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ULTRASOUND-ASSISTED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION APPROACH FOR PRECONCENTRATION OF ACYCLOVIR AS ANTIVIRAL DRUG IN DOSAGE FORMS PRIOR TO SPECTROPHOTOMETRIC DETERMINATION

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ABSTRACT. For acyclovir (ACV) determination in bulk and dosage forms, quick, sensitive, straightforward, and eco-friendly ultrasound-assisted dispersive liquid-liquid microextraction (UA-DLLME)-based spectrophotometric method has been created and validated. ACV with 1,2-naphthoquine-4-sulfonate (NQS) react in alkaline medium to produce a yellow-colored product, which is the basis of the newly created method. Investigation and optimization were done on the crucial experimental variables influencing ACV's extraction effectiveness. At λ_{max} = 495 nm, the minuscule organic droplets were detected. The linearity was present from 0.1 to 3.0 µg/mL under ideal circumstances, with a linear correlation coefficient of 0.9995. The detection limit was 0.03 µg/mL and quantification limit was 0.1 µg/mL. 22.50 was the enrichment factor. Relative standard deviation (RSD%) as accuracy at 2.0 µg/mL of ACV was 1.0%, with good recovery (99.30%). The developed UA-DLLME method was effectively used to determine the ACV in dosage forms, and the validation was evaluated. Results from the suggested technique for dosage forms and pure ACV were in excellent agreement with the results from the official method.

KEY WORDS: Ultrasound-assissted, Dispersive liquid–liquid microextraction, Acyclovir, Spectrophotometry, Dosage forms

INTRODUCTION

Acyclovir (ACV) is chemically named as 9-[(2-hydroxyethoxy)-methyl]-guanine). It is an antiviral medication utilised to treat herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) and varicella-zoster virus (herpes zoster and chicken pox) [1, 2].

ACV has been determined in pure form, dosage forms, and biological fluids using a variety of analytical methods, including chromatography [3-6], electrochemistry [7-10], spectrofluorimetry [11-13], flow injection [14], FT-IR [15], and spectrophotometry [13, 16-29]. The depicted analytical methods that have been described for the estimation of ACV appear to depend on the use of a practical instrument for the majority of these techniques. Additionally, a number of spectrophotometric techniques required cooling, buffer preparation, and/or incubation reaction times to finish the reaction. The trace detection of ACV using a green solvent is thus a green advancement in pharmaceutical analysis.

Due to environmental and financial concerns, miniaturization—which intended to reduce hazardous waste and produce safe products—has become a significant trend in the development of sample preparation. They benefit many economic aspects in addition to the environment and human wellbeing.

By employing a more contemporary method called dispersive liquid-liquid microextraction (DLLME), analytical chemists have attempted to minimise or completely eliminate the poisonous and extraction solvents. The quick and simple microextraction technique known as DLLME uses a green

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extractant and disperser solvents. With its ease of use, speed, and affordability, DLLME is a potent alternative sample preparation approach for the extraction and preconcentration of a variety of analytes [30].

The most common and widely used methods continue to be spectrophotometric techniques because of their accessibility and inherent simplicity. Additionally, compared to the other analysis techniques previously mentioned, they are thought to be more useful. Additionally unmatched are the sensitivity, adaptability, and accuracy of spectrophotometric approaches. It has been asserted that DLLME and spectrophotometry can be used in conjunction to identify amounts of analytes and other ingredients in medications [30-34].

As a result, the current research introduces for the first time the coupling of spectrophotometry and ultrasound-assisted DLLME (UA-DLLME) for the extraction and trace estimation of ACV in pure and dosage forms without the need for a complicated apparatus setup. The effect of reactant variables were investigated and optimised as important experimental variables that affect the ACV's extraction efficiency. The suggested method has undergone statistical validation for its accuracy, precision, sensitivity, selectivity, robustness, and ruggedness in accordance with ICH standards.

EXPERIMENTAL

Materials and reagents

The analytical reagent grade chemicals, solvents, and reagents utilised in the study, and all of the solutions were made from scratch each day. Pure sample of ACV with a purity (100.16 ± 0.47%) [1] was kindly supplied by Misr Pharmaceutical Co., Cairo, Egypt.

Pharmaceutical formulations containing ACV purchased from different pharmacy in the market; Zovirax 400 tablets labeled to contain 400 mg ACV/tablet (Glaxo Wellcome, London, UK) and acyclovir 200 tablets labeled to contain 200 mg ACV/tablet (Mempheis, Cairo, Egypt).

NQS (bought from Fluka, Germany), sodium bicarbonate, $NaHCO_3$ (El-Nasr company, Egypt) and without further refining, Triton X-114 (Fluka, Switzerland) was utilised. Cyclohexane, chloroform, carbon tetrachloride, dichloromethane, tetrachloroethylene and 1,2-dichloroethane, were inspected as extraction solvents and dispersive solvents (methanol, acetonitrile, acetone and ethanol) purchased from (Sigma-Aldrich, USA).

Apparatus

A 10 mm quartz cell-equipped Varian UV-Vis spectrophotometer (Cary 100 Conc., Australia) was used to produce each absorbance spectrum. To regulate the pH levels of solutions, an AD1000 model pH-meter (made by Adwa Instruments Kft., Szeged, Hungary) was used. Bidistilled water was obtained utilising a Milli-Q purification apparatus from Millipore in the United States. Sonication took place in an ultrasonic Jacuzzi (Dwarka, Delhi, India). The use of a centrifuge improved and eased the phase separation (HERMLE, Germany). All glass items were kept in HNO_3 (5.0%, v/v) for at least 24 hours before being rinsed and cleaned with bidistilled water.

Preparation of standard solutions

By dissolving 0.01 g of pure ACV in an alkaline solution of $NaHCO_3$ (0.1 M) in a 100 mL calibrated flask and sonicating for 10 min, a standard solution of ACV equating to 100 μg/mL was created. The solution was then attenuated to the proper concentration with $NaHCO_3$ solution and thoroughly mixed. The standard solutions were discovered to be stable for at least one week without change when stored in an amber-colored container and maintained in a refrigerator when not in use. To get the right concentration levels, serial dilution with the same solvent was used.

By dissolving 0.5 g of NQS in bidistilled water in to a 100 mL volumetric flask, a (0.5%, w/v) solution was created. Triton X-114 was dissolved in bidistilled water while being stirred in a 100 mL volumetric beaker to create an aqueous (1.0%, v/v) solution.

Preconcentration UA-DLLME procedure

Aliquots of the 0.1-3.0 µg/mL concentration levels of the standard ACV working solution (100 µg/mL) were added to NQS (1.0 mL, 0.5%, w/v) solution in a glass centrifuge tube. With bidistilled water, the amount was brought to 10 mL. Then, 1000 µL of disperser solvent (ethanol) and 500 µL of extractant solvent (chloroform) were both quickly introduced, followed by the addition of Triton X-114 (1.0%, v/v) (the surfactant) in the amount of 500 µL. The tubes were then immersed in an ultrasound bath and sonicated for two minutes. Then, for 5.0 min, the murky solution in the tube was created in a freezing ice bath. The centrifugation at 4000 rpm for 2.0 min of the solution was carried out. The minuscule organic droplets that had been scattered eventually settled. The top aqueous phase was eliminated using a syringe. The final step was to move the remaining phase to spectrophotometric analysis at λ = 495 nm versus a reagent blank that had undergone the same procedures but without the addition of ACV. Plotting the absorbance versus the final ACV content resulted in the calibration graph. It was possible to obtain the appropriate regression equation.

Applications to pharmaceutical formulations

Twenty tablets' worth of content were broken up, made into a fine powder, and weighed to calculate the average weight of one tablet. The powdered tablets that contained 10 mg of ACV were accurately weighed, dissolved in an alkaline solution of $NaHCO_3$ (0.1 M), sonicated for 10 min, and filtered. Then the filtrate was diluted to 100 mL to the proper concentration with an alkaline solution of $NaHCO_3$ (0.1 M) to produce a stock solution containing 100 µg/mL for the suggested method of analysis. The above-mentioned suggested procedures were then used to analyse a handy portion. Determine the nominal content of the tablets through the appropriate regression equation of the suitable calibration curve.

Stoichiometric relationship

By using the Job's technique of continuous variation [35] at the wavelength of maximum absorbance, the stoichiometric ratio of the derivative formed between ACV and NQS reagent was ascertained. Equimolar solutions were used in this technique; an ACV and NQS reagent solution at 1.0×10^{-3} mol/L. The total volume (2.0 mL) of the ACV and the reagent was maintained for a number of the solutions that were created. Following the aforementioned process, the ACV and NQS reagent were combined in different ratios and completed to volume in a 10 mL measuring flask containing $NaHCO_3$ solution (0.1 M). At the ideal wavelength, the absorbance of the prepared solutions was recorded.

RESULTS AND DISCUSSION

Absorption spectra

Because of its efficient reactivity with primary and secondary amines and its high reaction rate, a common spectrophotometric reagent (NQS) has been used as a colour development reagent in the spectrophotometric determination of many pharmaceutical compounds containing amino group at alkaline solution [36, 37]. The maximum absorbance of the reaction product with and without UA-DLLME was measured at λ_{max}= 495 and 490 nm, respectively. The suggested method is based on the derivatization reaction between ACV and NQS in alkaline medium (Figure 1).

Effect of NQS concentration

The effect of the NQS reagent on the absorbance intensity was investigated at various concentrations varying from 0.1% to 1.0% w/v. As the reagent concentration rose, the absorbance increased until it peaked at values between 0.4% and 0.6% w/v, after which it slightly decreased (Figure 2). Therefore, a concentration of NQS 0.5% w/v (2.0 mL) was used in all subsequent experiments.

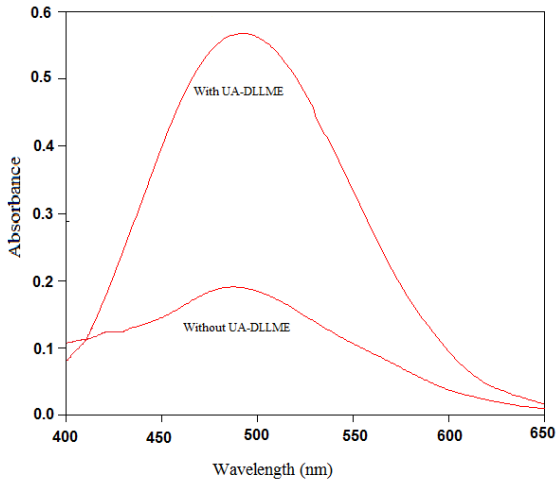

Figure 1. Absorption spectra for the reaction product of (3.0 µg/mL) ACV and NQS reagent with and without UA DLLME.

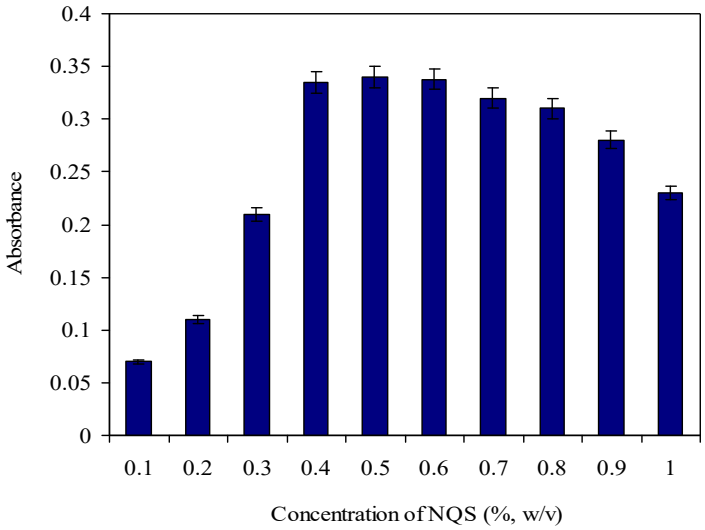

Figure 2. Effect of NQS reagent concentration on ACV-NQS derivative absorbance intensity. ACV concentration is (3.0 µg/mL).

Effect of alkaline solutions

Alkaline medium was required to produce the nucleophile from ACV and start the nucleophilic substitution process. Different inorganic bases and buffers were evaluated, including 0.1 M NaOH/0.2 M $NaHCO_3$ buffer, 0.1 M Na_2CO_3, 0.1 M Na_2HPO_4, 0.1 M $NaHCO_3$ and 0.2 M $NaHCO_3$/0.2 M $NaCO_3$ buffer. The best results were obtained with $NaHCO_3$ (0.1 M), whereas with other bases either high blank readings, non-reproducible findings, and/or weak sensitivity were noted, or precipitation of white colloid occurred upon diluting the reaction solution with organic solvent. The best quantity of $NaHCO_3$ for optimization studies was found to be 0.1 M.

The impact of pH on the absorbance of ACV-NQS product was examined in a different set of tests. The findings showed that the absorbances at pH < 5 were nearly zero, indicating that ACV has trouble reacting with NQS under acidity. This may have happened as a result of the amino group in ACV, which eliminates its ability to undergo nucleophilic substitution. The amino group of ACV transforms into the free-NH at pH > 5, which facilitates the nucleophilic substitution reaction. As a result, the absorbance rose quickly with increasing pH at this pH level. The pH range of 8–10 was where the highest absorption values were obtained. The absorbance of the solution clearly dropped when the pH exceeded 10. The rise in the quantity of hydroxide ions, which inhibits the condensation reaction between ACV and NQS, was likely to blame for this. The experiment was conducted at pH 9.0 in order to maintain the high sensitivity for ACV measurement.

Effect of type of extraction and dispersive solvents

Choosing the right type and amount of extraction solvent was crucial in the proposed method because it has a significant impact on the analyte's ability to be extracted. Because of their density and ability to extract the desired chemicals, various chlorinated solvents, including chloroform, dichloromethane, carbon tetrachloride, 1,2-dichloroethane, and tetrachloroethylene, were examined in this study as extraction solvents. The outcomes are shown in Figure 3. The outcomes demonstrated that using chloroform as the extraction solvent led to greater absorbance. Therefore, in subsequent trials, chloroform was chosen as the extraction solvent.

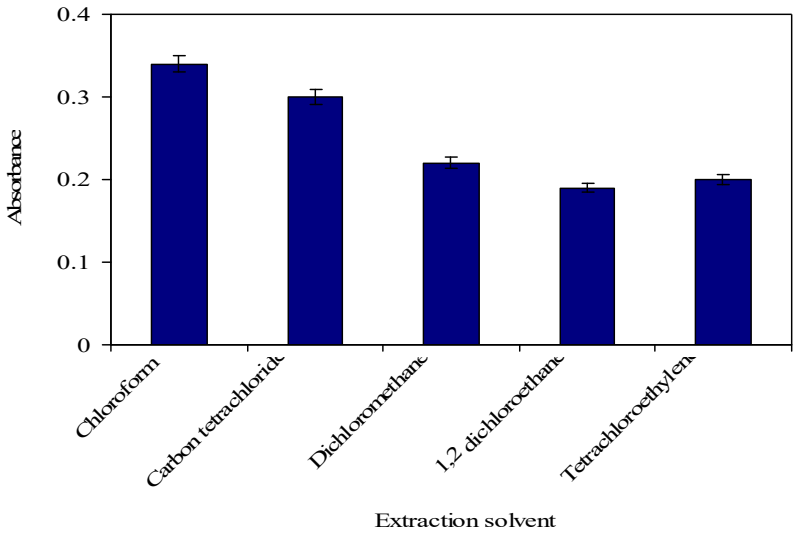

Figure 3. Effect of extraction solvent the ACV-NQS derivative absorbance intensity.

The choice of dispersive solvent is another crucial element in the UA-DLLME method. It should dissolve in extraction solvent and be combined with water, enabling the extraction solvent to disperse as minuscule particles in the aqueous phase, creating a cloudy solution (water/disperser solvent/ extraction solvent) [32–34]. As can be seen in Figure 4, different solvents including acetone, methanol, ethanol, and acetonitrile were examined. The findings demonstrated that using ethanol as the dispersive solvent led to greater absorbance.

As a result, the impact of volume of extraction and dispersive solvents on dispersion formation was researched and improved. In order to evaluate the effects of various chloroform volumes, ranging from 50 to 600 µL, a constant volume of dispersive solvent (1000 µL) ethanol was used. It was discovered that raising the chloroform volume to 400 µL improved the organic phase's absorbance. Therefore, for all future studies, 500 µL of chloroform was chosen as the ideal extraction solvent

volume. 500 to 2000 µL of various dispersive solvent volumes were investigated. Based on the findings, 1000 µL of ethanol was selected for the remaining trials because it produced the highest intensity. Therefore, a mixture of ethanol (1000 µL) and chloroform (500 µL) was used to extract the target analyte with the greatest efficiency.

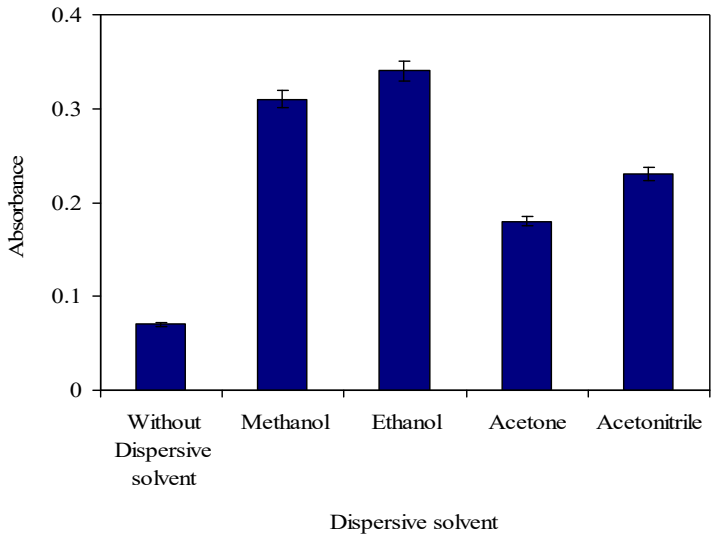

Figure 4. Effect of dispersive solvent on the ACV-NQS derivative absorbance intensity.

Effect of surfactant

The effects of various surfactants, including Triton X-114, Triton X-100, Tween 80, cetyltrimethylammonium bromide (CTAB), and sodium dodecyl sulfate (SDS), were measured. The findings showed that Triton X-114 had the highest absorbance and the best extraction efficiency, making it the ideal surfactant. The effect of Triton X-114 (1.0%, v/v) volume was evaluated by adding different amounts of the compound in the range of (100-600 µL). The results demonstrated that absorbance intensity increases as Triton X-114 volume grows. The gathered statistics show that Triton X-114 (500 µL) had the highest absorption.

Effect of ultrasonication time

When using the microextraction method, ultrasound energy significantly affects how the surfactant-rich phase disperses into the aqueous phase and increases extraction effectiveness [38, 39]. The effects of ultrasonication times between 1.0 and 5.0 min were studied. According to the findings, the extraction effectiveness was raised to 2.0 min. After this point, there was no appreciable change in the analytical signals. Therefore, 2.0 min was determined to be the ideal ultrasonication duration, which was sufficient for the surfactant to completely dissolve in the aqueous phase.

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 2.0 min at 4000 rpm.

Effect of ionic strength

To decrease the solubility of the analytes in the aqueous phase and increase their extraction into the organic phase, salt is introduced. To determine the influence of ionic strength on the efficacy of UA-DLLME extraction, a series of tests were conducted using an increase in the concentration of NaCl from 5.0 to 30% (w/v). It was found that increasing the NaCl concentration from 5.0 to 30% had no appreciable effect on absorption. These results led to no salt being used in any subsequent research.

Stoichiometric ratio

To ascertain the reaction's stoichiometry, we used Job's technique of the continuous variation [35] of equimolar solutions. The molar ratio that provided the greatest absorption was discovered to be (1:1) (ACV : NQS).

Linearity and sensitivity

Following the optimized experimental conditions, the relationship between the absorbance and concentration for ACV was quite linear in the concentration ranges 0.1–3.0 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. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 1. The apparent molar absorptivity of the resulting colored product and relative standard deviation were also calculated and recorded in Table 1. The limits of detection (LOD) and quantification (LOQ) were calculated according to the formula, $3 \times S_b/m$ and $10 \times S_b/m$, 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 0.03 and 0.10 µg/mL, respectively. The performance of the proposed procedure was assessed by calculating the enrichment factor (EF), which defined as the ratio between the calibration graph slopes with and without preconcentration procedure (EF = 22.50). The reliability and precision of the proposed system as the relative standard deviation (RSD%) was examined by applying ten replicate determinations of 2.0 µg/mL of ACV, and RSD% of the recovery was found to be 1.0%, which illustrate a good precision of the method[40].

Table 1. Analytical data for the determination of ACV using the methods without and with precocncetration.

Parameters	Without precocncetration	With precocncetration
Wavelengths (nm)	490	495
Linearity (µg/mL)	4.0-40	0.1–3.0
Molar absorptivity ε, (L/mol.cm) x 10^4	0.10904	2.5324
Sandal's sensitivity (ng cm^{-2})	206.53	8.893
Regression Equation [a]		
Intercept (*a*)	-0.0066	-0.0024
Slope (b)	0.0058	0.1305
Correlation coefficient (r)	0.9982	0.9995
Recovery% ± SD[b]	99.00±1.70	99.30±1.0
RSD%[b]	1.70	1.0
LOD (µg/mL)	1.20	0.03
LOQ (µg/mL)	4.0	0.1
EF [d]	-	22.50

[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.

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 ACV were prepared and analyzed in six replicates. The intra-day repeatability were performed in the same day and inter-day precision over five different days (for each level n = 6). The analytical results obtained from this investigation are summarized in Table 2. The low values of RE% and RSD% indicates good accuracy and precision of the proposed UA-DLLME method.

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

Taken concentration (µg/mL)	Recovery, %	Precision, RSD%	Accuracy, RE%	Confidence limit [a]
	Intra-day			
1.0	99.50	0.72	-0.50	0.995 ± 0.008
2.0	99.00	1.30	-1.0	1.98 ± 0.027
3.0	100.40	1.80	0.40	3.012 ± 0.057
	Inter-day			
1.0	98.40	1.10	-1.60	0.984 ± 0.011
2.0	101.0	1.50	1.0	2.02 ± 0.032
3.0	99.20	2.0	-0.80	2.976 ± 0.062

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

Robustness and ruggedness

The analysis was performed with altered conditions by taking three different concentrations of ACV and it was found that small variation of method variables did not significantly affect the procedures as shown by the RSD% values in the range of 0.60-2.0%. This provided an indication for the reliability of the proposed methods during its routine application for the analysis of ACV and so the proposed method was considered robust. The inter-analysts RSD% were in the range 0.90-2.20%, whereas the inter-instruments RSD% ranged from 0.70-2.50% suggesting that the developed method was rugged. The results are shown in Table 3.

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

Nominal concentration (µg/mL)	RSD%			
	Robustness		Ruggedness	
	Variable alerted[a]			
	Reagent volume	Centrifugation time	Different analysts	Different instruments
1.0	0.60	0.80	0.90	0.70
2.0	1.60	1.2	1.80	2.50
3.0	2.0	2.0	2.20	2.30

[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, recovery experiment was performed through standard addition technique. This study was performed by spiking three different levels of pure ACV (0.50, 1.0 and 1.50 µg mL^{-1}) to a fixed amount of drug in tablet powder (pre-analysed) and the total concentration was found by the proposed method. The determination with each level was repeated six times and the percent recovery was calculated from:

$$\% \text{ Recovery} = ([C_F - C_T]) / C_p \times 100 \quad (2)$$

where C_F is the total concentration of the analyte found, C_T is a concentration of the analyte present in the tablet preparation; C_P is a concentration of analyte (pure drug) added to tablets preparations. 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 in the assay. A statistical comparison of the results obtained from the assay of ACV by the proposed 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 [41]. 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 method for the determination of ACV in tablets (n = 6).

Samples	Taken drug in tablet (µg/mL)	Pure drug Added (µg/mL)	Zovirax tablets		Acyclovir tablets	
			Recovery (%)	RSD%	Recovery (%)	RSD%
	1.0	0.50	99.40	1.50	98.60	1.0
	1.0	1.0	100.70	1.90	99.50	1.40
	1.0	1.50	98.80	2.30	100.70	2.60
Mean recovery (%) ± SD			99.63 ± 0.97		99.60 ± 1.05	
Official method [1]			100.80±1.03		100.52±0.63	
t-value [a]			1.85		1.68	
F-value [a]			1.30		2.67	

[a]Average of six determinations. [b]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 and reported methods

The new strategy is contrasted with the other methods outlined [13, 16-29] in Table 5. The proposed method for determining ACV in pharmaceutical formulations is novel, sensitive, cost-effective, and selective. The revealed methods rely on crucial experimental parameters; some require strict pH control, which is time-consuming and laborious; other methods have a very small dynamic linear range and/or use expensive reagents or large amounts of organic solvents.

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

Methods	Wavelength (nm)	Beer's law (µg/mL)	Molar absorptivity (L/mol.cm) × 10^4	LOD (µg/mL)	Samples	Reference
Cerium(IV) in acidic medium	320	2.0-8	2.56	25.39	Pharmaceutical preparations	13
UV	252.8	1.0-20	1.5899	NA	Bulk and its pharmaceutical preparations	16
Ninhydrin-ascorbic acid at pH 5	540	10-30	4.1071	0.3	Dosage forms	17
UV	252	1.0-30	1.5899	NA	Bulk drug and pharmaceutical preparations	18

Perchloric acid-crystal violet	570	2.0-20	1.78	1.696	Pure form and pharmaceutical preparations	19
2,4-dinitrophenyl hydrazine	414	20-60	NA	NA	Tablet preparations	20
UV	253	2-20	1.3733	NA	Bulk and pharmaceutical dosage forms	21
Cerium(IV) ammonium sulfate/3-ethylbenzothiazolin 2-one hydrazone	630	5.0-50	0.41	0.18	Pharmaceutical formulations	22
Potassium persulfate/ 3-methylbenzothiazolin 2-one hydrazone	630	5.0-45	0.503	1.40		
N-bromosuccinimide / methyl orange	508	1.0-5.0	NA	0.2		23
Copper(II) in borax/sodium hydroxide buffer pH 9	290	112-1620	NA	NA	Pure and dosage forms	24
Cobalt(II) in 1% pyridine in methanol	287	112-1620	NA	NA		
3-Methyl benzothiazoline-2-one hydrazone /$FeCl_3$	616	20-200	0.0941	1.06	Pharmaceutical formulations	25
Folin–Ciocalteu in alkaline medium	760	50-450	0.0165	5.86	Bulk drug and formulations	26
Vanillin	470	2.0-10	NA	NA	Dosage form	27
p-Dimethylaminobenz-aldehyde	404	1.81-9.06	1.10	0.024	Bulk and dosage forms	28
UA-DLLME	495	0.1-3.0	2.5324	0.03	Pure and dosage forms	This work

NA: not available.

CONCLUSION

The application of the newly sensitive and environmentally friendly UA-DLLME method is described for the accurate quantification of ACV in both pure and dosage forms at alkaline pH. Spectrophotometric detection instrument that is relatively simple, economical, and straightforward to use for routine tests and is a widely accessible method of measurement in most laboratories. The developed method's relative independence from interference from common excipients in quantities greater than those found in pharmaceutical formulations is its most alluring aspect. The method's primary benefits were a low detection limit (0.03 μg/mL), excellent accuracy and precision in the recovery data, and a higher EF. For routine quality control assay of ACV in pure and dosage forms, thus the suggested validated technique representing a promising and helpful approach for the monitoring of ACV in dosage forms.

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