Asian Journal of Chemistry; Vol. 34, No. 5 (2022), 1119-1124

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2022.23611

A Simple and Reliable RPLC Method for Simultaneous Determination of Five β-Blocker Drugs in Pharmaceuticals and Human Plasma

HASSAN M. ALBISHRI[1,*], BASMAH H ALSHAMMARI[1] and DEIA ABD EL-HADY[2]

[1]Chemistry Department, Faculty of Science, King Abdulaziz University, Solimania, 80203 Jeddah, Saudi Arabia
[2]Chemistry Department, Faculty of Science, University of Jeddah, Osfan Road, P.O. Box 80327, 21589 Jeddah, Saudi Arabia

*Corresponding author: E-mail: hmalbeshri@kau.edu.sa

Received: 9 November 2021; *Accepted*: 30 December 2021; *Published online*: 20 April 2022; AJC-20761

A simple and reliable simultaneous determination of β-blocker drugs (acebutolol, pindolol, atenolol, nadolol and oxprenolol) was accomplished by reversed phase liquid chromatography-ultraviolet detection (RPLC/UV). The chromatographic separation was achieved on a SB-C18 ZORBAX® column (250 mm × 4.6 mm i.d., 5 micron), using a mobile phase consisted of 0.02 mol L^{-1} phosphate buffer (pH = 3.5)-acetonitrile (70:30, v/v). Isocratic elution was used at a flow rate of 1.0 mL min^{-1}. The UV detector was operated at 230 nm and the column temperature was maintained at 30 °C. Under optimal conditions, a good linearity in the range of 10-500 μg L^{-1} for five β-blocker drugs with the correlation of determinations (R^2) higher than 0.9971 was achieved. The proposed method was successfully applied to routine analysis of several β-blockers in pharmaceutical tablets. Moreover, the results obtained from this study demonstrated that the validated method can be successfully used to routine analyze the therapeutic concentrations of several β-blockers in human plasma. The LOD and LOQ for selected β-blockers were ranged within 0.050-0.538 and 0.152-1.794 μg L^{-1} & 0.192-0.845 and 0.641-2.562 μg L^{-1}, respectively, for pharmaceutical and plasma samples. Results of intra-day and inter-day precision expressed in terms of %RSD were found to be less than 2.0. Accuracy of the RPLC method was assessed by performing replicate analyses of selected β-blockers in samples against a calibration curve indicating high recoveries within 96.7-110.5%. This new method could be used for analysis of large sample series of five β-blockers in routine laboratory work.

Keywords: β-Blocker drugs, RPLC, Acebutolol, Pindolol, Atenolol, Nadolol, Oxprenolol, Matrices.

INTRODUCTION

β-Blockers or β-adrenergic blocking agents, are drugs that block norepinephrine and epinephrine (adrenaline) from binding to β-receptors on nerves [1]. Epinephrine and norepinephrine are secreted by nerves in the body as well as by the adrenal gland. They work as neuro-transmitters that might be active locally where they are secreted, or in another place in the body, once secreated to the blood. There are β- and α-receptors in the normal body. Regarding β receptors, three types exist, they regulate several functions relative on their position in the body [2]: (a) β_1 receptors are found in organs such as eye, heart and kidney; (b) β_2 receptors are located in the lungs, gastrointestinal tract, uterus, liver, blood vessels and skeletal muscle and (c) β_3 receptors are found in fat tissues. Moreover, β-blockers drugs, are broadly used to manage cardiac arrhythmia; they are used as a secondary prevention that retain the heart from further heart attacks, besides to their ability to treat the blood hypertension [3]. β-Blockers also affect the angiotensin-renin system in the kidneys and may cause a reduction in secretion of renin, which in turn decreases the demand of oxygen in the heart by dropping the volume of the extracellular cavity and elivate the capacity of the blood to carry oxygen [4]. Consequently, it is vital to develope a simple, trust worthy and highly precise method in order to quantify the β-blockers.

At present, numerous techniques including colorimetric methods [5,6], spectrophotometric methods [7-9], electrochemical determination [10] and high performance liquid chromatography [11-22] were available for β-blockers analysis. However, HPLC is the most common technique in the pharmaceutical industry and it also offers quick, automated and highly precise analytical methods for pharmaceuticals analysis. The

detection was performed using either ultraviolet (UV) detector [11-14] or fluorescence detector [15-19] or mass detector [20]. Recently, Yildirim *et al.* [21] published a review regarding the HPLC methods used in the last decade to separate the β-blockers or their enantiomers in different matrices. The HPLC methods were adopted to many dosage forms include tablets [22,23], capsules [24], ophthalmic dosage forms [25] and oral liquid preparations [26]. Moreover, β-blockers have been analyzed in biological samples such as urine [27,28], plasma [29-31], serum [32,33] and intestinal segment [34], as well as in environmental samples such as sewage [35] and natural waters [36]. The main trend in the accurate determination of β-blockers in pharmaceuticals is the direct injection of the sample solution into the instrument, after dilution with the HPLC-compatible organic solvents, for example, acetonitrile and methanol. However, application to complex biological samples needs simple protein precipitation with acetonitrile and methanol, which was preferred by some researchers for the pretreatment of plasma samples containing high amount of protein. Moreover, HPLC separations have been predominantly performed in RP mode, using C18 columns and mobile phases generally included ACN or MeOH as a strong organic modifier. Among detectors used, UV detection was mostly used since it is relatively cheap, reliable and available in most routine analysis laboratories.

Therefore, in present study, a simple and reliable isocratic RPLC-UV method was used for simultaneous determination of five β-blockers drugs *viz*. pindolol, atenolol, oxprenolol, nadolol and acebutolol in pharmaceutical tablets and plasma matrices. Potentially undesirable effects can these drugs have in overdose conditions. Hence, an presice observing of trace amounts of these drugs in biological samples is routenly indispensable. Furthermore, the required terms of validation [37] such as linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness were also investigated.

EXPERIMENTAL

Acebutolol chloride (analytical standard), pindolol (>98%), atenolol (> 98%), nadolol (> 98%) and oxprenolol (> 98%) were obtained from Sigma-Aldrich (USA). Methanol (purity ≥99.9%) was obtained from Fisher Scientefic (UK). Acetonitrile (purity ≥ 99.9%) was purchased from Aldrich (USA). Sodium dihydrogen orthophosphate and orthophosphoric acid with a purity of 85-88% were bought from BDH (UK). All the additional chemicals were of Analar grade. A Milli-Rx apparatus (Millipore, Milford, USA) was utilized in order to purify the water used throughout the work.

The Agilent HPLC system (USA) was equipped by means of a 1290 Quat pumping machine, a 1290 Sampler and a 1260 UV detector. Zorbax C18 column (250 mm × 4.6 mm i.d., 5 µm particle diameter) was used for the separation of analytes (Agilent, USA). The data and chromatograms were recorded with Agilent Chemstation Software. The solutions pH values were adjusted by a Jenway model 3510 pH-meter (Staffordshire, UK). A 50/60 kHz ultrasonic water bath, (Elma, D-78224, Germany) was used for the degassing of the mobile phase. The centrifuge was Kendro (Labofuge 200, Germany) and the vortex was from Falc Instruments (MIX 20, Italy).

Chromatographic conditions: The mobile phase composed of acetonitrile and a 0.02 M phosphate buffer solution (30:70, v:v) at pH 3.5. Isocratic elution mode with a flow rate of 1.0 mL min^{-1} was used. The solutions of analytes were injected in triplicate into the HPLC column with a constant injection volume of 20 µL. The detection system was set at a wavelength of 230 nm. Column temperature was maintained at 30 ºC.

Preparation of solutions: Stock solutions of pindolol, atenolol, nadolol, acebutolol and oxprenolol were prepared by dissolving the appropriate amount of each compound in methanol to achieve a concentration of 100 µg mL^{-1}. All stock solutions were stored in the dark at 4 ºC and diluted to the desired concentrations for preparation of the working solutions. All working solutions were freshly prepared. A phosphate buffer (0.02 mol L^{-1}) was prepared by dissolving 2.7 g of NaH_2PO_4 in 1 L water. The pH 3.5 was adjusted with 10 % (v/v) orthophosphoric acid. The mobile phase solution was ultrasonicated for 10 min and filtrated through a 0.45-µm membrane filter (Millipore, Bedford, MA, USA) prior to use.

Method validation: The proposed analytical method was validated according to the ICH guidelines of Q2 (R1) [37].

Selectivity and system suitability: The capability of the method to evaluate the analyte while its potential impurities is termed selectivity. System suitability testing was carried out on a freshly prepared standard solution of the five drugs to check the various factors such as number of theoretical plates (efficiency), capacity factor (k), separation or relative retention (α), resolution (Rs) and peak tailing (T) beside the relative standard deviation (RSD, %) was also examined at all stages of the method.

Linearity, LOD and LOQ: The working solutions were prepared from the stock solution of each drug so as to contain the drug in the range of 10-500 µg L^{-1}. The peak area *versus* concentration data were treated by least squares linear regression.

The sensitivity of method was measured in terms of limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ of the developed method were calculated from the standard deviation of the response and slope of the calibration curve of drugs using the formula as per ICH guideline:

$$\text{Limit of quantitation} = 10\times\frac{\sigma}{S}$$

$$\text{Limit of detection} = 3.3\times\frac{\sigma}{S}$$

where, "σ" is standard deviation of y intercepts of regression lines, "S" is slope of calibration curve.

Precision: Intra-day and inter-day evaluations were conducted to assess the precision of the proposed method. Intra-day and inter-day precision were conducted by performing three replicates of the three concentration levels 10, 30 and 50 µg L^{-1}.

Accuracy: Normally, accuracy is evaluated by conducting recovery studies. The accuracy of the method was judged by

employing the standard addition method, where sample having mixture of the five drugs were spiked at three diverse concentrations levels.

Robustness: The robustness of the current method was examined by a little variation in the flow rate within the range of 0.95-1.05 mL min^{-1} and column temperature within the range of 24.5-25.5 ºC. Furthermore, the effect of small change in pH (± 0.05) and mobile phase compositions (± 0.5%) around their optimal values were also evaluated.

Analysis of pharmaceutical tablets: Twenty tablets of atenolol (Brand name: Betaten*50, label claim: 50 mg atenolol per tablet which is available in Saudi Market), were superbly powdered. A powdered amount of the tablet corresponding to 50 mg of atenolol was conveyed into a 50 mL volumetric flask containing 30 mL methanol, sonicated for 0.5 h and diluted up to 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min. Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45 µm filter (Millipore, Milford, USA). The above stock solution was further diluted to get a sample concentration of 500 µg L^{-1}. A 20 µL volume of sample solution was injected into HPLC. The peak areas were measured and concentrations in the samples were determined using calibration curve of standard atenolol developed on the same HPLC system under the same conditions using linear regression equation.

Analysis of plasma samples: Human plasma samples were generous given from Unity of Blood Donation of King Abdulazuz University Hospital (Jeddah, Saudi Arabia). They were kept in the freezer at -18 ºC and allowed to defrost at room temperature before using. Liquid liquid extraction was conducted using 1.0 mL of methanol, which was added to 0.2 mL of the plasma, the tube was vortex-mixed for 1 min and then centrifuged at 5000 rpm for 5 min. Three concentrations of five β-blockes, namely; 50, 300 and 500 µg L^{-1} were added to the drug-free plasma in volumes not exceeding 2% of the plasma volume. A total of 20 µL was injected into the HPLC system.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions: Several methodical trials were performed to improve the chromatographic conditions for developing a simple RPLC-UV method for the analysis of five β-blocker drugs, namely; pindolol, atenolol, oxprenolol, nadolol and acebutolol. In this method, diverse mobile phase compositions with several solvents (acetonitrile, methanol and water) were considered. The results displayed better resolusion of the desired peaks in the presence of acetonitrile. By changing the concentration and volume percentage of the phosphate buffer against acetonitrile, it was found that the best mobile phase composed of 0.02 mol L^{-1} phosphate buffer and acetonitrile (70:30 v/v) giving the optimal baseline separation among the studied five drugs almost free from tailing. Phosphoric acid was used to adjust the pH values of mobile phase. The pH of mobile phase was selected to be 3.5, since above this value, the retention time was increased, while using mobile phase adjusted less than 3.5, the peaks were overlapped. Moreover, the other variable parameters (column temperature, wavelengths and injection volumes) were also studied. Increasing the flow rate from 0.5 to 1.2 mL min^{-1} showed a similar decrease in the retention time. The optimum flow rate was in 1.0 mL min^{-1}. In literature, a flow rate of 1.0 mL min^{-1} is usually chosen for β-blockers analysis even different mobile phases were used [11-14]. In present study, the optimum flow rate was also evaluated with the Van Deemter curve. Under the optimal conditions as described in experimental section, a representative chromatogram is shown in Fig. 1.

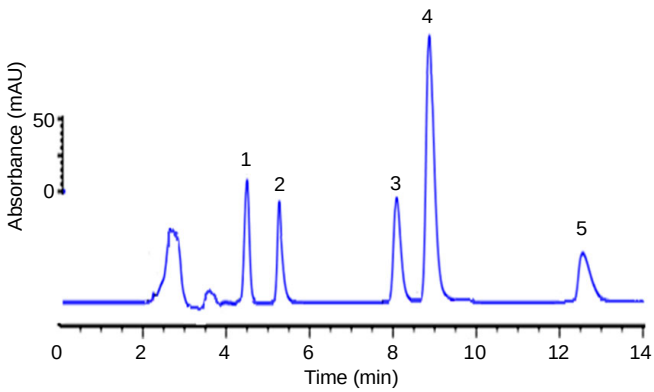

Fig. 1. A representative RPLC chromatogram for the separation of: (1) atenolol, (2) nadolol, (3) acebutolol, (4) pindolol and (5) oxprenolol under the optimal conditions as mentioned in the experimental section

Method validation: The described RPLC/DAD method was established using a simple isocratic mobile phase to provide a fast quality control evaluations of β-blockers in pharmaceuticals and plasma samples. The proposed analytical method was validated following the ICH guidelines of Q2 (R1) [37].

Selectivity and system suitability: Selectivity of the proposed RPLC method for the concurrent determination of pindolol, atenolol, oxprenolol, nadolol and acebutolol in plasma, since the detector used was UV, by comparing the similarity of chromatogram for each compound in the sample solution with the spectrum resulted from the standard solution using the same wavelength. Furthermore, the peak identity of each drug was firmly established by spiking five various concentrations of standard solutions in the studied matrix giving linearity equations similar to the calibration equations with standard at the same retention time. Results proved that β-blockers were free of interference and absence of any peak in plasma matches to that of any analyte.

The purpose of the system suitability test is to assure that the system constituents (reagents, columns, instruments, *etc.*) are acceptable for the intended analysis. Acceptance criteria of the system suitability test recommended by Food and Drug Administration (FDA) are tailing factor should be ≤ 2.0; theoretical plate number (N) should be > 2000; resolution (Rs) should be > 1.5 and capacity factor should be > 2. According to FDA criteria, selectivity factor is not an essential parameter. In addition, the RSD (%) value of the analytical peak area should be < 1.0 at all stages of the method. System suitability testing of proposed RPLC analytical method showed that the method was suitably to be used under the optimized conditions

for simultaneous determination of five β-blockers in pharmaceutical and plasma matrices (Table-1).

TABLE-1
SYSTEM SUITABILITY PARAMETERS FOR FIVE β-BLOCKERS UNDER OPTIMIZED CONDITIONS

Drug	N	k	α	R_S	T
Atenolol	19444	0.999	1.338	5.717	1.0
Nadolol	29744	1.886	1.198	15.096	1.1
Acebutolol	27566	2.595	1.123	3.412	1.82
Pindolol	27246	2.943	1.521	12.934	1.92
Oxprenolol	26711	4.581	–	–	1.75
Acceptance criteria	> 2000	Variable	> 1	> 1.5	< 2

*(N) number of theoretical plates, (k) capacity factor, (α) selectivity, (Rs) resolution and (T) peak tailing

Linearity, LOD and LOQ: The linearity of an analytical method is its ability, within a given range, to provide results that are directly proportional to the concentration of the analyte. The slope, intercept and correlation coefficient (R^2) are shown in Table-2. All the five drugs showed a good linearty in the range of 10 to 500 μg L^{-1}. For plasma, the calibration curve was done using the same working solutions followed by the pretreatment step. The calibration curves were also linear in the range of 10-500 μg L^{-1} for all analytes. The coefficients of determination (R^2) were greater than 0.9971 for all analytes.

Sensitivity of the method was tested by examining the limit of detection (LOD) and limit of quantification (LOQ) values. The LOD and LOQ for selected β-blockers were ranged within 0.050-0.538 and 0.152-1.794 μg L^{-1} & 0.192-0.845 and 0.641-2.562 μg L^{-1}, respectively, for pharmaceutical and plasma samples. These results are summarized in Table-2.

Precision: The experiment was repeated three times in a day (intraday precision) and the average %RSD values of the results were calculated. Similarly, the experiment was repeated on three consecutive days (interday precision) and the average %RSD values for peak area were calculated. Results of precision expressed in terms of %RSD were found to be < 1.0 intraday and < 2.0 interday as shown in Table-3.

Accuracy: Intraday and interday accuracy of the RPLC method was assessed by performing repeated analyses of selected β-blockers samples in plasma against a calibration curve. The results obtained from the intraday accuracy study at three concentrations (n = 3) specified high recoveries of β-blockers by the suggested method: 96.7-110.5% as shown in Table-3 indicating high accuracy of suggested method in human plasma.

Robustness and stability: Robustness is the measure of the capability of an analytical method to stay unpretentious by minor but deliberate disparities in method parameters. The current RPLC conditions set have been somewhat modified by the small changes of the flow rate and column temperature, as most operative means to estimate the current method's robustness. The selected variable parameters were column temperature (29.5, 30 and 30.5 ºC) and flow rate (0.95 mL min^{-1}, 1.00

TABLE-2
ANALYTICAL FEATURES OF THE STUDIED β-BLOCKER DRUGS

Parameter	Pharmaceuticals					Plasma				
	Pindolol	Atenolol	Nadolol	Acebutolol	Oxprenolol	Pindolol	Atenolol	Nadolol	Acebutolol	Oxprenolol
Linearity ranges (μg L^{-1})	10-500	10-500	10-500	10-500	10-500	10-500	10-500	10-500	10-500	10-500
Slope	52.854	14.995	10.882	19.371	15.231	7.806	12.363	8.376	13.020	8.201
Intercept	-27.832	-52.513	-50.915	-91.730	-54.407	22.230	49.027	30.335	50.397	0.194
R^2	0.9980	0.9982	0.9971	0.9992	0.9981	0.9980	0.9987	0.9975	0.9990	0.9988
LOD (μg L^{-1})	0.050	0.375	0.538	0.195	0.399	0.255	0.725	0.845	0.347	0.192
LOQ (μg L^{-1})	0.152	1.137	1.794	0.651	1.211	0.775	2.198	2.562	1.157	0.641

TABLE-3
ACCURACY AND PRECISION STUDIES FOR THE FIVE β-BLOCKER DRUGS IN PLASMA

Drugs	True concentration (μg L^{-1})	Found concentration (μg L^{-1}, n = 3)	Mean of recovery (%)	RSD%, Intra-day (n = 3)	RSD% Inter-day (n = 9)
Atenolol	10.0	10.8	108.0	0.60	1.32
	300.0	297.1	99.0	0.50	1.44
	500.0	510.4	102.1	0.30	1.73
Nadolol	10.0	11.0	110.0	0.27	1.43
	300.0	300.2	100.1	0.19	0.54
	500.0	520.4	104.1	0.12	1.21
Acebutolol	10.0	10.9	109.0	0.50	1.32
	300.0	290.4	96.8	0.35	1.34
	500.0	510.2	102.0	0.30	1.54
Pindolol	10.0	10.5	105.0	0.50	1.86
	300.0	290.6	96.9	0.44	1.84
	500.0	523.0	104.6	0.40	1.93
Oxprenolol	10.0	10.9	109.0	0.32	1.48
	300.0	284.0	94.7	0.90	1.52
	500.0	510.4	102.1	0.20	1.23

mL min^{-1} and 1.05 mL min^{-1}) as shown in Table-4. The results of capacity factor, resolution and column efficiency were evaluated with these small changes. Furthermore, the effect of small change in pH (± 0.05) and mobile phase compositions (± 0.5%) around their optimal values were also evaluated. It was found that the obtained results of β-blockers were superb under most conditions and remained unaffected by small deliberate changes of experimental parameters. Variation in the experimental parameters, as well as carrying out the experiment at room temperature, provided an indication of its reliability during normal use and concluded that the method was robust.

TABLE-4
ROBUSTNESS OF THE SELECTED β-BLOCKERS UNDER VARIOUS CONDITIONS OF FLOW RATE AND COLUMN TEMPERATURE

Drugs/parameters		0.95 mL min^{-1}	1.05 mL min^{-1}	24.5 °C	25.5 °C
Atenolol	N	19296	19824	19481	19698
	α	1.3071	1.298	1.357	1.375
	K	0.944	0.979	0.942	0.970
	R_S	5.449	5.355	5.332	5.752
Nadolol	N	29634	30682	30167	29477
	α	1.194	1.202	1.179	1.196
	K	1.831	1.891	1.829	1.894
	R_S	15.746	15.021	15.170	15.003
Acebutolol	N	26997	27904	27501	27827
	α	1.124	1.125	1.120	1.130
	K	2.513	2.45	2.615	2.659
	R_S	3.818	3.948	3.546	3.738
Pindolol	N	27434	27519	27880	27373
	α	1.506	1.51557	1.5367	1.518
	K	2.726	2.764	2.929	2.561
	R_S	12.955	12.284	12.886	12.570
Oxprenolol	N	28181	27795	27962	29158
	α	–	–	–	–
	K	4.702	4.028	4.3135	4.5120
	R_S	–	–	–	–

*Nominal values at flow rate 1.00 mL min^{-1} and column temperature 25 °C were indicated in Table-1.

The stability of sample solutions was tested by the proposed HPLC method over a period of 30 days. The freshly prepared solutions at room temperature and the 30 days stored samples in a refrigerator were analyzed. Increased biological plasma samples spiked with 50 μg L^{-1} of each β-blockers were subjected to deproteinization and stored at ambient temperature and in a freezer at 4 ºC for 30 days. Short time stability was assessed after 12 h at room temperature, 24 h of storage in a refrigerator and for long term assay after 5, 7 and 30 days refrigerated. Each sample was analyzed for intact β-blockers compounds once daily after a freeze thaw cycle for investigation of stability. Recovery% and RSD% of the stored samples were calculated and compared to that of freshly prepared samples. From the comparison of results, we can conclude that there were no degradation products and β-blockers were stable at 4 ºC for at least 30 days, indicating the possibility of using plasma samples over a period of 30 days at refrigeration without degradation.

Analysis of pharmaceutical tablets: Experimental results of the amount of atenolol in the selected commercial tablets, expressed as a percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients, which are normally present. The drug recovery was found to be 99.93% for atenolol five different lots of atenolol tablets were analyzed using the proposed procedures, the chromatogram is shown in Fig. 2.

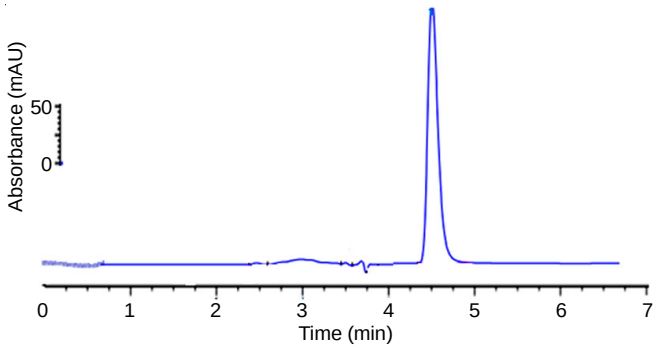

Fig. 2. Atenolol analysis in conventional tablet (Betaten*50) under the optimal conditions as mentioned in the experimental section

Analysis of plasma samples: The plasma samples were fortified with concentrations inside the linearity range of the five β-blockes. A representive chromatogram is shown in Fig. 3. The linearty, the recovery study, LOD and LOQ of the plasma samples are shown in Table-1.

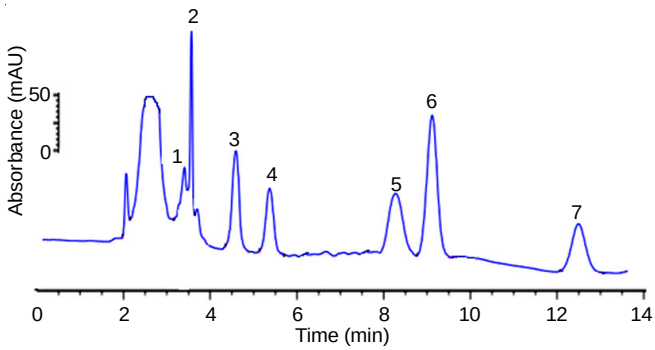

Fig. 3. Spiked plasma sample with the 5 drugs at 300 μg L^{-1} level. 1 and 2 from the plasma, 3) atenolol, 4) nadolol, 5) acebutolol, 6) pindolol and 7) oxprenolol under the optimal conditions as mentioned in the experimental section

Conclusion

A simple and reliable RPLC-UV method beneficial for the simultaneous determination of pindolol, atenolol, nadolol, acebutolol and oxprenolol was sensibly developed. The mobile phase composition and chromatographic conditions were optimized by witnessing conditions producing best baseline separations among peaks. The method was thoroughly validated, representing to be precise, sensitive, linear in the studied concentrations range, accurate and robust to determine of five β-blockers in pharmaceuticals and plasma samples. The proposed reliable RPLC method would be of use in routine quality control and varoius dosage form analysis. It is also a valuable tool in medicine and can be used in forensic toxicology.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. Y. Wu, L. Zeng and S. Zhao, *Biomolecules*, **11**, 936 (2021); https://doi.org/10.3390/biom11070936
2. R. Bruning, H. Dykes, T.W. Jones, N.B. Wayne and A.S. Newsome, *Front. Pharmacol*., **12**, 735841 (2021); https://doi.org/10.3389/fphar.2021.735841
3. N. Freemantle, J. Cleland, P. Young, J. Mason and J. Harrison, *BMJ*, **318**, 1730 (1999); https://doi.org/10.1136/bmj.318.7200.1730
4. P.A. James, S. Oparil, B.L. Carter, W.C. Cushman, J. Handler, C. Dennison-Himmelfarb, D.T. Lackland, M.L. LeFevre, T.D. MacKenzie, O. Ogedegbe, S.C. Smith Jr., L.P. Svetkey, S.J. Taler, R.R. Townsend, J.T. Wright Jr., A.S. Narva and E. Ortiz, *JAMA*, **311**, 507 (2014); https://doi.org/10.1001/jama.2013.284427
5. M.S. Mahrous, A.S. Issa and N.S. Ahmed, *Talanta*, **39**, 69 (1992); https://doi.org/10.1016/0039-9140(92)80052-F
6. N.A. Zakhari, S.M. Hassan and Y. El-Shabrawy, *Anal. Lett.*, **22**, 3011 (1989); https://doi.org/10.1080/00032718908052413
7. D. Pecanac, D. Radulovic, L. Zivanovic and S. Agatonovic-Kustrin, *J. Pharm. Biomed. Anal.*, **9**, 861 (1991); https://doi.org/10.1016/0731-7085(91)80014-Z
8. I. Panderi and M. Parissi-Poulou, *Int. J. Pharm.*, **99**, 327 (1993); https://doi.org/10.1016/0378-5173(93)90376-Q
9. R.A.S. Lapa, J.L.F.C. Lima, B.F. Reis, J.L.M. Santos and E.A.G. Zagatto, *Anal. Chim. Acta*, **366**, 209 (1998); https://doi.org/10.1016/S0003-2670(98)00108-1
10. S. Smarzewska and W. Ciesielski, *Anal. Methods*, **6**, 5038 (2014); https://doi.org/10.1039/c4ay00648h
11. A. Levent and Z. Sentürk, *Anal. Lett.*, **43**, 1448 (2010); https://doi.org/10.1080/00032710903502116
12. I. Ali, Z.A. Al-Othman, A. Hussain, K. Saleem and H.Y. Aboul-Enein, *Chromatographia*, **73**, 251 (2011); https://doi.org/10.1007/s10337-010-1891-4
13. S. Morante-Zarcero and I. Sierra, *Chirality*, **24**, 860 (2012); https://doi.org/10.1002/chir.22084
14. Z.A. Al-Othman, A. Al-Warthan, S.D. Alam and I. Ali, *Biomed. Chromatogr.*, **28**, 1514 (2014); https://doi.org/10.1002/bmc.3259
15. B. Yilmaz, S. Arslan and A. Asci, *J. Chromatogr. Sci*., **50**, 914 (2012); https://doi.org/10.1093/chromsci/bms090
16. M.M. Hefnawy, M. Al-Shehri, M.A. Abounassif and G.A.E. Mostafa, *J. AOAC Int.*, **96**, 976 (2013); https://doi.org/10.5740/jaoacint.11-191
17. N.M. Najib, N. Idkaidek, A. Adel, B. Mohammed, S. Al-Masri, I. Admour, S.M. Alam, R. Dham and Qumaruzaman, *Biopharm. Drug Dispos.*, **26**, 1 (2005); https://doi.org/10.1002/bdd.416
18. J. Tomková, P. Ondra, E. Kocianová and J. Václavík, *Biomed. Chromatogr.*, **31**, e3911 (2017); https://doi.org/10.1002/bmc.3911
19. J.V. Shah, D.P. Patel, P.A. Shah, M. Sanyal and P.S. Shrivastav, *Biomed. Chromatogr.*, **30**, 208 (2016); https://doi.org/10.1002/bmc.3537
20. M.G. Santos, I.M.C. Tavares, V.B. Boralli and E.C. Figueiredo, *Analyst*, **140**, 2696 (2015); https://doi.org/10.1039/C4AN02066A
21. S. Yildirim, C. Erkmen and B. Uslu, *Crit. Rev. Anal. Chem.*, **52**, 131 (2020); https://doi.org/10.1080/10408347.2020.1791043
22. E.A. Abdel Hameed, R.A. Abdel Salam and G.M. Hadad, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **141**, 278 (2015); https://doi.org/10.1016/j.saa.2015.01.035
23. N.S. Mohammed, *Int. J. Pharm. Sci. Res.*, **8**, 2706 (2017).
24. S. Ahmed, A. Alqurshi and A.-M.I. Mohamed, *Talanta*, **184**, 296 (2018); https://doi.org/10.1016/j.talanta.2018.03.008
25. F.A. Ibrahim, H.M. Elmansi and S.A. El Abass, *Ann. Pharm. Fr.*, **77**, 302 (2019); https://doi.org/10.1016/j.pharma.2019.02.005
26. L. Zahálka, L. Matysová, Z. Šklubalová, S. Klovrzová and P. Solich, *Chromatographia*, **76**, 1553 (2013); https://doi.org/10.1007/s10337-013-2457-z
27. B. Yilmaz and S. Arslan, *Sep. Sci. PLUS*, **1**, 4 (2018); https://doi.org/10.1002/sscp.201700023
28. S. Ansari and M. Karimi, *Med. Chem. Res.*, **26**, 2477 (2017); https://doi.org/10.1007/s00044-017-1947-1
29. K. Farhadi, M. Firuzi and M. Hatami, *Mikrochim. Acta*, **182**, 323 (2015); https://doi.org/10.1007/s00604-014-1336-0
30. H. Sereshti and S. Bakhtiari, *RSC Adv.*, **6**, 75757 (2016); https://doi.org/10.1039/C6RA11363J
31. S.T. Ulu and Z. Aydogmus, *J. Chromatogr. Sci.*, **50**, 615 (2012); https://doi.org/10.1093/chromsci/bms056
32. M. Silva-Gracia, A. Köppl, S. Unholzer and E. Haen, *Biomed. Chromatogr.*, **31**, e3968 (2017); https://doi.org/10.1002/bmc.3968
33. T.A. Rodina, E.S. Mel'nikov, A.I. Dmitriev, S.A. Belkov, A.V. Sokolov, V.V. Arkhipov and A.B. Prokof'ev, *Pharm. Chem. J.*, **51**, 1111 (2018); https://doi.org/10.1007/s11094-018-1750-4
34. M.S. Kaynak, E. Buyutuncel, H.J. Cagler and S. Sahin, *Trop. J. Pharm. Res.*, **14**, 163 (2015); https://doi.org/10.4314/tjpr.v14i1.23
35. M.L. Wilde, K. Kümmerer and A.F. Martins, *J. Braz. Chem. Soc.*, **23**, 1732 (2012); https://doi.org/10.1590/S0103-50532012005000035
36. A. Sarafraz-Yazdi, M.R. Abedi and Z. Es'haghi, *J. Liq. Chromatogr. Relat. Technol.*, **36**, 750 (2013); https://doi.org/10.1080/10826076.2012.673212
37. B. Gajra, M. Patel and D.A. Patel, *Int. J. Pharm. Innov.*, **1**, 45 (2011).