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DEVELOPMENT OF AN EFFICIENT DISPERSIVE LIQUID-LIQUID MICROEXTRACTION APPROACH COMBINED WITH SPECTROPHOTOMETRY FOR DETERMINATION OF ANTIVIRAL DRUG, VALACYCLOVIR HCl IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT. A new, fast, sensitive, simple, and green dispersive liquid–liquid microextraction (DLLME)-based spectrophotometric method for the determination of valacyclovir HCl (VAL) in pure form and pharmaceutical formulations has been developed and validated. The developed method is based on the formation of a coloured product through the reaction of VAL and 1,2-naphthoquine-4-sulfonate (NQS) at pH = 9. The important experimental parameters affecting the extraction efficiency were investigated and optimized. The tiny organic droplets had a wavelength of λ = 505 nm. At the optimum conditions, linearity ranged from 0.06 to 2.0 μg/mL, with a linear correlation coefficient of 0.9995. The limits of detection and quantification were 0.02, and 0.06 μg/mL, respectively. The enhancement factor was 36.87. Good recovery as accuracy (99.50%) and relative standard deviation (RSD) as precision were 1.20%, respectively. The developed DLLME method was successfully applied to the determination of VAL in pharmaceutical formulations, and the validity was assessed by applying the standard addition technique. The results obtained by the proposed method for the pure VAL and commercial tablets agreed well with those obtained by the official method.

KEY WORDS: Valacyclovir HCl, Spectrophotometry, 1-2-Naphthoquine-4-sulfonate, Dispersive liquid–liquid microextraction, Pharmaceutical formulations

INTRODUCTION

The molecular name for valacyclovir HCl (VAL) is [(S)-2-[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy]ethyl-2-amino-3-methylbutanoate]. VAL is a prodrug that is in vivo transformed to acyclovir. The viral DNA polymerase is inhibited by VAL's action mechanism, which also involves involvement in the termination of the viral DNA chain. Herpes simplex types 1 (HSV-1) and 2 (HSV-2) varicella-zoster virus infections, herpes zoster (shingles), and herpes B are all treated with VAL [1-3].

The review of the literature revealed that a number of analytical techniques, including high-performance liquid chromatography [4-7], electrochemistry [8-11], spectrofluorimetry [12-14], and spectrophotometry [15-27], have been reported for the determination of VAL in pharmaceutical formulations and biological fluids. According to the illustrated analytical techniques that have been reported for the measurement of VAL, it appears that the majority of these techniques rely on the use of a practical instrument like HPLC. Additionally, several spectrophotometric procedures needed buffer preparation time, incubation reaction time, and/or cooling to complete the reaction. Thus, a green trend in analytical chemistry is the trace detection of VAL in a single step using a non-hazardous organic solvent.

Due to environmental and economic considerations, miniaturization, which aimed to reduce hazardous waste and produce safe products, has emerged as a significant trend in the development

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of sample preparation. They affect not just the environment and human health, but also various economic factors [28].

With the use of a more contemporary technology known as dispersive liquid-liquid microextraction (DLLME), analytical chemists have attempted to reduce or eliminate the use of dangerous and volatile extraction solvents. A green extractant and disperser solvents with excellent miscibility in both the extractant and aqueous phase are used in the quick and easy microextraction method known as DLLME.

Due to their availability and inherent simplicity, spectrophotometric techniques continue to be the most popular and widely used approaches. They are also seen as a more practical option to the other analytical methods previously described. The sensitivity, adaptability, and precision of spectrophotometric approaches are also unmatched. It has been claimed that DLLME and spectrophotometry can be used together to determine medicinal ingredients and other analytes' traces [28-32].

As a result, the present study presents for the first time the coupling of DLLME with spectrophotometry for the extraction and trace estimation of VAL in pure form and pharmaceutical preparations without needing any complex equipment setup. The significant experimental variables that affect the VAL's extraction efficiency were examined and optimised, including the effect of reactant variables, the type and volume of extraction and dispersive solvents, the extraction speed, and the impact of ionic strength. According to ICH guidelines, the suggested technique has undergone statistical validation for its accuracy, precision, sensitivity, selectivity, robustness, and ruggedness [33].

EXPERIMENTAL

Apparatus

Each absorbance spectrum was created using a Varian UV-Vis spectrophotometer (Cary 100 Conc., Australia) outfitted with a 10 mm quartz cell. With a scanning speed of 200 nm/min and a bandwidth of 2.0 nm in the 200-900 nm wavelength range, this spectrophotometer has a wavelength accuracy of 0.2 nm. An AD1000 model pH-meter (Adwa Instruments Kft., Szeged, Hungary) combined with a glass electrode was utilised to control the pH-values of the solutions. A Milli-Q purification device (Millipore, USA) was utilised to obtain deionized/bidistilled water that was used for the preparation of solutions. The phase separation was enhanced and facilitated by the use of a centrifuge (HERMLE, Germany). Prior to the experiment, all glass objects were rinsed and cleaned with bidistilled water after being stored in HNO_3 (5.0% v/v) for at least 16 hours.

Materials and reagents

All of the substances—chemicals, solvents, and reagents—used in this work were of the analytical or pharmaceutical reagent grade, and all of the solutions were made from scratch each day. A pure sample of VAL was kindly supplied by EVA Pharma S.A.E., Cairo, Egypt, with a purity of 99.30 ± 1.20% by applying the official method [1]. Valysernex tablets, labelled to contain 1000 mg VAL per tablet, product of EVA Pharma, Cairo, Egypt. Valtrovir tablets, labelled to contain 1000 mg VAL per tablet, product of Hikma Pharma, Amman, Jordan. 1,2-Naphthaoquine-4-sulfonate (NQS) was purchased from Fluka, Germany, M.wt. 260.20 g/mol with purity 98.0%. Sodium bicarbonate, $NaHCO_3$ (El-Nasr company Egypt). Triton X-114 (Fluka, Buches, Switzerland) was used as the non-ionic surfactant without further purification. Chloroform, carbon tetrachloride, dichloromethane, tetrachloroethylene, and 1,2-dichloroethane were inspected as extraction solvents and methanol, ethanol, acetone and acetonitrile as dispersive solvents, and purchased from Sigma Aldrich, St. Louis, USA.

Preparation of standard solutions

A solution of $NaHCO_3$ (0.1 M) was prepared by dissolving 0.83 g in 100 mL of bidistilled water. A stock standard solution of VAL equivalent to 100 µg/mL was prepared by dissolving 0.01 g of pure VAL in an alkaline solutionof $NaHCO_3$ (0.1 M) in a 100 mL measuring flask, diluted to the mark with the $NaHCO_3$ solution,and mixing well. The standard solutions were found stable for at least one week without alteration when kept in an amber colored bottle and stored in a refrigerator when not in use. Serial dilution with the same solvent was performed to obtain the appropriate concentration ranges. A solution of 0.5% (w/v) of NQS was prepared by dissolving 0.5 g in bidistilled water; then, it was transferred into a 100-mL volumetric flask and diluted to the mark with bidistilled water and mixed well. An aqueous (1.0%, v/v) solution of Triton X-114 was prepared by dissolving 1.0 mL of Triton X-114 in 100 mL of bidistilled water in 100 mL volumetric flask with stirring.

General procedures

Aliquots of the standard VAL working solution (100 g/mL) in the concentration ranges (0.06-2.0 g/mL) were mixed with 1.0 mL of 0.5% w/v) NQS solution in a conical-bottom glass centrifuge tube. Bidistilled water was used to bring the volume up to 10 mL. Then, a 1000 µL mixture of ethanol (extractant solvent) and chloroform (disperser solvent) was rapidly injected, followed by 500 µL of Triton X-114 (surfactant) (1% v/v). Then, the cloudy, turbid solution in the tube was formed in an ice bath for 5.0 min. To speed up phase separation, the solution was centrifuged at 3500 rpm for 3.0 min. The dispersed, tiny organic droplets were subsequently sedimented at the bottom of the centrifuge tube. Using a syringe, the upper aqueous phase was removed. Finally, the remaining phase was transferred into a 1-cm quartz cell for spectrophotometric measurement (λ = 505 nm) against a reagent blank that had been treated similarly but not with VAL. The calibration graph was constructed by plotting the absorbance versus the final concentration of VAL. The corresponding regression equation was derived.

Applications to pharmaceutical formulations

The contents of twenty tablets were crushed, finely powdered, and weight, and the average weight of one tablet was determined. An accurate weight of the powdered tablets equivalent to 10 mg of VAL was dissolved in an alkaline solutionof $NaHCO_3$ (0.1 M) with shaking for 5.0 min and filtered using a Whatman No. 42 filter paper. The filtrate was diluted to the mark with an alkaline solutionof $NaHCO_3$ (0.1 M) in a 100-mL measuring flask to give a 100 µg/mL stock solution for analysis by the proposed method. A convenient aliquot was then subjected to analysis by the DLLME procedures described above. Determine the nominal content of the tablets using the corresponding regression equation of the appropriate calibration graph.

Stoichiometric relationship

The stoichiometric ratio of the formed derivative between VAL and NQS reagent was determined by applying Job's method of continuous variation [34] at the wavelength of maximum absorbance. In this method, equimolar solutions were employed: a 1.0 x 10^{-3} M standard solution of VAL and NQS reagent were used. A series of solutions were prepared in which the total volume of VAL and the reagent was kept at 2.0 mL. The drug and reagent were mixed in various complementary proportions (0 : 10, 1 : 9, ... , 9 : 1, 10 : 0, inclusive) and completed to volume in a 10 mL calibrated flask containing $NaHCO_3$ solution (pH 9), following the above-mentioned procedure. The absorbance of the prepared solutions was measured at the optimum wavelength.

Validation procedure

The proposed method was validated according to ICH guidelines [33] concerning linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, and ruggedness.

RESULTS AND DISCUSSION

Absorption spectra

A common spectrophotometric reagent (NQS) has been used as a colour development reagent in the spectrophotometric determination of many pharmaceutical compounds containing amino groups in an alkaline solution because of its effective reactivity with primary and secondary amines and its high reaction rate [35, 36]. The proposed method is based on the derivatization reaction between VAL and NQS in an alkaline medium and the maximum, absorbance of the reaction product with and without DLLME was recorded at 500 and 505 nm, respectively (Figure 1). Thus, the suggested mechanism of the reaction product of VAL with NQS is shown in Scheme 1.

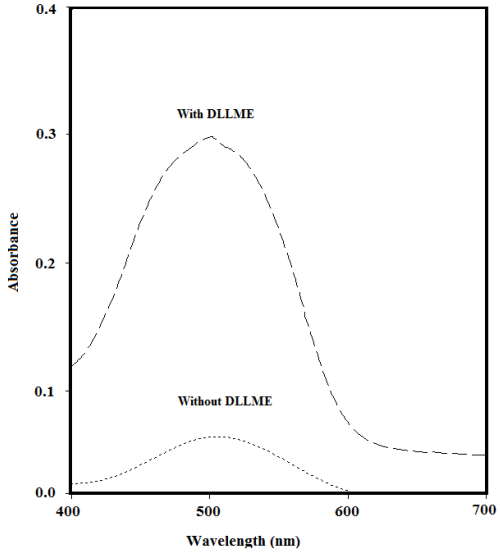
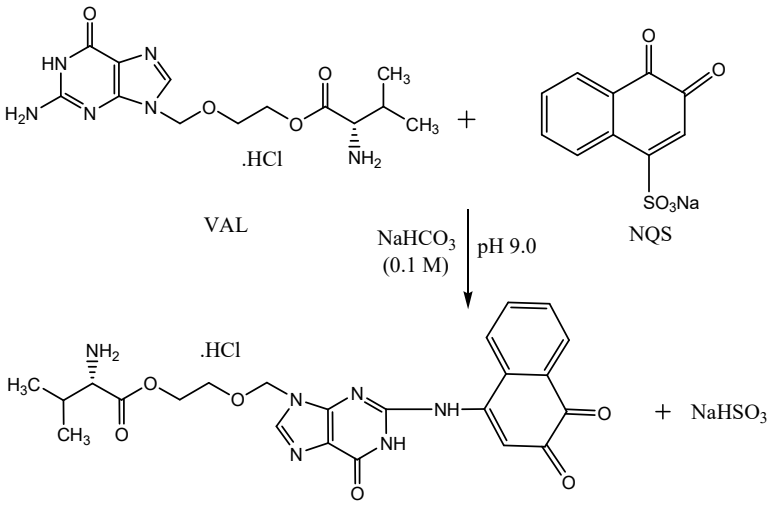


Figure 1. Absorption spectra for the reaction product of (2.0 µg/mL) VAL and NQS reagent with and without DLLME.

Optimization of the reaction conditions

The reaction conditions' parameters were changed one at a time while keeping the others constant, and the effects on the colored product's absorbance were observed. The ideal circumstances for the created method were investigated and improved.



Scheme 1. Suggested chemical reaction of VAL and NQS.

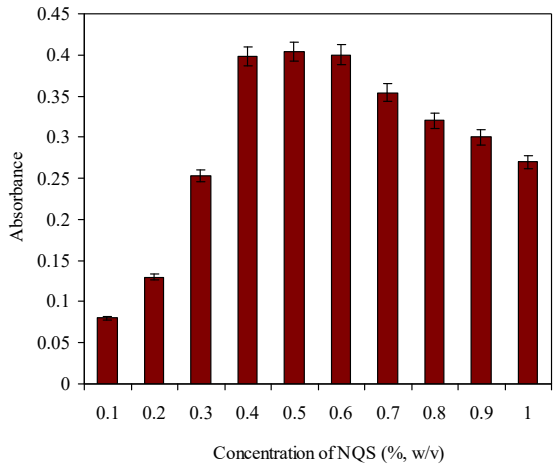


Figure 2. Effect of NQS reagent concentration on the absorbance intensity of VAL-NQS derivative. VAL concentration is (2.0 µg/mL).

Effect of NQS concentration

Different concentrations ranging from 0.1% to 1.0% w/v were used to study the impact of the NQS reagent on the absorbance intensity. The absorbance grew as the reagent concentration increased, peaking at concentrations between 0.4% and 0.6% w/v, after which the absorbance marginally dropped (Figure 2). Therefore, in all future studies, a concentration of NQS 0.5% w/v (1.0 mL) was utilised.

Effect of alkaline solutions and pH

To generate the nucleophile from VAL and activate the nucleophilic substitution reaction, an alkaline medium was necessary. Sodium hydroxide, sodium carbonate, disodium hydrogen phosphate, sodium bicarbonate, and 0.2 M $NaHCO_3$/0.2 M Na_2CO_3 buffer were among the inorganic bases and buffer solutions tested. The best results were obtained with $NaHCO_3$ (0.1 M), whereas with other bases either precipitation of white colloid occurred when the reaction solution was diluted with organic solvent, high blank readings, non-reproducible results, and/or weak sensitivity were observed. The optimum concentration for optimising $NaHCO_3$ concentration was found to be 0.1 mM (Figure 3a).

In a separate series of experiments, the influence of pH on the absorbance of the VAL-NQS product was investigated. The results revealed that the absorbances at pH < 6 were close to 0, indicating that under acidity, VAL has difficulty reacting with NQS (Figure 3b). This could be because the amino group of VAL exists as a hydrochloride amine salt, removing the ability for nucleophilic substitution. At pH > 6, the absorbance increased rapidly as the amino group of VAL converted to free-NH rather than HCl salt, facilitating the nucleophilic substitution reaction. The maximum absorption values were attained in the pH range of 8–10. The absorbance of the solution clearly decreased when the pH exceeded 10. This was most likely due to an increase in the amount of hydroxide ion, which inhibits the condensation reaction between VAL and NQS. In order to maintain the high sensibility required for the determination of VAL, the experiment was carried out at pH 9.0.

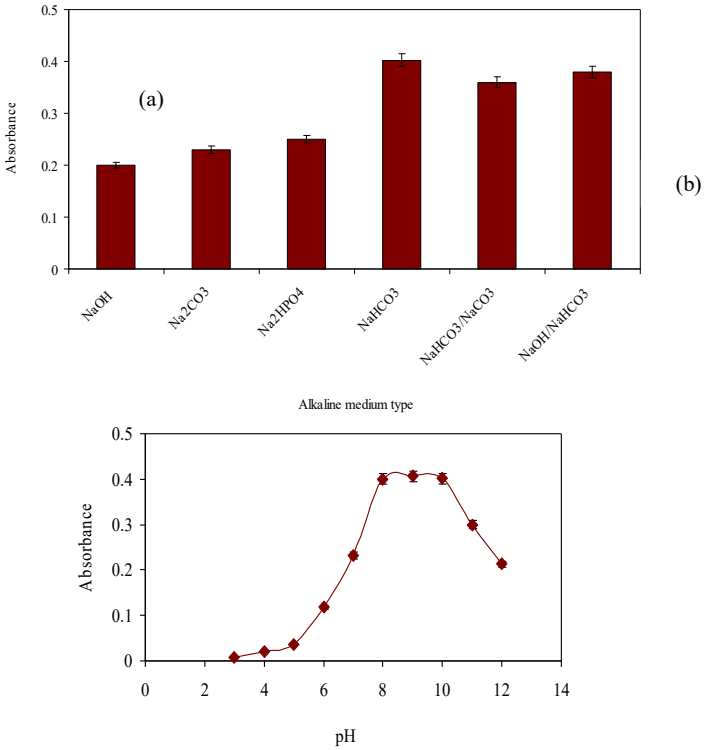


Figure 3. Effect of (a) alkaline medium type and (b) pH on the absorbance intensity of VAL-NQS derivative; concentrations of Val (2.0 µg/mL) and NQS (0.5%, w/v).

Effect of type of extraction, dispersive solvents, and surfactants

In the DLLME technique, choosing the type and volume of extraction solvent was an essential factor that had a large effect on the extraction performance of the analyte. In the present work, different chlorinated solvents such as chloroform, carbon tetrachloride, dichloromethane, 1,2 dichloroethane, and tetrachloroethylene were examined as extraction solvents because of their density and capacity to extract the chemicals of interest. The results are shown in Figure 4a. The results showed that higher absorbance was obtained when chloroform was used as the extraction solvent. So, chloroform was selected as the extraction solvent in further experiments.

In addition, the choice of dispersive solvent is an important parameter in the DLLME technique. It should be miscible in water and soluble in the extraction solvent, allowing the extraction solvent to be distributed as tiny particles in the aqueous phase, forming a cloudy solution (water/disperser solvent/extraction solvent) [30-32]. Therefore, various solvents like, acetone, acetonitrile, methanol, and ethanol were examined, as shown in Figure 4b. The results showed that higher absorbance was obtained when ethanol was used as the dispersive solvent.

Consequently, the effect of the volume of extraction and the dispersive solvents on the formation of dispersion was studied and optimized. Therefore, the effect of different volumes of chloroform was tested in the range of (50-600 µL) at a constant volume of dispersive solvent (1000 µL ethanol). It was found that the absorbance of thecollected organic phase was enhanced by increasing the chloroform volume up to 400 µL. Hence, 500 µL of chloroform was chosen as the optimum extraction solvent volume for all the subsequent studies. Different volumes of dispersive solvents ranging from 500 to 2000 µL were examined. The results show that 1000 µL of ethanol gave highest intensity and was chosen for the subsequent experiments. The best extraction efficiency of the target analyte was therefore achieved using a mixture of ethanol (1000 µL) and chloroform (500 µL).

Different types of surfactants were used in order to measure the impact of each surfactant, including Triton X-114, Triton X-100, Tween 80, cetyltrimethylammonium bromide (CTAB), and sodium dodecyl sulphate (SDS) (Figure 4c). Triton X-114 was chosen as the ideal surfactant since the results indicated that it had the highest absorbance and the best extraction efficiency. Also. By adding various amounts of Triton X-114 in the range of (100-600 µL), the effect of Triton X-114 (1% v/v) volume was assessed. The findings showed that as Triton X-114 volume increased, absorbance intensity rose. The collected data demonstrate that (500 L) Triton X-114 had the maximum absorption.

Effect of centrifugation speed and time

Centrifugation was tested at speeds ranging from 1000 to 5000 rpm for periods of 1.0 to 10 min. According to the data, the greatest absorption was attained in 3.0 min at 3500 rpm.

Effect of ionic strength

A salt is added to reduce analytes' solubility in the aqueous phase and increase their extraction into the organic phase. A series of tests were carried out by increasing the concentration of NaCl from 5 to 30% (w/v) in order to evaluate the impact of ionic strength on the effectiveness of DLLME extraction. It was discovered that raising the amount of NaCl from 5% to 30% had no discernible impact on absorbance. Based on these findings, no salt was added during any following studies.

Stoichiometric ratio

Job's method of the continuous variation [34] of equimolar solutions was employed to determine the stoichiometry of the reaction. The results show that the molar ratio that gave maximum absorbance was found to be (1:1) (VAL: NQS).

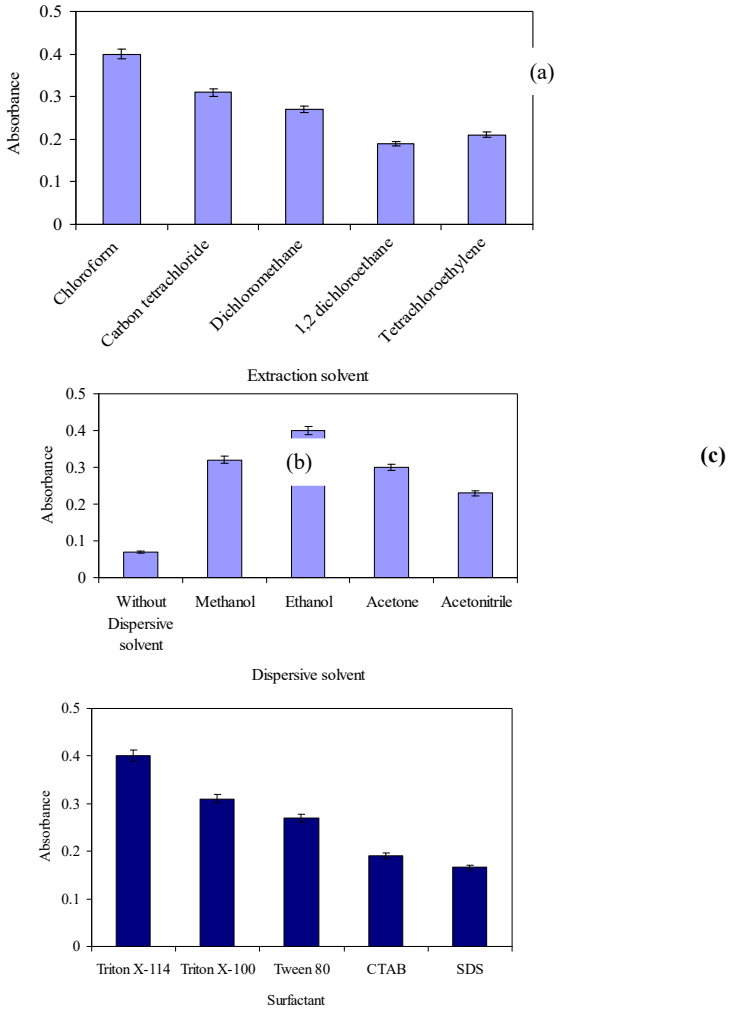


Figure 4. Effect of (a) extraction solvent, (b) dispersive solvent and (c) surfactant type on the extraction efficiency of VAL with NQS.

Linearity and sensitivity

Following the optimised experimental conditions, the relationship between the absorbance and concentration for VAL was quite linear in the concentration ranges of 0.06–2.0 µg/mL. The calibration graph is described by the equation:

$$A = a + b\,C \quad (1)$$

where A = absorbance, a = intercept, b = slope, and C = concentration in µg/ml, obtained by the method of least squares. The correlation coefficient, intercept, and slope for the calibration data are summarised in Table 1. The apparent molar absorptivity of the resulting colored product and

the relative standard deviation were also calculated and recorded in Table 1. The limits of detection (LOD) and quantification (LOQ) were calculated according to the formulas, 3 × Sb/m and 10 × Sb/m, respectively, where Sb and m are the standard deviation of the blank and the slope of the calibration graph, respectively. Therefore, LOD and LOQ were found to be 0.02 and 0.06 µg/mL, respectively. The performance of the proposed DLLME procedure was assessed by calculating the enrichment factor (EF), which is defined as the ratio between the calibration graph slopes with and without the preconcentration procedure (EF = 36.87). The reliability and precision of the proposed DLLME system as measured by the relative standard deviation (RSD%) were examined by applying ten replicate determinations of 1.0 µg/mL of VAL, and the % of the recovery was found to be 1.20%, which illustrates the good precision of the method.

Table 1. Analytical data for the determination of VAL using the methods without and with DLLME.

Parameters	Without DLLME	With DLLME
Wavelengths (nm)	500	505
Linearity (µg/ml)	2.0-50	0.06–2.0
Molar absorptivity ε, (L/mol.cm) x 10^4	0.1842	7.9493
Sandal's sensitivity (ng cm^{-2})	195.87	4.54
Regression Equation [a]		
Intercept (*a*)	-0.0009	0.0064
Slope (b)	0.0052	0.1917
Correlation coefficient (r)	0.9984	0.9995
Recovery%±SD[b]	99.20±2.30	99.50±1.20
RSD%[b]	2.30	1.20
LOD (µg/mL)[c]	0.60	0.02
LOQ (µg/mL)[c]	2.0	0.06
EF[d]	-	36.87

[a]$A = a + bC$, where C is the concentration in µg/mL, A is the absorbance units, a is the intercept, b is the slope. [b]SD, standard deviation; RSD%, percentage relative standard deviation. [c]LOD, limit of detection; LOQ, limit of quantification. [d]Enrichment Factor was calculated from the slope of calibration curve with and without DLLME.

Accuracy and precision

To evaluate the accuracy as percent relative error (RE%) and precision as relative standard deviation (RSD%) of the proposed methods, solutions containing three different concentrations of VAL were prepared and analysed in six replicates. The intra-day repeatability was performed on the same day, and the inter-day precision was measured over five different days (for each level n = 6). The analytical results obtained from this investigation are summarised in Table 2. The low values of RE% and RSD% indicate good accuracy and precision of the proposed DLLME method.

Robustness and ruggedness

The analysis was carried out under altered conditions with three different concentrations of VAL, and it was discovered that small variations in method variables did not significantly affect the procedures, as evidenced by RSD% values ranging from 0.70 to 2.30%. This provided an indication for the reliability of the proposed methods during their routine application for the analysis of VAL, and so the proposed DLLME method was considered robust. The inter-analyst RSD% were in the range of 0.50-2.10%, whereas the inter-instrument RSD% ranged from 0.80–2.50%, suggesting that the developed method was rugged. The results are shown in Table 3

Table 2. Intra-day and Inter-day accuracy and precision data for VAL obtained by the proposed DDLME method.

Taken conc. (µg/mL)	Recovery, %	Precision, RSD%	Accuracy, RE%	Confidence limit [a]
	Intra-day			
0.5	98.50	0.86	-1.50	0.493 ± 0.004
1.0	99.10	1.10	-0.90	0.991 ± 0.011
1.5	99.40	1.30	-0.60	1.491 ± 0.021
	Inter-day			
0.5	99.00	0.95	-1.0	0.495 ± 0.005
1.0	98.30	1.75	-1.70	0.983 ± 0.018
1.5	99.60	2.20	-0.40	1.494 ± 0.035

[a]Confidence limit (mean ± standard error) at 95% confidence level and five degrees of freedom (t = 2.571), (n = 6).

Table 3. Results of method robustness and ruggedness (all values in RSD%) studies

Nominal concentration (µg/mL)	RSD%			
	Robustness		Ruggedness	
	Variable alerted [a]			
	Reagent volume (n = 3)	Centrifugation time (n = 3)	Different analysts (n = 3)	Different instruments (n = 3)
0.5	0.70	0.85	0.50	0.80
1.0	1.40	1.60	1.90	1.70
1.5	2.10	2.30	2.10	2.50

[a]Volume of (0.5%, w/v) NQS reagent is (2.0 ± 0.2 mL) and centrifugation time is (3.0 ± 0.5 min) (after adding reagent) were used.

Recovery studies and application on pharmaceutical formulations

To ascertain the accuracy, reliability, and validity of the proposed methods, a recovery experiment was performed using standard addition techniques. This study was performed by spiking three different levels of pure VAL (0.50, 1.0 and 1.50 µg/mL) onto a fixed amount of drug in tablet powder (pre-analyzed), and the total concentration was found by the proposed DLLME method. The determination at each level was repeated six times, and the percent recovery was calculated from:

$$\% \text{ Recovery} = ([C_F - C_T])/C_p \times 10 \quad (2)$$

where C_F is the total concentration of the analyte found, C_T is the concentration of the analyte present in the tablet preparation; C_P is the concentration of the analyte (pure drug) added to the tablet preparation. The results of this study, presented in Table 4, revealed that the accuracy of the developed method was unaffected by the various excipients present in tablets, like (glucose, lactose, sucrose, starch, alanine, and albumin) which did not interfere with the assay. A statistical comparison of the results obtained from the assay of VAL by the proposed DDLEM method and the official method [1] by applying the Student's t-test for accuracy and F-test for precision (Table 4), the calculated t-value and F-value at 95% confidence level did not exceed the tabulated values for five degrees of freedom [37]. Hence, no significant difference between the proposed method and the reported method at the 95 % confidence level with respect to accuracy and precision.

Table 4. Application of the developed DLLME method for the determination of VAL in tablets (n = 6).

Samples	Taken drug in tablet (µg/mL)	Pure drug Added (µg/mL)	Valysernex tablets		Valtrovir tablets		Official method [1]
			Recovery (%)	RSD%	Recovery (%)	RSD%	
	0.50	0.50	99.10	1.45	99.30	1.20	
	0.50	1.0	98.50	2.40	100.50	0.90	
	0.50	1.50	101.50	1.50	99.00	2.0	
Mean recovery% ± SD			99.70 ± 1.59		99.60 ± 0.79		9.77 ± 0.86
t-value [a]			0.08		0.33		
F-value [a]			3.42		1.19		

[a]The theoretical values of *t* and *F* are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).

Comparison between the proposed method and reported methods

Table 5 contrasts the new DLLME approach with the other methods that have been described [15-27]. The suggested approach is brand-new, sensitive, economical, and selective for VAL determination in pharmaceutical formulations. The disclosed methods depend on important experimental parameters. A few methods demand strict pH control, which is laborious and time-consuming; other methods have a very small dynamic linear range, and/or employ expensive reagents or huge amounts of organic solvents.

Table 5. Comparison of the developed DLLME method with the reported spectrophotometric methods for determination of VAL.

Methods	Wavelength (nm)	Beer's law (µg/mL)	Molar absorptivity (L/mol.cm) × 10^4	LOD (µg/mL)	Samples	Reference
Zero order UV method	254	5.0-25	NA	NA	Bulk and tablet	[15]
Brucine-sodium periodate ($NaIO_4$)	543	5.0-110	0.4642	0.995	Pharmaceutical formulations and human body fluids	[16]
3-methyl-2-benzothiazo-linone-hydrazone (MBTH)/$FeCl_3$	644	2.0-45	0.9761	0.366		
Sodium acetate	251	1-80	1.23247	NA	Pharmaceutical dosage forms	[17]
Phosphate buffer pH 5.0	251	1-80	1.36221	NA		
Phosphate buffer pH 7	252	1-80	1.070308	NA		
borate buffer pH 9.0	253	1-80	1.102742	NA		
0.1 N NaOH	265	1-80	1.070308	NA		
Paradimethylamino cinnamaldehyde	524	10-100	NA	NA	Bulk and its pharmaceutical formulations	[18]
Phenyl hydrazine hydrochloride/hexacyano ferrate(III) in acidic medium	520	2-10	2.66	0.111	Pure and pharmaceutical dosage forms	[19]
Fe^{3+}/1,10-phenanthroline	510	5-25	0.506	0.4276		
MBTH/Fe^{3+}	630	5-25	0.817		Bulk and tablet dosage form	[20]
MBTH/ $NaIO_4$	624	2-10	2.83			
UV/0.1 M sulfuric acid	255	4-12	NA	3.7458	Pharmaceutical dosage forms	[21]
2,5-Dichloro-3,6-dihydroxy-1,4-benzoquinone	450	20-100	0.22	NA	Pharmaceutical formulations	[22]

UV/0.1 M HCl	255	5-25	1.0910	NA	Bulk and tablet dosage form	23
p-Dimethyl aminobenzaldehyde	388	100-500	0.3463	NA	Bulk and pharmaceutical dosage forms	24
Vanillin	428	20-100	0.2842	NA		
UV	274	5-50	2.69167	4.50	Bulk and tablet dosage forms	25
UV	252	4-24	NA	0.315	Pharmaceutical dosage form	26
Quinalizarin	565	0.5-16	1.9365	0.15	Pure form and pharmaceutical formulations	27
Alizarin red S	540	1.0-20	1.4126	0.30		
DLLME	505	0.06-2.0	7.9493	0.02	Pure form and pharmaceutical formulations	This work

NA: not available.

CONCLUSION

The present work describes the application of the developed DLLME method combined with spectrophotometric technique for the quantification of VAL in its pure form and pharmaceutical formulations. The proposed method is simple, rapid, cost-effective, sensitive, accurate, robust, and uses less toxic and green chemicals (Triton X-114). The most attractive feature of the developed method is its relative freedom from interference by the usual diluents and excipients in amounts higher than their normal existence in pharmaceutical formulations. The main advantages of the method were the low limit of detection, the good accuracy and precision revealed by the recovery data, and the higher EF. Therefore, the proposed validated method could be useful for routine quality control assays of VAL in pure form and pharmaceutical formulations.

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