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Parallel Reaction Monitoring Mode for Atenolol Quantification in Dried Plasma Spots by Liquid Chromatography Coupled with High-Resolution Mass Spectrometry

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Abstract: In this study, we reported a rapid, sensitive, robust, and validated method for atenolol quantification in dried plasma spots (DPS) by liquid chromatography with high-resolution mass spectrometry (LC-HRMS) using parallel reaction monitoring mode (PRM). Aliquots of 25 µL human plasma were placed onto Whatman 903 Cards and air-dried. Disks (3.2 mm internal diameter) were punched, and a 100 µL working internal standard solution was added to each sample and then incubated on a shaker for 15 min at 40 °C, followed by rapid centrifugation (10,000× *g*, 10 s). The supernatant was transferred into 300 µL vials for subsequent LC–HRMS analysis. After chromatographic separation, atenolol and the internal standard were quantified in positive-ion parallel reaction monitoring mode by detection of all target product ions at 10 ppm tolerances. The total time of the analysis was 5 min. The calibration curve was linear in the range of 5–1000 ng/mL with interday and intraday precision levels and biases of <14.4%, and recovery was 62.9–81.0%. The atenolol in DPS was stable for ≥30 days at 25 and 4 °C. This fully validated method is selective and suitable for atenolol quantitation in DPS using LC–HRMS.

Keywords: dried plasma spot; DPS; DBS; atenolol; PRM; LC–HRMS; parallel reaction monitoring

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1. Introduction

Cardiovascular diseases are defined as disorders related to the heart and blood vessels, e.g., hypertension, heart attack, and heart failure, and are some of the leading causes of the mortality worldwide [1]. In the Russian population, there has been a 21.9% increase in the prevalence of arterial hypertension [2]. To treat such diseases, physicians use a combination of cardiovascular therapies. Such therapies generally include various hypotensive drugs (diuretics, angiotensin II receptor antagonists [ARA-II], or β-blockers), lipid-lowering medicines (statins or ezetimibe), antiplatelet drugs (salicylic acid or clopidogrel), and antidiabetic drugs (metformin or glibenclamide) [3,4]. Atenolol is a selective (cardioselective) β1-adrenoblocker that was approved worldwide as a hypertension medication many years ago [5,6]. Atenolol offers the advantage of once-daily dosing in contrast to alternatives (β-blockers, such as metoprolol and propranolol) [7].

The dried blood spot (DBS) technique is now broadly used with the most extensively reported medication monitoring for toxicological and therapeutic purposes. The main advantages of DBS over whole blood revolve around the noninvasiveness of sampling (finger prick versus venipuncture), smaller volumes requirement (less than 100 µL for 3–4 spots), and more accessible storage and transport conditions [8,9]. This technique has several benefits in comparison with current venous blood-sampling methods: because the samples are dried blood spots, there are no biohazards, and therefore the samples can even be transferred in sealed packages to an appointed laboratory at no additional cost; drug monitoring can be carried out at any time; sample stability is significantly higher for

DBS than for venous blood; and patients can self-collect samples at home without special training [1,10]. Nonetheless, patients generally cannot collect samples correctly, which leads to incorrect data processing in 19% of cases [11]. The percentage of dissatisfactory samples can reach 30% [11,12]; hence, sampling in a laboratory is preferable.

The most significant parameter affecting the accuracy of the analysis is the hematocrit of the blood, which affects the viscosity of the blood, resulting in a change in the size of the DBS for a given volume [13]. One possible way to minimize the effect of blood hematocrit is the use of internal standards (ISs) directly added to the spot [14–16]. Another way to eliminate the potential hematocrit effect that we employed in our research is the use of dried plasma spots (DPS). On the other hand, this approach also implies that the concentration of the target analyte is relatively low, thereby requiring a sensitive assay for detection and quantification. Accordingly, mass spectroscopy is currently the most suitable technique for measuring drug concentrations in DPS [9,10,17].

Several instrument-based approaches are usually utilized for quantifying substances, depending on the mass spectrometer [18,19]. Most of biological samples are complex, and therefore it is not surprising to find several compounds with very similar masses in the matrix. In these cases, chromatography can help to separate these isobars; however, they can still influence the simulation result if the isobar is not completely separated from the analyte. With a triple quadrupole mass spectrometer, a technique known as multiple reaction monitoring mode (MRM), also known as selected reaction monitoring, can alleviate such potential problems [20,21]. In this technique, the first quadrupole device is configured to transmit a molecular ion (based on its m/z), the analyte is fragmented in the second quadrupole, and the third quadrupole is configured to transmit one or more known fragment ions (based on m/z) from the analyte [18]. The resultant "fingerprint" of the fragment ions also served to confirm or even determine the structure of a molecule. The transition with the highest intensity is used for the quantitation; usually, one or two confirm the analyzed substance. High-resolution mass spectrometry (HRMS) allows the detection of analytes by means of accurate mass at $\leq$10 ppm variation. HRMS enhancement enables the addition of the parallel reaction monitoring (PRM) method [22], initially designed for targeted proteomics [23,24]. In PRM, all ions of a target product are detected in a single mass analysis, which allows the determination of a "fingerprint" of the studied substance with high resolution. The ability to sum up signals from different fragments to increase the intensity of the resulting peak is an excellent advantage of PRM compared to MS/MS, where only one transition is detected. This capability helps when dealing with small amounts of samples and the low concentrations of substances typical for DPS. Nonetheless, to date, only three studies involved liquid chromatography (LC) coupled with MS/MS to measure atenolol concentrations in DBSs in either time-of-flight or MRM mode [1,25,26]. Therefore, the purpose of this study was to develop and validate a rapid and robust method of LC–HRMS in PRM mode for atenolol quantification in DPS with a one-step extraction procedure.

2. Materials and Methods

2.1. Materials

Atenolol and metoprolol (analytical standard grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Their structures are shown in Figure 1. Methanol and acetonitrile of LC–mass spectrometry (MS) grade were acquired from AppliChem Panreac (Barcelona, Spain). LC–MS grade water was obtained using a Milli-Q system from Millipore Corp. (Bedford, MA, USA). Whatman 903 Protein Saver Cards were purchased from Bio-Sciences Corp. (Westborough, MA, USA). Human plasma was collected from healthy volunteers with approval by the human ethics committee of the Institute of Chemical Biology and Fundamental Medicine (Novosibirsk, Russia).

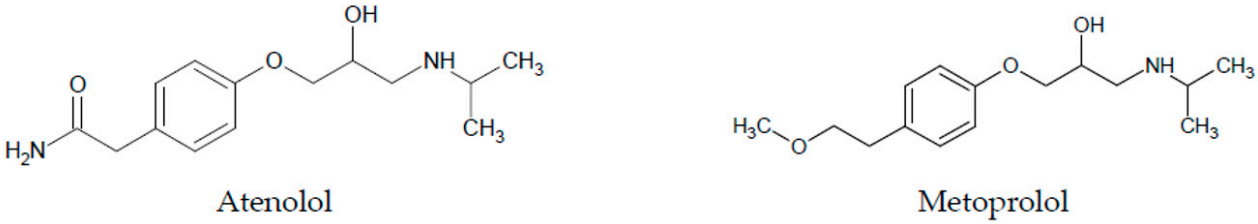

Figure 1. Structures of atenolol and metoprolol.

2.2. The LC–HRMS Method

The LC–HRMS system consisted of a DIONEX UltiMate 3000 chromatograph (Thermo Fisher Scientific, Inc., Waltham, MA, USA) coupled to a Q Exactive HF Orbitrap mass spectrometer (Thermo Fisher Scientific). The analysis was performed on a ProntoSil-120-3-C18 (75 × 2 mm, 3 µm) analytical column (EcoNova, Novosibirsk, Russia) at 40 °C. The total run time of this analysis was 5 min. Separation was performed via gradient elution at a flow rate of 200 µL/min.

The gradient consisted of water (eluent A) and methanol (eluent B), both containing 0.1% of formic acid, and was implemented as follows: the initial eluent was 5% B, which was increased to 50% for 1 min, then increased to 65% from minute 1.00 to minute 1.01, next increased to 80% from minute 1.01 to minute 1.40, and finally increased to 100% from minute 1.40 to minute 2.50.

The column was washed with 100% B in the next 2.5 min, followed by equilibration with the initial mobile phase (5% B) for the next run. The final composition of the mobile phase was employed for additional 2.5 min before equilibration of the column with the initial mobile phase (5% B) for the next run. All data were acquired and analyzed in Xcalibur 4.2.47 software (Thermo Fisher Scientific).

The optimal parameters of MS were the spray voltage of 4200 V, sheath and auxiliary gas pressure of 9 and 2 arbitrary units, respectively, and capillary temperature of 320 °C. The analytes were detected in positive-ion PRM mode. The general parameters of PRM were set as follows: in-source CID, 0.0 eV; polarity, positive; default charge, 1; dynamic RT, off; and inclusion, on. Full MS/MS scans were acquired with an AGC target value of 2E5, a resolution of 30000, normalized collision energies (NCE) of 40 eV, and a maximum ion injection time of 100 ms. Each target was monitored with a m/z 0.4 isolation window. The inclusion list consisted of two lines with the following settings: m/z 267.1680 (~3 ppm error relative to theoretical mass) with NCE of 40 eV for atenolol, and m/z 268.1907 (less than 1 ppm error relative to theoretical mass) with NCE of 40 eV for metoprolol, used as an IS. Several fragment ions' m/z values were chosen for detection and quantification: 74.0604, 116.1069, 145.0644, 190.0858, 225.1227, and 267.1696 for atenolol, and 159.0803, 191.1066, and 268.1906 for the IS.

2.3. Preparation of Calibration Standards and of Method Validation Samples

A stock solution of atenolol was prepared in methanol at 1 mg/mL. Fresh working solutions were made by diluting the stock solution with methanol to the following concentrations: 10,000, 5000, 2500, 1000, 500, 250, 100, and 50 ng/mL. Finally, the calibration standards of atenolol were prepared by 10-fold dilution of the respective working solutions in 1.5 mL Eppendorf tubes containing human plasma to attain final concentrations of 1000, 500, 250, 100, 50, 25, 10, and 5 ng/mL.

For preparing the 1 mg/mL IS stock solutions, 12 mg of metoprolol was dissolved in 1.2 mL of methanol. The IS working solution was obtained by dilution with a mixture of MeOH and H_2O (60:40, v/v) to achieve a final concentration of 0.2 ng/mL. The stock solutions were kept at −22 °C, and working solutions were prepared immediately before use.

Quality control (QC) solutions were obtained by diluting the respective working solutions of atenolol in human plasma at three levels: 800, 400, and 80 ng/mL. Solutions of QCs were also prepared in MeOH to measure the recovery of atenolol.

2.4. Sample Preparation and the Extraction Procedure

Plasma spots were produced via direct spotting of 25 µL of calibration standards onto Whatman 903 cards by means of an automatic pipettor. The resultant samples were air-dried at room temperature for at least 3 h before processing. Next, disks (3.2 mm internal diameter) were punched from the center of each DPS sample and transferred to 1.5 mL Eppendorf tubes. After that, 100 µL of a working IS solution was added to each disk and used as an extraction solvent. The tubes were vortexed for 15 min at 40 °C and centrifuged for 10 s at 10,000× *g*. Next, each extract was placed into a 300 µL vial for subsequent LC–HRMS analysis.

Blank DPS samples were prepared via spotting of 25 µL of atenolol-free plasma onto Whatman 903 cards and were treated in the same way as above.

For measuring the recovery of atenolol, 5 µL of plasma containing a QC was placed onto Whatman 903 cards. The whole spot was then cut out, transferred into a 1.5 mL Eppendorf tube, and extracted with 200 µL of a working IS solution. After that, the other extraction steps were carried out as described above.

To estimate the matrix effect, 5 µL of pure plasma was spotted onto a Whatman 903 card, whole spots were punched out into 1.5 mL Eppendorf tubes, and 200 µL of a working IS solution was added; the procedure was then completed as outlined above.

2.5. Linearity

Calibration standards were prepared in quadruplicate (artificial replicates; the artificial samples prepared and analyzed in an identical way in parallel; hereafter identified as replicates). A calibration curve was constructed with eight concentrations (1000, 500, 250, 100, 50, 25, 10, and 5 ng/mL). A calibration plot of the analyte/IS peak area ratio versus nominal atenolol concentration was built, and equally weighted linear regression was applied.

2.6. Accuracy

Inter- and intraday accuracy and precision of the assay were determined by the analysis of replicates (n = 6) of calibration standards at three concentrations within the range of 5–1000 ng/mL on 3 consecutive days. These levels were defined as low, medium, and high concentrations (80, 400, and 800 ng/mL, respectively). Accuracy was calculated as relative error (percentage bias), and precision was calculated as percentage relative standard deviation (%RSD). Results of the three concentrations were considered acceptable if they were in the $\leq$15% range in accordance with the European Medicines Agency guidelines [27].

2.7. The Recovery and Matrix Effect

To measure recovery and matrix effect, atenolol at each concentration was added in equal volumes (5 µL) to MeOH-based and DPS samples.

The recovery was evaluated by comparing the peak area ratios of compounds extracted from DPS samples with the peak area of compounds added into blank DPS extracts. Recovery was determined at three concentrations (low, medium, and high), using six replicates, as performed for the determination of accuracy and precision.

To evaluate the suppression or enhancement effects in DPS extracts, matrix effects were tested. Atenolol at each concentration (5 µL) was added into six replicates of blank DPS extracts derived from 5 µL of pure plasma, and the results were compared with those from standards that were added into a pure solvent.

2.8. Stability

A series of DPS was prepared with atenolol at three concentrations (800, 400, and 80 ng/mL), and each sample was divided into two parts. One part was stored at room temperature in a postal envelope for a bench-top stability assay; the other was kept in the same package at 4 °C for refrigeration stability evaluation. As described above, the samples were extracted and analyzed on days 7 and 30. The purpose of the stability test was to assess the storage time of atenolol in DPS.

2.9. Application of Method to Volunteer Spot Samples

The developed analytical method was applied to a series of DPS samples collected from a selected healthy adult male volunteer. The volunteer did not have any hematological, renal, or significant cardiac disease. Blood samples were collected both pre-dose and following an oral administration of a 50 mg atenolol tablet at 1, 2, 4, 6.5, 8.5, 10.5, and 23 hours in K3.EDTA vacutainer collection tubes (Zhejiang Gongdong Medical Technology Co., Ltd. Taizhou, China). The blood samples were immediately centrifuged for 10 min at 3200 rpm and 4 °C, and the plasma was collected in new Eppendorf tubes. The collected plasma samples were stored at −20 °C until analysis by the proposed analytical method. On the day of the analysis, the plasma samples were thawed, and 25 µL were spotted onto Whatman 903 cards. The subsequent procedure was performed as described in Section 2.4.

3. Results and Discussion

3.1. The Extraction Procedure

We previously conducted experiments to optimize the parameters of atenolol extraction from DPS [28] by the MRM method. In this study, we utilized these optimized extraction parameters to develop and validate a quantitative assay of atenolol by HRMS. In brief, extraction was carried out by means of 100 µL of a MeOH:H_2O mixture (60:40, v/v) containing the IS for 15 min at 40 °C in a shaker at 900 rpm. The only change was a reduction of extraction volume from 200 µL [28] to 100 µL for concentrating the samples with a small amount of analyte.

3.2. The LC–HRMS Method

The PRM method was selected to quantify atenolol by the HRMS technique. The ions in question (the M + 1 isotope $[M + 1 + H]^+$ of atenolol, and the protonated molecule $[M + H]^+$ of metoprolol) have very similar m/z values: 268.1737 and 268.1907, respectively [29]. To avoid their interference with each other, we set the exact masses of atenolol and IS in the inclusion list with an accuracy of 10 ppm. During the analysis, these compounds were fragmented, followed by the detection of all ions. Thus, both whole-molecule and product ions were detected simultaneously with the increased accuracy. The use of several fragment ions in the PRM method for quantitative analysis allows both the qualification of the analytes' structure and the summing up of the peak areas for increasing the intensity of the signal and also the sensitivity of the assay. For the quantitative determination of atenolol, based on our experiments, several m/z values were chosen: 74.0604, 116.1069, 145.0644, and 190.0858. The whole-molecule ion [1,30,31] (m/z 267.1696) as well as a known fragment ion (m/z 225.1227; $C_{11}H_{16}N_2O_3$) [32] were used also.

The delta of the observed mass of the atenolol whole molecule compared with the theoretical one was found to be −2.6 ppm. For the IS, several fragments were selected, with m/z 159.0803, 191.1066, and 268.1906 [33]. The delta of the observed mass of the metoprolol whole molecule compared with the theoretical one turned out to be −0.4 ppm. The mass spectra and chemical structures of atenolol and of the IS are presented in Figures 2 and 3.

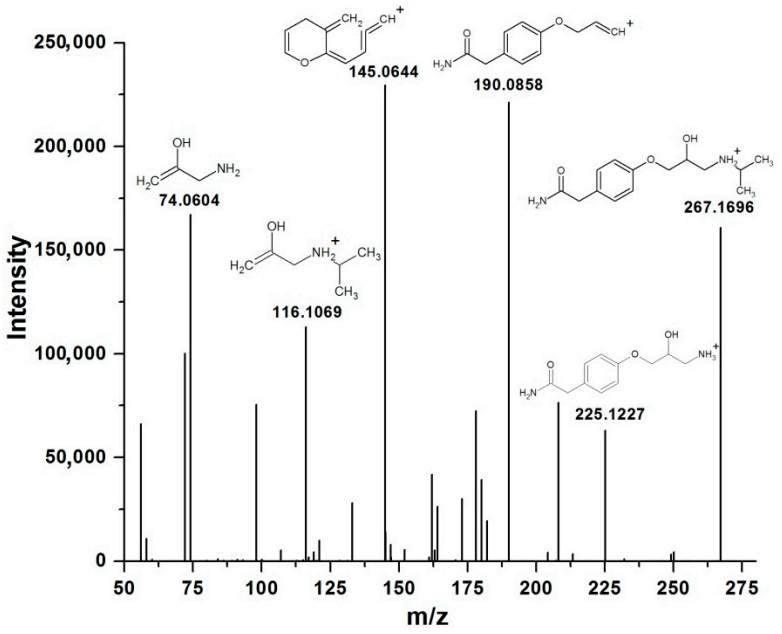

Figure 2. The mass spectrum of atenolol as registered by the PRM method. Masses of fragment ions of atenolol are identified.

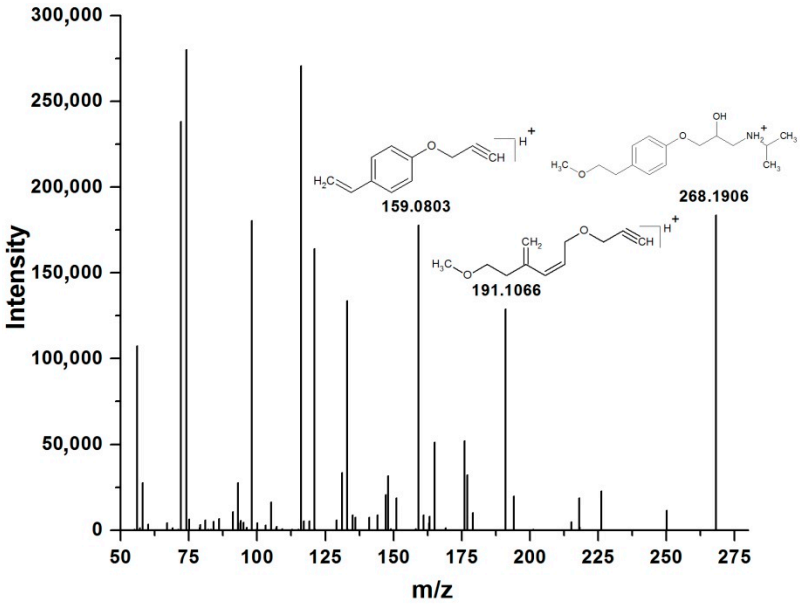

Figure 3. The mass spectrum of the IS as recorded by the PRM method. Fragment ions of the IS employed in this work are identified, and their masses are indicated.

3.3. Linearity

The linearity of the newly developed method was estimated by means of a standard calibration curve with eight concentrations, on the basis of four biological and two technical replicates. A plot of the peak area ratio against analyte concentration was constructed next. These calibration standards fulfilled the specified acceptance criteria: tolerance of ±20% was accepted for the lowest concentration, and ±15% for the others. The calibration curve was linear within the 5–1000 ng/mL range when linear regression ($y = 0.2403x$) with equal weighting was utilized. A coefficient of the determination (R^2) of 0.9941 was obtained during the validation of the method. The minimal concentration with tolerance of ±20% and a signal-to-noise ratio of >10 was defined as the lower limit of quantitation (LLOQ). No signal was detectable in the blank DPS sample.

Representative chromatograms of limits of quantitation (LOQs) of DPS and IS are shown in Figure 4.

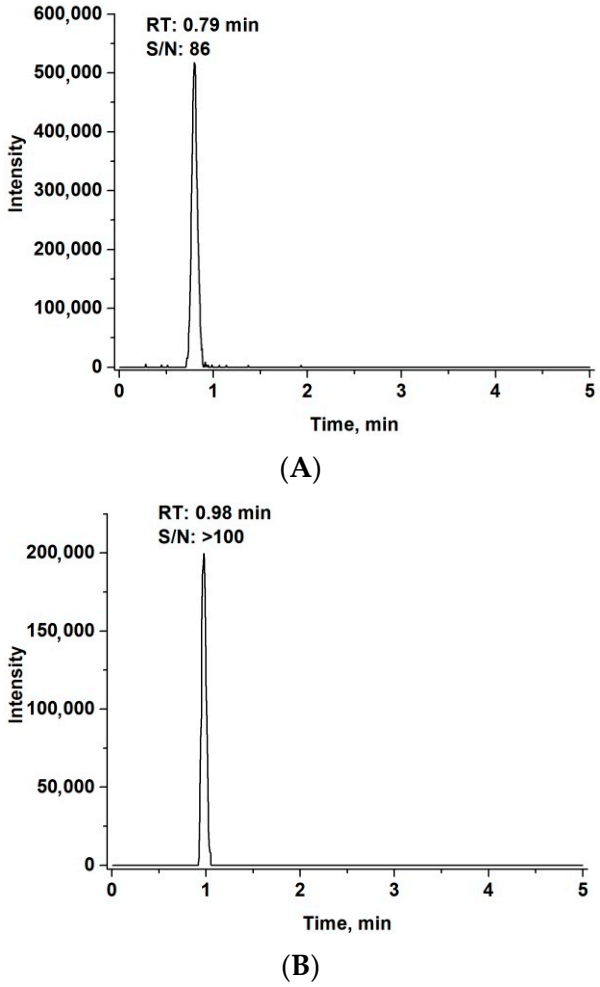

Figure 4. LC–HRMS chromatograms of (**A**) the LOQ of atenolol (5 ng/mL) and (**B**) the IS (0.2 ng/mL) with corresponding retention times (RT) and signal-to-noise ratios.

3.4. Accuracy

To assess the interday and intraday precision and accuracy, three QCs (80, 400, and 800 ng/mL) were used. The interday precision and accuracy were determined by repeated analyses (performed on three successive days). Precision within and between days was calculated as percentage relative standard deviation (%RSD), while accuracy was computed as percentage bias. The results were within the acceptable range for both interday and intraday analysis; accuracy and precision were less than 15% at all QC levels and less than 20% for the LLOQ, as presented in Table 1.

Table 1. Intraday and interday accuracy and precision.

Concentration, ng/mL	Interday		Intraday	
	%Bias	%RSD	%Bias	%RSD
5	16.6	13.8	12.9	11.0
80	14.4	13.5	8.5	7.5
400	4.3	5.8	6.0	6.9
800	8.0	6.1	6.3	8.9

3.5. The Recovery and Matrix Effect

The recovery was evaluated to check the extraction efficiency of the newly developed method. The results are listed in Table 2. The recovery and matrix effect of atenolol varied from 62.9% to 81.0% and from 65.4% to 79.4%, respectively. Although the method's overall efficiency, which is the recovery multiplied by the matrix effect, was ~40–60%, the one-step extraction procedure enabled us to reduce the sample preparation time. Furthermore, even this overall efficiency allows the detection of a 5 ng/mL analyte (LLOQ). Perhaps this is because as the concentration of atenolol decreased, the overall efficiency of the method increased.

Table 2. Results on the extraction recovery and matrix effect.

Concentration, ng/mL	Recovery, %	Matrix Effect, %
80	81.0	79.4
400	62.9	71.0
800	66.5	65.4

3.6. Stability

To evaluate stability, samples were kept both in a refrigerator at 4 °C and at room temperature for 7 and 30 days, and the results were compared with those from freshly prepared samples. The data in Table 3 show that DPS samples were stable for 30 days both at room temperature and at 4 °C.

Table 3. Stability of atenolol in DPS at 4 and 25 °C.

Concentration, ng/mL	1 Day		7 Days		30 Days	
	%Bias	%RSD	%Bias	%RSD	%Bias	%RSD
25 °C						
80	9.1	7.1	9.4	13.7	12.8	11.1
400	3.9	13.3	14.3	14.8	14.0	11.5
800	6.6	21.5	8.8	11.3	9.8	8.3
4 °C						
80	9.1	7.1	13.0	11.9	6.3	12.9
400	3.9	13.3	23.7	14.4	10.3	14.8
800	6.6	21.5	13.8	13.7	12.0	9.4

3.7. Application of the Method

To test a proposed method in a real-time situation, we quantified atenolol in DPS prepared from the healthy volunteer's blood plasma after administration of 50 mg atenolol. All samples were analyzed twice. The mean pharmacokinetic profile of atenolol is shown in Figure 5, which indicates the suitability of the presented method for pharmacokinetic studies of atenolol in humans. The area under the plasma concentration–time curve from zero hour to infinity ($AUC_{0\text{-inf\_obs}}$), half-life ($t_{1/2}$), the time point of C_{max} (T_{max}), and maximum concentration in plasma (C_{max}) for atenolol were 3577 ± 456 ng/mL·h, 12.7 ± 2.1 h, 2 ± 0 h, and 442 ± 21 ng/mL, respectively. The calculated pharmacokinetics parameters and pharmacokinetic profile of atenolol obtained in this study were similar to those reported in other works [26,30,34,35].

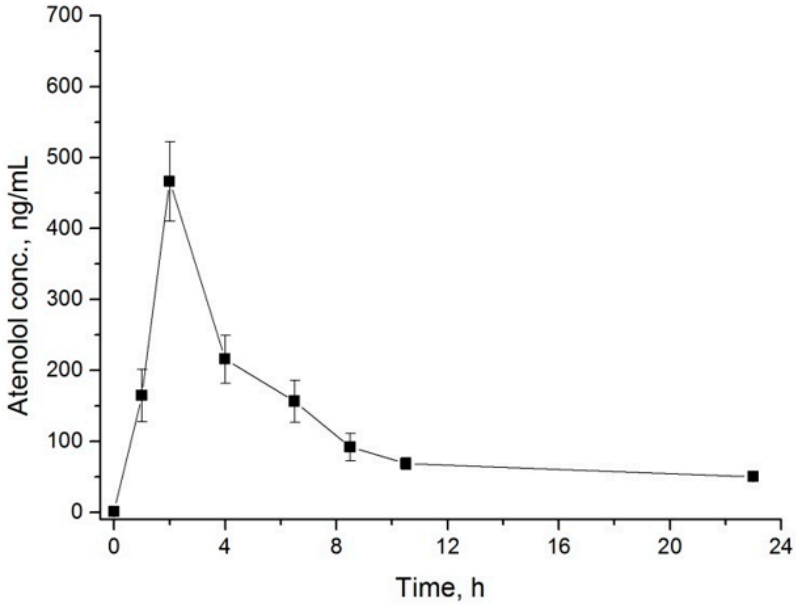

Figure 5. The mean plasma concentration–versus–time plot of atenolol after single oral administration of 50 mg to a healthy human volunteer.

4. General Discussion

To the best of our knowledge, only five studies report the development of a method for atenolol measurement in dried spots. Two methods were employed in the MRM mode using transition m/z 267.1→190.1 [1] or 267.1→116.1 [36] for atenolol quantification. In the other cases, researchers applied the HRMS technique by extracting the atenolol ion from a mass scan at m/z 267.1703 with tolerance 1 ppm [26] or 5 ppm [25,37]. Nevertheless, no one has used the PRM method for atenolol detection. Meanwhile, PRM has properties similar to those of MRM: it allows the detection of a molecule not only by exact mass but also by structure, with much greater accuracy than that of the MRM method.

The authors of the aforementioned studies used different procedures for atenolol extraction. Extraction with 300 µL of a solvent followed by vortexing for 1 min, sonication for 30 min at 40 °C, drying, and reconstitution with 150 µL of methanol:water (40:60, v/v) containing 0.1% of formic acid was applied in refs. [25,37]. A similar method with 500 µL of an extraction solvent, sonication for 15 min, drying, and reconstitution with 100 µL of acetonitrile:water (20:80, v/v) was used in ref. [1]. In other studies, researchers have dispensed with the stage of solvent evaporation to dryness and sample reconstitution. For instance, in ref. [26], the extraction involved 200 µL of a solvent, vortexing for 1 min, and sonication for 30 min. A shorter procedure was employed for pre-cut DBS: sonication for 5 min with 50 µL of water, followed by the addition of 50 µL of acetonitrile and second sonication for 5 min [36]. The advantage of our method is the avoidance of sonication and of the stage of reconstitution. Instead of an ultrasonic bath, we proposed using a shaker as more common equipment and only for 15 min. Moreover, in an ultrasonic bath, sonication can be uneven, thereby yielding different degrees of extraction. Our method is devoid of this possible disadvantage, is simple and short, and consists of one stage only.

Among the parameters of the methods being developed in this field, the LOQ and linear range are both essential for possible practical application. Therefore, researchers have tried to improve these parameters. Because different authors use different amounts of a sample (in this context, different spot sizes), different volumes of an extraction solvent, and different volumes of aliquots for MS, the LOQ actually is analyzed with different amounts of a sample. To compare real sensitivity among the published methods, it is necessary to take into account these parameters and recalculate the LOQ for the amount of a substance used for analyses (Table 4). The blood sample volume was estimated according to ref. [38], and the blood plasma sample volume was calculated in accordance with refs. [39,40]. The

amount of a sample analyzed as an LOQ (Table 4; the amount of a sample analyzed as an LOQ, pg) was computed via the following formula:

$$(\text{estimated sample volume} \times \text{LOQ} \times \text{aliquot for MS})/\text{final solvent volume}$$

where the estimated sample volume is the blood volume corresponding to dried spot size (Table 4; blood sample volume estimate, µL); the LOQ is lowest quantifiable concentration of the atenolol (Table 4; LOQ, pg/µL); the aliquot for MS is the volume utilized for the MS analysis (Table 4; the aliquot for MS, µL); and the final solvent volume is the total volume of the extraction solvent (Table 4; final solvent volume, µL).

Table 4. Amounts of a sample at the LOQ level.

Spot Size	Blood Sample Volume Estimate, µL	LOQ, pg/µL	Aliquot for MS, µL	Final Solvent Volume, µL	Amount of Sample Analyzed as LOQ, pg	Linear Range, ng/mL	Reference
6 mm	13	25	5	100	16.25	25–12,500	[1]
5 mm	9	25	5	200	5.63	25–1500	[26]
8 mm	23	10	20	150	30.67	10–1500	[25]
Pre-cut	2	2.4	2	100	0.10	2.4–24	[36]
8 mm	23	10	20	150	30.67	10–1500	[37]
3.2 mm	1.6 *	5	5	100	0.43	5–1000	Current study

* plasma volume.

The amount of a sample analyzed as an LOQ was lower only in one report [36] than in our study; specifically, the LOQ was only 2 times lower than ours was. In addition, in that study, the linear range was very narrow (2.4–24 ng/mL) [36] and applicable to trace analysis. In other studies, the LOQ was higher: 10 ng/mL [25,37] to 25 ng/mL [1,26], as was the amount of the sample analyzed as an LOQ. In addition, the linear range was extended to 1500 ng/mL [25,26,37] and even up to 12,500 ng/mL [1].

The method proposed in this study was developed to show the PRM technique's advantages over the MRM method for any possible practical application. The LOQ and linear range of our method was 5 and 5–1000 ng/mL, respectively. The method was successfully applied to the real DPS samples, and the atenolol concentration varied in the 40–442 ng/mL range. In various studies on biological samples, atenolol concentration varied in the following ranges: after oral administration of 50 mg of atenolol, the range was 15–420 ng/mL in ref. [30]; the range was 15–450 ng/mL [34], 20–325 ng/mL [26], 25–525 ng/mL [6], and 20–650 ng/mL after oral administration of 100 mg of atenolol [35,41]. Thus, the validated method's LOQ and linear range fulfilled the requirements for practical application. Moreover, the signal-to-noise ratio at the achieved LOQ in the validated method proved to be 86, and a further decrease in concentration was limited only by tolerance >20%. Therefore, we could reduce the LOQ by improving this parameter in further studies.

5. Conclusions

This work demonstrated the advantages of the PRM method in LC–HRMS; this method helped us to achieve an LOQ of 5 ng/mL. The PRM method was validated in terms of precision, accuracy, selectivity, and linearity within the 5–1000 ng/mL analyte range. In addition, the DPS samples containing atenolol showed good stability after storage for 30 days in a postal envelope at room temperature. This HRMS method was designed to improve the accuracy of the atenolol detection (without compromising the other method parameters) in order to simplify cardiovascular drugs' monitoring and improve the convenience of this approach in preclinical or clinical pharmacokinetic studies.

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