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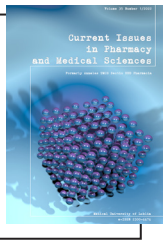

Determination of β-blocking receptor drugs in silica gel TLC systems with the mobile phase containing surfactant

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ABSTRACT

Eight drugs blocking beta-adrenergic receptors activity (acebutolol, alprenolol, atenolol, oxprenolol, labetalol, metoprolol, propranolol and sotalol) were investigated through the use of the thin-layer technique with it should be the mobile phase containing surfactant. Assessment of the effect of surfactant presence and 1-propanol concentration in the mobile phase on the retention and separation of investigated solutes was then carried out wherein the influence of the surfactant concentration on the zone shape properties (asymmetry and tailing coefficient) was investigated. The method was applied for the quantitative analysis of the chosen solutes, and the LOD and LOQ values of chosen were determined. These were as follows: acebutolol – 1.11 and 3.36 μg/spot, metoprolol 1.45 μg/spot, 4.4 μg/spot. The chosen system is environmentally friendly due to using silica gel plates and only 5% of propanol in water.

IINTRODUCTION

Beta-adrenergic blocking drugs are of great interest due to their clinical efficacy in treating hypertension, ischemic heart disease, congestive heart failure and arrhythmias. Ahlquist noticed that catecholamines affect the body by activating β receptors. Therefore, it has become the impulse for synthesising and pharmacological evaluation of β receptor antagonists [1]. Currently, β receptor antagonists include about 50 compounds based on the structure aryloxypropanolamine and 1-aryl-2-alkyloaminoethanol [2,3]. However, structural differences result in different physical and chemical properties and pharmacological effects [4].

Due to their great application, the qualitative and quantitative analyses of the abovementioned group of drugs were performed using many chromatographic techniques. A review on applying high-performance liquid chromatography systems to the analysis of beta-blockers in biological samples was presented in [5]. In [6], the HPLC system linked with Quadrupole Orbitrap High-Resolution Mass Spectrometry was applied to the resolution of 27 beta-blockers and their metabolites from milk powder samples. In [7], the column-switching (LiChrospher RP-4 and Phenomenex Gemini Phenyl Hexyl 110 A) HPLC-DAD systems were applied, together with gradient of the mobile phase for separation various classes of drug, including beta-blockers (metoprolol, timolol, bisoprolol, propranolol, carvedilol and nebivolol) and their metabolites.

A review on the separation of beta-blockers with the use of thin-layer systems was presented by Gumieniczek and Berecka in [8]. Moreover, Ogrodowczyk and Marciniec applied a normal phase TLC system as one of the methods (UV, FT-IR, MS) to examine six beta-blockers (acebutolol, alprenolol, atenolol, metoprolol, pindolol and propranolol) [9]. This technique linked with densitometry was applied for the validated analysis of chosen beta-blockers (atenolol, acebutolol, propranolol, and bisoprolol) in pharmaceutical formulation by Krzek and Kwiecień [10].

Due to the basic character of beta-blockers, the ion-pairing or micellar system can be successfully used to enhance the separation capability. Gallegos and his co-workers proposed a new approach involving ion-pairing RP-HPLC systems to investigate the timolol in human plasma [11]. This technique, together with the micellar mobile phase systems, was applied to the determination of various beta-blockers by M. C. Garcıa-Alvarez-Coque and her team [e.g., 12-16]. The effect of the surfactant, its kind, organic solvent, and column type on the separation of beta-blockers and other compounds were presented in [12,14]. The presence of surfactant in the mobile phase also affected the peak shape and the mixture separation [13].

Micellar RP-HPLC, combined with gradient mobile phase elution was applied to separate the beta-blockers mixture in [15]. The authors also utilised the Drylab® software

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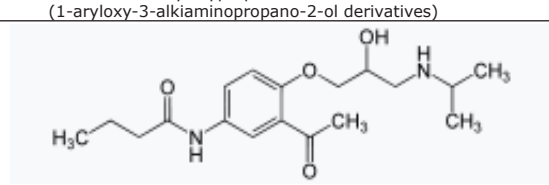
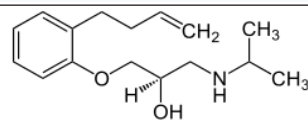
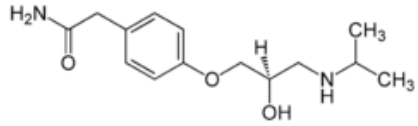
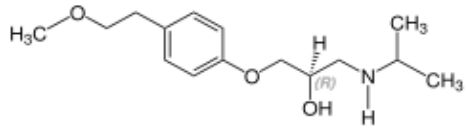
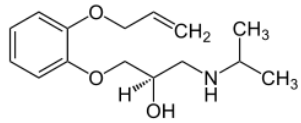
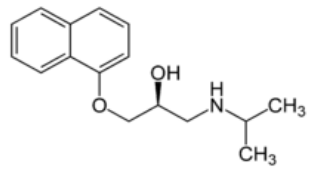
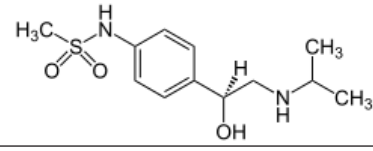
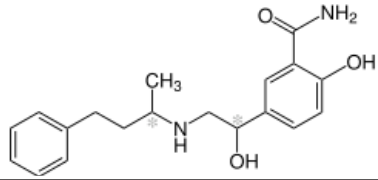
to optimise the gradient conditions and successfully applied it to the determination of beta-blockers in the urine sample. The isocratic and gradient elution were also used to separate several drug classes (beta-blockers, sulfonamides, and flavonoids) [16].

Drug determination due to economic reasons should be cheap, easy to use in routine analysis and respond to green chemistry postulates (be nature friendly). The first postulate is fulfilled with thin-layer chromatography linked to modern detection techniques. At the same time, the application of HILIC systems enriched with surfactants satisfied the second. Thus, our work aimed to determine the possibility of a TLC normal phase system containing surfactant to analyse several beta-blockers.

MATERIALS AND METHODS

All investigated β-blockers were purchased from the following sources: acebutolol hydrochloride, alprenolol, atenolol and labetalol from ICN, Biomedicals Inc, (ICN, Biomedicals Inc, Aurora, Ohio, USA), metoprolol tartare was from Zakłady Farmaceutyczne (Zakłady Farmaceutyczne w Starogardzie Gd., Poland), oxprenolol (coretal), propranolol and sotalol were the gift from P.Z. Comindex (P.Z. Comindex, Rzeszów, Poland). The sodium cholate and sodium dodecylsulphate were from Sigma -Aldrich (St. Louis, MO, USA). The chemical structures and physicochemical properties of investigated solutes are presented in Table 1.

Table 1. Chemical structures and physicochemical properties of the investigated β-blockers

Solute	Chemical structure	pKA*	Log P**
1-aryloxypropanolamines (1-aryloxy-3-alkiaminopropano-2-ol derivatives)			
acebutolol (second generation β1 selective)		9.65	1.43
alprenolol (first generation, nonselective)		9.67	2.59
atenolol (second generation β1 selective)		9.67	0.57
metoprolol (second generation β1 selective)		9.67	1.80
oxprenolol (coretal) (first generation, nonselective)		9.67	2.44
propranolol (first generation, nonselective)		9.67	3.03
1-aryl-2-alkiloamino ethanol derivatives			
sotalol (first generation, nonselective)		9.43	0.85
1-hydroxy-1-arylethylamino derivatives			
labetalol (third generation, non-selective)		9.8	1.73

*Log P and **pKa values were found using the Drug-bank website (www.drugbank.com)

The mobile phase component (propan-1-ol) was obtained from Avantor Performance Materials (Poland SA, Gliwice, Poland). This manufacturer was also the source of methanol. The redistilled water was produced in the Department of Physical Chemistry. The silica-gel 60 F_{254} 10×20 chromatographic plates were received from Merck (Darmstadt, Germany).

The test solution was prepared by dissolving 1 mg of the investigated β-blockers in 1 mL of methanol. The test solutions were stored in the refrigerator. The samples were applied onto the sorbent layer using a Camag TLC Sampler 4 (ATS 4, Camag, Muttenz, Switzerland) in forms of bands of 6 mm length. The volume of applied compound was 2 µL per band. The ready to use plates (silica-gel 60 F_{254}, Merck) 10×20 were cut using the chromatographic plate cutter into 10×10 cm size. They were then activated at 105°C for 15 minutes and left to cool in a desiccator before use. The chromatograms were developed in a horizontal 10×10 DS chamber (Chromdes, Lublin, Poland). The eluent distances for the majority of chromatograms were 45 mm, except for solute quantitative determination (95 mm). After development, the eluent was evaporated from the fume hood plate. The localisation of the solute spots was determined with VU light (254 nm), using a TLC Scanner (Camag, Muttenz, Switzerland) or TLC Visualiser (Camag,Muttenz, Switzerland).

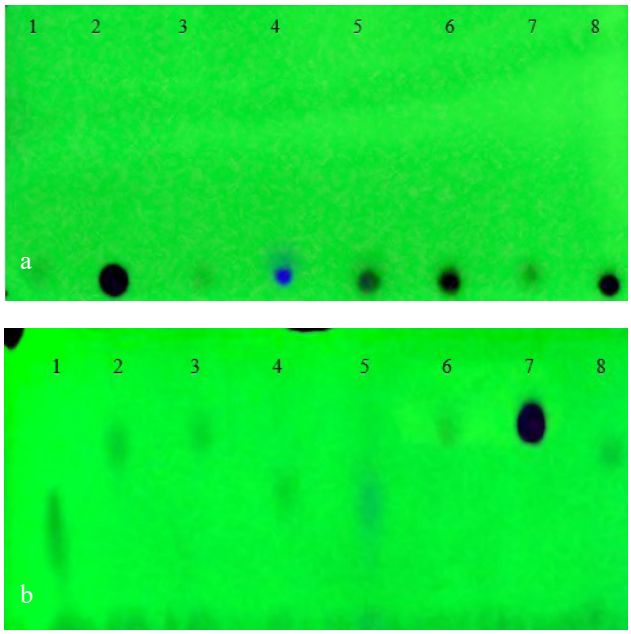
RESULTS AND DISCUSSION

Due to their physicochemical properties, interactions between β-blocker molecules and silica gel result in strong retention. Several possibilities may be applied to diminish this. For example, a buffer of high pH value could be employed as a mobile phase component, although such a procedure damages the stationary phase. Another approach is to utilize an ion-pairing agent in the eluent [11]. Moreover, some authors use the surfactant HPLC mode [12-16]. Unfortunately, the presence of the abovementioned mobile phase additives reduce solute retention. Of note, TLC systems containing surfactants were broadly explored by Sumina *et al*. [17].

Sodium dodecyl sulphate is the most popular anionic surfactant. However, since sodium cholate (NaC) is bile salt of a large structure with a rigid and hydrophobic steroid core, it is used less frequently in research. In addition, the amphiphilic properties of NaC are different from common aliphatic surfactant molecules [18]. These properties have found wide use in developing micelle-based drug delivery systems [19].

The effect of surfactant

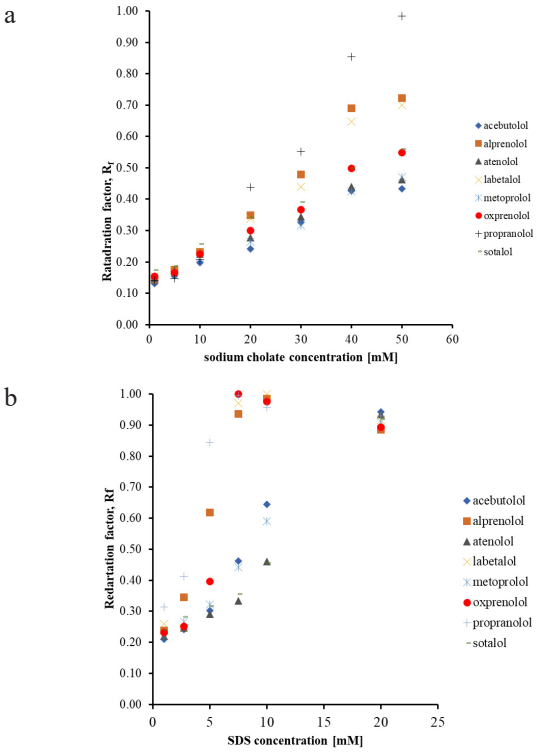
By coincidence, we have noticed that adding the surfactant to the mobile phase decreases the retention of the investigated beta-blockers, which results in the separation of solute zones. The comparison of two chromatographic systems without (a) and with surfactant (b) is presented in Figures 1a, b. The eluent consisted of propan-1-ol, and water (5:95 v/v) was applied in both systems. A high content of sodium cholate (30 mM) was used as the mobile phase additive in the second system.



The investigated solutes: 1 – metoprolol, 2 – acebutolol, 3 – atenolol, 4 – oxprenolol, 5 – sotalol, 6 – labetalol, 7 – alprenolol, 8 – propranolol

Figure 1 a,b. Comparison of the chromatogram photos developed without (a) and with surfactant (b) in the mobile phase (5% of propanol in distilled water): (a), 5% of propanol in distilled water and (b) sodium cholate (30 mM). Stationary phase: silica gel

This observation led to a deeper investigation of the effect of surfactant content and its kind in the eluent on solute retention. The results are presented in Figures 2 a, b. The experiments used sodium cholate and sodium dodecylsulphate as the mobile phase components.



(a) – sodium cholate (concentration range 1-50 mM), (b) – sodium dodecylsulphate (concentration range 1-20 mM). The mobile phase: 5% of propanol in distilled water. Stationary phase: silica gel

Figure 2 a,b. The effect of the surfactant concentration and its type on the solute retardation factor

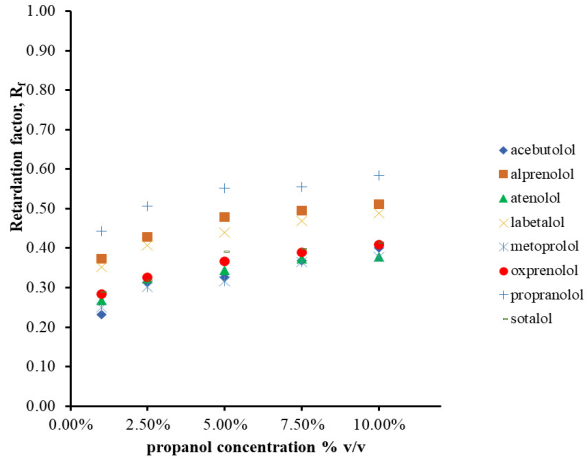
The presence of surfactant (sodium cholate or sodium dodecyl sulphate) diminishes solute retention. Both surfactants differ in the aggregation number and critical micelle concentration (CMC) values. The aggregation number in water at 25°C is 2-3 and 62 for sodium cholate and SDS, respectively. CMC is 9-15 and 7-10 for sodium cholate and SDS, correspondingly, at the same conditions [20]. Thus, their effect on solute retention varies. The relationships solute R_f *vs*. sodium cholate concentration in such a mobile phase are flatter than for a system containing SDS.

Considering the first system with sodium cholate, a small surfactant additive (1-5 mM) has an insignificant effect on the solute retentions. This concentration range does not separate the tested solute. However, the enhancement of surfactant concentration range from 5-10 mM slightly decreases the solute retentions. Moreover, application of the mobile phases containing more than 20 mM of sodium cholate enhances R_f values and improves the solute separation. Unfortunately, a concentration higher than 30 mM surfactant deteriorates the distinguishing zone. The order of the solute retention increasing for 30 mM of sodium cholate is as follows: propranolol > alprenolol > labetalol > sotalol > oxprenolol > atenolol > acebutolol > metoprolol. This order is consistent with the log P diminution for the first generation β-blockers (propranolol, alprenolol, oxprenolol, sotalol) and is not consistent with the and remaining group representatives (second generation β_1 selective drugs; metoprolol, acebutolol and atenolol) (for log P see Table 1).

The SDS presence in the mobile phase has a different effect on solute retention compared with sodium cholate. The R_f vs. SDS concentration plots for the investigated compounds are steeper. Furthermore, applying a system with a smaller amount of SDS (1-5 mM) decreases beta-blocker retention in a more significant mode than a system containing sodium cholate of the same concentration range. This outcome may indicate that the eluotropic strength of eluent with SDS is higher than sodium cholate, and the interactions between this surfactant and beta-blockers are stronger than sodium cholate are stronger than for the sodium cholate system. Also, it is worth adding that SDS can more strongly modify the stationary phase surface. In addition, the increasing surfactant content in the mobile phase lowers solute retention and improves their zone separation. However, a SDS content in the mobile phase higher than 10 mM significantly results in weak retention and diminishes the zone separation. In contrast, quite good separation is observed for systems containing 7.5 mM of SDS. The order of the solutes for this SDS content is as follows when starting from the highest retardation factor: oxprenolol and propranolol > labetalol > alprenolol > acebutolol > metoprolol > sotalol > atenolol. This order is not consistent with the log P decrease presented in Table 1. Unfortunately, the applied eluent does not affect the baseline separation of the investigated solutes. It turns out that the presence of SDS at a higher concentration in the mobile phase worsens the zone compared to the system containing sodium cholate. Thus, this surfactant was applied for future investigation.

Influence of propan-1-ol concentration on the solute retention

One of the factors affecting the separation of mixtures in TLC is the composition of the mobile phase. The concentration of the propan-1-ol in the mobile phase was investigated. The range of this organic modifier was from 1-10 % v/v. The effect of the propan-1-ol content on the solute retention and separation is presented in Figure 3.

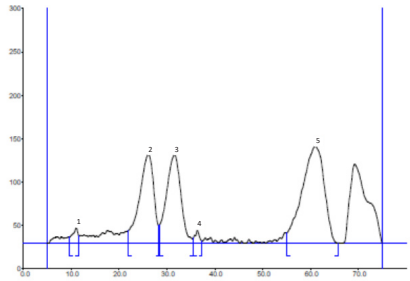


Mobile phase: various concentration of 1-propanol in distilled water, sodium cholate (30 mM). The stationary phase: silica gel

Figure 3. The effect of concentration of 1-propanol in the mobile phase on the retention β-blockers

Increasing the 1-propanol content in the mobile phase results in higher elution strength and reduces solute retention. Unfortunately, the latter is not related to improving compound zone separation for all propan-1-ol concentrations. Thus, the best separation of the tested mixture was obtained when 5% v/v of this alcohol was in the mobile phase. In contrast, a higher concentrations of the mobile phase organic modifier (7.5 and 10% v/v) worsen the zone distinguishing.

Since the optimal composition of the mobile phase (30 mM of sodium cholate and 5% of propanol in distilled water) was determined thus, this system was applied to separate the solute mixture containing various generations of β-blockers (metoprolol (second generation β1 selective drug), propranolol and sotalol (both first generation, non-selective β blocking drugs)). The chromatogram is presented in Figure 4.

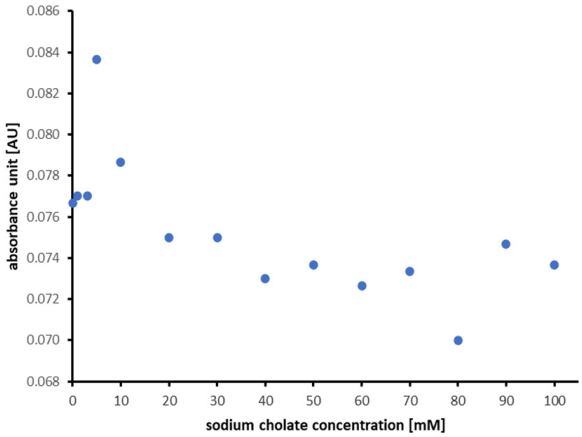


The mobile phase: 5% of propanol in distilled water, 30 mM of sodium cholate. Stationary phase: silica gel Si-60 HPTLC F_{254}plates

Figure 4. TLC-chromatogram of separation mixture of metoprolol (1), sotalol (2) and propranolol (5); peaks (1) and (4) unknown (possible contamination)

Determination of the mode of analysis

We wondered whether the chosen surfactant concentration was in submicellar or micellar range. Thus, we decided to determine the critical micelle concentration of sodium cholate. To this end, we prepared 14 solutions of various surfactant content (0-100 mM) in the propanol-water (5+95 v/v). To each, 600 µL of azorubine solution (0.02% w/v water solution w/v) was added. The effect of sodium cholate amount on the azorubine absorption of the visible light (510 nm) was then investigated. The measurement was based on the observation of the changes of the solution absorptivity. The solution with surfactant content equal to CMC now in the micelles form, results in enhancement or deterioration of this property. Considering the system with azorubin, both increase and decrease of absorptivity was noticed. The results are presented in Figure 5.



Sodium cholate range 0-100 mM, azorubine (0.02% w/v in water) 600 µL in each sample. The visible light wavelength: 510 nm. The presented absorbance data are average from three measurements

Figure 5. Spectrophotometric determination of CMC for the eluent containing sodium cholate. The mobile phase composition: propanol (5%) and water

We saw two abrupt changes in the solution absorbance (Figure 5). The first one, maximal, is observed at a concentration of 5 mM of sodium cholate, while the second minimal is at 80 mM of this surfactant. Thus, we can state that sodium cholate solutions exhibit two values of CMC. The first is sometimes presented as a "noncritical multimerisation concentration", and it refers to the formation of sodium cholate multimers (small aggregates) [21-23]. The fact of two values of CMC for bile salts (e. g. sodium cholate) is known from the literature [21-23]. What is more, the literature shown that the radius of the sodium cholate micelle depends on the surfactant concentration [24]. Thus, the change in the micelle radius can result in enhanced or diminished system absorptivity.

Thus, it turned out that the optimal sodium cholate concentration chosen for further investigation (30 mM) is within the range between two CMC values determined for this surfactant (5 mM and 80 mM). Since the first value refers to the formation of small aggregates, while the second value relates to stable micelle formation, the system used in our research is in the submicellar range.

The effect of the surfactant concentration on the solute shape

In the next step of our investigation, the impact of the surfactant concentration on the solute zone shape was determined with regard to its peak asymmetry (A_s) and tailing (T_f) factors. Table 2 shows the effect of sodium cholate concentration on the average values of A_s and T_f factors of all investigated solutes. Both investigated factors (A_s and T_f) describe the zone shape. The best Gaussian zones are observed when A_s and T_f are close to 1.0. Considering the effect of the sodium cholate on the average asymmetry and tailing factors, the closest to the ideal zones were obtained for the systems containing 30 or 40 mM of sodium cholate. Lower and higher concentrations of the surfactant resulted in zone fronting.

Table 2. The effect of surfactant concentration on the average solute asymmetry and tailing factors

Factor	Sodium cholate concentration [mM]					
	5	10	20	30	40	50
A_s	0.90 ±0.06	0.91 ±0.06	1.04 ±0.29	0.95 ±0.05	1.00 ±0.24	0.91 ±0.11
T_f	0.80 ±0.13	0.81 ±0.13	0.71 ±0.27	0.98 ±0.15	1.00 ±0.48	0.82 ±0.22

As = (a+b)/2a, where: b is the distance from the peak midpoint (perpendicular from the peak highest point) to the trailing edge of the peak measured at 10% of peak height (left peak half-width) and a is the distance from the leading edge of the peak to the peak midpoint (perpendicular from the peak highest point) to the trailing edge of the peak measured at 10% of peak height (right peak half-width)

Tf = b/a, where: b is the distance from the peak midpoint (perpendicular from the peak highest point) to the trailing edge of the peak measured at 10% of peak height (left peak half-width) and a is the distance from the leading edge of the peak to the peak midpoint (perpendicular from the peak highest point) to the trailing edge of the peak measured at 10% of peak height (right peak half-width)

Replicability of the measurement

The conditions presented below were also tested for the quantitative determination of the chosen compounds. Before this step, repeatability of measurement and inter-day reproducibility was investigated. For the first purpose, metoprolol and acebutolol solutions were applied using a Camag TLC Sampler 4 (ATS 4). The volume of each sample was 2 µl (2µg/spot). The plate was then developed, and after the mobile phase evaporation, the solute spot localisation was determined under VU light (222 nm) using a TLC Scanner 4 (Camag). The graphs of the obtained peaks for metoprolol are presented in Figure 6. Regarding metoprolol, the average migration distance was 15.92±0.04 mm (% RSD = 0.27%), the average zone area was 3406.9±150.9 (% RDS = 4.42%), and the average zone height was 117.35±2.71 (% RSD = 2.31%). Considering acebutolol, the average migration distance was 18.78±0.32 mm (% RDS = 1.74%), the average zone area was 12817.39±200 (%RSD = 1.56%), and the average zone height was 578.07±4.04 (% RSD = 0.7%). Moreover, the inter-day reproducibility for metoprolol was determined as % RSD. In terms of average zone height, this was 6.31%, while the zone area was 5.45. The same parameters for acebutolol were 2.66% (mean zone height) and 2.83% (average zone area).

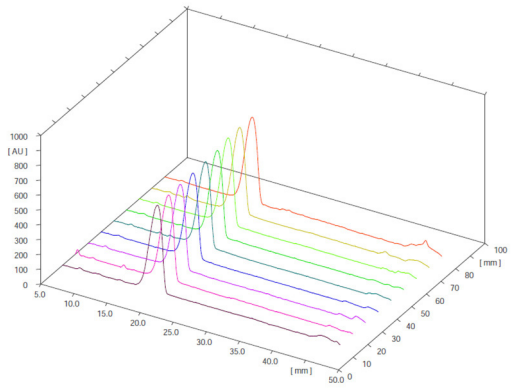


Figure 6. The replicability of the metoprolol sample development

Quantitative analysis

A calibration curve was prepared for the metoprolol, acebutolol, propranolol and sotalol samples. In undertaking this, various amounts of these solutes were applied to the chromatographic plate. After the development, the plates were evaporated and scanned using a TLC scanner (Camag) and 222 nm as the light wavelength. Figure 7 shows the systems of chromatograms used to prepare calibration curve for metoprolol. At the same time, the quantification data for chosen beta-blockers are presented in Table 3. The method was linear, with the derived R^2 values of 0.9978, 0.9982, 0.9990 and 0.9890 for metoprolol, acebutolol, propranolol and sotalol, respectively. The range for the detection was determined from 0.4-18 µg/spot for metoprolol, 0.4-16 µg/spot for acebutolol, 0.4-12 µg/spot for propranolol, and 2-12 µg/spot for sotalol. The determined limit of detections (LOD) and limit of quantification (LOQ) were 1.45 and 4.40 µg/spot for metoprolol, 1.11 and 3.36 µg/spot for acebutolol, 0.50 and 1.52 µg/spot for propranolol, 1.49 and 4.52 µg/spot for sotalol.

Table 3. Method quantification

Solute	Linear equation	R^2	Linear range µg/spot	LOD µg/spot	LOQ µg/spot
Metoprolol	Y= 679.85x+352.2	0.9978	0.4-18	1.45	4.40
Acebutolol	Y = 2171x+ 2341	0.9982	0.4-16	1.11	3.36
Propranolol	Y = 4683x+352,3	0.9990	0.4-12	0.50	1.52
Sotalol	Y = 1568x+5454	0.9890	1.0-12	1.49	4.52

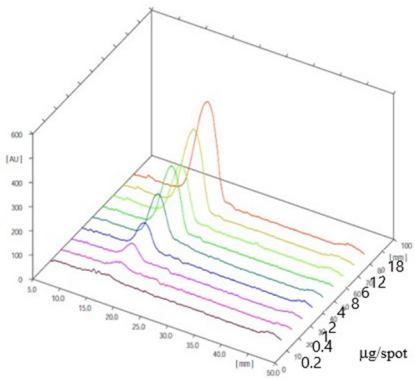


Figure 7. The systems of chromatograms used to prepare calibration curve for metoprolol

In the last step, the method was applied to compare the manufacturer-declared and the determined content of the investigated solutes. The results are presented in Table 4. In all studies, the error was below 4%, which demonstrates that this method can be used for quantitative research.

Table 4. Quantitative analysis in drug

Solute	Drug content [mg]	Determined content [mg]	Error [%]
Metoprolol	50	48.42	3.1%
Sotalol	80	79.85	0.18%
Propranolol	10	10.23	2.3

CONCLUSIONS

In this work, eight beta-blockers (sotalol, acebutolol, metoprolol, labetalol, alprenolol, atenolol, oxprenolol and propranolol) were investigated via TLC on silica-gel 60 F_{254} plates under eco-friendly (propan-1-ol, water and sodium cholate) conditions for the first time. Furthermore, the influence of propanol concentration and various surfactants was examined. The outcome of this activity was that the application of less popular amphiphile sodium cholate was found to result in better beta-blocker zone separation than the use of the more popular surfactant sodium dodecyl sulphate, SDS.

For the first time, such a system was used to quantify three β-blockers. The results show that TLC can be successfully used in all laboratories. Furthermore, this technique allows quick and inexpensive separation of a mixture of tested substances and their quantitative analysis.

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