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## Development of Cloud Point Extraction for Preconcentration and Spectrophotometric Determination of Entecavir as Antiviral Drug in Pharmaceutical Formulations and Application to Content Uniformity Testing

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### Abstract

Entecavir (ENT), an antiviral medication, has been identified using two simple, sensitive, accurate, and precise spectrophotometric approaches that have been developed and verified. In order to produce a yellow-coloured Schiff's base product that can be detected at a maximum wavelength of 402 nm, ENT reacts with p-dimethylaminobenzaldehyde (p-DMAB) in an acidic solution. This reaction is the basis for method A. Under ideal conditions, the concentration range of 20-400 μg mL$^{-1}$ agrees with Beer's law due to its excellent correlation coefficient ($r^2$ = 0.9988) and low relative standard deviation (RSD% = 1.80). The method B approach uses cetyltrimethylammonium bromide (CTAB) and Triton X-114 as surfactants at a maximum wavelength of 415 nm to measure the amount of the yellow colour product using a cloud point extraction (CPE) methodology. Beer's law was seen to be obeyed in the concentration range of 0.1-3.0 μg mL$^{-1}$, with a $r^2$ value of 0.9995 and an RSD% of 1.50. The ideal reaction conditions include the molar ratio, solvent type, reagent concentration, and reaction time. The detection and quantification limits were calculated. With the regularly used excipients and additives, no interaction was noticed. The suggested techniques for measuring ENT in its pharmaceutical formulations were successfully applied, and the findings for pure ENT and commercial tablets were in good agreement with those from the reported approach.

*Keywords:* Entecavir; p-dimethylaminobenzaldehyde; Spectrophotometry; Cloud point extraction; Dosage forms; Content uniformity.

## 1. Introduction

An antiretroviral drug is entacavir (ENT). It is a guanosine nucleoside analogue that has selective efficacy against human immunodeficiency virus (HIV) and the hepatitis B virus (HBV). Additionally, it is used to treat HIV patients who have HBV infection as well as to prevent HBV reinfection following liver transplantation. Additionally, it prevents viral replication by preventing transcription, DNA replication, and reverse transcription [1, 2]. Entecavir monohydrate is chemically designated as 2-amino-1,9-dihydro-9-[(1S,3R,4S)-4-hydroxy-3- (hydroxymethyl)-2-methylenecyclopentyl]-6H-purin-6-one, monohydrate. (Fig. 1).
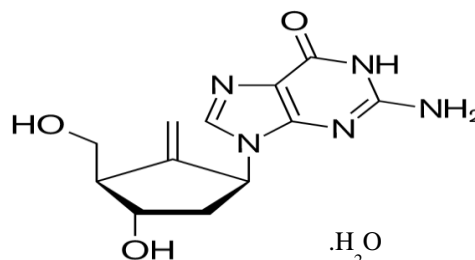


**Fig. 1.** The chemical structure of entecavir monohydrate.

The quantitative determination of ETV has been reported using a variety of analytical techniques, including spectrophotometry [3–11], spectrofluorimetry [11], electrochemistry [12], HPLC using a UV detector [10, 13–15], LC-MS/MS [16–18], capillary zone electrophoresis [19], and derivative spectroscopy assisted FTIR [20]. Some spectrophotometric techniques limit their selectivity, while others call for high-temperature heating. Furthermore, chromatographic methods increased costs and had a detrimental impact on the environment because they required complex equipment and large amounts of organic solvents.

The practise of "green chemistry" involves using less or stopping the manufacturing of substances that are hazardous to the environment and human health [21]. Cloud point extraction (CPE) is a green method because (a) it uses cheap, diluted solutions of surfactants as the extractor media, economical reagents, and produces few laboratory residues; and (b) surfactants are not toxic, volatile, or easily flammable, unlike the organic solvents used in liquid-liquid extraction.

In order to preconcentrate hydrophilic analytes using CPE, such as ENT, some modified conditions are required. When compared to a single surfactant, mixed micelles used in the CPE technique exhibit synergism because of their higher surface activity and capacity for co-stabilization and co-sensitisation [22]. Mixed surfactants with different charges are used to produce both perfect hydrophobic and non-ideal electrostatic interactions in the same extraction system. When ionic and non-ionic surfactants are combined in a small proportion in the same extraction solution, ideal hydrophobic and non-ideal electrostatic interactions may develop. It has been shown that the extraction efficiency of both organic and inorganic compounds can be improved by combining cationic (cetyltrimethylammonium bromide, or CTAB), and nonionic (Triton X-114) surfactants [23-27]. To the best of our knowledge, preconcentration of ENT for applications of CPE has never been documented. Following extraction and preconcentration using CPE, a spectrophotometric approach using Schiff's base reaction coupling p-dimethylaminobenzaldehyde has been effectively employed for spectrophotometric detection of several drug source ingredients [28–31].

In order to recognise ENT as a highly sensitive process, the current work aims to develop a new CPE technique for pre-concentration of ENT in pharmaceutical formulations prior to its determination using spectrophotometry. The procedure is based on the formation of Schiff's base product with *p*-dimethylaminobenzaldehyde (*p*-DMAB) in an acidic solution and the ionic surfactant cetyltrimethylammonium bromide (CTAB), followed by extraction with Triton X-114. The factors affecting the efficiency of the procedure were systematically studied and optimised. This method has been approved in accordance with ICH criteria [32] and has also been used to assess the uniformity of the content of the examined tablet formulations in accordance with USP guidelines [33].

## 2. Experimental

### 2.1. Apparatus

Every absorbance spectrum was created using a Varian UV-Vis spectrophotometer (Cary 100 Conc., Australia) outfitted with a 10 mm quartz cell. Ultrasonicator (Cole-Parmer, Chicago, USA); computerised analytical balance (Meltter Toledo, Glattbrugg, Switzerland); thermostatically controlled water bath (Germany); vortex mixer (Gemmy Industrial Co. Taiwan, China). A centrifuge was used by the researchers (Isolab, GmbH, Germany). Water that had been deionized was produced using Milli-Q (Millipore, USA). The software GraphPad InStat version 3.05 ® was used for all calculations and statistics. Laboratory glassware was cleaned and rinsed with bidistilled water after being submerged in a diluted $HNO_3$ solution for the night. Samples were stored in polypropylene bottles prior to the study.

### 2.2. Chemicals and reagents

All reagents used were of analytical grade (El Nasr Chemical Co., Abu Zaabal, Cairo, Egypt).

*p*-dimethylaminobenzaldehyde (*p*-DMAB) (0.5%, w/v) was prepared in 0.5 mol $L^{-1}$ sulfuric acid [27]. Different surfactants (Triton X-100, Triton X-114, Tween 20, Tween 80, STAP, SDS) (10%, v/v) were prepared by diluting 10 ml with distilled water in a 100-mL volumetric flask. $Na_2SO_4$ (5.0%, w/v). Cetyltrimethylammonium bromide (CTAB) (0.01 M) was prepared by dissolving 0.3644 g in 100 mL of distilled water. 0.5 mol $L^{-1}$ of $H_2SO_4$, HCl, $HNO_3$, $H_3PO_4$ and $CH_3COOH$ were prepared by proper dilution.

Entecavir monohydrate (ENT, 99.80 %) was kindly supplied by Evapharma, Egypt. Different dosage forms available in the Egyptian market were analysed: Tecavir tablets, labelled to contain 1.0 mg of ENT, provided by Evapharma, Egypt.

The stock solution of ENT was prepared by dissolving precisely weighed 0.1 g of drug in 25 mL of methanol in a 100-mL volumetric flask and completing to the mark with methanol to get the 1000 μg $mL^{-1}$ required working standard solution. To generate a working standard solution in the range of the calibration curves, further methanol dilutions were made.

### 2.3. General procedure for method A (without CPE)

Experimental standard ENT drug solutions corresponding to (100-2000 µg mL$^{-1}$) were placed into 5-mL volumetric flasks, and 1.0 mL of (0.5%, w/v) *p*-DMAB was added. The solutions were well combined, allowed to stand for 15 minutes at ambient temperature (25 ± 5°C), and then diluted with methanol to the desired concentration. The yellow product's absorbance was assessed at 402 nm in comparison to a reagent blank. After creating the calibration curve and deriving the regression equation, the absorbance was plotted against the final ENT concentration.

### 2.4. General procedure for method B (with CPE)

In a 15-mL centrifuge tube, Schiff's base solutions prepared at various ENT concentrations (0.1-3.0 µg mL$^{-1}$) were transferred, and then 1.0 mL each of Triton X-114 (10%, v/v), and CTAB (0.01 mol L$^{-1}$), and 2.0 mL Na$_2$SO$_4$ (5%, w/v) were added. After that, the volume was completed with distilled water up to 15 mL, and the tubes were transferred to the thermostatic water bath device. The finished product was equilibrated for 15 minutes at 40 °C in a water bath. For 5.0 min. at 4000 rpm, centrifugation was utilised to hasten the phase separation. After the mixture was refrigerated in an ice bath to increase the viscosity of the surfactant-rich phase, the aqueous phase was extracted using a syringe pipette. The phase containing a high concentration of surfactants was diluted with 0.5 mL of methanol. Finally, 0.5 mL of methanol was added to dissolve the micelle layer, and the absorbance at 415 nm of the methanolic surfactant-rich phase was then measured in a quartz cell against the appropriate reagent.

### 2.5. Application to dosage forms

Twenty of the aforesaid ENT-containing pills were weighed precisely, ground in a mortar to a fine powder, and thoroughly mixed. A precisely weighed amount of the ground tablets, equivalent to 10 mg of ENT, was added to a 100-mL volumetric flask along with about 80 mL of methanol. After that, the flask underwent 20 minutes of sonication [27], was finished with the same solvent, and was then filtered. The initial batch of filtrate was discarded. Aliquots of this solution were placed in a succession of 5-mL volumetric flasks, and analysis was performed as usual.

## 3. Results and discussion

### 3.1. Absorption spectra

A yellow colour results from the chemical reaction of the substance p-dimethylaminobenzaldehyde (p-DMAB) with aromatic or aliphatic amino groups in an acidic medium [27]. The current effort focuses on the development of precise, repeatable, and sensitive enough spectrophotometric methods, both with and without CPE, for identifying ENT as an antipsychotic agent in pharmaceutical formulations and bulk powder. The procedure, which is based on the creation of a yellow Schiff-based coloured product following the elimination of water, may be detected at 402 nm and 415 nm, respectively, without CPE and with CPE (Fig. 2). When sulfuric acid and the main amino group of ENT interact with *p*-DMAB's aldehyde moiety, this product is created.
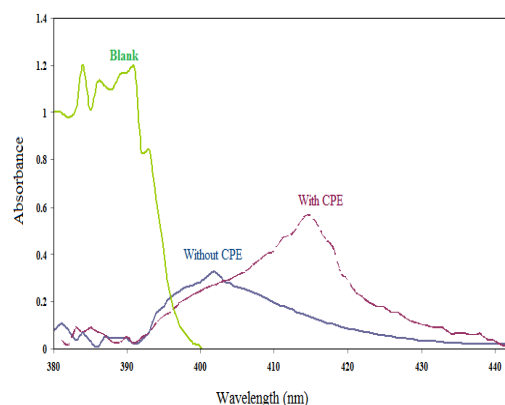


**Fig. 2.** Absorption spectra of ENT before and after reaction with *p*-DMAB (0.5%, w/v) reagent, with and without CPE.

### 3.2. Optimisation of reaction variables
### 3.2.1. Method A (without CPE)

Investigated were the experimental parameters (volume of *p*-DMAB, type and concentration of acid, reaction temperature and time, and the impact of solvent dilution).

#### 3.2.1.1. Effect of volume of p-DMAB

The effect of the coupling reagent *p*-DMAB (0.5%, w/v) concentration was investigated by adding a number of quantities (0.2-2.0 mL) along with 1.0 mL of ENT solution (300 µg mL$^{-1}$). The volume of 1.0 mL of *p*-DMAB coupling reagent is excellent for the subsequent procedures since it produces the greatest absorbance, as shown in Fig. 3.
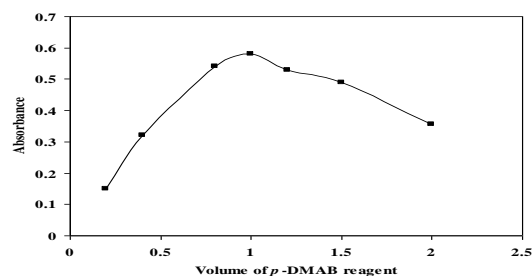


**Fig. 3.** Effect of volume of *p*-DMAB reagent (0.5%, w/v) on the absorption intensity of the Schiff's base-ENT colored product (ENT; 300 µg mL$^{-1}$).

### 3.2.1.2. Effect of type of acids

The effect of several acids on the level of coloured product absorption was examined using hydrochloric acid, nitric acid, sulfuric acid, and o-phosphoric acid (0.5 mol L$^{-1}$). Since sulfuric acid had the highest absorption intensity, it was found to be the best acid (Fig. 4).
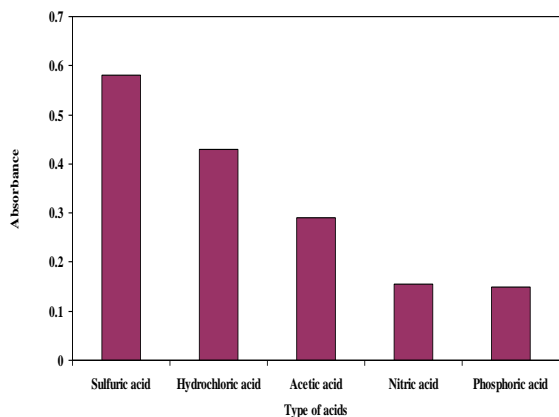


**Fig. 4.** Effect of different acids on the absorption intensity of the Schiff's base-ENT colored product (ENT; 300 μg mL$^{-1}$).

### 3.2.1.3. Effect of sulfuric acid concentration

The greatest absorbance intensity was found to be attained at the acid concentration of 0.5 mol L$^{-1}$. Different sulfuric acid concentrations ranging from (0.4-1.0 mol L$^{-1}$) were used for the overall test process.

### 3.2.1.4. Effect of reaction time, temperature and stability of the Schiff's base product

The manufacture of the yellow-coloured product and the condensation process between the ENT and *p*-DMAB have both been optimised for the effects of time and temperature. The instantaneous occurrence of full colour production and the fact that the maximum absorption intensity was attained after 15 minutes and remained constant for at least 40 minutes after dilution at room temperature (25 ± 5°C) were both discovered.

### 3.2.2. Method B (with CPE)
### 3.2.2.1. Effect of surfactants

The type of surfactant used in CPE is a crucial factor because of its low cloud point temperature (CPT) and high density. Triton X-114 is one of the non-ionic surfactants that is widely used in CPE. This is due to its advantages, which include high commercial purity, a low cloud point temperature, low toxicity, low cost, and a high density of the phase rich in surfactants that enables phase separation by centrifugation. Within the Triton X-114 concentration range of (1.0-15%, v/v), the effect of non-ionic

surfactant concentration on CPE efficiency was examined. To improve the absorbance of the Schiff's base-coloured product, the Triton X-114 concentration was increased to a maximum of (10%, v/v). When surfactant concentrations are raised above 10% (v/v), absorbance significantly decreases. This is explained by an increase in the volume and viscosity of the micellar phase. The extraction efficiency of Schiff's base product was low because there weren't enough molecules of the surfactant to quantitatively entrap it at concentrations below this cutoff. In order to determine how Triton X-114 (10%, v/v) dosages affected CPE effectiveness, multiple doses between 0.2-2 ml were used. With 1.0 mL of Triton X-114 (10%, v/v), the product's maximal absorbance was obtained. Therefore, 1.0 mL of Triton X-114 (10%, v/v) was used for the studies that followed (Figs. 5 and 6).

The influence of ionic surfactant (CTAB) concentration on the CPE and determination of ENT was examined in the concentration ranges of $1.0 \times 10^{-4}$ to $1.0 \times 10^{-1}$ mol L$^{-1}$. By increasing CTAB concentration to $1.0 \times 10^{-2}$ mol L$^{-1}$, the absorbance improved and was steady at higher concentrations.
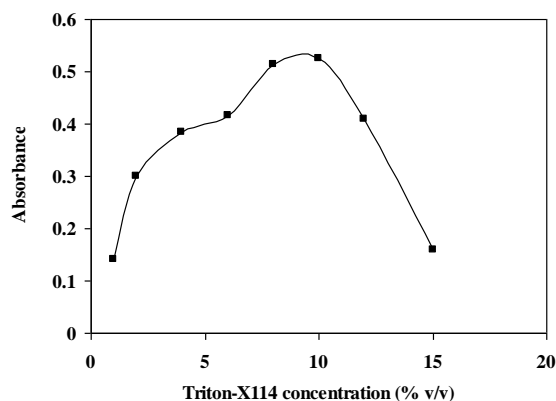


**Fig. 5.** Effect of Triton X-114 concentration on the absorption intensity of the Schiff's base-ENT colored product (ENT; 3.0 μg mL$^{-1}$).
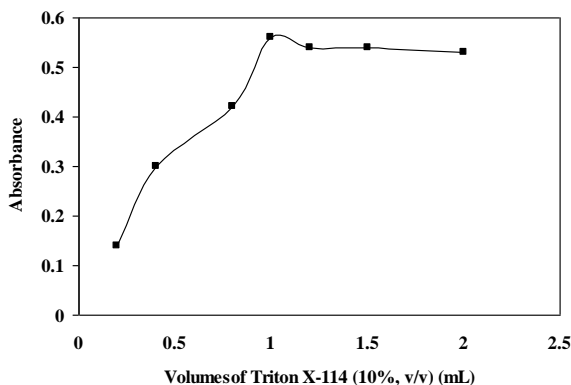


**Fig. 6.** Effect of Triton X-114 (10%, v/v) volume on the absorption intensity of the Schiff's base-ENT colored product (ENT; 3.0 μg mL$^{-1}$).

### 3.2.2.2. Effect of salt

Due to the salting-out phenomenon, the addition of an electrolyte with an appropriate concentration to an aqueous solution of the surfactant micellar system speeds up phase separation and increases micellar concentration in the surfactant-rich phase. As a result, the volume of the surfactant-rich phase will decrease as a result of the salt addition, increasing the pre-concentration factor while also making the surfactant-rich phase more viscous [27]. Several electrolytes (KCl, NaCl, and $Na_2SO_4$) were studied at a concentration of (5% w/v) of each salt in order to determine the best salt to use and the right concentration of it. The most effective salt type and amount to achieve the best extraction efficiency and distribution ratio (D) was found to be 2.0 ml of $Na_2SO_4$.

### 3.2.2.3. Effects of incubation time and equilibration temperature

Between 5.0 and 30 minutes, the impact of incubation time and equilibration temperature was investigated. Only a 15-minute incubation period was needed before the separation technique. The effect of the equilibration temperature was investigated by altering the temperature in the range of 30-70 °C. The results demonstrated that the extraction effectiveness and maximum absorbance of the Schiff's base-coloured product were maintained at a temperature range of 40–45°C. Schiff's base-coloured product degrades and extracts less well at higher temperatures. Instead of affecting micelle production, centrifugation settings often influence the rate of phase separation. This led to the conclusion that 5.0 min at 4000 rpm was the appropriate centrifugation duration for complete separation and a successful CPE operation.

### 3.2.2.4. Effect of diluting solvents

In order to lower the viscosity of the surfactant-rich phase generated after CPE and make the absorbance measurements easier, a variety of solvents, including water, dimethyl formamide, dichloromethane, ethanol, and methanol, were examined as diluent solvents. It was found that the phase high in surfactants was freely soluble in methanol. As a result, methanol was selected as the diluent solvent. The surfactant-rich phase was thus finished with methanol to a volume of 0.5 mL. In this way, a preconcentration factor of 30 was achieved using the indicated CPE method.

### 3.2.2.5. Investigation of the molar ratio

Using Job's method of continuous variation, the stoichiometric ratio of the ENT and *p*-DMAB reagent was determined using ($3.3 \times 10^{-2}$ mol $L^{-1}$) master equimolar solutions of both chemicals [34]. In Job's

method, various volumes of the reagent solution and ENT solution are mixed in a series of 10 mL volumetric flasks before the proposed methods are applied. The volumes of the ENT solution and reagent solution range from (0.1-0.9 mL) to (0.1-0.9 mL), respectively. The results shown in Fig 7 show that the molar ratio, which was found to be 1:1, was in agreement with the reaction mechanism hypothesis. A yellow-coloured product is produced when the primary amine group of ENT interacts with the aldehyde moiety of *p*-DMAB in an acidic environment and water is removed. Fig. 8 depicts the expected reaction process.



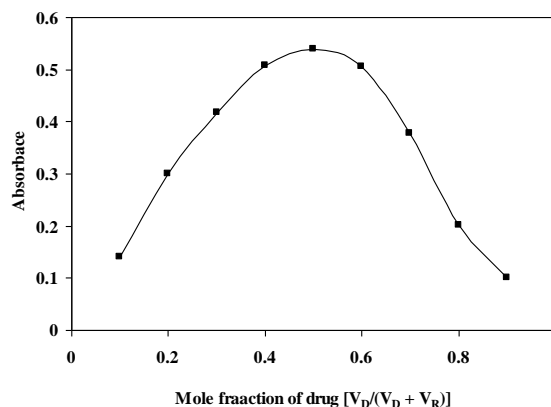**Fig. 7.** Plot of Job's method for the determination of molar ratio of ENT and *p*-DMAB reagent using $3.3 \times 10^{-2}$ mol $L^{-1}$ equimolar solutions.
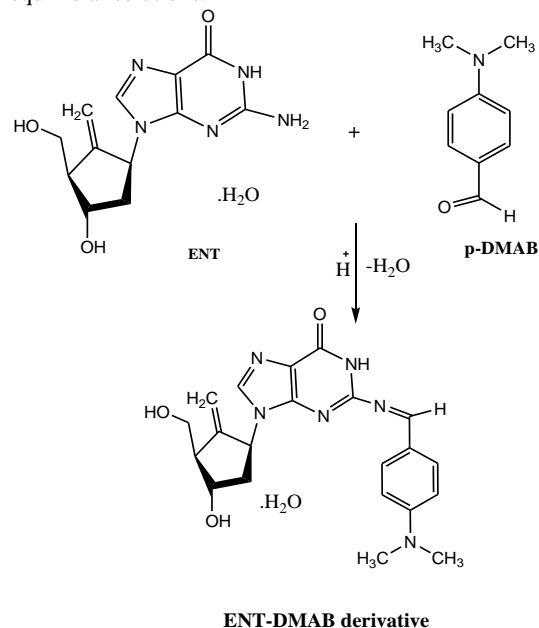


**Fig. 8.** The suggested reaction mechanism between ENT and *p*-DMAB reagent.

### 3.3. Validation of the proposed methods
#### 3.3.1. Linearity

Under ideal reaction conditions, a standard calibration graph for ENT has been produced by

looking at a series of ENT concentrations and reducing the relative error by taking the mean of three values for each concentration. In the concentration ranges of 20–400 μg mL$^{-1}$, the relationship between ENT concentration and absorbance was mainly linear in the absence of CPE (Method A). With a correlation coefficient of ($r^2$ = 0.9988), the linear equation was A = 0.042+0.0007C, where A is the absorbance and C is the ENT concentration (in μg mL$^{-1}$). CPE (0.1-3.0 μg mL$^{-1}$) was used to find the equation 0.1788C + 0.0143 ($r^2$ = 0.9995) (Method B). Table 1 lists the analytical characteristics of the indicated processes, both with and without CPE. The improvement factor, which is determined by comparing the slopes of the calibration graphs before and after the preconcentration operation with and without CPE, was also close to 255.43. Methods A and B were found to have relative standard deviations (RSD) and relative errors of 1.80% and 1.50%, respectively, for six repeat measurements of 300 and 2.0 μg mL$^{-1}$ of ENT.

### 3.3.2. Limits of detection and quantitation

The least concentration at which the analyte can be reliably identified for ENT (LOD) was used to calculate the limits of detection. The limit of quantification (LOQ) was defined as the lowest concentration that can be measured with appropriate accuracy and precision. To determine LOQ and LOD, the formulas listed below were utilised [32]:

$$LOD = 3.3 \ s/k$$
$$LOQ = 10 \ s/k$$

Where *s* is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte, and *k* is the sensitivity, namely the slope of the calibration graph. The outcomes are displayed in Table 1. The formula showed that the LOD and LOQ, respectively, were 6.0 and 20 μg mL$^{-1}$ without CPE and 0.03 and 0.1 μg mL$^{-1}$ with CPE. The percentage recoveries of pure ENT attained using the published approach and the reported procedure are contrasted in Table 1 [10]. Statistical analysis [35] was used to assess the validity of the proposed method by comparing the outcomes of the recommended approach with those of the stated method. There is no noticeable difference in terms of accuracy and precision when contrasting the proposed and documented methods using the estimated Student's t-test and variance ratio F-test (Table 1).

**Table 1**
Analytical parameters for the analysis of ENT by the proposed methods (A and B).

| Parameters | Method A Without | Method B With |
|---|---|---|

| | CPE | CPE |
|---|---|---|
| λ max (nm) | 402 | 415 |
| Calibration range (μg mL$^{-1}$) | 20-400 | 0.1-3.0 |
| Molar absorptivity ε, (L mol$^{-1}$ Cm$^{-1}$) | 5.654 × 10$^2$ | 5.256 × 10$^4$ |
| Regression equation [a] | | |
| Slope (b) | 0.0007 | 0.1788 |
| SD of slope (Sb) | 0.007 | 0.012 |
| Intercept (a) | 0.042 | 0.0143 |
| SD of the intercept (Sa) | 0.01 | 0.009 |
| SD of residual ( Sy/x) | 0.01 | 0.013 |
| Correlation coefficient ($R^2$) | 0.9988 | 0.9995 |
| Limit of detection (μg mL$^{-1}$) | 6.0 | 0.03 |
| Limit of quantification, (μg mL$^{-1}$) | 20 | 0.1 |
| Reproducibility (RSD, %) (*n*=6) | 1.80 (300 μg mL$^{-1}$) | 1.50 (2.0 μg mL$^{-1}$) |
| Calculated *t*-value [d] | 0.91 | 0.84 |
| Calculated *F*-value [d] | 1.68 | 2.26 |
| Preconcentration factor | - | 30 |
| Enrichment factor | - | 255.43 |

[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; RE%, percentage relative error.
[c] LOD, limit of detection; LOQ, limit of quantification; ε, molar absorptivity.
[d] The theoretical values of *t* and *F* at P= 0.05 are 2.571and 5.05, respectively, at confidence limit at 95% confidence level and five degrees of freedom (*p*= 0.05).

### 3.3.3. Accuracy and precision

Three repeat experiments on pure ENT medicine solution at three distinct concentration levels (within the working range) were done to assess the precision and accuracy of the suggested approaches. The accuracy and precision of the proposed method were determined using the relative error percentage (RE%) and the relative standard deviation percentage (RSD%). Using the equation below, we can get the relative error % as follows:

$$\% \ R.E. = \left[ \frac{found - taken}{taken} \right] \times 100$$

The intra-day results for Method A and Method B, respectively, are found to be within -1.0 - 1.2% and 0.90- 1.50%, and within -1.60- -0.80% and 1.10-2.0%, respectively. Inter-day results for Method A are found to range between -1.20 and 0.50% and between 0.60 and 1.40%, whereas those for Method B are found to range between -1.0 and 0.40% and between 0.72 and 1.80%, respectively (Table 2). The results demonstrated that the developed methods had good reproducibility and repeatability. The RSD% was less than 2.0% in every instance, indicating the great repeatability and reliability of the suggested

strategy. The proposed methodology's level of precision was sufficient for ENT quality control analysis.

**Table 2**
Evaluation of intra-day and inter-day accuracy and precision for ENT obtained by the proposed methods.

| Method | Taken ($\mu g$ $mL^{-1}$) | Recovery % | Accuracy RE % [a] | Precision RSD % [a] | Confidence Limit [b] |
|---|---|---|---|---|---|
| | | | | | Intra-day |
| A | 100 | 99.0 | -1.0 | 0.90 | 99.0 ± 0.936 |
| | 200 | 99.40 | -0.60 | 1.15 | 198.80 ± 2.40 |
| | 300 | 101.20 | 1.20 | 1.50 | 303.60 ± 4.782 |
| B | 1.0 | 98.40 | -1.60 | 1.10 | 0.984 ± 0.011 |
| | 2.0 | 101.0 | 1.0 | 1.50 | 2.02 ± 0.032 |
| | 3.0 | 99.20 | -0.80 | 2.0 | 2.976 ± 0.062 |
| | | | | | Inter-day |
| A | 100 | 98.80 | -1.20 | 0.60 | 98.80 ± 0.622 |
| | 200 | 99.50 | -0.50 | 1.0 | 199.0 ± 2.09 |
| | 300 | 100.50 | 0.50 | 1.40 | 301.50 ± 4.43 |
| B | 1.0 | 99.50 | -0.50 | 0.72 | 0.995 ± 0.008 |
| | 2.0 | 99.00 | -1.0 | 1.30 | 1.98 ± 0.027 |
| | 3.0 | 100.40 | 0.40 | 1.80 | 3.012 ± 0.057 |

[a] Mean of three determination, RSD%, percentage relative standard deviation; RE%, percentage relative error.
[b] Mean ± standard error, Confidence limit at 95% confidence level and five degrees of freedom (t = 4.303).

### 3.3.4. Robustness

The volume of *p*-DMAB, the concentration of sulfuric acid, and the reaction time were the three variables that were tested for the robustness of the designed assay. The recovery% was computed each time one parameter changed throughout the test while the other parameters remained the same. Small adjustments in each investigated parameter showed little to no effect on the intensity of the product's absorption, as shown by the obtained recoveries (97.50–99.20%) and standard deviations (0.52-1.0).

### 3.3.5. Specificity and effect of excipients

Searching for any interference from the excipients of the typical tablet was done to evaluate the specificity of the suggested method. By adding pure ENT at known concentrations to a tablet solution that had already been tested, the conventional addition approach was used. By comparing the concentration of the spiking mixtures

with the previously determined value, the recovery of the added ENT was computed (Table 3). Strong recovery values for the suggested method showed that excipients did not interfere with it, indicating that the method has a high selectivity.

**Table 3**
Results of recovery experiments by standard addition method for the determination of ENT in tablets using the proposed methods.

| Method | Taken drug ($\mu g$ $mL^{-1}$) | Pure drug Added ($\mu g$ $mL^{-1}$) | Tecavir tablets Total found ($\mu g$ $mL^{-1}$) | Recovery [a] (%)± SD |
|---|---|---|---|---|
| A | 100 | 50 | 149.40 | 99.60 ± 0.62 |
| | | 100 | 197.80 | 98.90 ± 0.80 |
| | | 150 | 251.0 | 100.40 ± 1.10 |
| B | 1.0 | 0.50 | 1.503 | 100.20 ± 0.32 |
| | | 1.0 | 1.972 | 98.60 ± 0.46 |
| | | 1.50 | 2.483 | 99.30 ± 0.59 |

[a] Average of three determinations.SD; standard deviation.

### 3.4. Analytical applications

A 95% confidence level t-test and variance ratio F-test were used to compare the results to those of the previously reported technique [10], which was used to discover the ENT tablet formulation. The values of the proposed and reported approaches did not differ significantly. This illustrates the high degree of precision and accuracy of the assay for the chemical under study (Table 4).

**Table 4**
Comparison between the proposed assay and the reported method for the determination of the antiviral drug ENT in Tecavir tablet.

| Parameters | Proposed methods | | Reported method [11] |
|---|---|---|---|
| | Method A | Method B | |
| % Recovery ± SD [a] | 99.60 ± 0.96 | 99.70 ± 0.55 | 99.30 ± 0.70 |
| t-value [b] | 0.57 | 1.0 | |
| F-value [b] | 1.88 | 1.62 | |

[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).

### 3.5. Content uniformity test

The developed method was applied to the content uniformity test, which is a time-consuming procedure when using standard experiment techniques, due to the highly accurate suggested

assay's ability to quickly determine the content of the drug in only one tablet obtained with sufficient precision. Ten other tablets were analysed using the same method that was used to investigate the ENT medication in tablets. Chapter 905: Uniformity of Dosage Units of the United States Pharmacopoeia has been used to check the contents for uniformity [33]. The computed acceptance value (AV) for dosage forms was found to be significantly less than the highest permissible acceptance value (L1). The results of the homogeneity of the commercial preparation's composition are shown in (Table 5).

**Table 5**

Results of content uniformity testing of ENT drug tablets using the proposed CPE method.

| Dosage form no. | Tecavir tablets % labeled claim |
|---|---|
| 1 | 98.20 |
| 2 | 98.70 |
| 3 | 99.00 |
| 4 | 98.0 |
| 5 | 98.60 |
| 6 | 98.80 |
| 7 | 99.20 |
| 8 | 98.20 |
| 9 | 98.70 |
| 10 | 99.10 |
| **Mean** | **98.65** |
| **SD** | **0.41** |
| **Acceptance value (AV) [a]** | **0.98** |
| **Max. allowed AV(L1)** | **15.0** |

[a] Acceptance value = $2.4 \times$ SD.

*3.6. Comparison of the proposed method with other methods*

Comparison the presented CPE method with some reported spectrophotometric methods for estimation of ENT shown in Table 6. The main advantages of the developed method were low LOD, high molar absorpitivity, and high preconcentration factor (PF). The repeatability of the method is good.

**Table 6.**

Comparison between the presented CPE method with reported spectrophotometric methods for determination of ENT.

| Reagent | Wavelength (nm) | Beer's law ($\mu$g mL$^{-1}$) | Molar absorpitivity (L.mol$^{-1}$.cm$^{-1}$) | References |
|---|---|---|---|---|
| 3-amino phenol in acidic media | 437 | 0.4-2.0 | N.D [a] | [3] |
| Ninhydrin | 580 | 5-60 | $3.59 \times 10^3$ | [4] |
| Ascorbic acid | 535 | 5-80 | $6.4 \times 10^3$ | |
| p-Benzoquinone | 395 | 10-60 | $4.36 \times 10^3$ | |
| Folin-Ciocalteau | 750 | 8-80 | $5.74 \times 10^3$ | [5] |
| 3-methyl-2-benzothiazolinone hydrazone HCl/FeCl$_3$ | 650 | 6-60 | $4.1887 \times 10^4$ | |
| p-dimethyl amino benzaldehyde | 430 | 1-10 | $1.389 \times 10^4$ | |
| 1, 2-Napthoquinone-4-sulfonate sodium (NQS)/alkaline medium | 450 | 0.6-3.0 | $1.358 \times 10^5$ | [6] |
| p-dimethylamino cinnamaldehyde (PDAC) under acidic conditions | 520 | 1-10 | $4.380 \times 10^4$ | |
| 1,10-Phenanthroline/FeCl$_3$ | 500 | 2-50 | $0.499 \times 10^4$ | |
| UV | 253 | 2.5-40 | ND | [7] |
| UV | 254 | 3-18 | ND | [8] |
| UV | 267 | 2-18 | $1.54 \times 10^4$ | [9] |
| First order derivative spectroscopy | 227 | | $3.02 \times 10^4$ | |
| Area Under Curve | 262-272 | | $1.94 \times 10^4$ | |
| p-DMAB (without CPE) | 402 | 20-400 | $5.654 \times 10^2$ | The present work |
| (with CPE) | 415 | 0.1-3.0 | $5.256 \times 10^4$ | |

ND: Not detedcted

## 4. Conclusion

In the investigation being presented, p-DMAB was used as the coupling agent to combine ENT and produce a yellow result. The presented work has the advantages of being sensitive, simple, rapid, reliable, and accurate for the examination of the antiviral drug (ENT) in its bulk and commercial tablets. The samples don't need to be pre-treated or extracted, which also saves time. The suggested assay is also suitable for use in regular assays, quality control checks of the ENT antiviral medication, and assessments of the uniformity of tablet content.

## 5. Conflicts of interest

The authors declare that they have no competing interests.

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