_{do} and C_{tm} are the concentrations of two compounds, dorzolamide hydrochloride and timolol maleate, in binary mixtures calculated as g 100 ml^{-1}. A denotes the absorbance of the mix-

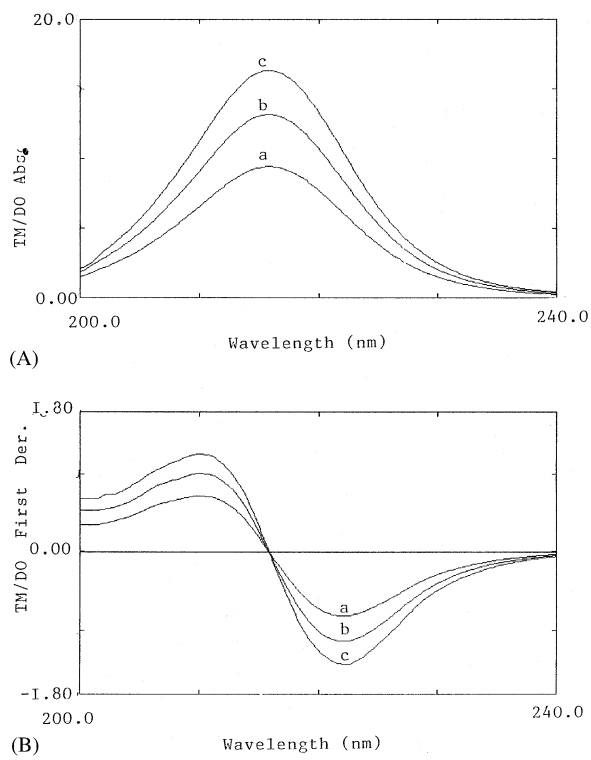

Fig. 3. (A) Ratio spectra of the timolol maleate (a) 10.0 μg ml^{-1}; (b) 13.8 μg ml^{-1}; and (c) 24.6 μg ml^{-1}, where 20.0 μg ml^{-1} dorzolamide hydrochloride used as divisor in methanol. (B) First ratio derivative spectra of the timolol maleate (a) 10.0 μg ml^{-1}; (b) 13.8 μg ml^{-1}; and (c) 24.6 μg ml^{-1}, where 20.0 μg ml^{-1} dorzolamide hydrochloride used as divisor in methanol.

Table 1
Analytical data of the calibration graphs for the determination of dorzolamide hydrochloride and timolol maleate by the proposed methods

Parameters	Dorzolamide Hydrochloride		Timolol Maleate	
	First der. spec.	Rat. der. spec.	First der. spec.	Rat. der. spec.
Range (μg ml^{-1})	8.0–30.0	8.0–30.0	3.0–24.6	3.0–24.6
Detection limits (μg ml^{-1})	0.034	0.032	0.64	0.65
Regression equation (Y)[a]				
Slope (b)	1.5×10^{-3}	2.6×10^{-4}	3.2×10^{-3}	1.1×10^{-4}
S.D. on slope (S_b)	1.7×10^{-5}	2.8×10^{-6}	6.8×10^{-4}	2.4×10^{-5}
Intercept (a)	8.4×10^{-4}	1.5×10^{-2}	2.7×10^{-5}	5.8×10^{-4}
S.D. on intercept (S_a)	3.2×10^{-6}	3.9×10^{-4}	6.4×10^{-6}	3.1×10^{-5}
S.E of estimation (S_e)	6.8×10^{-4}	9.2×10^{-5}	7.4×10^{-4}	6.1×10^{-3}
Correlation coefficient (r)	0.9996	0.9990	0.9975	0.9991
RSD (%)[b]	0.9	1.5	2.3	1.7
% Range of error[b] (95% confidence limit)	0.08	0.05	0.07	0.18

[a] $Y=a+bC$ where C is concentration in μg ml^{-1} and Y in absorbance units.
[b] Five replicate samples.

ture solution, and represent the values of A_1^1 (1%, 1 cm) for ingredients. The subscripts 1 and 2 refer to $\lambda_1=\lambda_{max}=250.3$ nm of dorzolamide hydrochloride compound and $\lambda_2=\lambda_{max}=315.8$ nm of timolol maleate compound, respectively.

By the fact that there was no official method for the analysis of dorzolamide hydrochloride and timolol maleate in binary mixture. Therefore, the Vierordt's method was chosen as the analytical reference method. Table 4 shows the experimental parameters obtained by using the zero-order absorption spectra for the standard solutions of dorzolamide hydrochloride and timolol maleate. The application of the Vierordt's method of two-component analysis for the determination of these drugs in authentic mixtures and in tablets gave satisfactory results (Table 2).

3.4. Linearity, sensitivity and selectivity

The calibration graphs were constructed for the proposed methods from data points over the concentration range cited in Tables 1 and 4. The latter presents the results of the statistical analysis of the experimental data, the regression equations calculated from the calibration graph, along with the standard deviation of the slope (S_b) and the intercept (S_a) on the ordinate and the standard deviation of residuals (S_e). The linearity of the calibration graphs and conformity of the ^{1}D and ^{1}DD measurements of the proposed methods to Beer's law are proved by the high values of the correlation coefficients (r) of the regression equations. The detection limits (LOD) [13] for first derivative spectrophotometry were 0.034 μg ml^{-1} for dorzolamide hydrochloride, and 0.64 μg ml^{-1} for timolol maleate while the quantification limits (LOQ) [14] were 0.91 μg ml^{-1} for dorzolamide hydrochloride, 1.06 μg ml^{-1} for timolol maleate. The ratio derivative spectrophotometry, detection limits (LOD) were 0.032 μg ml^{-1} for dorzolamide hydrochloride, and 0.65 μg ml^{-1} for timolol maleate while the quantification limits (LOQ) were 1.08 μg ml^{-1} for dorzolamide hydrochloride and 1.14 μg ml^{-1} for timolol maleate, respectively. The other method, Vierordt's method, detection limits (LOD) were 0.92 μg ml^{-1} for dorzolamide hydrochloride and 0.51 μg ml^{-1} for timolol maleate; while the quantification limits (LOQ) were 1.82 μg ml^{-1} for dorzolamide hydrochloride and 1.67 μg ml^{-1} for timolol maleate.

3.5. Accuracy and precision

The utility of these methods was verified by means of a recovery assay in the presence of

blank. Eye drops containing 20.0 mg dorzolamide hydrochloride and 5.0 mg timolol maleate were prepared and processed according to the proposed methods. Recovery were determined by comparison with the corresponding standard solution. The specificity of the 'zero-Crossing' derivative spectrophotometry, ratio first derivative spectrophotometry and Vierordt's method were tested by examining the possible interference of pharmaceutical excipients in the assay procedure. Sodium citrate, mannitol, and benzalkonium chloride exhibited no interference. The relative standard deviations were found to be less than 1.5% indicating reasonable repeatability and accuracy of the two methods (Table 2).

Developed methods were applied to two different batches of commercial formulations. A summary of the results is shown in Table 3. The results of developed methods for the same preparations were compared by Student's t-test. The calculated (experimental) t-values and F-values did not exceed the tabulated (theoretical) values in the test, indicating that there was no significant difference between the methods compared.

4. Conclusion

The two developed methods can be used for simultaneous determination of dorzolamide hydrochloride and timolol maleate in their binary mixtures in pharmaceutical formulations. The

Table 2
Assay results of dorzolamide hydrochloride and timolol maleate in laboratory-made mixtures

Sample	Recovery (mean ± S.D.) (%)[a]					
	Dorzolamide hydrochloride			Timolol maleate		
	First der. spectr	Ratio der. spectr.	Vierordt's met.	First der. spectr	Ratio der. spectr.	Vierordt's met.
Synthetic mixtures	99.1 ± 1.5	99.9 ± 0.8	98.1 ± 0.5	98.5 ± 1.3	98.9 ± 0.4	100.5 ± 1.7
	$t = 1.72$ (2.26)[b]	0.97		0.84	1.68	
	$F = 2.50$ (3.18)[b]	1.14		1.78	2.14	

[a] Mean and relative standard deviation for ten determinations; percentage recovery from the label claim amount.
[b] Values in parentheses are the theoretical values at $P = 0.95$. Theoretical values at 95% confidence limits $F = 3.18$; $t = 2.26$.

Table 3
Assay of dorzolamide hydrochloride and timolol maleate in eye drops preparations

Sample	Recovery (mean ± S.D.) (%)[a]					
	Dorzolamide hydrochloride			Timolol maleate		
	First der. spectr.	Ratio der. spectr.	Vierordt's met.	First der. spectr	Ratio der. spectr.	Vierordt's met.
Batch no 1[c]	102.5 ± 1.67	97.9 ± 0.84	100.7 ± 1.87	100.5 ± 1.42	101.6 ± 1.37	100.5 ± 1.7
	$t = 2.02$ (2.26)[b]	1.92		1.17	1.00	
Batch no 2[c]	99.5 ± 1.23	99.9 ± 1.92	101.4 ± 0.91	99.0 ± 1.03	97.9 ± 0.93	97.1 ± 1.83
	$t = 1.42$	0.55		1.71	0.47	

[a] Mean and relative standard deviation for ten determinations; percentage recovery from the label claim amount.
[b] Theoretical values at 95% confidence limit; $t = 2.26$.
[c] Commercial eye drops are the product of MSD Pharm. Ind., Turkey; each per ml drops was labeled to contain 20.0 and 5.0 mg of dorzolamide hydrochloride and timolol maleate, respectively.

Table 4
Experimental parameters calculated for the simultaneous determination of dorzolamide hydrochloride and timolol maleate in binary mixture by Vierordt's method

λ (nm)	Dorzolamide hydrochloride		Timolol maleate	
	α_1	α_2	β_1	β_2
λ_1: 250.3	1250.0	–	1848.4	–
λ_2: 315.8	–	428.7	–	365.7
Linearity range ($\mu g\ ml^{-1}$)	8.0–30.0		3.0–24.6	

zero-crossing derivative spectrophotometry is more rapid and simple than ratio derivative spectrophotometry, while the ratio derivative spectrophotometry has greater sensitivity and accuracy. These proposed methods could be regarded as a useful alternative to the chromatographic techniques (HPLC) in the routine quality control of pharmaceutical formulations, allowing qualitative and quantitative information to be simultaneously and rapidly achieved with a relatively inexpensive instrumentation. In the absence of an official monograph these developed methods can be used for the simultaneous determination of the cited drugs in pharmaceutical preparations.

References

[1] B.K. Matuszewski, M.L. Constanzer, E.J. Woolf, T. Au, H. Haddix, J. Chromatogr. B 653 (1994) 77.
[2] M.L. Constanzer, C.M. Chavez, B.K. Matuszewski, J. Pharm. Biomed. Anal. 15 (1997) 1001.
[3] M.L. Satuf, J.C. Robles, H.C. Goicoechea, A.C. Oliveri, Anal. Lett. 32 (1999) 2019.
[4] R.C. Tim, R.A.: Kautz, B.L. Karger, Electrophoresis 21 (2000) 220.
[5] J.R. Carlin, R.W. Walkar, R.O. Davies, R.K. Ferguson, W.J.A. Vandenheuvel, J. Pharm. Sci. 69 (1980) 1111.
[6] M.S. Lennard, S. Parkin, J. Chromatogr. B 338 (1985) 249.
[7] K. Kubota, H. Nakamura, E. Koyama, T. Yamada, K. Kikuchi, T. Ishizaki, J. Chromatogr. B 533 (1990) 255.
[8] S.P. Kulkarni, P.D. Amin, J. Pharm. Biomed. Anal. 23 (2000) 983.
[9] B.R. Patel, J.J. Krischbaum, R.B. Poet, J. Pharm. Sci. 70 (1981) 336.
[10] D.J. Mazzo, P.A. Snyder, J. Chromatogr. 438 (1988) 85.
[11] D.J. Mazzo, J. Chromatogr. 299 (1984) 503.
[12] J.M. Garcia, O. Hernandez, A.I. Jimenez, F. Jimenez, J.J. Arias, Anal. Chim. Acta 317 (1995) 83.
[13] Nomenclature, symbols, units and their usage in spectrochemical analysis, II. Spectrochim. Acta B 33 (1978) 242.
[14] Guidelines for data acquisition and data quality evaluation in environmental chemistry, Anal. Chem. 52 (1990) 2242.
[15] J.J. Baldwin, G.S. Ponticello, P.S. Anderson, M.E. Christy, M.A. Murcko, W.C. Randall, H. Schwam, M.F. Sugrue, J.P. Springer, P. Gautheron, J. Grove, P. Mallorga, M.P. Viader, B.M. McKeever, M.A. Navia, J. Med. Chem. 32 (1989) 2510.