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Extraction Procedure Optimization of Atenolol from Dried Plasma Spots

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Authors' contributions

This work was carried out in collaboration between both authors. Author VVK designed the study, wrote the protocol and managed the literature searches. Author AAC managed analyses, performed the statistical analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: Atenolol is one of the β-blockers widely used for the treatment of hypertension and other cardiovascular diseases. To simplify the methods for determining of drugs concentrations in blood and plasma the dried spots assays (dried blood spots or dried plasma spots) could be used. In this case high sensitive detector like mass-spectrometer is required as well as high level of drug recovery from dried spot. In this study the extraction of atenolol from dried plasma spots (DPS) was studied to offer the optimum parameters of extraction method.
Study Design: Short research articles.
Place and Duration of Study: Core Facility of Mass Spectrometric Analysis, Institute of Chemical Biology and Fundamental Medicine SB RAS, between January and October 2019.
Methodology: The organic extraction method was chosen for evaluation as the most suitable for LC-MS assay. Several parameters: % of organic solvent, presence or absence of 0.1% formic acid,

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time, volume and temperature of extraction were investigated to find the best combination for atenolol recovery from DPS for further LC-MS analysis.
Results: Results showed that the solvent composition and temperature has main influence on the extraction. The effect of extraction time and volume of solvent have no significant influence on atenolol recovery. Pure acetonitrile is the worst solvent for atenolol extraction from DPS. The solvents: MeOH:H_2O (60:40, v:v), MeOH:0.1% FA in H_2O (60:40, v:v), ACN:0.1% FA in H_2O (50:50, v:v) or ACN:MeOH (60:40, v:v) provide the best recovery of atenolol. The optimum extraction temperature is 40°C, time of extraction is 15-30 min and volume of solvent - 200-300 μL.
Conclusion: Several solvents acceptable for LC-MS analysis with optimized recovery parameter from DPS can be used for routine extraction of atenolol.

Keywords: Dried plasma spot; DPS; DBS; atenolol; LC-MS/MS; extraction.

1. INTRODUCTION

Cardiovascular diseases are the leading cause of mortality in the world [1]. The main attributable risk factor of death is hypertension. The hypertension is also responsible for 50% of the cases of ischemic and cerebrovascular heart diseases [2,3]. For hypertension treatment various classes of antihypertensive drugs have been developed: diuretics, calcium channel blockers, α blockers, β blockers, etc. [2]. For example, atenolol was among the 200 most prescribed drugs in the United States in 2003 with 4th rank.

Atenolol is a representative of β blockers class. The chemical structure of atenolol, [4-(2-hydroxy-3-isopropylaminopropoxy) phenyl acetamide], is presented at Fig.1. It is used for the treatment of cardiovascular disease including hypertension, angina pectoris and arrhythmia [4].

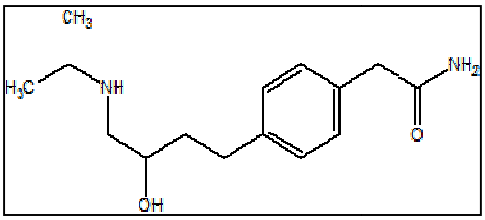


Fig. 1. Chemical structure of atenolol

The use of dried blood spots (DBSs) can simplify the methods for determining the concentrations of drugs [2]. The DBS sampling technique is suitable even for small children [5] and is ideal for routine clinical testing [4]. DBS helps with recruitment of subjects for preclinical or clinical studies [6] due to simpler storage and easier transfer by mail to the assigned analytical laboratory [2].

The DBS technique minimized the risk of infection with of infectious pathogens, including HIV [7]. In addition, DBS offer an easier transfer and simpler storage with minimum unnecessary costs [8,9]. The above advantages, coupled with the possibilities of mass spectrometry, allow the use of this methodology for various applications, including preclinical or clinical pharmacokinetic studies [6,10-13] and therapeutic drug monitoring [14,15].

The hematocrit is one of the reasons why incorrect values of drug concentration could be obtained using DBS. Using the DPS can solve this problem. In several works the DBS assay was used for atenolol determination [2], but there are no studies for atenolol quantification in DPS. Moreover only in one recent research authors discuss the atenolol extraction from DBS [16]. The aim of this study was to find the optimized parameters of atenolol extraction from DPS.

2. MATERIALS AND METHODS

2.1 Reagents

Atenolol, formic acid (FA) and Whatman 903 Protein Saver Card were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and methanol of LC-MS grade were purchased from Panreac AppliChem (Barcelona, Spain). Water was purified by means of a Milli-Q system from Millipore Corp. (Bedford, USA). Nitrogen gas (ultrapure, >99.9%) was produced by an Agilent 5183-2003 nitrogen generator (Agilent Technologies, USA).

2.2 Equipment and HPLC-MS/MS Conditions

Mass spectrometry analysis was carried out in the Core Facility of Mass Spectrometric Analysis (ICBFM SB RAS). Chromatographic separation

of the samples was achieved using a Agilent 1200 HPLC (Agilent Technologies, USA) equipped with Zorbax Eclipse XBD-C18 guard column (4.6 × 12.5 mm, 5 μm) followed immediately by EcoNova ProntoSil-120-3-C18 (2 × 75 mm, 3 μm) analytical column (EcoNova, Russia). The column oven temperature was set to 30°C. Sample injection volume was 10 µl. The flow rate was 0.4 mL/min and the gradient was composed of water containing 0.1% (v/v) formic acid (eluent A) and acetonitrile containing 0.1% (v/v) formic acid (eluent B). The mobile phase was initiated at 50% B and increasing to 90% B by 3.0 min, returning to 50% B by 0.1 min and held until 5.0 for washing. The total run time was 5 min. The autosampler temperature was held at 4°C.

MS/MS detection was performed on an Agilent 6410 QQQ mass spectrometer (Agilent Technologies, USA). Analytes were detected in positive ionization mode using multiple reaction monitoring. The capillary voltage was set to 4000V, and the gas temperature was set to 300°C. The nebulizer gas pressure and flow were 30 psi and 8 L/min, respectively. Dwell time was set to 200 ms. The ion transitions for atenolol were m/z 267.2→145.1 (collision energy 25 V, fragmentor voltage 135 V) as a quantifier; m/z 267.2→190.1 (collision energy 15 V, fragmentor voltage 135 V) and m/z 267.2→225 (collision energy 7 V, fragmentor voltage 135 V) as qualifiers. Signal output was captured and processed with the MassHunter software v.3.0. All LC-MS measurements were performed in duplicate.

2.3 Preparation of Samples

Stock solution and working samples were prepared in same way as described in work [9]. Briefly, atenolol was dissolved in acetonitrile to prepare a 10 mg/mL stock solution. The atenolol stock solution was diluted with acetonitrile to prepare intermediate stock solution that was added to blank rat plasma to create working solution with atenolol concentration of 1,000 ng/mL. All stock and working solutions were freshly made on the day of the analysis and were stored at 4°C before use. The working samples with final plasma concentration of atenolol of 1,000 ng/mL (each consisting of 25 μL of rat plasma) was placed on a Whatman 903 Protein Saver Card (GE Healthcare, USA) to fill the circles on the card and was air dried completely overnight. After that, 3.2 mm circles of DPS were cut out by means of a DBS Puncher, and each circle was placed in a 1.5 mL Eppendorf tube.

2.4 Solvents Preparation

Five different types of solvents were prepared. The first one: MeOH:H_2O mixture from 50% to 100% of MeOH (v:v) with 10% step. The second one: MeOH: 0,1% of FA in H_2O mixture from 50% to 100% of MeOH (v:v) with 10% step. The third one: ACN:H_2O mixture from 50% to 100% of ACN (v:v) with 10% step. The fourth one: MeOH:0,1% of FA in H_2O mixture from 50% to 100% of ACN (v:v) with 10% step. The last one: MeOH:ACN mixture from 0% to 100% of MeOH (v:v) with 10% step.

2.5 Extraction Procedure

The organic extraction method was used to optimize the extraction parameters. In general, organic solvent directly adds to DPS samples and then extraction is carried out under certain conditions. All experiments were conducted with three replicates.

2.5.1 Solvent selection

The 300 μL of solvent was added to 3.2 mm circles of DPS placed in 1.5 mL Eppendorf tube. Samples were incubated on a shaker (TS-100C; BioSan, Latvia) at 900 rpm for 1 h at 30 °C. After centrifugation for 10 s at 1000 ×g, 250 μL of the solution was transferred to a 300 μL vial for further LC-MS analysis.

2.5.2 Extraction time selection

The extraction was carried out as for solvent selection but with different extraction time: 15 min, 30 min, 45 min, 60 min, 75 min and 90 min.

2.5.3 Extraction temperature selection

The extraction was carried out as for solvent selection but with different extraction temperature: 30°C, 40°C, 50°C, 60°C, 70°C and 80°C.

2.5.4 Solvent volume selection

The different solvent volume: 200 μL, 300 μL, 400 μL, 600 μL, 800 μL and 1000 μL was added to 3.2 mm circles of DPS placed in 1.5 mL Eppendorf tube. Samples were incubated on a shaker (TS-100C; BioSan, Latvia) at 900 rpm for

1 h at 30°C. After centrifugation for 10 s at 1000×g, solutions were transferred to a new Eppendorf tubes. The solvent was evaporated to dryness using Labconco SpeedVac systems (Labconco, USA). Samples were reconstituted in 100 μL of MeOH and transferred to a 300 μL vial for further LC-MS analysis.

3. RESULTS AND DISCUSSION

Among three extraction methods (organic extraction, liquid-liquid extraction and protein precipitation) the organic extraction method is most suitable for extraction from DPS.

Protein precipitation or liquid-liquid extraction are a two-step extraction methods where in first step the blood or plasma spot extracted into the aqueous phase and then proteins from the DBSs precipitated by acetonitrile or target substance extracted in water-immiscible organic solvent, respectively. Organic extraction is a one-step extraction method that simply adds organic solvent directly to DPS samples [16]. In this case, proteins and red blood cells remain inside the stain, and the target substance is extracted into a solvent. Acetonitrile and methanol are most suitable solvents for using in further LC-MS analysis. Therefore for atenolol extraction from DBS researchers used 150 μL, MeOH:H_2O (70:30, v/v) [17], 300 μL, MeOH [18], 200 μL, MeOH:H_2O (60:40, v/v) [4], 500 μL of ACN:MeOH (1:2, v/v).

At the first stage of this study, the selection of the solvent providing the greatest recovery was carried out, since there is no data on extraction atenolol from a DPS, and researchers used different solvents with different volumes for extraction from a DBS. Different types of solvents consisting of a mixture of MeOH or ACN with water in the presence or absence of 0,1% of FA as well as different MeOH:ACN mixtures were prepared (Table 1).

To compare the effectivity of atenolol extraction by solvents, all these experiments were carried out under the same conditions in triplicate. Each sample was analyzed twice by the LS-MS method. The results are shown in Fig. 2. Almost all solvent mixture showed approximately the same efficiency, excluding 100% ACN (Fig. 2b, 2d, 2f).

The adding 0,1% of FA to the solvent leads to a slight increase (by 15–20%) in the recovery of atenolol (Fig 2a,2b). For MeOH:H_2O mixture a tendency for a recovery decrease with an increase in % of MeOH was observed (Fig. 2a,2c).

No significant changes in the MeOH:ACN mixture from 100 to 50% of MeOH were detected (Fig 2e); the greatest efficiency was demonstrated at 60% of ACN with a subsequent decrease in atenolol recovery with a further increase % of ACN (Fig. 2f).

These data do not correlate quite with the work [16] where the ratio ACN:MeOH (1:2, v/v) was selected for atenolol extraction from DBS. From other hand, the difference in recovery is negligible: 1201 ± 40 (signal area) for 60% of ACN and 1112 ± 71 (signal area) for 40% of ACN.

Since solvents showed approximately the same extraction efficiency, excluding 100% ACN, 4 solvents were chosen to optimize other extraction parameters: MeOH:0,1% FA in H_2O (60:40, v/v), MeOH:H_2O (60:40, v/v), ACN: 0,1% FA in H_2O (50:50, v/v) and MeOH:ACN (40:60, v/v).

The next step was to determine the optimum extraction time. The extraction was carried out with different times from 15 min to 90 min with 15 min steps for each chosen solvent mixture (Fig. 3).

Significant differences in the efficiency of extraction of atenolol with increasing incubation time were not found (Fig 3), as well as with an increase in the volume of solvent (Fig. 4).

Table 1. Solvent composition

MeOH:H_2O, % MeOH	MeOH:H_2O, (0,1% FA), % MeOH	ACN:H_2O, % ACN,	ACN:H_2O, (0,1% FA), % ACN,	MeOH:ACN, % MeOH	MeOH:ACN, % ACN
100	100	100	100	100	100
90	90	90	90	90	90
80	80	80	80	80	80
70	70	70	70	70	70
60	60	60	60	60	60
50	50	50	50	50	50

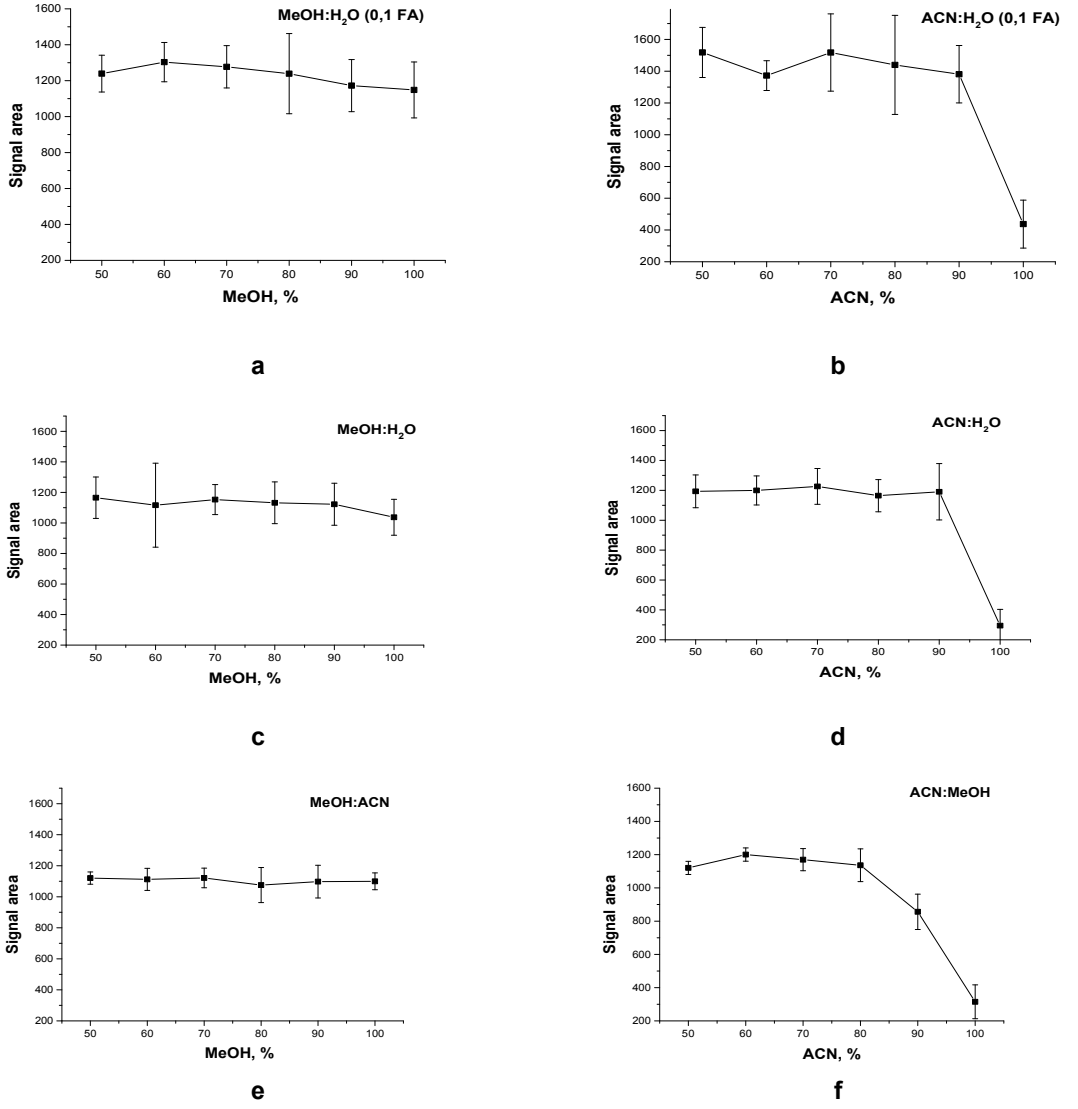
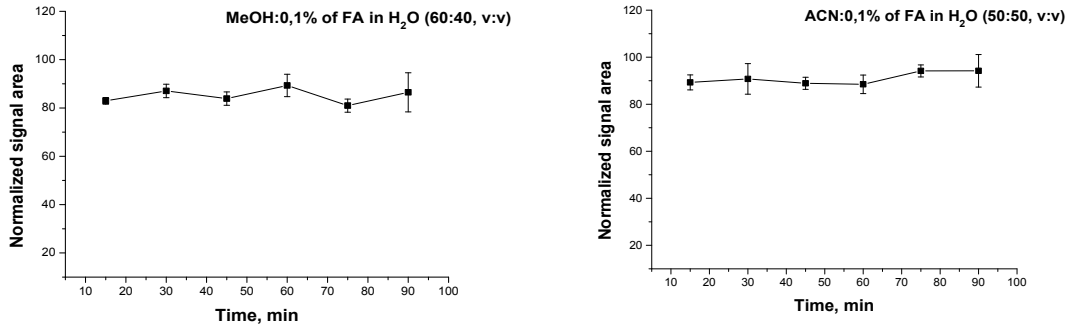


Fig. 2. Atenolol recovery from DPS at the concentration 1000 ng/ml by different solvents: MeOH: 0,1% of FA in H_2O mixture (a), ACN: 0,1% of FA in H_2O mixture (b), MeOH:H_2O mixture (c), ACN:H_2O mixture (d), MeOH:ACN mixture (e and f)



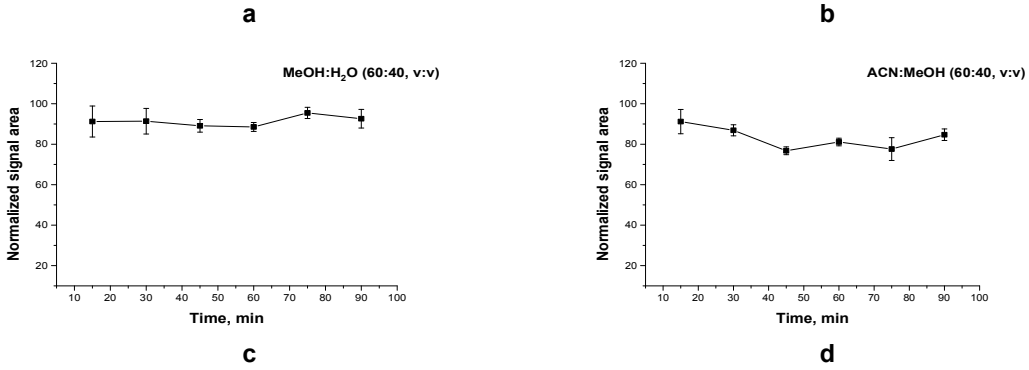


Fig. 3. Normalized atenolol recovery from DPS at the concentration 1000 ng/ml at different extraction times: MeOH: 0,1% of FA in H_2O mixture (a), ACN: 0,1% of FA in H_2O mixture (b), MeOH: H_2O mixture (c), MeOH:ACN mixture (d)

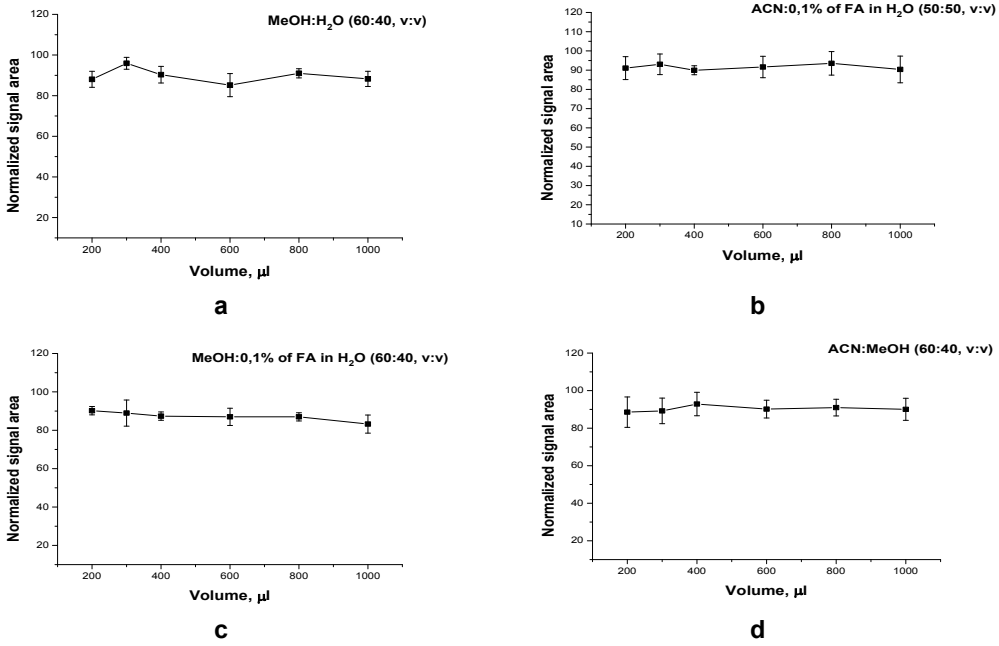
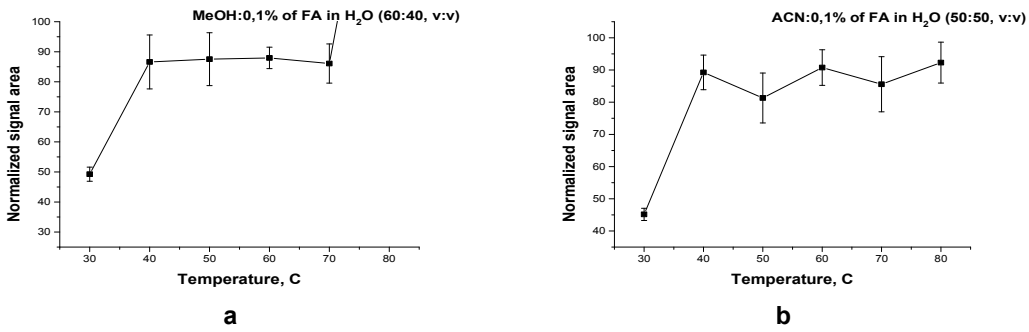


Fig. 4. Normalized atenolol recovery from DPS with different solvent volume: MeOH:0,1% of FA in H_2O mixture (a), ACN:0,1% of FA in H_2O mixture (b), MeOH:H_2O mixture (c), MeOH:ACN mixture (d)



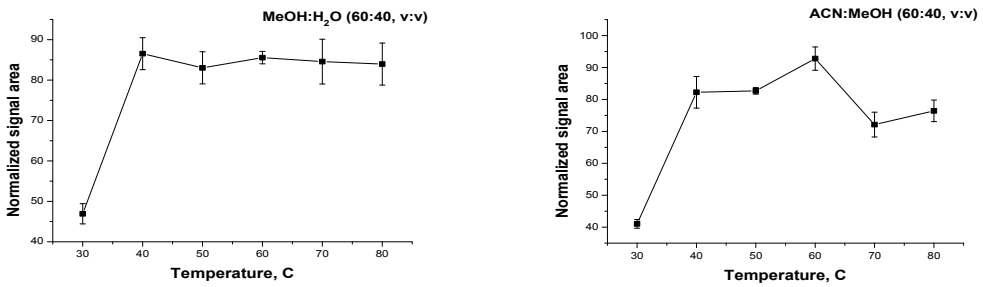


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Fig. 5. Normalized atenolol recovery from DPS at different temperature: MeOH:0,1% of FA in H_2O mixture (a), ACN:0,1% of FA in H_2O mixture (b), MeOH:H_2O mixture (c), MeOH:ACN mixture (d)

In this work, to compare different volumes of solvent, the samples were evaporated to dryness and reconstructed in the same volume. For a one-step extraction method without this procedure, an increase in solvent volume will lower the signal level. This in turn will adversely affect the lower detection limit of the substance. The volume 200-300 μL will be enough for the extraction of atenolol from a 3 mm disk of DPS.

Temperature rise from 30°C to 40°C increased recovery by about 2 times. Whereas further temperature raises leads to the evaporation of solvents from closed tubes, to loss of solvent volume and as a results the increase of measurement error. It was most appreciable for the MeOH:ACN solvent, since such mixture has a high fluidity.

Therefore, extraction at high temperature cannot be carried out using the MeOH:ACN mixture as a solvent. As seen in Fig. 5, an extraction temperature of 40°C is sufficient for the effective extraction of atenolol from DPS. This is consistent with data from work [18] where the authors sonicated samples of DBS for 30 mins at 40°C.

4. CONCLUSION

In this study, extraction method was optimized for the determination of atenolol in DPS samples. The method was tested in terms of extraction dependence on temperature, time, as well as type and volume of solvent. It was shown that the optimal extraction parameters are temperature of 40°C, incubation time up to 30 minutes, 200-300 μL of solvent. Subject to further LS-MS analysis, mixtures of MeOH with H_2O or ACN can be used as solvents. Pure ACN is not a suitable solvent for the extraction of atenolol. Add of 0,1% of FA to solvents mixtures slightly increase the recovery of atenolol from DPS, but without reliable confirmation. For better optimization, it is necessary to carry out additional experiments with detailed parameterization in the range established in this work.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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