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Ion-Pair Complex of Ketotifen Fumarate with New Reagent and its Extraction by LLE and DLLME

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ABSTRACT

A novel, simple, fast, and reproducible liquid-liquid extraction and dispersive liquid-liquid micro extraction (DLLME) combined with UV-visible spectrophotometric methods were developed utilizing Sodium (E)-4-chloro-5-((2-hydroxynaphthalen-1-yl) diazenyl)-2-methylbenzenesulfonate (SCMS) as a reagent for the estimation of ketotifen fumarate (KTF) in a pure state and in the pharmaceutical dosage form. The suggested methods are based on the formation of the red ion-pair complex of ketotifen fumarate (KTF) with SCMS reagent in britton pH=2 buffer. The extracted red complexes showed absorbance maxima at 490 nm. Beer's law is obeyed in the concentration ranges (5.0-50.0 mg/L) for (LLE) and (1.0-10.0 mg/L) for (DLLME), respectively. The molar absorptivities 1.36×103 and 15.8×103 L.mol-1.cm-1 for LLE and DLLME procedures, respectively, as well as the Sandell`s sensitivity, 0.31 and 0.26 µg.cm-2 for both methods, which indicated the high sensitivity of the suggested methods. The percent relative standard deviation (RSD%) 1.8-5.1% and 0.5-4.6 % for LLE and DLLME refer to the high precision of the proposed method.

Key words: Ion-pair complex, Ketotifen fumarate, LLE, DLLME, Extraction

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INTRODUCTION

Ketotifen fumarate, as known [10H- benzo(4,5) cyclohepta(1,2-b)thiophen-10-one,4,9-hydro-4-(1-methyl-4-piperidinylidine)-(E)-2-butenedioate, (KTF)] (Figure 1) [1]. It contains anti-allergic and anti-histaminic properties [2,3]. It's prescribed for asthma prevention, hay fever symptom relief, and urticaria alleviation; it's also utilized to treat allergic disorders like rhinitis and conjunctivitis [4]. A variety of techniques and methods to assay the ketotifen fumarate in various samples such as body fluids and pharmaceutical formulations. From these techniques, HPLC [5-11], TLC [12], capillary electrophoresis [13], spectrofluorimetry [14], potentiometric titration [15,16], coulometric titration [17], chemiluminescence [18-20] and UV-Vis spectrophotometry [4,19-23]. The dispersive liquid-liquid micro extraction (DLLME) has many advantages like rapid, low cost, and safety [24,25]. The aim of the work is the determination and extraction of ketotifen fumarate in pure and pharmaceutical formulations using an ion-pair complex with a new reagent followed by liquid-liquid extraction (LLE) and dispersive liquid-liquid micro extraction (DLLME).

Experimental

Apparatus

A double beam UV-Vis spectrophotometer (cintra 5) was utilized for absorption intensity and spectra measurements using (1 cm) quartz cell. Also, the pH

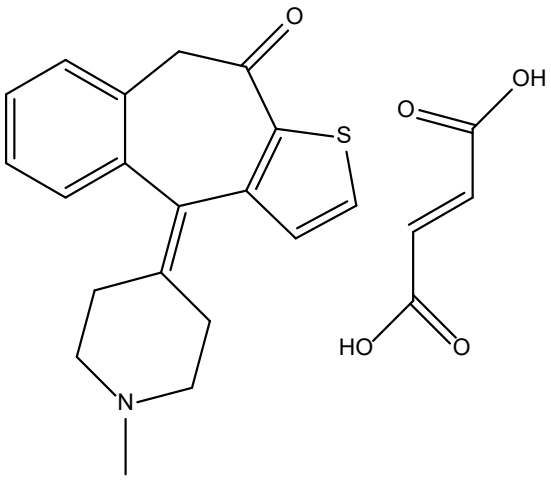

Figure 1: Ketotifen fumarate (KTF) structure.

measurements were achieved by pH-meter DW-9421 from the Philips instrument. To separate the organic phase from the aqueous phase, a hermle Z-300 centrifuge (Germany, Wehingen) was utilized.

MATERIALS AND METHODS

Ketotifen fumarate (KTF, purity 99.8%) was obtained as a gift sample from the state company for drug industries and medical appliances, Samara-Iraq(SDI). Sodium (E)-4-chloro-5-((2-hydroxynaphthalen-1-yl) diazenyl)-2-methylbenzenesulfonate (SCMS) reagent was synthesized according to Asmaa et al [26]. A stock solution of (KTF) 0.05 gm was weighed and dissolved in the 100 mL of 0.1N HCl solution to prepare 500 $mg.l^{-1}$. Also, a stock solution of SCMS (500 mgl^{-1}) was prepared by dissolving 0.05 gm in 100 mL of D.W in the volumetric flask (100mL). Sulphate buffer (pH 2) was prepared by dissolving 132.1 g of ammonium sulphate in the 14 mL concentrated sulfuric acid and completing the volume to 500 mL with D.W in the volumetric flask 500 ml [27].

Assay procedure for dosage forms

Five tablets of KTF, 10 mg (asmafort, UAE & astomide, Syria) were weighed and powdered. The amount weighed was dissolved in the 100 mL of 0.1 N HCl solutions in the volumetric flask. The content was mixed well and filtered through a filter paper to remove the insoluble compounds.

General procedure of LLE

A 5.0-50 mg. L-1 of KTFstandard solution was added to a 15 mL volumetric flask stoppered tube including 2 mL SCMS (100 mg. L-1). The volume was completed to 5 mL with sulphate buffer (pH 2). The mixture was shaken for 5 min, then heated for 10 min at 30 0C. Chloroform 5 mL was added to the mixture and centrifuged at 4000 rpm for 5 min. The organic phase that contains the ion-pair complex was separated using a syringe and the absorbance at 490 nm was measured against a blank.

General procedure of DLLME

A 1.0–10.0 $mg.L^{-1}$ of KTF standard solution was added to a 15 mL volumetric flask containing 2 mL SCMS (20 $mg.L^{-1}$) and 2 mL of sulphate buffer (pH=2). The volume was completed to 10 mL with D.W. The mixture was shaken for 5 min, then heated for 10 min at 30 °C. A cloudy solution was created by rapidly injecting 200 µl chloroform as an extraction solvent and 800 µl acetone as a dispersive solvent into the mixture utilizing a microsyringe. For 5 min, the mixture was centrifuged at 4000 rpm. A microsyringe was utilized to get the red ion-pair complex and the absorbance at 490 nm was measured against a blank.

RESULTS AND DISCUSSION

In the presence of the SCMS reagent anion (A-), ketotifen fumarate (KTF+) binds to form a red-colored an ionic pair complex. The red complex`s absorbance is measured utilizing a spectrophotometer in solution pH 2 at λmax 490 nm against a blank, the spectrum of the complex is shown in Figure 2.

Optimization of liquid-liquid extraction (LLE)

The ion-pair complex of ketotifen fumarate was separated and extracted using the LLE approach, and the absorption was measured at 490 nm. Various volumes (1-8) of phosphate buffer were studied, and the results showed that pH=2 is the optimum (Figure 3). The sulphate buffer is the best among the investigated buffer solutions (phosphate, suphate, and KCl+HCl) (Table 1). The extraction solvents (chloroform, tetrachloromethane, and dichloromethane) were investigated (Table 2). The results showed that chloroform was the best extraction solvent for the separation of ion-pair complex. A water bath was used to study temperatures ranging from 25 to 50 °C, and it was discovered that 30 °C had the highest

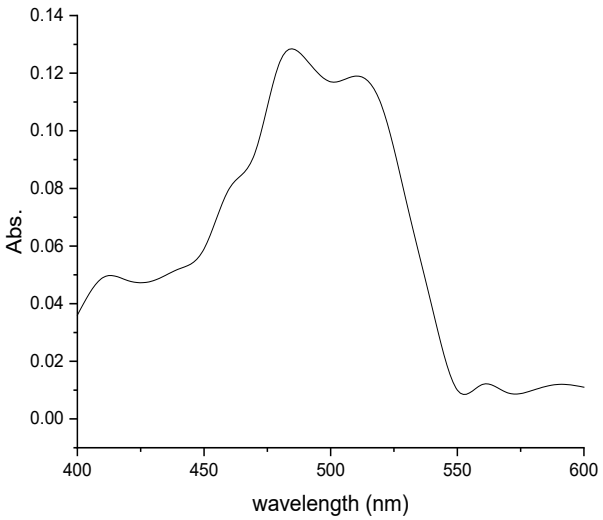

Figure 2: Spectrum of the resulting ion-pair complex.

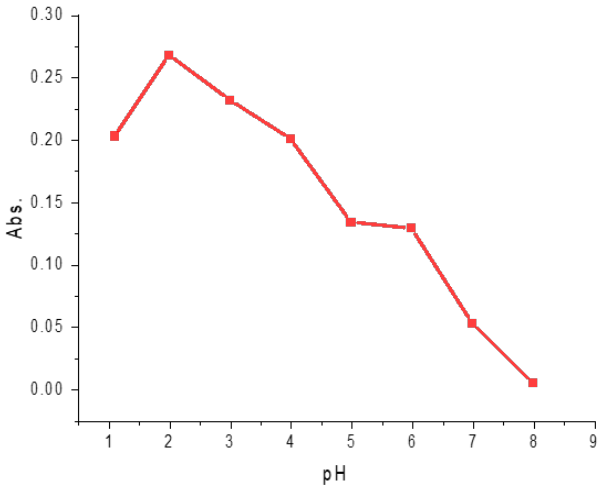

Figure 3: Effect of phosphate buffer.

Table 1: Effect of buffer type.

Type of buffer	Abs.
phosphate	0.273
Sulphate	0.54
KCl+HCl	0.268

absorption value at 490 nm (Figure 4). From 5.0 to 30 minutes were needed to extract and separate the complex, with 10.0 minutes having the highest absorption value at 490 nm (Figure 5). The effect of the reagent volume was also studied and it is found that the best volume was 2.0 ml (Figure 6). The influence of number and time rotation on the extraction of ion-pair complex in the centrifuge is crucial. 4000 rpm at 5 min gives the highest absorbance as shown in the Tables 3 and 4.

Two approaches were used to denote the ratio of KTF and SCMS reagent molar ratio and continuous variation techniques. It was demonstrated that the ratio for both procedures was 1:1 (KTF drug+SCMS reagent) (Figures 7 and 8). The effect of excipients such as glucose, starch, and others was achieved and it was found that they have no effect on the determination and extraction of the drug, as shown in the Table 5.

Table 2: Effect of solvent type.

Type of solvent	Abs.
$CHCl_3$	0.54
CCl_4	0.32
CH_2Cl_2	0.519

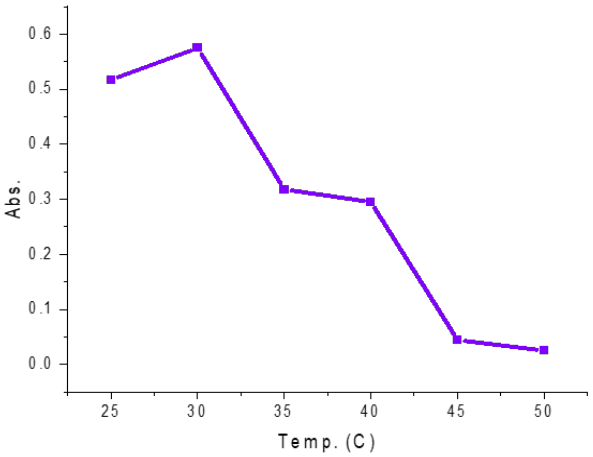

Figure 4: Effect of temperature.

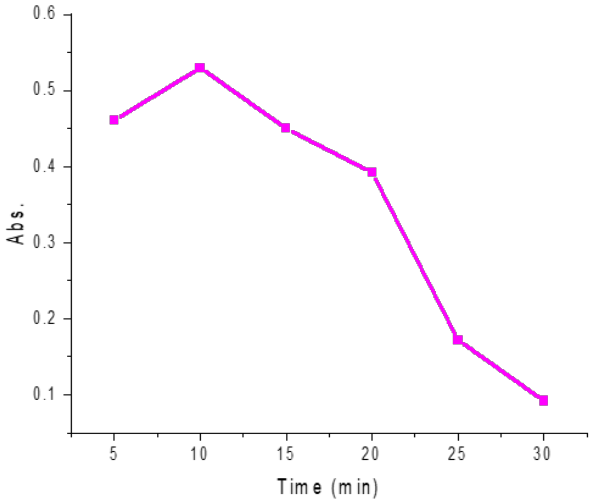

Figure 5: Effect of incubation time.

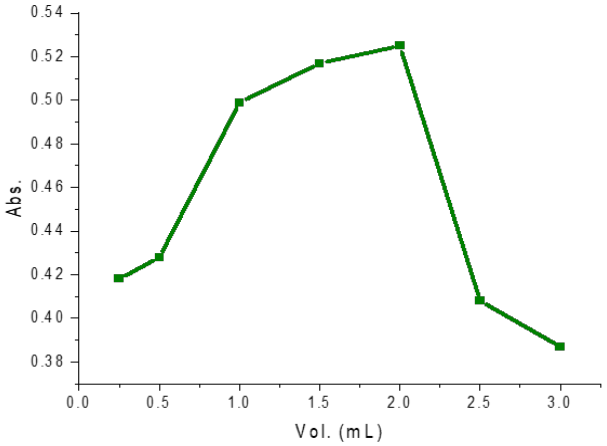

Figure 6: Effect of reagent volume.

Table 3: Effect of rotation number.

Rotation No.	Abs.
1000	0.432
2000	0.473
3000	0.491
4000	0.54

Table 4: Effect of the rotation time.

Rotation Time	Abs.
1	0.238
2	0.456
3	0.492
4	0.514
5	0.541

Calibration curve

An analytical method is shown to be linear if it can produce test findings that are proportionate to the concentration of analyte in the sample. Over the concentration range of 5.0 to 50 mg/L, the KTF calibration curve was linear (Figure 9).

Optimization of dispersive liquid-liquid microextraction (DLLME)

DLLME method was utilized for extraction and assessment of the KTF drug and study of perfect conditions for the complexation of ketotifen fumarate drug and SCMS reagent. The influence of extraction solvents such as chloroform, dichloromethane, and tetrachlorocarbon was achieved (Table 6). The results showed that the ideal extraction and dispersive solvents for the best extraction were chloroform and acetone, respectively (Table 7).

The absorbance of a series of volumes of extraction solvent (200-500 µl) was investigated and it was found that a volume of 200 µl gives the highest absorbance value at 490 nm (Figure 10). The influence of dispersive solvent on the extraction was also investigated. The volume of (800 µl) gave the highest value of absorbance (Figure 11). Ion-pair complex formation between KTF drug and SCMS reagent was studied. A series of reagent volumes(0.25-3.0 mL) were added. The reagent volume 2.0 mL gives the highest absorbance (Figure 12). The

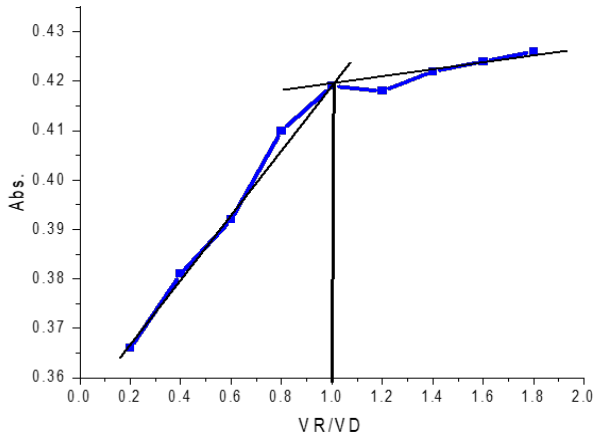

Figure 7: Mole ratio method of KTF.

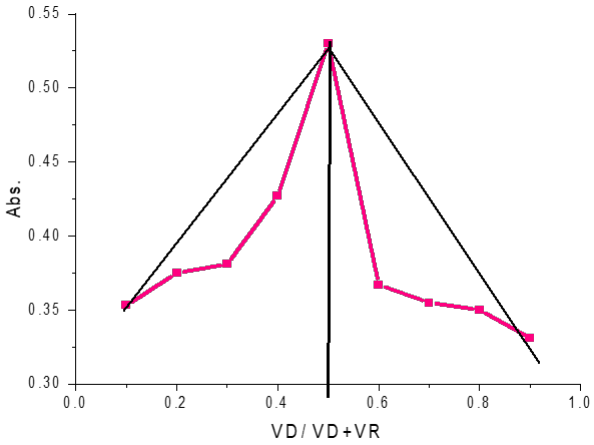

Figure 8: Continuous variation method of KTF.

Table 5: Effect of excipients on the extraction of KTF.

Excipients type	Reco %
Galactose	88.8
Maltose	86.2
Sucrose	90
Glucose	93
Talic acid	93
Ribose	97
Starch	91

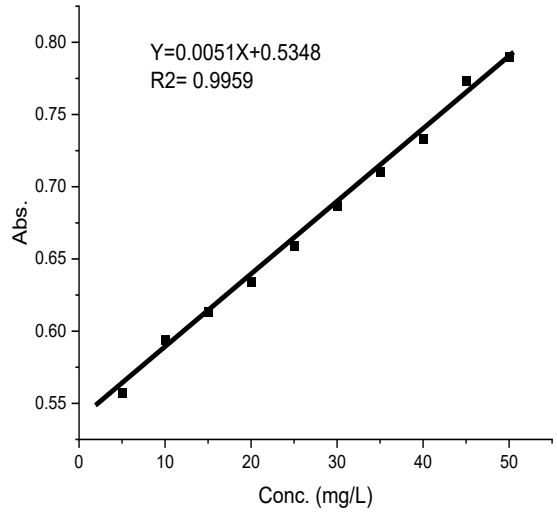

Figure 9: Calibration curve of KTF using LLE.

Table 6: Effect of extraction solvent.

Type of solvent	Abs.
$CHCl_3$	0.2
CH_2Cl_2	0.187
CCl_4	--

Table 7: Effect of dispersive solvent.

Type of dispersive solvent	Abs.
Ethanol	0.461
Methanol	0.201
Acetone	0.598

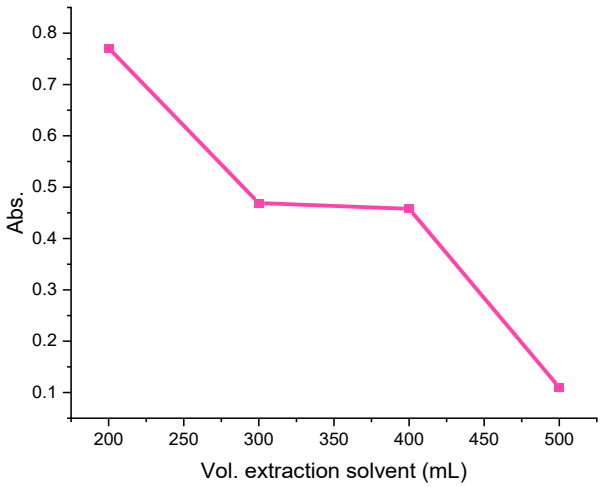

Figure 10: Effect of extraction solvent volume.

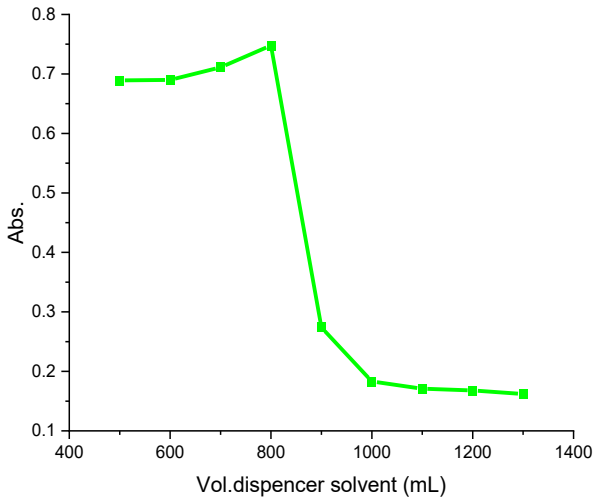

Figure 11: Effect of dispersive solvent volume.

influence of centrifuge speed and incubation time plays a vital role in the extract of the ion-pair complex. A 4000 rpm and 5.0 min give the greatest value of the absorbance (Figures 13 and 14).

In the DLLME, the influence of the possible presence of associated materials such as starch, sucrose, galactose, talic acid, ribose, and fructose on the absorbance of the ion-pair complex was investigated. It was observed that none of these substances does interferes (Table 8).

Linearity and range

The Beer's law range, regression equation, molar

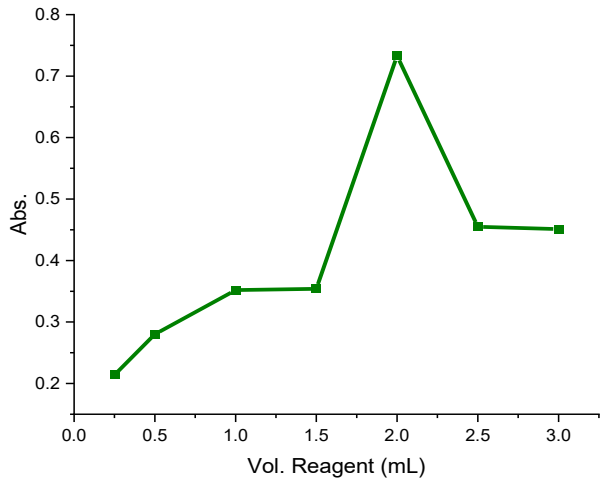

Figure 12: Effect of reagent volume.

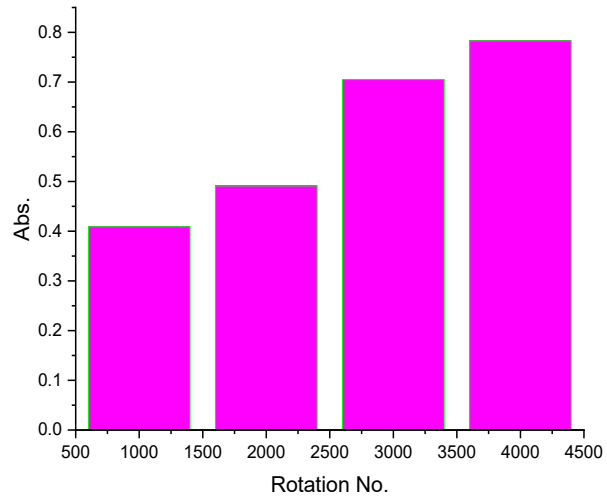

Figure 13: Effect of rotation number.

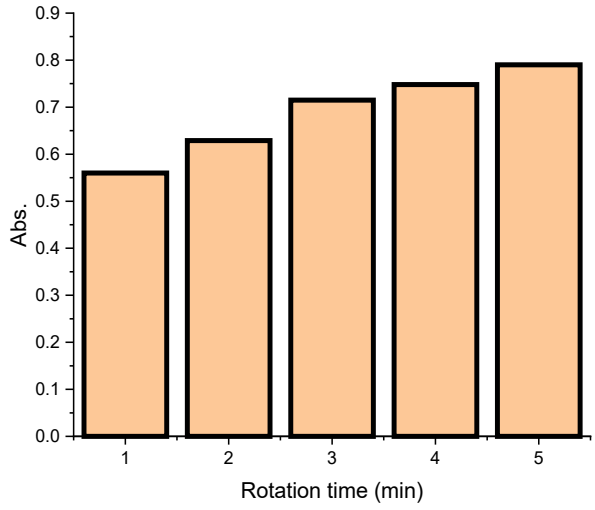

Figure 14: Effect of rotation time.

absorptivity, Sandell's sensitivity, and correlation coefficient for the two the suggested procedure are given in (Table 9). A linear relationship was achieved between the absorbance at λmax and the concentration of the KTF drug in the range (1.0- 10.0) mg.L-1 for the DLLME method in the final measured volume of 10ml (Figure 15). Regression analysis of the Beer's law plots at λmax reveals a good correlation. The high molar absorptivities of the resulting ion-pair complex indicate the high sensitivity of the procedure.

Validation of the method

The validity of the two methods for the analysis of KTF in its pure state and in its pharmaceutical formulations was investigated. The results obtained for pure drugs are given in the Table 10. The accuracy and precision of the

Table 8: Effect of excipients on the extraction of KTF.

Excipients type	Reco %.
Galactose	93.3
Maltose	93.4
Sucrose	92.1
Glucose	96.8
Talic acid	95.6
Ribose	99.3
Starch	100.7

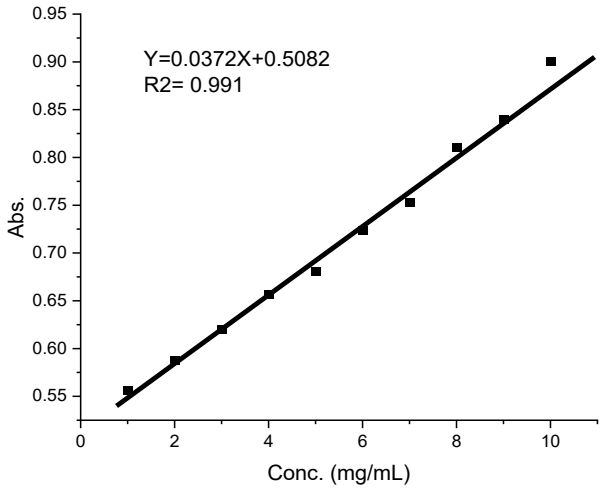

Figure 15: Calibration curve of KTF using DLLME.

Table 9: Analytical and statistical parameters of LLE and DLLME.

Parameter	LLE	DLLME
λ max(nm)	490	
Color	Red	
Linearity range mg/L	5.0-50	1.0-10.0
Molar absorptivity (L.mol^{-1} cm^{-1}), ε	1.36×103	15.8×103
Sandell's sensitivity µg/cm^2	0.31	0.26
Correlation coefficient (R_2)	0.9959	0.991
Regression equation	Y=0.0051X+ 0.5348	Y=0.0372X+ 0.5082
Slope(b)	0.0051	0.0372
Intercept(a)	0.5348	0.5082
Limit of detection mg /L LOD	0.92	0.93
Limit of quantification mg/L LOQ	2.81	2.81
C.L. for the slope at 95%	0.0051 ± 1.156×10-4	0.0372 ± 0.00125
C.L. for Intercept at 95%	0.5348 ± 0.00359	0.5082 ± 0.00773
*C.L. for the X1 mg/L at 95%	5.33 ± 0.0302	2.84 ± 0.207
*C.L. for the X2 mg/L at 95%	10.27 ± 0.0010	4.71 ± 0.38
*C.L. for the X3 mg/L at 95%	16.51 ± 0.0107	6.77 ± 0.71

Table 10: Application of the suggested methods (LLE & DLLME) for the evaluation of KTF.

Drug	Conc. of drug µg/mL-1		Relative Error%	Recov %	Average Recov%	RSD% (n=3)
	Taken	Found				
Liquid-liquid extraction						
Astomide	5	5.01	0.26	100.26	97.08	3.6
	10	9.29	-7.06	92.9		3.3
	15	14.73	-1.7	98.1		3.4
Asmartfone	5	4.86	-2.6	97.3	96.9	5.1
	10	9.5	-5	95		1.8
	15	14.83	-1.06	98.6		2.7
Dispersive liquid-liquid microextraction						
Astomide	3	2.85	-4.96	94.9	97.4	4.6
	5	4.85	-2.86	97.1		0.51
	7	7.01	0.26	100.2		3.86
Asmartfone	3	2.89	-3.53	96.4	97.2	3.35
	5	4.83	-2.66	96.7		3.87
	7	6.91	-2.1	98.6		1.39

Table 11: Comparison of the linearity, recovery, and LOD with previous studies.

Method	Linearity mg/L	LOD mg/L	Ref.
Titrimetric method	2-18	0.39	[28]
Spectrophotometric method	5-35	0.06	[29]
Turbidity method	0.1-50 mM	1.136	[30]
Charge transfer complex	10-100	1.5	[31]
Spectrophotometric method	50-250	-	[32]
Fluorometric method	1-45 mm	29.785	[33]
HPLC method	10-35	-	[34]
HPLC method	0.005-0.1515	0.001	[10]
LLE method	5-50	0.92	Present work
DLLME method	1-10	0.93	Present work

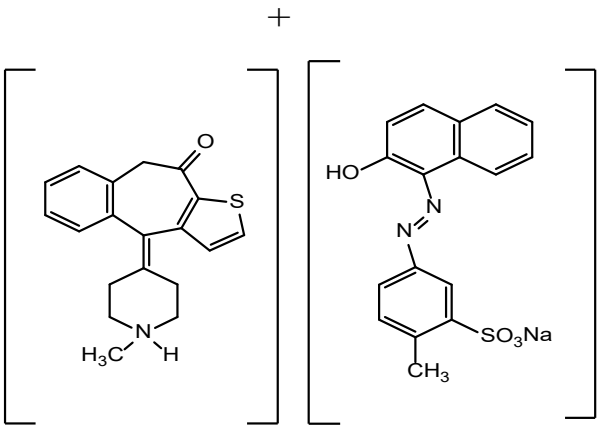

Figure 16: The suggested structure of ion-pair complex KTF.

methods were examined by analyzing three replicates of the drug. The low values of relative standard deviations (RSD%) indicate the best precision and reproducibility of the methods. The results of the analysis of dosage forms are given in the Table 11. The results were reproducible with low RSD% values. The average percent recoveries (Rec.%) obtained were quantitative (92.9%-100.26%) indicating good accuracy of the methods (Figure 16).

CONCLUSION

Simple, rapid, and low-cost LLE and DLLME methods are used for the extraction of red ion-pair complex between KTF drug and SCMS reagent, combined with UV-Visible spectrophotometry. The suggested LLE and DLLME methods have been successfully utilized to quantify and extract KTF drug in both pure and pharmaceutical formulations.

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