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ORIGINAL RESEARCH ARTICLE

Optimization of dispersive liquid–liquid microextraction (DLLME) and spectrophotometric analysis of sodium diclofenac in pharmaceutical formulations using Box-Behnken design (BBD)

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ABSTRACT

A green and efficient Dispersive Liquid-Liquid Microextraction (DLLME) method coupled with spectrophotometry was developed and validated for the determination of sodium diclofenac in pharmaceutical samples. The method is based on the formation of an ion-pair complex to facilitate extraction. Critical extraction parameters, including pH, type and volume of extraction/disperser solvents, and centrifugation parameters, were systematically optimized using a Box-Behnken Design (BBD). This BBD approach enabled a comprehensive evaluation of the influence of these variables on extraction efficiency. Under optimal conditions, the method demonstrated excellent linearity within the range of 1.0-16.0 mg/L (R^2 = 0.9982). The limits of detection (LOD) and quantification (LOQ) were determined to be 0.326 mg/L and 0.987 mg/L, respectively, indicating good sensitivity. Comparative validation against a standard HPLC method, assessed using F-test and T-test, showed no statistically significant difference, thereby confirming the proposed method's accuracy and reliability. Overall, the proposed DLLME method offers a simple, efficient, environmentally friendly, and cost-effective approach for the accurate quantitative analysis of sodium diclofenac in pharmaceutical formulations.

Keywords: microextraction; sodium diclofenac; green method; box-behnken design; dllme method

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1. Introduction

Sodium 2-[(2,6-dichlorophenyl)amino]phenylacetate, or diclofenac sodium, is a nonsteroidal anti-inflammatory medication widely used to treat inflammation in the human body, rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and sports injuries[1,2] (**Figure 1**). Diclofenac is typically found in sodium or potassium salts, which are water-soluble and can be easily taken orally. This drug is used to treat numerous inflammatory conditions and alleviate pain associated with gout, bursitis, headaches, and menstrual cramps. The sodium and potassium salts of diclofenac are also effective in reducing fever and relieving aches[3]. According to an investigation by Alok and colleagues, diclofenac sodium (DS) alleviates pain by blocking the conversion of arachidonic acid into prostaglandins, which are key players in the inflammatory response. Notably, DS stands out from other nonsteroidal anti-inflammatory drugs used to treat rheumatoid arthritis and osteoarthritis, because it has significantly fewer gastrointestinal side effects[4].

Various analytical techniques have been reported in the literature for the assay of diclofenac sodium in pharmaceutical formulations. These techniques include Solid-state microfabricated potentiometric[5],

thermo gravimetric analysis[6], Flow Injection Analysis[7], HPLC[8-10], fluorimetry[11], and UV/VIS spectrophotometry[12-15]. Notably, the field of "green analytical chemistry" has seen significant improvements in developing rapid, straightforward, efficient, and environmentally friendly strategies for drug extraction. One such approach is Dispersive Liquid-Liquid Microextraction (DLLME), which is based on the formation of a cloudy solution when an appropriate mixture of extraction and disperser solvents is added to an aqueous phase[16-20]. Cloud point extraction (CPE) and salt-induced homogeneous liquid-liquid microextraction (SIHLLME) are examples of several innovative approaches being explored[21-25]. In this work, the extraction of sodium diclofenac coupled with spectrophotometry was investigated using dispersive liquid-liquid microextraction (DLLME). A Box-Behnken design (BBD) and Central composite design (CCD) were used to optimize the effects of the type and volume of extraction solvent, pH value, type and volume of dispersive solvent, and rotation parameters. In this study, the Box-Behnken Design (BBD) was used to identify an optimized response surface. BBD is an experimental design that requires fewer experiments and is more practical and feasible compared to other designs[26-29]. In this method, the ion-pair complex was utilized for determining and extracting sodium diclofenac using DLLME. This work demonstrates the effective integration of DLLME preconcentration, utilizing ion-pair complex formation with Rhodamine 6G, followed by spectrophotometric detection at 508 nm. The comprehensive optimization of critical extraction parameters (pH, type and volume of extraction/disperser solvents, centrifugation) using Box-Behnken Design (BBD) ensures an efficient and robust method. This systematic design-of-experiments approach provides a deeper understanding of variable interactions compared to traditional one-variable-at-a-time methods. Furthermore, this method aligns with the principles of green analytical chemistry by utilizing microextraction, which significantly reduces solvent consumption and waste generation compared to conventional extraction techniques. This makes it an environmentally friendly alternative for the analysis of sodium diclofenac. The developed method offers a simple, efficient, and cost-effective approach for routine quantitative analysis of sodium diclofenac in commercial pharmaceutical samples.

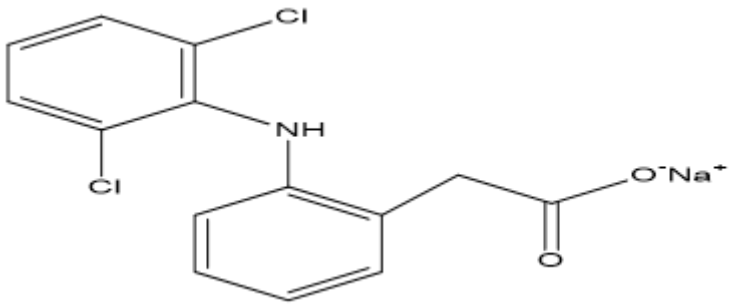

Figure 1. Sodium salt of Sodium Diclofenac structure.

2. Experimental

2.1. Instrument and software

Absorbance measurements were taken at 508 nm using a single-beam UV-visible spectrophotometer (Analyticjena, Germany) with 1 cm quartz cells. To speed up the extraction process, a Beckman model TJ-6 centrifuge (Germany) was used. In order to investigate the influence of various parameters on the extraction efficiency of diclofenac using the DLLME method, the BBD method was performed (Design Expert software, version 13, State-Ease, Inc., United States).

2.2. Reagents and solutions

Rhodamine 6G (95%, Sigma-Aldrich) and sodium diclofenac (purity 99.6%, SDI Company, Samara-Iraq) were used in this study. A stock solution of sodium diclofenac (100 mg/L) was prepared by dissolving 0.01 g

in 100 mL of 0.1 N NaOH solution. Similarly, a stock solution of Rhodamine 6G (100 mg/L) was prepared by dissolving 0.01 g in 100 mL of deionized water (DW). Buffer solutions (pH 5, 8, and 10) were prepared according to reference[30].

2.3. Assay procedure for dosage forms

One tablet each of sodium diclofenac, 100 mg (Olfen, Acino, Switzerland), (Refen retard, Hemofarm, Serbia), and (Voldic, Pharma International, Jordan), were weighed and powdered. The powdered samples were then dissolved in 100 mL of 0.1 N NaOH solutions in separate volumetric flasks. Each solution was mixed well and filtered through filter paper to remove insoluble compounds.

2.4. DLLME procedure

In a 15 mL glass centrifuge tube, a series of sodium diclofenac solutions (1.0-16.0 mg/L) was mixed with 1.5 mL of Rhodamine 6G reagent (20 mg/L). Then, 1.0 mL of phosphate buffer (pH 10) was added, and the solution was brought to a final volume of 10 mL with distilled water. A cloudy solution was generated using a micro-syringe by rapidly injecting 800 µL of dichloromethane (extraction solvent) and 1200 µL of methanol (disperser solvent). The mixture was centrifuged at 2500 rpm for 6 minutes. The resulting orange ion-pair product was diluted to 2 mL with ethanol and then transferred to a 1 cm quartz microcell. The absorbance was measured at a wavelength of 508 nm against a blank (**Figure 2**).

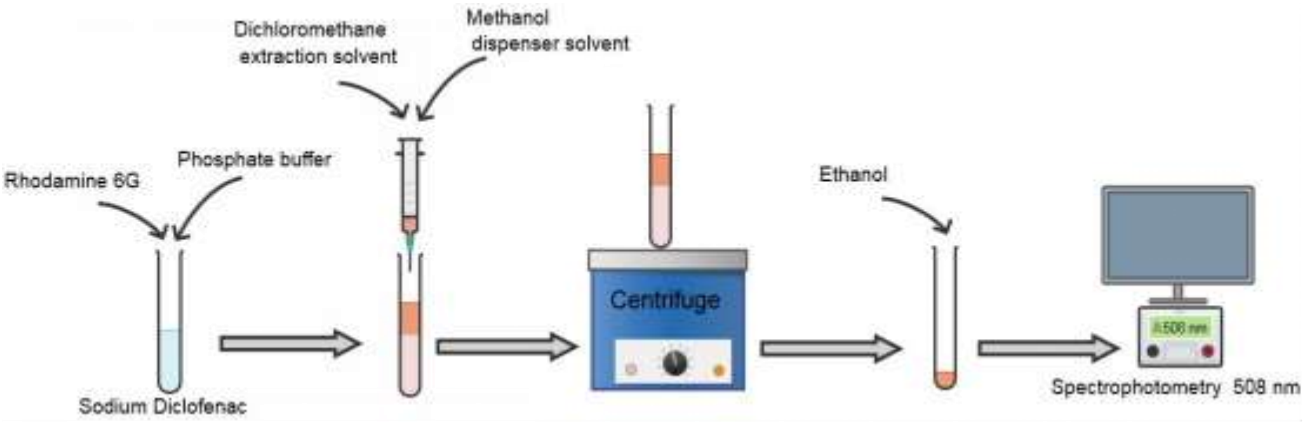

Figure 2. DLLME Procedure for determination of Sodium Diclofenac.

3. Results and discussion

Ion-pair extraction is a technique that involves combining an aqueous solution of carboxyl compounds with a suitable reagent, typically a dye, to generate an ion pair between the negatively charged carboxyl compounds and a countercharge reagent. The resulting ion-pair product is distinguished by its solubility in organic solvents, such as chloroform. Additionally, the resulting compound is characterized by its colored nature. The spectrophotometric technique can be employed to provide an estimation. Ion-pair products are formed by connecting carboxyl drugs in their anionic state (sodium diclofenac) with a suitable cationic reagent (Rhodamine 6G reagent). The following equation (1) can be used to express this:

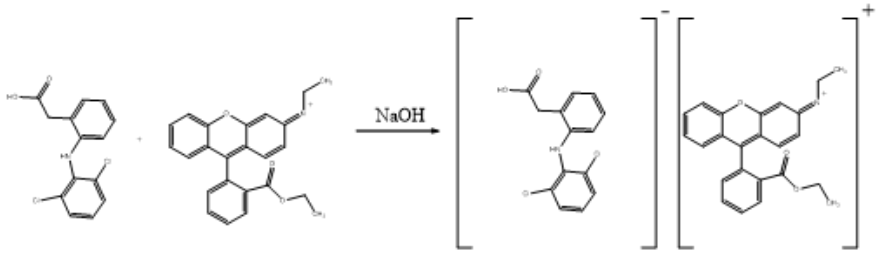

Figure 3. Ion-pair complex of sodium diclofenac with Rhodamine 6G reagent.

The distinct absorption maximum at 508 nm confirms the formation of a new ion-pair complex between sodium diclofenac and Rhodamine 6G. This complex, formed with the highly colored Rhodamine 6G dye, retains chromophoric properties, absorbing light in the visible spectrum. The 508 nm wavelength, corresponding to the complex's complementary color, is vital for spectrophotometric determination. This stable, quantifiable interaction allows for accurate and sensitive measurement of sodium diclofenac. The ion-pair product of sodium diclofenac was determined using the DLLME method, and the absorbance was measured at 508 nm (the maximum wavelength for the product) (**Figure 3**).

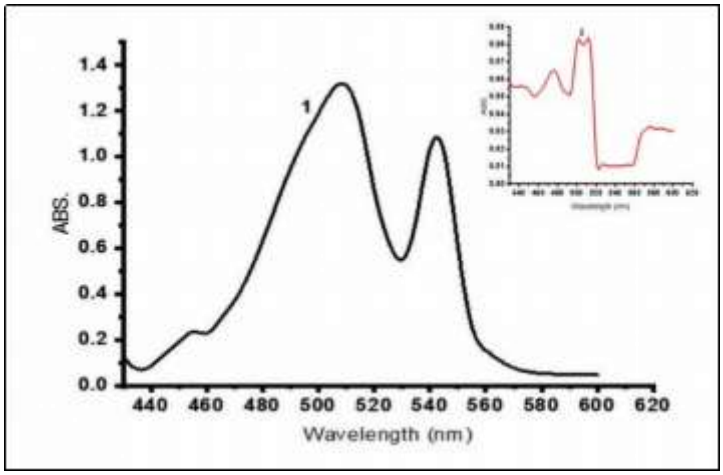

Figure 4. UV-visible spectrum of sodium diclofenac complex.

3.1. Optimization of DLLME method

In conjunction with UV-Vis spectrophotometry, the DLLME technique was employed to identify the optimal conditions for extracting the sodium diclofenac complex with Rhodamine 6G reagent at a wavelength of 508 nm. The most critical factors in these complex formation processes are the pH, the type and volume of extraction solvent, the type and volume of dispersive solvent, and the reagent volume. Accordingly, a comprehensive investigation was conducted. The addition of phosphate buffer was necessary to control and maintain the pH of the sample at the optimal level (pH 10) during the Dispersive Liquid-Liquid Microextraction process. The pH is a critical factor in the ion-pair complex formation between the negatively charged carboxyl compounds (sodium diclofenac) and a cationic reagent (Rhodamine 6G), which is essential for effective extraction. The buffer ensures that the pH remains stable throughout the extraction, leading to consistent and optimal complex formation and subsequent extraction efficiency.

The impact of chloroform, carbon tetrachloride, and dichloromethane on the extraction process was investigated. The results demonstrated that dichloromethane was the most effective solvent for drug testing. The resulting data are presented in **Table 1**. Also, the impact of various dispersive solvents, including ethanol, methanol, acetone, and acetonitrile, was examined. The findings indicated that methanol was the most effective dispersion solvent for sodium diclofenac, as illustrated in **Table 2**. Dichloromethane proved to be the most effective extraction solvent due to its suitable polarity, which allows for efficient dissolution of the formed ion-pair complex between sodium diclofenac and Rhodamine 6G. Its higher density than water is also crucial for Dispersive Liquid-Liquid Microextraction (DLLME), facilitating clear phase separation and collection of the analyte-rich organic phase. Methanol was identified as the optimal disperser solvent because of its excellent miscibility with both the aqueous sample and the extraction solvent. This property enables the rapid formation of a fine cloudy dispersion, maximizing the interfacial area between phases and thereby enhancing the mass transfer efficiency of the ion-pair complex into the organic solvent.

Table 1. Selection type of extraction solvent.

Extraction Solvent Type	Abs.
CH_2Cl_2	0.431
CCl_4	0.353
$CHCl_3$	0.326

Table 2. Selection type of dispersive solvent.

Dispersive Solvent Type	Abs.
Methanol	0.547
Acetone	0.132
Acetonitrile	---
Ethanol	0.432

The extraction of the sodium diclofenac product requires a specific volume of Rhodamine 6G reagent. The highest absorbance of the drug was observed when a volume of 1.5 mL of Rhodamine 6G was used, as illustrated in **Figure 5**.

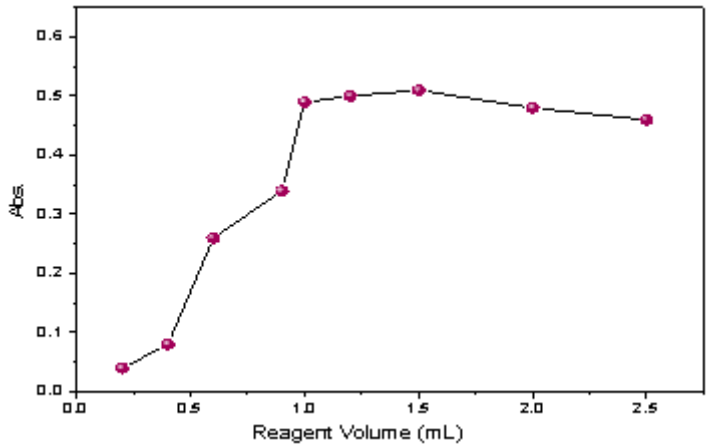

Figure 5. Effect of reagent volume.

3.2. The other effective conditions

On the other hand, we studied the effect of other parameters such as pH value, extraction solvent volume, dispersive solvent volume, and rotation number and time using a Design-Expert 13 program. The program designed and applied the model for a series of experiments in a laboratory. After conducting these experiments, the finding that this model was significant is shown in **Table 3**. The results show that the P-values were less than 0.0001, indicating that the model terms are significant. In this case, B, A^2, B^2, C^2, D^2, and E^2 are significant model terms. The significant linear term for B (Extraction solvent Vol., p-value < 0.0001) indicates that the volume of the extraction solvent has a dominant and direct linear effect on the extraction efficiency. This means that the changing this volume leads to a proportional change in the amount of sodium diclofenac extracted. The significant quadratic terms for A^2 (pH^2), B^2 (Extraction solvent $Vol.^2$), C^2 (Dispersive solvent $Vol.^2$), D^2 (Rotation $number^2$), and E^2 (Rotation $time^2$), all with p-values < 0.0001, are highly important. These quadratic effects signify a non-linear relationship between each respective factor and the extraction efficiency, implying that there is an optimal point or range for each of these parameters.

Table 3. show the ANOVA for the response surface of the Quadratic model.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.5636	20	0.0282	32.08	< 0.0001	significant
A-pH	0.0007	1	0.0007	0.7758	0.3889	
B-Extraction solvent Vol.	0.0369	1	0.0369	41.97	< 0.0001	significant
C-Dispersive solvent Vol	0.0010	1	0.0010	1.14	0.2986	
D-Rotation number	0.0004	1	0.0004	0.4248	0.5220	
E-Rotation time	0.0006	1	0.0006	0.6681	0.4233	
AB	0.0001	1	0.0001	0.0778	0.7831	
AC	4.749E-06	1	4.749E-06	0.0054	0.9421	
AD	0.0000	1	0.0000	0.0154	0.9026	
AE	0.0006	1	0.0006	0.6434	0.4319	
BC	6.250E-06	1	6.250E-06	0.0071	0.9336	
BD	9.000E-06	1	9.000E-06	0.0102	0.9204	
BE	0.0002	1	0.0002	0.2584	0.6168	
CD	0.0005	1	0.0005	0.6040	0.4462	
CE	0.0001	1	0.0001	0.1050	0.7493	
DE	1.040E-06	1	1.040E-06	0.0012	0.9729	
A^2	0.0416	1	0.0416	47.30	< 0.0001	significant
B^2	0.0390	1	0.0390	44.45	< 0.0001	significant
C^2	0.0435	1	0.0435	49.54	< 0.0001	significant
D^2	0.0409	1	0.0409	46.59	< 0.0001	significant
E^2	0.0270	1	0.0270	30.68	< 0.0001	significant
Residual	0.0176	20	0.0009			
Cor Total	0.5812	40				

R^2= 0.9698
R^2_{Adj}= 0.9395
R^2_{Pred}= 0.9586
Adeq precision= 16.2913(Adeq precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable

To obtain the greatest absorbance, optimizing the selected significant extraction process variables is important. The results show that the optimal conditions to achieve the best extraction are as follows: pH 10, extraction solvent volume 800 µL, dispersive solvent volume 1200 µL, and rotation at 2500 rpm for 6 minutes, respectively, as shown in **Figures 6(a-i)**. The 3D response surface plots in **Figure 4** were instrumental in visualizing the complex interactions between these critical parameters and their combined effect on extraction efficiency. The characteristic dome-shaped surfaces, exemplified by **Figure 4(a)** for pH and extraction solvent volume, clearly identified the optimal region for maximum absorbance. The apex of each "dome" directly pinpointed the precise combination of plotted variables yielding the highest extraction efficiency. By interpreting these contours and peaks, we were effectively guided to identify the optimal operating conditions for parameters such as pH, extraction and dispersive solvent volumes, rotation number, and rotation time, thereby ensuring the method's peak performance.

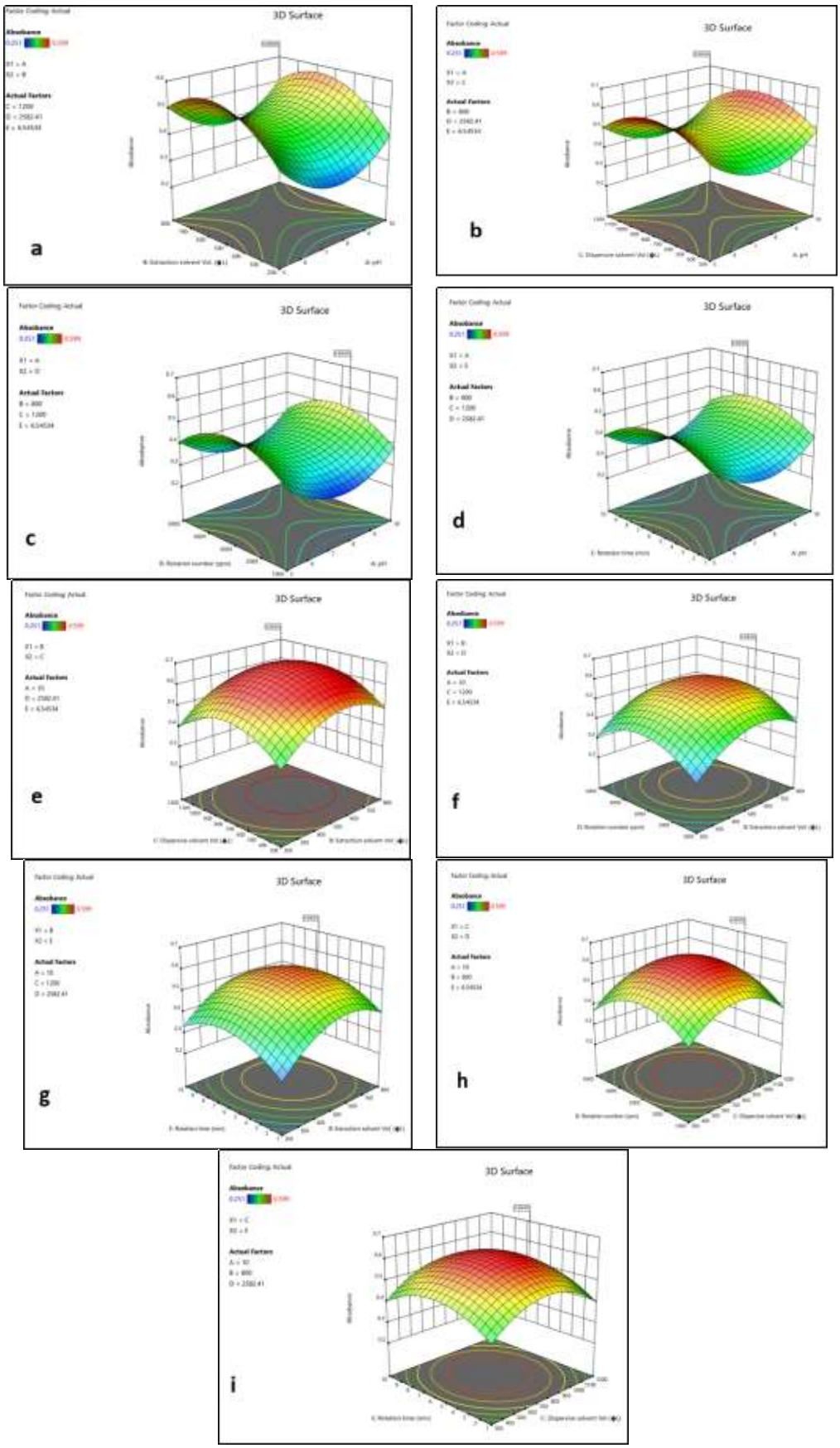

Figure 6 (a-i). 3-D response surface plots illustrating the effects of: (a) pH and extraction solvent volume, (b) pH and dispersive solvent volume, (c) pH and rotation speed, (d) pH and rotation time, (e) dispersive and extraction solvent volumes, (f) rotation speed and extraction solvent volume, (g) rotation time and extraction solvent volume, (h) rotation speed and dispersive solvent volume, and (i) rotation time and dispersive solvent volume.

The normal probability plots (**Figures 7a and 7b**) confirm the good linear relationship between predicted and observed experimental results, validating the Box-Behnken Design (BBD) model. This linearity signifies normally distributed residuals, crucial for the statistical reliability of ANOVA. It indicates the quadratic model accurately captures underlying relationships and effectively predicts the response. Furthermore, it assures that errors are random and unbiased, providing confidence in the identified optimal conditions.

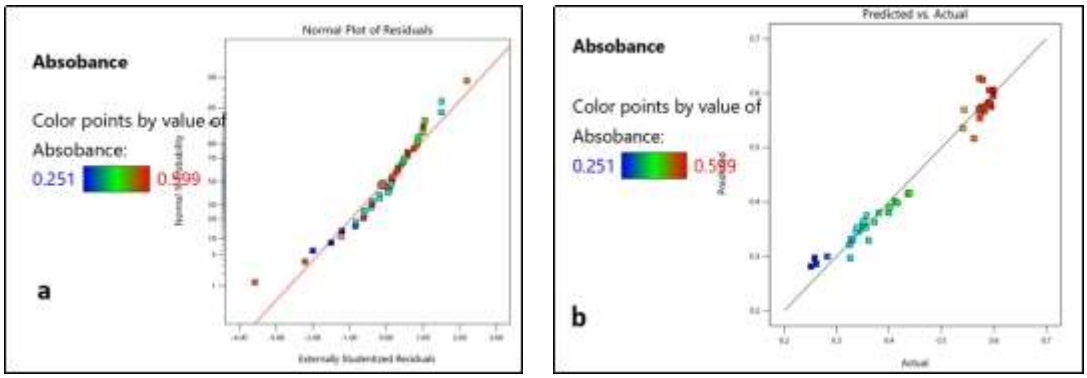

Figure 7(a and b). The normal plots for sodium diclofenac drug using the DLLME method.

The ratio of the medication to the Rhodamine 6G reagent was expressed using the continuous variation method and the mole-ratio method. The process demonstrated a 1:1 ratio (sodium diclofenac: Rhodamine 6G reagent), as illustrated in **Figures 8 and 9**. The observed optimal volume of 1.5 mL for Rhodamine 6G reagent signifies the precise amount needed to achieve maximum ion-pair complex formation and subsequent extraction efficiency, ensuring that the reaction is not limited by reagent availability without introducing excess that could hinder the process. Furthermore, the crucial 1:1 reaction ratio between sodium diclofenac and Rhodamine 6G, confirmed by both the continuous variation and mole-ratio methods, provides fundamental insight into the reaction mechanism, establishing that one molecule of diclofenac precisely interacts with one molecule of the reagent. This defined stoichiometry is vital for method robustness, as it guarantees consistent and complete complexation under optimal conditions, thereby contributing significantly to the reproducibility and reliability of the analytical procedure.

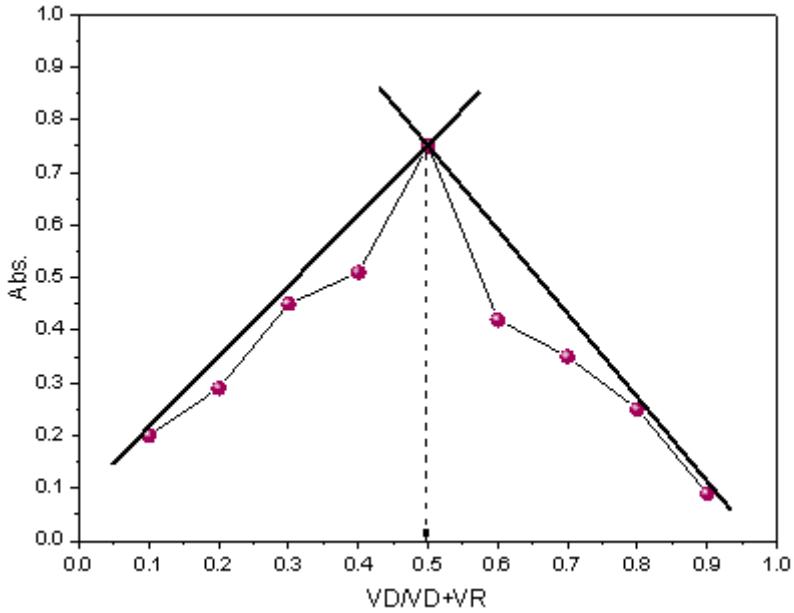

Figure 8. Continuous variation method of Sodium Diclofenac.

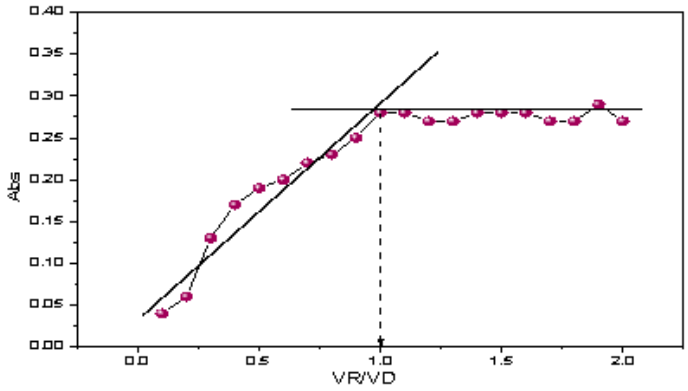

Figure 9. Mole-ratio method of Sodium Diclofenac.

The impact of various compounds on the analytical process, including glucose, fructose, sucrose, and other sugars that may be present in pharmaceutical preparations, was examined. The influence of sugar interference on the sodium diclofenac extraction procedures was found to be insignificant, as evidenced by the data presented in **Table 4**.

Table 4. Extraction recovery with different interference compound.

Rec. %	Comp.
-	Drug without excipients
93.7	Galactose
89.9	Maltose
91.1	Sucrose
92.4	Glucose
97.8	Talic acid
93.3	Ribose
99.2	Starch

3.3. Calibration curve and statistical treatments

Once the optimal conditions for extracting the drug product (Sodium Diclofenac) had been established, a calibration curve was constructed by plotting the absorbance of the drug against its solution concentration. The concentration range of sodium diclofenac was determined to be 1.0-16.0 mg/L, as illustrated in **Figure 10**, using the regression equation Y = 0.0996X + 0.0064 and R^2 = 0.9982 **(Table 5)**.

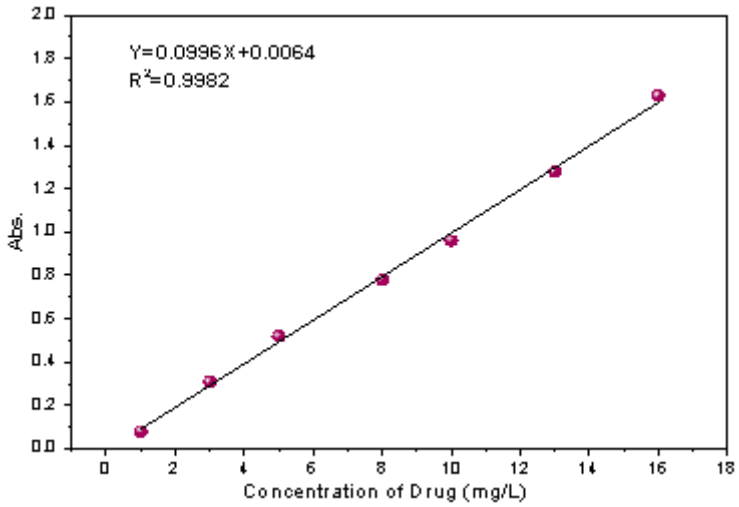

Figure 10. Calibration curve of sodium diclofenac by DLLME method.

Table 5. Analytical parameters of the DLLME method.

Parameter	DLLME method
λ max(nm)	508
color	Red
linearity range mg/L	1.0-16.0
Molar absorptivity (L.mol^{-1} cm^{-1}), Ɛ	31.6×10^{3}
Sandell's sensitivity µg/cm^{2}	1×10^{-2}
Correlation coefficient (R^2)	0.9982
Regression equation	Y= 0.0996X- 0.0064
Slope(b)	0.0996
Intercept(a)	0.0064
Limit of detection mg /L (LOD)	0.326
Limit of quantification mg/L(LOQ)	0.987

4. Accuracy and precision

The experiments for accuracy and precision were conducted using three concentrations of sodium diclofenac: 5, 10, and 15 mg/L, with three replicates for each, under optimal DLLME conditions. These concentrations fall within our established linearity range of 1.0-16.0 mg/L. The method demonstrated good accuracy and precision, evidenced by a recovery rate of 99.3% for sodium diclofenac.

To further validate the method, an F-test and a two-tailed T-test were employed to compare our proposed DLLME method with standard HPLC method. The purpose of these statistical tests was to determine if there was a significant difference between the two methods. The results of the statistical analysis, presented in **Table 6**, showed that the calculated T-values and F-values for determining sodium diclofenac in different pharmaceutical formulations were less than their respective critical values at a 95% confidence interval and (n-2) degrees of freedom. This indicates that there is no statistically significant difference between our proposed DLLME method and the standard HPLC method. This standard HPLC method, as described in Reference[31] (Emara et al.), was properly developed and validated according to US-FDA guidelines, lending strong credibility to its use as a benchmark.

Table 6. Accuracy and precision of DLLME procedure of sodium diclofenac.

Drug	DLLME method							
	Con. µg/mL		Relative Error%	Rec. %	Av. Rec.%	T-value	F-value	RSD%
Sodium diclofenac	5	5.23	4.6	104.6	99.3	0.06	2.5	0.39
	10	10.64	-4.5	95.5				1.28
	15	15.78	-2.2	97.8				0.94
Critical value at 95% confidence limit, T = 2.77, F= 19								

4.1. Application to commercial Pharmaceutical Dosage Forms

Three commercially available pharmaceutical formulations—Olfen-100SR (capsules), Voldic, and Refen Retard (tablets)—were used to evaluate the sodium diclofenac content via DLLME. According to **Table 7**, high accuracy and acceptable results were achieved.

Table 7. DLLME application for Sodium Diclofenac analysis in commercial pharmaceuticals.

drug	Conc. of drug mg/L		DLLME method			
	Taken	Found	Relative Error%	Rec. %	Av. Rec.%	RSD% (n=3)
Olfen-100SR (Swiss)	5	5.33	6.6	106.6		1.08
	10	10.29	-8.8	91.1	97.8	1.5
	15	15.58	-4.2	95.8		0.49
Voldic (Jorden)	5	5.07	1.4	101.4		0.51
	10	10.770	-1.4	96.2	98.1	0.67
	15	15.967	-3.3	96.7		0.72
Refen Retard (Serbia)	5	4.87	-2.6	97.4		0.82
	10	10.695	-13.1	86.8	92.8	0.14
	15	15.946	-5.4	94.6		0.54

4.2. Comparison with reported methods

In the proposed method, the Dispersive Liquid-Liquid Microextraction (DLLME) of sodium diclofenac was performed using 800 µL of dichloromethane as the extraction solvent and 1200 µL of methanol as the disperser solvent. This approach, coupled with spectrophotometric detection at 508 nm, resulted in a low LOD, LOQ, and a good linearity range of 1.0-16.0 mg/L (R^2 = 0.9982). The limits of detection (LOD) and quantification (LOQ) were calculated to be 0.326 mg/L and 0.987 mg/L, respectively. Many other methods have been reported for the determination of sodium diclofenac. Compared with these existing methods, the proposed DLLME method simplifies the analytical process and can be effectively used for the determination of sodium diclofenac in market pharmaceutical formulations (**Table 8**). The method offers a simple, efficient, environmentally friendly, and cost-effective approach for quantitative analysis. When comparing this method to the Digital image-based (webcam) flame emission spectrometric method[32], while it has a linearity range of 24.9–124.5 mg/L, our proposed DLLME method offers superior sensitivity, achieving a lower LOQ of 0.987 mg/L compared to both[32] (2.1 mg/L) and[34] (1.765 µg/mL). The advantage of this method lies in its greenness; unlike Gas Chromatography[35,36] or HPLC[37], which often require larger volumes of organic solvents, this DLLME method presents a significantly greener alternative. Finally, the simplicity and cost-effectiveness of spectrophotometry make DLLME method a more accessible choice compared to complex and often more expensive chromatographic or advanced spectrometric techniques.

Table 8. Comparison of the linearity and LOD, LQO with previous studies.

Method	Linearity	LOD	LQO	Ref.
Digital image-based (webcam) flame emission spectrometric method (Indirect determination)	24.9–124.5 mg/L	0.6 mg/L	2.1 mg/L	[32]
Derivative UV Spectrophotometric (First Derivative method)	1-15 µg/mL	0.31 µg/mL	0.96 µg/mL	[33]
Manipulating Ratio Spectra Spectrophotometric method (Ratio spectra peak to peak measurement)	2.0–24.0 µg/mL	0.583 µg/mL	1.765 µg/mL	[34]
Gas Chromatography	0.25–5 µg/mL	0.05 µg/mL	0.15 µg/mL	[35]
Linear Sweep Voltammetry (LSV)	5–35 µg/mL	1.6 µg/mL	4.8 µg/mL	[35]
Gas Chromatographic method	2-10 µg/mL	0.5 µg/mL	1.5 µg/mL	[36]
Solid-Phase Extraction-HPLC	6.4–16 µg/L	0.12 µg/L	0.39 µg/L	[37]
DLLME-Spectrophotometric - Box-Behnken Design (BBD)	1.0-16.0	0.326	0.987	Present work

5. Conclusion

This study successfully developed a simple, efficient, environmentally friendly, and cost-effective method for the spectrophotometric determination of sodium diclofenac in commercial pharmaceutical formulations. The method relies on ion-pair complex formation combined with optimized dispersive liquid-liquid microextraction (DLLME), with key parameters systematically optimized using a Box-Behnken design (BBD). The findings confirm DLLME as a highly effective technique for the accurate quantification of sodium diclofenac, presenting a valuable alternative for routine analytical applications.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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