Journal of Reports in Pharmaceutical Sciences 2018, 7(1), 79-87

Salt-Assisted Liquid-Liquid Extraction followed by High Performance Liquid Chromatography for Determination of Carvedilol in Human Plasma

Alireza Pourhossein and Kamal Alizadeh*

Department of Chemistry, Lorestan University, Khorramabad 6813717133, Iran

ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 2017-10-05
Revised: 2017-12-01
Accepted: 2017-12-10
ePublished: 2017-12-16

***Keywords*:**
Salting out assisted liquid-liquid chromatography
Carvedilol
HPLC
Human Plasma

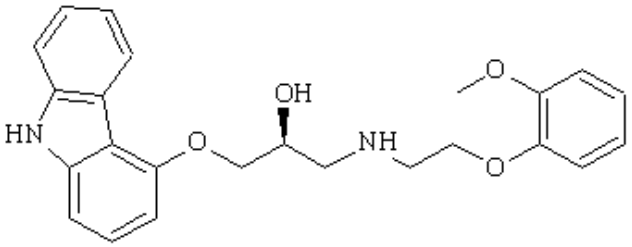
A B S T R A C T

A fast and simple method for extraction of carvedilol in human plasma samples based on salting out assisted liquid-liquid extraction (SALLE) is described. The method involving extraction of carvedilol with water-miscible organic solvent acetonitrile when solvent phase separation occurs using NaCl as a salt. The extracted phase was analyzed by high- performance liquid chromatography with ultra violet detection at 240 nm. The procedure has been optimized with respect to type and amount of salt, volume of sample, extraction solvent and the pH of solution. In the optimal condition the linear calibration range was 5-500 µg L^{-1} and the correlation coefficient was 0.9965. The limit of detection and limit of quantification were 1.0 µg L^{-1} and 3.3 µg L^{-1}, respectively and a relative standard deviation of 3.5 % for five replicates were obtained. In spiking experiments on real samples, the average recoveries found by the present method were between 96.0% and 105.0%.

*Corresponding Author: Kamal Alizadeh, E-mail: Alizadehkamal@yahoo.com

Introduction

Carvedilol (3- (9H-carbazol-4-yloxy)- 2-hydroxypropyl [2-(2-methoxyphenoxy) ethyl] amine) (scheme 1) which is used for the treatment of hypertension, ischemic heart disease and congestive heart failure [1]. It is an antihypertensive agent with beta and alpha -1-adrenergic receptor blocking activities [2, 3]. It has been prescribed as an antihypertensive agent and an angina agent [4, 5]. Carvedilol is a powerful anti-oxidant; it can protect major organs like heart, kidneys, brain, vasculature and so on from damage and hence is used in the treatment of chronic hypertension [6]. On oral dosing of racemic carvedilol, the unbound fraction of S (-)-enantiomer is higher than that of the R(+)-enantiomer and hence S(-)- gets distributed predominantly in all tissues like heart, lungs, liver, kidneys and is metabolized faster than R(+)-enantiomer [7].



Scheme 1. Structure of Carvedilol.

Several methods have been developed for the extraction and preconcentration of different drugs from biological sample matrices, such as liquid–liquid extraction (LLE) [8] solid-phase extraction (SPE) [9, 10], liquid-phase microextraction (LPME) [11, 12], solid-phase microextraction (SPME) [13, 14] and dispersive liquid-liquid microextraction (DLLME) [15–18]. LLE and SPE are time-consuming and expensive methods. In LLE high volume of toxic and hazardous organic solvents are required. LPME has the following disadvantages: fast stirring tending to break up the organic drop, air bubble formation, time-consuming extraction and equilibrium could not be attained after a long time in most cases. SPME is also expensive, its fiber is fragile and has limited lifetime and sample carry-over can be a problem. In DLLME, the choice of the extraction solvent is its main drawback and solvents with the densities higher than water are required and further, they are not often compatible with reverse phase HPLC. In addition, the high density extraction solvents, being mostly halogenated, are generally hazardous to laboratory personnel and the environment [19, 20]. Another extraction procedure, namely salting out assisted liquid liquid extraction, utilizes a phase separation phenomenon in a homogeneous solution and a very small collected phase is resulted. This method has been used for extraction and preconcentration of the selected analytes from aqueous samples [21, 22]. In SALLE addition of an electrolyte (or electrolyte mixtures) allows the weakening or the disruption of the inter-molecular forces between the organic and the aqueous phases. This means its main effect is in the separation of water-miscible organic solvents by the formation of a biphasic system [23]. Here we develop a suitable extraction method for determination of carvedilol in human plasma samples with high recovery. For that purpose, we take advantage of the general knowledge on SALLE and optimizing these approaches to fit our specific goal. The optimized procedure is successfully utilized in combination with HPLC for the determination of carvedilol in real samples.

Materials and Methods

Chemicals

Carvedilol used as standard was purchased from Sigma-Aldrich (Steinheim, Germany). LC-grade acetonitrile were obtained from Dae-Jung (South

Korea). Sodium chloride, magnesium phosphate, potassium phosphate monobasic and ammonium sulfate and sodium hydroxide were purchased all from Merck (Darmstadt, Germany).

Instrumentation

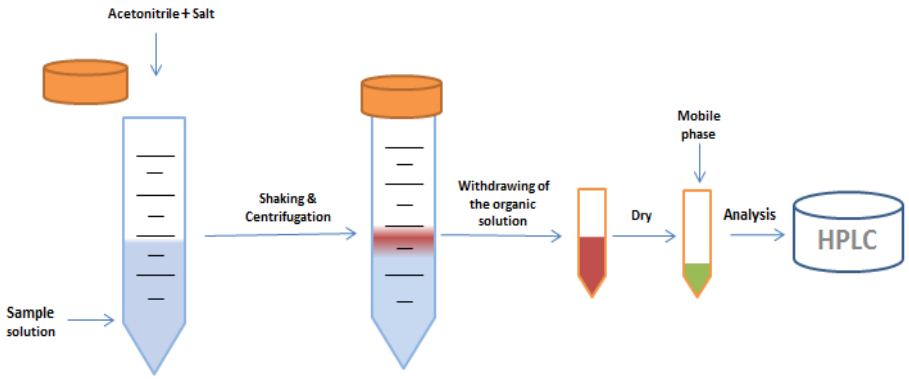
Analysis of the carvedilol was performed on a Knauer HPLC system (Berlin, Germany) equipped with a Smartline-1000 binary pumps and a Smartline-UV-2500 detector variable wavelength programmable, an on-line solvent vacuum degasser and manual sample injector fitted with a 20 µL injection loop. Chromatographic separation was achieved on a C8 column (15 cm × 4.0 mm with 5 µm particle size) from Waters (Milford, MA, USA). Centrifugations were performed with a Heraeus centrifuge model biofuge (Germany). All pH measurements were made at 25 ± 1℃ with Metrohm instrument Model 744 (Switzerland) using combined glass electrode.

Chromatographic analysis

The mobile phase consisted of 70% buffer containing 2.72 g L^{-1} potassium phosphate monobasic and 30% acetonitrile. The pH of the aqueous buffer in the mobile phase was adjusted to pH 2.0. A mobile phase flow-rate of 0.75 mL min^{-1} was used in isocratic elution mode and the detection was performed at the wavelength of 240 nm.

Experimental Procedure for the SALLE

5 ml of the sample solution was spiked with standard solution containing the target analytes and transferred to a 15 ml screw capped test tube. The pH value of the solution was adjusted to 8 by adding appropriate amounts of 0.1 M NaOH and then 1.5 ml acetonitrile and 1.5 gr NaCl were added. Thereafter, the solution was shaken gently for 1 min. The solution was centrifuged at 4000 rpm for 6 min, which resulted in phase separation. Then, the upper organic phase was carefully withdrawn using 1 ml micro-syringe and quantitatively transferred to a vial, and was then dried under blowing nitrogen at room temperature. The residue was re-dissolved in the 500 µL of mobile phase followed by vortexing for 1 min. Thereafter it was filtered with a 0.2 µm nylon filter and injected to the HPLC system. Schematic illustration of the SALLE procedure is represented in scheme 2.



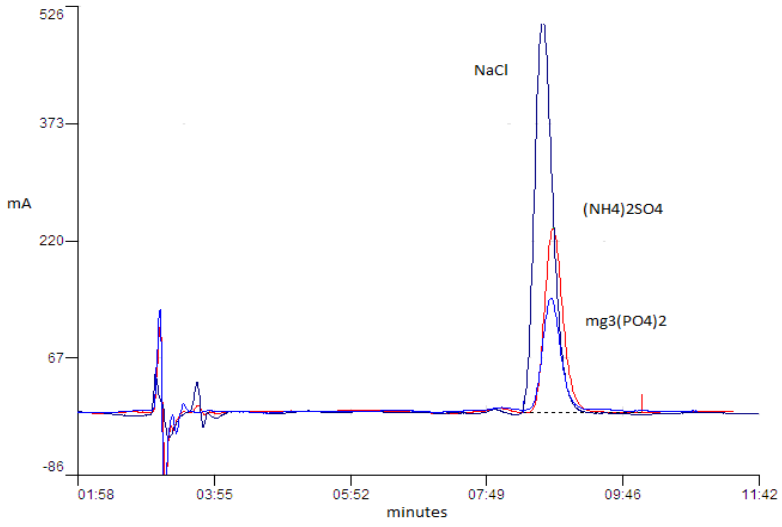
Scheme 2. Experiment setup of the salting out assisted liquid-liquid extraction procedure using HPLC-UV technique.

Results and discussion

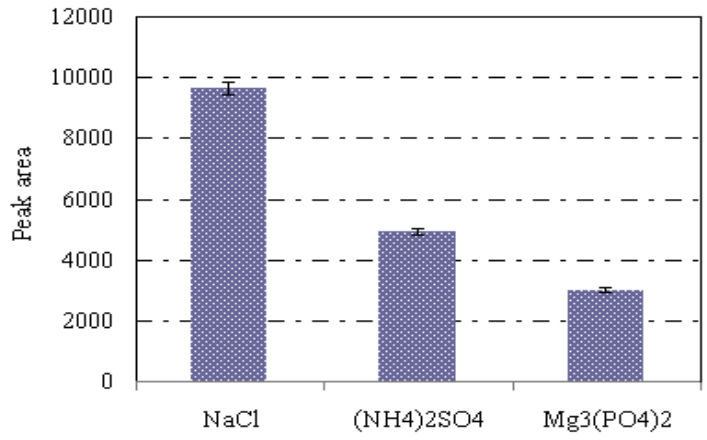
There are some affecting factors in the extraction of carvedilol by SALLE, such as concentration of extraction solvent, type and amount of salt, pH of sample solution and sample volume. The optimization of the SALLE procedure was carried out using spiked aqueous samples with 100 µg l^{-1} of carvedilol. Then the method was applied for extraction of target analyte from human blood plasma and recovery was used to evaluate the extraction efficiency in human plasma samples.

Selection of salt type

Influence of salt type and its concentration on the extraction recovery was also studied. Three salts including sodium chloride, magnesium phosphate and ammonium sulfate were investigated as the salting-out reagents. According to obtained results from Fig. 1A, sodium chloride is the most appropriate salt. The same results will be revealed from the HPLC-UV chromatogram as represented in Fig. 1B. In the next step, different amounts of NaCl between 0 and 3 gr were added into samples while the other conditions were kept constant to evaluate the influence of the salt addition on the performance of SALLE. Fig. 2 shows that the analyte concentration increased as the salt concentration increased up to 1.5 gr. The addition of salt might reduce the solubility of the target analytes in the sample solution. However, when the concentration of NaCl was higher than 1.5 gr, a slight decrease in the extraction efficiency was observed with the increase of NaCl concentration. It is probably related to increase the viscosity of aqueous phase which reduces the mass transfer of the analyte from aqueous to organic phase. Thus, 1.5 gr of NaCl was used in the subsequent studies.



(A)



(B)

Fig. 1. Effect of (A) salt type on the extraction of carvedilol from aqueous sample and (B) HPLC-UV chromatogram (λ=240 nm) (solvent: acetonitrile, salt amounts=1.5 g).

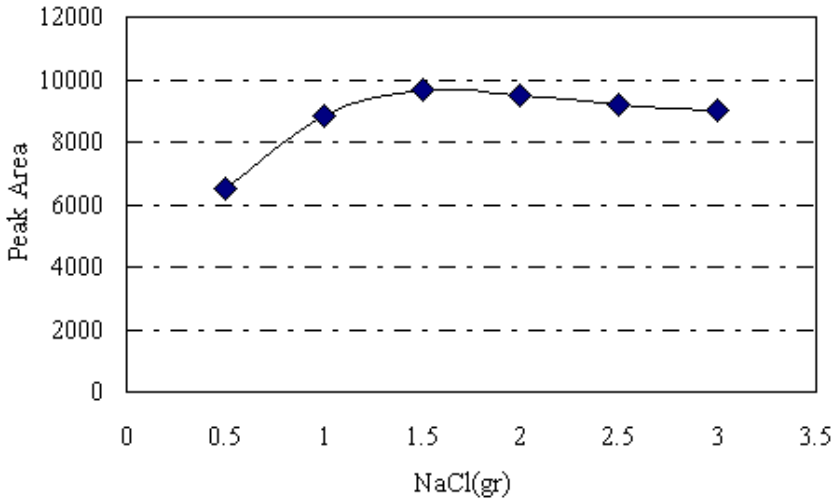


Fig. 2. Influence of NaCl concentration on the recovery of carvedilol from aqueous sample (pH=8.0, volume of acetonitrile=1.5 ml).

Selection of the water miscible organic solvent and effect of its volume

Several water-miscible organic solvents such as methanol, ethanol and acetonitrile (ACN), were examined as organic phases. ACN was the only water miscible solvent that resulted in clear phase separation. With the other organic solvents examined a phase-separation was not observed. Thus, ACN was selected as organic solvent. After that the volume of extraction solvent has important role and could influence the extraction performance of SALLE. The influence of the acetonitrile volume on the extraction recovery is shown in Figure 3. It can be seen that the recoveries of carvedilol increased with the volume of acetonitrile from 1.0–1.5 mL and then decreased. With low volumes, the interface between the acetonitrile and the aqueous phases was not clear and the collection of the organic layer was difficult. On the other hand, with volumes upper to 1.5 ml, dilution of target analyte can be occurred and led to decrease of its peak area. Based on the experimental results, 1.5 ml acetonitrile was selected as the optimum volume in all the subsequent experiments.

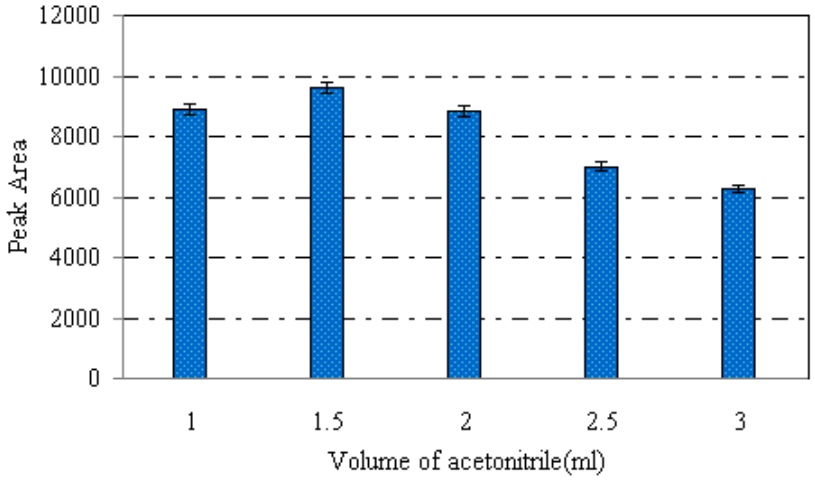


Fig. 3. Effect of volume of acetonitrile on the extraction of carvedilol (pH=8.0, NaCl =1.5 g).

The pH of the *aqueous phase*

Carvedilol exhibited a typical weak base pH-dependent solubility profile in water with a high solubility at low pH (545.1–2591.4 µg/mL within the pH range 1.2–5.0) and low solubility at high pH (5.8–51.9 µg/mL within the pH range 6.5–7.8) [24]. The effect of sample pH on the extraction efficiency was studied in the range of 1–10. As seen from Fig. 4, an increase in pH leads to an increase in carvedilol extraction up to pH 8.0 and then leads to a slight decrease. Therefore the pH of 8.0 was selected for further study.

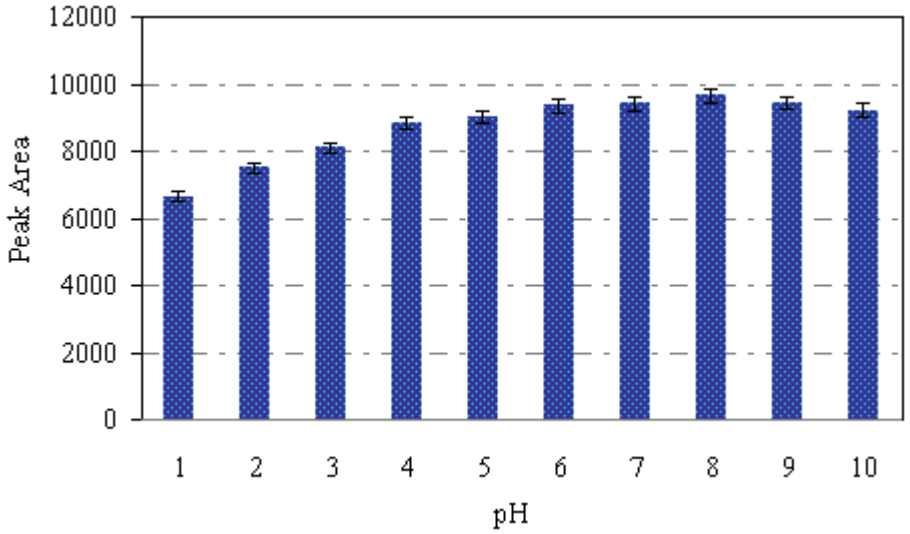


Fig. 4. Effect of pH of sample solution on the extraction of carvedilol (solvent: acetonitrile, NaCl =1.5 g).

Figures of merit

Validation parameters of the proposed method such as linearity, the limit of detection (LOD), the limit of quantitation (LOQ), precision and accuracy were evaluated under the optimized conditions. The linearity of the method was determined by extracting and injecting standard solutions of carvedilol at different concentrations. In the optimal condition the linear calibration range was 5-500 µg L^{-1}, the r-square value of the calibration curve was 0.9965 which confirmed the linearity of the method. The LOD and LOQ were 1 µg L^{-1} and 3.3 µg L^{-1}, respectively. The precision based on the relative standard deviation (RSD) of the peak area for a 5 µg l^{-1} solution of carvedilol was calculated to be 3.5% for five replicates. The accuracy of the method was investigated by determining the relative recovery of spiked carvedilol in plasma samples at three concentration levels. Table 1 lists the obtained relative recoveries from the analysis of spiked samples. As can be seen, relative recoveries were in the range of 96.0–105%.

Table 1. Obtained accuracy of carvedilol for spiked plasma samples using SALLE method.

Added (µg l^{-1})	Found (µg l^{-1})	Recovery (%)	RSD (%)
2.0	2.1	105	4.0
5.0	4.8	96	3.5
10.0	10.3	103	3.1

Real sample analysis

The applicability of proposed method was evaluated by analyzing of spiked human plasma samples. Three different human plasma samples were collected from Imam Reza Hospital (Kermanshah, Iran). After collection, blood samples were gently and carefully mixed and immediately centrifuged in a refrigerated centrifuge at 3000 rpm for 10 min. then the plasma was separated and stored at –30 °C in dark until analysis. The samples were spiked by carvedilol (100 µg l^{-1}) before extraction process. The pH of each plasma sample was adjusted utilizing HNO_3 and NaOH and the optimized experimental conditions were applied on each sample. To study the matrix effect of the different plasma samples on the current method, relative recoveries were determined by spiking them with carvedilol at three different concentration levels (Table 1). The related HPLC chromatogram has been shown in fig. 5. Figure 5 indicate that carvedilol could be determined selectively without interference from plasma components. The chromatogram indicates the high sensitivity and selectivity of the developed method.

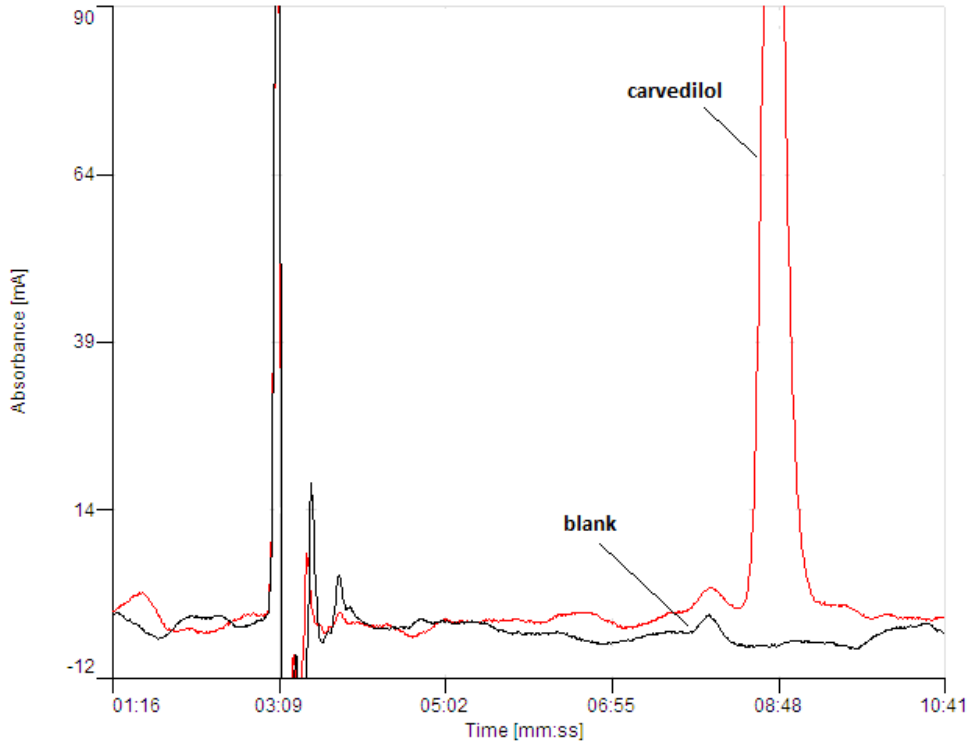


Fig. 5. HPLC chromatogram of carvedilol in spiked human plasma sample (spiked carvedilol=30 µg l^{-1}).

Comparison with other works

In order to show the analytical advantages of the proposed method for the determination of carvedilol, some details were compared with reported methods in literature [۲۵-۲۹] and these results are shown in Table 2. As it can be seen, considerable LOD and relatively wide dynamic range were obtained. In addition, in the reported methods, toxic solvents or expensive analytical instruments have been used for extraction and determination of carvedilol. In contrast, in the proposed method, a rapid, benign and simple salting out assisted microextraction was utilized for preconcentration and extraction of carvedilol. No hazardous material was used in this sample pre-treatment method. In addition, an inexpensive and sensitive analytical instrument was applied for determination.

Table 2. Comparison of the proposed methodology with reported methods.

Method	Sample	LOD (µg/l)	Linear rang (µg/l)	Ref.
DLLME-HPLC[a]	Human Urine, Human Plasma	4, 14	50-750, 20-1000	25
SPE-CE[b]	Human Urine	50	50-500	26
Synchronous fluorimetry	Pharmaceutical preparations	1	5-100	27
LLE-HPLC[c]	Human serum	2.5	5-500	28
Ionic liquid phase microextraction spectrofluorimetry	Human Urine, Human Plasma, Pharmaceutical preparations	1.7	10-250	29
This work	Human plasma	1.0	5-500	-

[a]Dispersive liquid-liquid microextraction.
[b]Solid phase extraction-capillary electrophoresis.
[c]Liquid-liquid extraction.

Conclusion

In this work, a rapid and simple extraction based on SALLE in combination with HPLC has been proposed for the determination of carvedilol in human plasma. The influence of affecting variables on the extraction efficiency were investigated and optimized. Under optimum conditions good recovery of analyte was achieved and full validation was carried out. This study shows interesting perspectives for the application of SALLE for the monitoring of carvedilol in real biological matrices.

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

References

[1] Sweetman SC. Martindale: The complete drug reference, 35th Ed., p. 1114, Pharmaceutical Press, London 2007.

[2] Nichols AJ, Gellai M and Ruffolo RR. Studies on the mechanism of arterial vasodilation produced by the novel antihypertensive agent, carvedilol, J. Fundam.Clin. Pharmacol. 1991;5:25-38.

[3] DeMey C, Breithaupt K, Schloos J, Neugebauer G, Palm S and Belz G. Dose-effect and pharmacokinetic-pharmacodynamic relationships of the beta 1-adrenergic receptor blocking properties of various doses of carvedilol in healthy humans, Clin. Pharmacol. Ther. 1994;55:329-337.

[4] Packer M, Fowler BM, Roecker BE, Coats JSA, Katus AH, Krum H, Mohacsi P, Rouleau LJ, Tendera M, Staiger C, Holcslaw LT, Amann-Zalan I and DeMets LD. Effect of carvedilol on the morbidity of patients with severe chronic heart failure: results of the carvedilol prospective randomized cumulative survival (COPERNICUS) study, Circulation. 2002;106:2194-2199.

[5] Ruffolo RR, Boyle DA, Brooks DP, Feuerstein GZ, Venuti RP, Lukas MA and Poste G. Carvedilol: A novel cardiovascular drug with multiple actions, Cardiovasc. Drug Rev. 1992;10:127-157.

[6] Lamprecht G, Gruber L, Staschitzky K, and Lindner W. Enantioselective Analysis of (R)- and (S)-Carvedilol in Human Plasma by High Performance Liquid Chromatography. Chromatographia. 2002;56:25-29.

[7] Oldham HG and Clarke SE. In Vitro Identification of the Human Cytochrome P450 Enzymes Involved in the Metabolism of R(+)- and S(-)-Carvedilol. Drug Metabolism and Disposition. 1997;25:970-977.

[8] Wilson JF, Smith BL, Toseland PA, Watson ID and Williams WJ. A survey of extraction techniques for drugs of abuse in urine. Forensic Sci. Int. 2001;119:23–27.

[9] Scheurer J and Moore CM. Solid-phase extraction of drugs from biological tissues, a review, J Anal. Toxicol. 1992;16:264–269.

[10] Whittington D and Kharasch ED. Determination of morphine and morphine glucuronides in human plasma by 96-well plate solid-phase extraction and liquid chromatography–electrospray ionization mass spectrometry, J. Chromatogr. B. 2003;796:95–103.

[11] Ho TS, Pedersen-Bjergaard S and Rasmussen KE. Liquid-phase microextraction of protein-

bound drugs under non-equilibrium conditions, Analyst. 2002;127:608-13.
[12] He Y, Vargas A and Kang YJ. Headspace liquid-phase microextraction of methamphetamine and amphetamine in urine by an aqueous drop, Anal. Chim. Acta. 2007;589:225–230.
[13] Gorynski K, Gorynska P, Gorska A, Harężlak T, Jaroch A, Jaroch K, Lendor S, Skobowiat C and Bojko B. SPME as a promising tool in translational medicine and drug discovery: From bench to bedside, Journal of Pharmaceutical and Biomedical Analysis. 2016;130:55-67.
[14] Juntinga L, Penga C and Suzukib O, Solid-phase microextraction (SPME) of drugs and poisons from biological samples, Forensic Sci. Intern. 1998;97:93-100.
[15] Shamsipur M and Fattahi N. Extraction and determination of opium alkaloids in urine samples using dispersive liquid–liquid microextraction followed by high-performance liquid chromatography, J. Chromatogr. B. 2011;897: 2978–2983.
[16] Tehrani MS, Givianrad MH and Mahoor N. Surfactant-assisted dispersive liquid–liquid microextraction followed by high-performance liquid chromatography for determination of amphetamine and methamphetamine in urine samples, Anal. Methods. 2012;4:1357–1364.
[17] Lorenzo Ferreira RA, Ares Fuentes AM, Regenjo Vázquez M, CarroDíaz AM, Fernández Sobrino AM and Férnandez Gómez P. Dispersive liquid–liquid microextraction (DLLME) optimization for determination of designer drugs (cathinones) and drugs abuse in oral fluid, Toxicol. Lett. 2016;258:183-184.
[18] Rodríguez MP, Pezza HR and Pezza L. Ultrasound-assisted dispersive liquid-liquid microextraction of tetracycline drugs from egg supplements before flow injection analysis coupled to a liquid waveguide capillary cell, Anal. Bioanal. Chem. 2016;408:6201-6211.
[19] Ahmadi-Jouibari T, Fattahi N, Shamsipur M and Pirsaheb M. Dispersive liquid–liquid microextraction followed by high-performance liquid chromatography–ultraviolet detection to determination of opium alkaloids in human plasma, J. Pharm. Biomed. Anal. 2013;85:14–20.
[20] Ahmadi-Jouibaria T, Fattahi N and Shamsipur M. Rapid extraction and determination of amphetamines in human urine samples using dispersive liquid–liquid microextraction and solidification of floating organic drop followed by high performance liquid chromatography, J. Pharm. Biomed. Anal. 2014;94:145–151.
[21] Farajzadeh MA, Sheykhizadeh S and Khorram P. Salting-out homogeneous liquid–liquid extraction in narrow-bore tube: extraction and preconcentration of phthalate esters from water, J. Sep. Sci. 2013;36:939–946.
[22] Zhao FJ, Tang H, Zhang QH, Yang J, Davey AK and Wang J. Salting-out homogeneous liquid–liquid extraction approach applied in sample preprocessing for the quantitative determination of entecavir in human plasma by LC–MS, J. Chromatogr. B. 2012;881–882:119–125.
[23] Valente IM, Goncalves LM and Rodrigues JA. Another glimpse over the salting-out assisted liquid–liquid extraction in acetonitrile/water mixtures, J. Chromatogr. A. 2013;1308:58-62.
[24] Hamed R, Awadallah A, Sunoqrot S, Tarawneh O,Nazzal S, Al Baraghthi T, Al Sayyad J, and Abbas A. pH-dependent solubility and dissolution behavior of carvedilol—case example of a weakly basic BCS class II drug, AAPS Pharm. Sci. Tech. 2016;17:418–426.
[25] Zamani-Kalajahi M, Fazeli-Bakhtiyari R, Amiri M, Golmohammadi A, Afrasiabi A and Khoubnasabjafari M. Analysis of losartan and carvedilol in urine and plasma samples using a dispersive liquid–liquid microextraction isocratic HPLC–UV method. Bioanalysis. 2012; 4: 2805–2821.
[26] Mazzarino M, De La Torre X, Mazzei F, Botre F. Rapid screening of beta-adrenergic agents and related compounds in human urine for anti-doping purpose using capillary electrophoresis with dynamic coating. J Sep Sci. 2009;32:3562–3570.
[27] Xiao Y, Wang HU, Han J. Simultaneous determination of carvedilol and ampicillin sodium by synchronous fluorimetry. Spectrochim Acta, Part A. 2005;61:567–73.
[28] Gannu R, Yamsani VV and Rao YM. New RP-HPLC method with UV-detection for the determination of carvedilol in human serum. J Liq Chromatogr Rel Technol. 2007;30:1677–685.
[29]Zeeb M and Mirza B. Ionic liquid phase microextraction combined with fluorescence spectrometry for preconcentration and quantitation of carvedilol in pharmaceutical preparations and biological media. DARU Journal of Pharmaceutical Sciences. 2015;23:30-39.

