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Ultrasound-assisted dispersive liquid-liquid microextraction with HPLC-UV for the simultaneous determination of Diclofenac potassium and Indomethacin in serum and plasma samples: Experimental design and optimization

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In this work, a rapid, simple, and environmentally friendly method based on ultrasound-assisted dispersive liquid-liquid microextraction (UADLLME) was proposed for simultaneous determination of diclofenac potassium and indomethacin in serum and plasma samples. High performance liquid chromatography with UV detection (HPLC-UV) was used. The expermental conditions, including pH of sample solution, type of extraction solvent, time of ultransound, centrifugation condition and ionic strength were investigated and optimized. After screening out the factors with insignificant effect, the remaining factors were optimized using the Central Composite Design. Under the optimal conditions, detection limit were found as 1.09 and 2.18 ng mL-1 for diclofenac potassium and indomethacin respectively and relative standard deviations (RSD) of the analysis less than 3% (n= 5) and detection. Mean recoveries of both in human plasma and serum samples were in the ranges of 92–99%. UADLLME - HPLC-UV was successfully applied for the simultaneous determination of diclofenac potassium and indomethacin in human plasma and serum samples.

Keywords: Ultrasound-assisted emulsification-microextraction, diclofenac potassium, indomethacin, Human serum and plasma samples, HPLC-UV.

INTRODUCTION

Diclofenac potassium and indomethacin are nonsteroidal anti-inflammatory drugs (NSAIDs). They have been widely used to treat fever and a variety of conditions that cause pain and inflammation [1]. Beside of their widly use, there are unwanted side effects such as indigestion, ulcers and bleeding parts of the gastrointestinal tract along with liver, kidney and heart problems [2-4]. Therfore, monitoring NSAID drug concentrations are considered an important issue in pharmacokinetic and medicine studies for improving the toxicological management of long-term NSAID therapy [5-7].

Several chromatographic methods have been described for determination of NSAIDs in biological samples, such as capillary electrophoresis (CE) [8-10], high-performance thin-layer chromatography (HPTLC) [11], high-performance liquid chromatography [12-14] and gas chromatography (GC) [15-17]. Sample preparation methods such as Dispersive liquid–liquid extraction(DLLE) [18, 19], dispersive liquid–liquid microextraction based on solidification of floating organic droplets (DLLE-SFO) [14], solid-phase extraction(SPE) [13], hollow fiber-based liquid phase microextraction (HF-LPME) [17, 20], and stir bar-sorptive extraction (SBSE) [21] are needed when biological samples are to be analysis for NSAIDs.

Dispersive liquid-liquid microextraction (DLLME) was reported by Rezaee et al. [22] as an effective method among the microextraction methods for preconcentration and separation of organic and inorganic specimens. It has several advantages including simplicity of operation, rapidity, high recovery, low consumption of organic solvents, simplicity of experiment, and low cost [23]. In ultrasound assisted -DLLME (UA-DLLME), the mixture a microvolume of solvents is rapidly injected into the sample to extract analytes. Mass transfer process in the above extraction procedure was accelerated by ultrasonic radiation, caused to introduce a new method named. The consequence is a very efficient and fast analyte extraction. After mass transfer, the two phases can be readily separated by centrifugation [22].

George E. P. Box (1950s) introduced response surface methodology (RSM) -a factorial design based method for collection of statistical techniques- that has been used in the modeling and optimization of some processes [24-25]. Different types of RSM such as three-level factorial design, central composite design (CCD), and Box-Behnken design (BBD) and have different properties and

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characteristics. Different types of CCD are such as Central Composite Circumscribed (CCC), Central Composite Inscribed (CCI), and Central Composite Face-centered (CCF). Finally, it is a good way to graphically illustrate the relation between different experimental variables and the response(s) [25].

The goal of this work is simultaneous determination of diclofenac potassium and indomethacin in human serum and plasma samples by HPLC-UV after preconcentration by UA-DLLME. Experimental variables affecting the extraction efficiency, including pH of sample solution, volume of extraction and dispersive solvent, and ultrasound time were considered and optimized using the central composite design.

EXPERIMENTAL

Materials and Reagents

All analytical-reagent grade of the drugs (>99%) were purchased from Sigma Aldrich (Steinheim, Germany). The stock solutions (500 ng mL^{-1}) were prepared by dissolving appropriate amount of each drug in methanol. The working solutions were prepared by diluting of the stock solutions with methanol. Methanol (HPLC-grade) was purchased from Merck (Darmstadt, Germany). Deionized water produsd by Milli-Q system (Millipore, Bedford, MA, USA). All of the standard solutions were stored at 4°C and brought to ambient temperature just prior to use. Throughout the experimental runs, all the solvents, calibration, and real samples were filtered through 0.45 μm nylon filter membranes (Varian, USA).

Instrumentation

The chromatography measurements were carried out by a KNAUER HPLC system equipped with a micro vacuum degasser, HPLC column (C18 250 mm×4.6mm, 5μm), and UV detector set to recorde absorbance at 254 nm. The pH was measured using a pH meter (Metrohm 827, Switzerland) combined with a glass electrode. A 320R Hettich centrifuge (Germany) and a digital 10P ultrasonic bath (Sonorex, Germany) were also used. The MINITAB 16 was used for experimental design, analysis and subsequent regression analysis.

Extraction procedure

The real samples in this study were collected from human serum and plasma samples orthopedic patient volunteers at Taleghani medical center (Abadan, Iran) and then stored at 5-8°C until analysis (female, age 27 ± 3.1 years; and male, age 24 ± 5.0 years). Human samples were prepared using the UADLLME method. To aliquots of 1 mL human sample a solution containing 200 μL of 1% TCA was added for protein precipitation. 200 μL of sample was placed in centrifuge vial and 100 μL of 0.01 M phosphate buffer (pH= 4.5) was added. Then, 80 μL of n-hexane and 10 μL of methanol were injected into the sample solution and shaken manually. The vial was immersed in an ultrasonic, sonicated for 2 min, and shaken manually. A cloudy solution was centrifuged for 6 min at 3000 rpm in order to disrupt the emulsions and separate both phases. After centrifugation extraction, the organic phase on the bottom of the tube was collected with a Hamilton microsyringe. Finally, 10 μL of the obtained mixture was injected into the separation system.

RESULTS AND DISCUSSION

Calibration line preparation for caffeine analysis

In order to establish a sensitive and simple analytical method for the simultaneous analysis of selected NSAIDs, all affecting experimental variables were investigated and optimized. These variables were pH, type and volume of extraction solvent, type and volume of dispersive solvent, time of ultrasound, conditions of centrifuging step, ionic strength were studied and optimized. After screening out the factors with insignificant effect, the remaining factors were optimized using the Central Composite Design.

Optimization of chromatographic condition

The main of this work is to HPLC determination of diclofenac potassium and indomethacin after extraction and preconcentration by UADLLME. Two variables including type of mobile phase and column oven temperature were optimized with the hope to find both analytes. Mixtures of acetonitrile/water, acetonitrile/methanol/water, and methanol/ water with different pH values were studied. The best symmetry of the peak shapes was found in the mobile phase containing methanol and water with pH value of 4.5. Formic acid was used to adjust pH of the mobile phase in all experiments. It was found that during the chromatograic analysis increasing the ratio of water to methanol caused to elute efficiently the analytes from the column. Effect of column oven temperature was also studied in the range of 20-30 °C with the selected mobile phase and flow rate of 1.0 mL min-1. According to the results, temperature of 25 °C was found to be optimal and used in the subsequent analysis. It should be mentioned that changing the flow rate of mobile phase did not affect the chromatographic peaks. Scheme of the gradient used in the HPLC analysis

are presented in Table 1.

Table 1. Scheme of the gradient used in the HPLC analysis.

Time(min)	%H_2O (pH 3.5)	%MeOH
0	40	60
2	45	55
3	45	55
5	40	60
7	30	70
10	30	70

Optimization of the extraction parameters using one-at-a time method

The extraction efficiency of UADLLME method depends on some important analytical parameters. In order to optimize the experimental parameters on the response, two methods were applied. The variables pH of the sample solution, type and volume of extraction solvent, type and volume of dispersive solvent, centrifugation time, ultrasound extraction time, and ionic strength were investigated and optimized using one-factor-at-a time.

The variables pH, type of…..were studied and optimized pH of sample solution

pH of the sample solution is one of the factors studied in this study. Effect of pH on the response of drugs, phosphate buffers in the range of 2.0 - 6.0 were investigated. According to the obtained results, it can be concluded that response of drugs were increased when the sample pH was decreased to 4.5. It was found, at low pH, the considered drugs were not in ionic form in solution. The results are shown in Fig. 1. Finally, pH of 4.5 was select as the optimum pH sample solution for the following experiments.

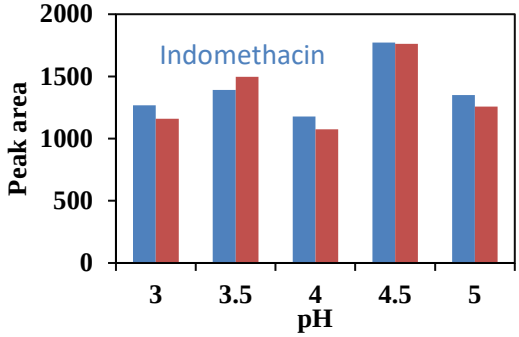


Fig. 1. Effect of pH sample solution on the response of drugs (200 (ng mL^{-1})).

Selection of extraction solvent and dispersive solvent

One of the most important analytical parameter in UADLLME methods selection of suitable extraction solvent [23]. The extraction solvent has to meet some properties such as lower density than that of water, low solubility in water, and high extraction capability of the target analytes. Different extraction solvents including n-hexane, 1-octanol, chlorobenzene, and dichloromethane and different dispersive solvents including methanol, acetonitrile, ethanol and acetone were studied. Among them, n-hexane was chosen as the best extraction solvent and methanol was chosen as the best dispersive solvent, because it had higher recoveries in comparison with the others. To obtain the highest response, volume of the extraction solvent and dispersive solvent had to be optimized. Finally, volume of them extraction and dispersive solvents was changed in the range of 10.0 to 100.0 µL and 5.0 to 40.0 µL respectively. The results are shown in Fig. 2.a-b. The optimum volumes of extraction solvent and dispersive solvents for both drugs were found 80.0 µL and 10 µL respectively.

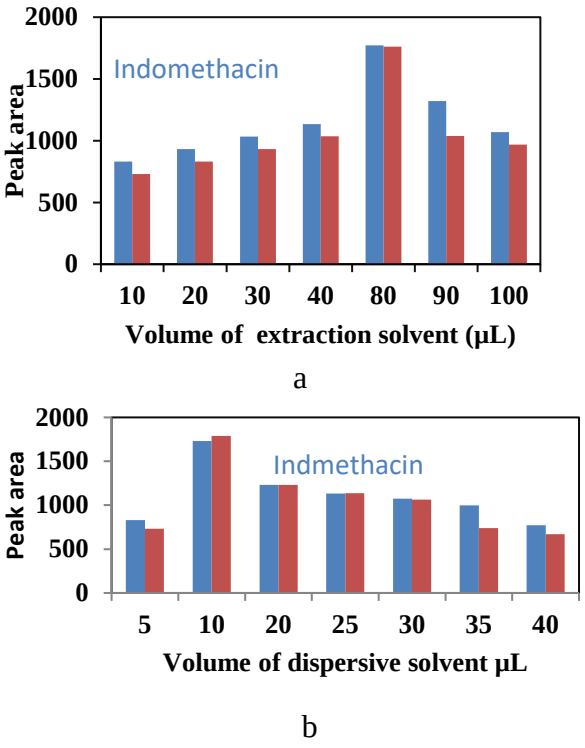


Fig. 2. Effect of extraction solvent volume on the recoveries of drugs. Conditions: sample solution, 5 mL of 200 (ng mL-1) of each drugs; pH sample solution: 4.5.

In this method, to improve the homogeneity, effect of time and temperature of ultrasound to help mixing the solvents and sample solution were investigated with a series of experiments. Ultrasound radiation might affect recoveries due to its own affecting on both emulsification and mass transfer process. Temperature affects organic solvent solubility in water and distribution coefficients as well as the emulsification phenomenon. Time and temperature was studied in the range of 0-5 min and different temperatures ranging from 20 ºC - 35 ºC. Maximum response were obtained after

ultrasonication for 2 min and 25 ºC no improvement was achieved by further ultrasonication. Ultrasound could generate the emulsion quickly and make rapidly a very large contact surface area between the extraction phase and the aqueous phase. Finally, 2 min was found to be the optimum time. It is clear from the results shown in this fig. that emulsification at ambient temperature helps to reach higher response. Therefore, this temperature was taken in the extraction step. At lower temperature, response decreased due to decrease in mass transfer phenomenon.

Effect of centrifugation condition

Centrifugation was required to break down the emulsion and accelerate the phase-separation process. In this method, extraction time is defined as the interval time between injection of the dispersive and extraction solvents to the sample and the start of centrifugation. Centrifugation time was investigated in the range of 1-7 min, whereas centrifuging rate was kept at 3000 rpm. Fig.3. shows that response of the drugs were increased by increasing centrifugation time up to 6 min and decreased after that. This time was chosen at the best.

Ionic strength

Effect of ionic strength varies in different extraction methods and, therefore, it should be study. Influence of ionic strength was investigated by adding different amounts of NaNO3, NaCl, and KH_2PO_4 0–10% (w/v) to the aqueous drugs solution to be extracted. The results indicated that the response was approximately constant at different ionic strengths. Finally No significant variation was seen in the extraction efficiencies of target analytes.

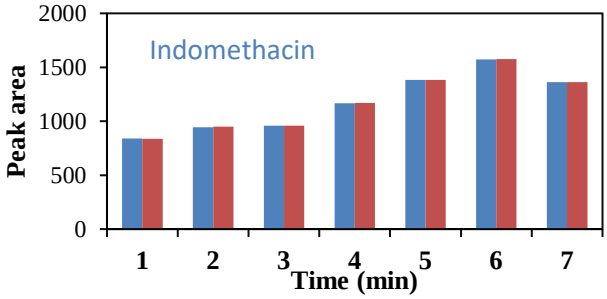


Fig. 3. Effect of centrifugation extraction time on the response of drugs. Conditions: sample solution, 5 mL of 200 (ng mL-1) of each drugs; pH sample solution: 4.5; volume and type of extracting solvent: 1-octanol, 80.0 µL; ultrasonication extraction time: 2 min, ultrasound temperature 25 0C.

Optimization of the variables using the Central composite design

According to the results obtained from study of one-factor-at-a time, four independent variables including volume of extraction solvent (X1), dispersive solvents (X2), pH (X3), time of ultransound (X4) were found to be significant. These parameters were further studied by the Central composite design. A set of 46 runs were chosen based on this design and performed randomly. Variables, assigned levels, and the corresponding Central composite design are shown in Table 2 and Table 3. The response variable for diclofenac potassium (Y1) indomethacin (Y2) and the tested variables were related by the following equations.

Table 2. Factors and their levels in Central Composite design.

Factors	Level		
	Low	Center	High
	-1	0	1
$V_{\text{extraction µL}}$ X_1	70	80	90
$V_{\text{dispersive µL}}$ X_2	5	10	15
pH* X_3	3.0	4.0	5.0
Time X_4	1	2	3

*Time of ultransound (min)

Table 3. Factors and their levels in Central Composite design and obtained result for each run.

	Variables				Absorbance	
Run no	X1	X2	X3	X4	Y1	Y2
1	0	0	0	-2	0.737	1.109
2	1	1	1	-1	0.652	0.878
3	0	0	-2	0	1.098	1.347
4	0	0	0	0	0.999	1.4985
5	-1	-1	-1	1	0.642	0.963
6	0	0	0	0	0.881	1.3215
7	1	1	-1	-1	1.003	1.5045
8	-1	1	1	1	0.996	1.494
9	0	0	0	0	1.001	1.5015
10	-1	1	1	-1	0.885	1.3275
11	1	-1	1	1	1.007	1.5105
12	0	0	0	0	0.989	1.4835
13	0	-2	0	0	0.994	1.491
14	0	0	2	0	1.003	1.5045
15	-1	1	-1	-1	1.012	1.518
16	-1	1	-1	1	0.862	1.257
17	-1	-1	1	-1	0.659	0.9885
18	1	-1	-1	1	1.022	1.533
19	0	2	0	0	1.007	1.5105
20	1	-1	1	-1	0.994	1.491
21	-1	-1	-1	-1	0.555	0.8325
22	1	1	-1	1	0.863	1.2945
23	0	0	0	0	1.036	1.554
24	0	0	0	2	0.645	0.9675
25	-2	0	0	0	0.83	1.215
26	0	0	0	0	0.872	1.308
27	1	1	1	1	0.544	0.816
28	1	-1	-1	-1	0.909	1.181
29	2	0	0	0	0.779	1.003
30	-1	-1	1	1	0.871	1.196
31	0	0	0	0	1	1.381

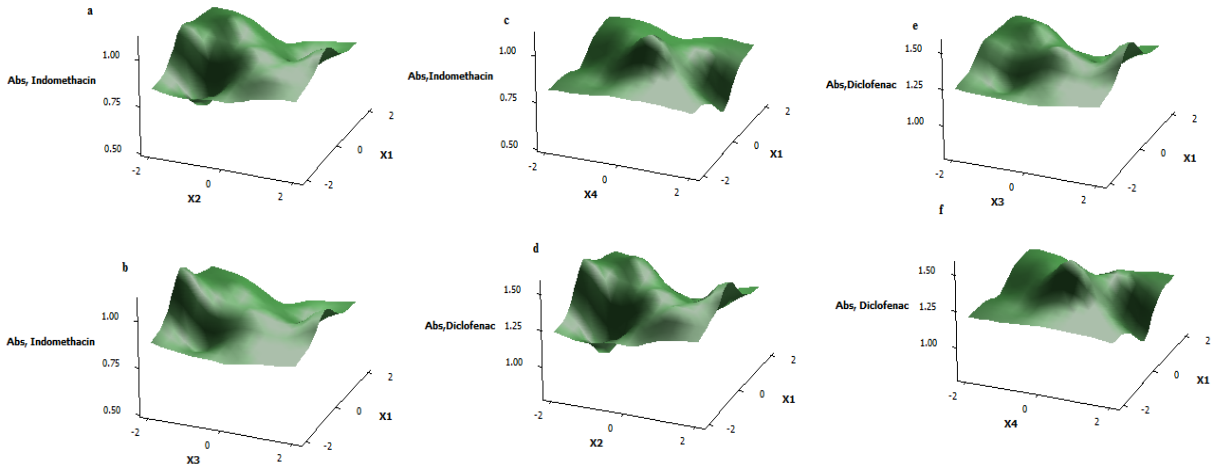


Fig. 4. Response surfaces plot for the Central composite design for indomethacin (a) (x_1) V $_{extraction}$ µL – (x_2) V $_{dispersive}$ µL, (b) (x_1) V $_{extraction}$ µL – (x_3) pH, (c) (x_1) V $_{extraction}$ µL – (x_4) time of ultransound, and Central composite design for diclofenac (d) (x_1) V $_{extraction}$ µL – (x_2) V $_{dispersive}$ µL, (e) (x_1) V $_{extraction}$ µL– (x_3) pH, (f) (x_1) V $_{extraction}$ µL – (x_4) time of ultransound.

Analysis of variance (ANOVA) was applied to evaluate the statistical significance of the model. F-test was used to estimate the statistical significance of all terms in the polynomial equation within 95% confidence interval [24-25]. The resulted model had R2 and F-value of 0.99 and, respectively. The summery of the ANOVA is shown in Table 4. The result showed the value of R2 of 0.8. This finding means that the stabilished model was able to explain 0.81 of the results (or of the variability of the response). After generation of the polynomial equations that relate the absorbance to the independent variables, genetic algorithm was employed to optimize of the process. Response surface curves facilitate investigating the interaction between the independent variables and finding the optimal level for each variable, as well. These curves are represented in Fig. 4. All of factors found effective in one-factor at a time study were appear in the eq. 1 and, eq. 2 therefore, had significant effect on the response. Based on the resulted model, it was found that volume of extraction solvent, dispersive solvents , pH , and time of ultransound were found to be significant effect on the response.

Optimum values of the tested variables for analysis of both analytes were found to be as follows: volume of extraction solvent µL (X1=79 µL), volume of dispersive solvent µL (X2=10 µL), pH (X3=4.3), and time of ultransound (X4=2.2 min).

Analytical features of proposed method

Under the optimal conditions, analytical features of the proposed method including limit of detection (LOD), limit of quantification (LOQ), dynamic range, enrichment factor(EF), and relative standard deviations (RSD) were investigated. Results are shown in Table 4. A good linear relationship is displayed between the corresponding peak areas and the concentrations of the both drugs based on the correlation coefficients.

Comparison of the parameters obtained in this work with those reported in the literature is given in Table 6. It is obvious that analytical features of the proposed procedure are comparable or better than the others reported for diclofenac potassium and indomethacin determination.

Chromatograms of solutions containing mixture of both under the optimal conditions are shown in Fig.6a-b. Chromatograms two-dimensional chromatograms of the extracted indomethacin (50 ppb) and diclofenac (10 ppb) in serum samples and indomethacin (200 ppb) and diclofenac (100 ppb) in plasma samples after spiking.

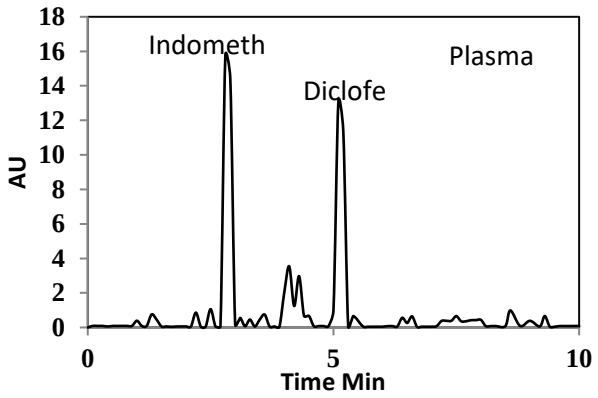
Table 4. Statistical parameters and figures of merit for determination of analytes in samples by applying UADLLME method.

Drugs	LOD (ng mL^{-1})	Dynamic range (ng mL^{-1})	EF*	LOQ (ng mL^{-1})	RSD (%)
Indomethacin	2.18	5-500	210	6.09	1.11
Diclofenac	1.09	5-1000	2800	3.55	2.07

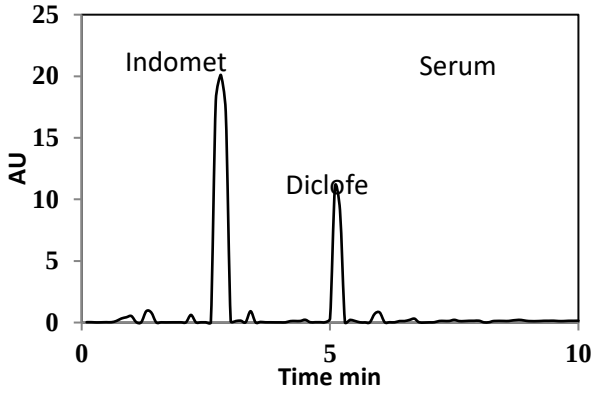
*Average Enrichment factor

Table 6. comparison of this study and the reported different methods for the determination of diclofenac and Indomethacin

Method	Appratus	Analyte	LOD ngmL^{-1}	RSD %	EF	Ref
HF-LPME	HPLC–DAD	Diclofenac	52.9	1.3	1060	12
DLLME-SFO	HPLC	Diclofenac	5.2	-	-	14
USAEME	HPLC-DAD	Diclofenac, Indomethacin	1.09, 2.18	1.11, 2.007	210,-2800	



a.



b

Fig. 6. Chromatograms two-dimensional chromatograms of the extracted indomethacin (50 ppb) and diclofenac (10 ppb) in serum samples and indomethacin (200 ppb) and diclofenac (100 ppb) in plasma samples after spiking.

Application of the proposed method to real samples

To evaluate performance of the proposed method, determination of diclofenac potassium and indomethacin in human serum and plasma samples was carried out under the optimized conditions. The results are collected in Table 7. Mean recoveries in human samples were in the ranges of 92–99%. The recoveries demonstrated that the matrixes have negligible effect on the quantification of these compounds and the method is accurate within the desired range. The obtained results revealed ability of the proposed method for the determination of diclofenac potassium and indomethacin in human serum and plasma samples.

Table 7. Added and Found indomethacin and diclofenac concentrations (ng mL^{-1}) in serum samples (R1-R3) and plasma sample (R4-R6).

Samples		Indomethacin	Diclofenac
R1*	Added	0	0
	Found	45.1	68.9
	Recovery%		
R2*	Added	50.0	10.0
	Found	96.5	73.2
	Recovery%	98.54	92.77
R3*	Added	100.0	50.0
	Found	144	112.8
	Recovery%	99.3	94.94
R4**	Added	0	0
	Found	9.0	12.5
	Rcovery%		
R5**	Added	200.0	100.0
	Found	206.7	110.1
	Rcovery%	98.56	97.86
R6**	Added	200	5
	Found	206.7	18.2
	Rcovery%	98.56	96.70

*Serum ** plasma(n=3)

CONCLUSIONS

A new method has been proposed for the simultaneous determination of diclofenac potassium and indomethacin in human serum and plasma samples using HPLC-UV after optimization by UADLLME. The proposed method has advantages such as; simplicity of operation, low consumption of organic solvents, good reproducibility and gives a precise, highly sensitive and selective procedure with good LODs.

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REFERENCES

1. R. Liu, J.L. Zhou, A. Wilding, *Journal of Chromatography* A, **1022**(1), 179 (2004).
2. C.K.S. Ong, P. Lirk, C.H. Tan, R.A. Seymour, *Clinical medicine & research*, **5**(1), 19 (2007).
3. U. Kotowska, J. Kapelewska, J. Sturgulewska, *Environmental Science and Pollution Research*, **21**(1), 660 (2014).

4. E. Dinç, C. Yücesoy, F. Onur, *Journal of pharmaceutical and biomedical analysis*, **28**(6), 1091 (2002).
5. M.M. Sena, Z.F. Chaudhry, C.H. Collins, R. J. Poppi, *Journal of pharmaceutical and biomedical analysis*, **36**(4), 743 (2004).
6. G. Escandar, A. Bystol, A. Campiglia, *Analytica Chimica Acta*, **466**(2), 275 (2002).
7. L. Moberg, G. Robertsson, B.Karlberg, *Talanta*, **54**(1), 161 (2001).
8. C Simó, A Gallardo, J San Román, C Barbas, A Cifuentes, *Journal of Chromatography B*, **767**(1), 35 (2002).
9. Z. Shihabi, M. Hinsdale, *Journal of Chromatography B*, **683**(1), 115 (1996).
10. F.K. Główka, M. Karaźniewicz, *Analytica chimica acta*, **540**(1), 95 (2005).
11. T.K. Save, D. Parmar, P.V. Devarajan, *Journal of chromatography B*, **690**(1), 315 (1997).
12. M.R Payán, M.Á.B. López, R.Fernández-Torres, J. L. Pérez Bernal, M. C. Mochón., *Analytica chimica acta*, **653**(2), 184 (2009).
13. A. Bakkali, E. Corta, L.A. Berrueta, B. Gallo, F. Vicente, *Journal of Chromatography B*, **729**(1-2), 139 (1999).
14. D.S.M. Shukri, M.M. Sanagi, W.A.W. Ibrahim, N.N.Z. Abidin, H.Y. Aboul-Enein, *Chromatographia*, **78**(15-16), 987 (2015).
15. D. Borrey, E. Meyer, W. Lambert, S. Van Calenbergh, C. Van Peteghem, A.P. De Leenheer, *Journal of Chromatography A*, **910**(1), 105, (2001).
16. A. Azzouz, E. Ballesteros, *Journal of Chromatography B*, **891**(1), 9 (2012).
17. A. Sarafraz-Yazdi, A. Amiri, G. Rounaghi, Eshtiagh-Hosseini, *Journal of Chromatography B*, **908**(1), 67 (2012).
18. D.S.M. Shukri, M.M. Sanagi, W.A.W. Ibrahim, N.N.Z. Abidin, H.Y. Aboul-Enein, *Chromatographia*, **78**(15-16), 987 (2015).
19. U. Alshana, N.G. Ertaş, N. Göğer, *Food chemistry*, **138**(2), 890 (2013).
20. M.R. Payán, M.Á.B López,. R. Fernández-Torres, M.V. Navarro, M.C. Mochón, *Talanta*, **79**(3), 911 (2009).
21. P.L. Kole, J. Millership, J.C.McElnay, *Journal of pharmaceutical and biomedical analysis*, **54**(4), 701 (2011).
22. M.D.Luque de Castro, F. Priego-Capote, *Talanta*. **72**, 321 (2007).
23. H. Chen, J. Ying, H. Chen, J. Huang, L. Liao, *Chromatographia*. **68**, 629 (2008).
24. M. Ghaedi, H. Mazaheri, S. Khodadoust, S. Hajati, M.K. Purkait, *Spectrochimica Acta Part A*, **135**, 479 (2015).
25. N. Chamkouri, A. Niazi, V. Zare-Shahabadi, *Spectrochimica Acta Part A*, **156**, 105 (2016).

ЕДНОВРЕМЕННО ОПРЕДЕЛЯНЕ НА ДИКЛОФЕНАК-КАЛИЙ И ИНДОМЕТАЦИН С ПРОБИ ОТ СЕРУМ И ПЛАЗМА С ПОМОЩТА НА ЕДНОВРЕМЕННИ УЛТРАЗВУКОВА ДИСПЕРСИОННА ТЕЧНО-ТЕЧНА МИКРО-ЕКСТРАКЦИЯ И HPLC-UV. ПЛАНИРАН ЕКСПЕРИМЕНТ И ОПТИМИЗАЦИЯ

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(Резюме)

В тази работа се предлага бърз, прост и екологично съобразен метод за едновременното определяне на диклофенак-калий и индометацин в серум и плазма. Методът се основава на ултразвукова дисперсионна течно-течна микроекстракция. Определянето става с високо-ефективна течна хроматография с UV-датчик. Експерименталните условия (pH на разтворите, вида екстрагент, времетрането на ултразвуковото третиране, условията на центрофугиране и йонната сила) са изследвани и оптиирани. След отсяването на незначителните, останалите фактори са оптимизирани чрез централен композиционен план. Границите на откриване на диклофенак-калий и индометацин при оптималните условия са съответно 1.09 и 2.18 ng mL^{-1} . Относителното стандартно отклонение (RSD) при анализите беше под 3% (n = 5). Средните добиви в човешка плазма и серум бяха в интервала 92–99%.