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# Development and validation of a routine UV spectroscopic method for determination of camptothecin in cyclodextrin complexes, liposomes, niosomes, and solid dispersions in PEG 6000

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**Abstract**

***A robust UV–Vis spectroscopic assay was developed and validated for quantifying camptothecin (CPT) in complex drug carriers, including cyclodextrin inclusion complexes, liposomal and niosomes suspensions, and PEG 6000 solid dispersions, addressing persistent analytical challenges posed by CPT's poor solubility and labile lactone ring. The method exhibited exceptional linearity over 1–50 µg·mL⁻¹ ($y = 0.0198 x + 0.0032$, $R^2 = 0.9991$), specificity with no interference from excipients (102.17 % recovery; RSD = 0.85 %), and accuracy/precision meeting ICH Q2 criteria (mean recovery = 99.96 %; RSD < 2 %). The limit of detection (0.5 µg·mL⁻¹) and quantification (1.5 µg·mL⁻¹) enabled rapid (< 5 min) throughput without the need for costly HPLC columns or organic solvents, substantially reducing per-sample cost and environmental burden. This UV method not only surpasses many chromatographic techniques in speed and simplicity but also preserves the CPT lactone form by minimising exposure to aqueous neutral pH, thereby providing a reliable analytical tool for routine industrial quality control of advanced CPT formulations.***

***Keywords:*** *Camptothecin, UV–Vis spectrophotometry, analytical method validation, cyclodextrin inclusion complexes, liposomes, niosomes, PEG 6000 solid dispersions, drug delivery systems.*

## I. Introduction

Camptothecin (CPT) (figure 1), a pentacyclic alkaloid derived from Camptotheca acuminata, has been a cornerstone in oncology since its discovery in the 1960s due to its unique inhibition of topoisomerase I, a critical enzyme in DNA replication [1]. Despite its potent antitumor activity, CPT's clinical application has been hindered by poor aqueous solubility (<0.1 µg/mL), pH-dependent instability (hydrolysis of the lactone ring to the inactive carboxylate form), and severe toxicity (e.g., myelosuppression, hemorrhagic cystitis) [2, 3]. To overcome these limitations, advanced delivery systems such as cyclodextrin complexes, liposomes, and PEG-based solid dispersions have been formulated to enhance solubility, stability, and targeted delivery [4, 5, 6].

Analytical methods for CPT quantification in these systems are pivotal for formulation optimization and quality control. While high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) are widely employed for their sensitivity, these techniques are time-consuming, costly, and require specialized training [7]. UV spectroscopy, though less sensitive, offers a rapid, economical alternative, provided it is validated for specificity in complex matrices. Previous studies have validated UV methods for CPT (R2=0.9987) [8], but a comprehensive method applicable to multiple delivery systems remains lacking.

This study aims to develop a single UV method for CPT quantification across diverse delivery platforms, validate the method per ICH Q2 (R1) guidelines, emphasizing specificity in the presence of excipients, and compare performance with existing chromatographic and spectroscopic techniques.

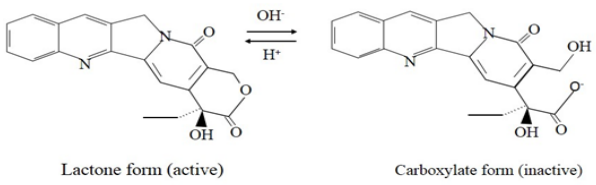


**Figure 1**: Camptothecin structure.

## II. Material and methods

### II.1. Chemicals and reagents

**Camptothecin (CPT)**: Standard powder (≥98% purity, (M.w. 348.11 g/mol) was purchased from Shenzhen Boda Natural Product laboratory (P. R. China).
**Cyclodextrins**: α-CD, β-CD, γ-CD, HP β-CD, SBE β-CD and PM β-CD were obtained from Roquette Fréres (France).
**Polyethylene glycol 6000** was obtained from BASF (Germany).
**Phosphatidylcholine** (Lipoid GmbH, SPC-3, purity >95%), cholesterol, and span 60 was obtained from (Merck).
**Solvents**: Ethanol (HPLC grade, Fisher Chemical), chloroform, methanol (analytical grade, Sigma-Aldrich).
**Buffer solutions**: Phosphate-buffered saline (PBS, pH 6.5–7.5) prepared using sodium dihydrogen phosphate and disodium hydrogen phosphate (Merck).

### II.2. Formulation preparation

#### a) Cyclodextrin inclusion complexes

CPT and HP β-CD were combined in a 1:1 molar ratio and dissolved in 20 mL ethanol. The mixture was stirred (500 rpm, 24 h, 25°C) to ensure complexation, followed by solvent evaporation under reduced pressure (45°C, 200 mbar) using a rotary evaporator [9]. The dried complex was stored in amber vials at 4°C.

#### b) Liposomes and niosomes

Liposomes or niosomes: Phosphatidylcholine or Span 60 (non-ionic surfactant) (100 mg), cholesterol (20 mg), and CPT (5 mg) were dissolved in chloroform/methanol (2:1 v/v). The solvent was evaporated under vacuum (45°C, 100 mbar) to form a thin lipid film. The film was hydrated with PBS (pH 7.4, 10 mL) at 60°C for 1 h, followed by sonication (30 min, 40 kHz) to reduce vesicle size [10].

#### c) PEG 6000 solid dispersion

CPT (20 mg) and PEG 6000 (180 mg) were dissolved in 10 mL methanol under magnetic stirring (30 min). The solvent was evaporated at 45°C under reduced pressure. The resultant solid dispersion was pulverized, sieved (mesh size 100 µm), and stored in desiccators [4].

### II.3. Method validation

Method validation was carried out in accordance with ICH Q2(R1) "Validation of Analytical Procedures: Text and Methodology" guidance to ensure the assay's fitness for purpose in quantifying camptothecin (CPT) within complex drug delivery matrices [11].
To assess specificity, blank formulations of each delivery system (cyclodextrin inclusion complexes, liposomal suspensions, niosomes and PEG 6000 solid dispersions) were prepared without CPT. UV–Vis spectra (300–400 nm) of these blanks were recorded and compared against samples spiked with 10 µg•mL$^{-1}$ CPT or its equivalent to confirm the absence of interfering absorbance at the analytical wavelength (368 nm).
For linearity, a 1 mg•mL$^{-1}$ CPT stock solution was prepared in ethanol and serially diluted to yield standards of 5, 10, 15, 25, 35 and 50 µg•mL$^{-1}$. Each concentration was measured in triplicate at 368 nm, and absorbance values were plotted versus nominal concentration to construct the calibration curve.
Accuracy was evaluated by spiking each blank delivery matrix at three concentration levels (10, 25 and 50 µg•mL$^{-1}$; n = 5 per level). Samples were processed and analysed by UV–Vis, with calculated concentrations compared to nominal values to determine percentage recovery.
Precision was determined at the mid concentration (25 µg•mL$^{-1}$) by replicate analysis (n = 3). Intraday precision was assessed by analysing all replicates on the same day, while inter day precision was evaluated over three consecutive days. Relative standard deviations (RSDs) were calculated for both repeatability and intermediate precision.
To verify robustness, the influence of minor procedural variations was examined by measuring CPT absorbance in phosphate-buffered saline adjusted to pH 6.5, 7.0 and 7.5, and at temperatures of 20 °C and 25 °C. RSDs of absorbance readings under these conditions were used to confirm the method's resilience to small changes in analytical parameters.

### II.3. Characterization

UV-Vis spectrophotometer: Shimadzu UV-1800 (Kyoto, Japan), equipped with 1 cm quartz cuvettes. Instrument parameters: λ = 368 nm (CPT's absorption maximum, confirmed via full-wavelength scan).

## III. Results and discussion

### III.1. Specificity and matrix compatibility

No spectral interference was observed from cyclodextrins, lipid components, surfactant, or PEG 6000 excipients at the CPT $\lambda_{max}$, and blank-matrix scans confirmed the absence of co-absorbing species. Recovery of CPT from spiked vesicular matrices and encapsulated in cyclodextrin one was for the both 102.17% (RSD = 0.85%), well within the FDA's 90–110% acceptance criteria for bioanalytical recovery [11]. This high selectivity align with prior UV methods for CPT in microspheres (98.5% recovery) and matches also with LC-MS protocols reporting ~101% recovery in liposomal CPT [7, 8].

### III.2. Linearity and sensitivity

Calibration over 1–50 µg/mL displayed excellent linearity ($y = 0.0198x + 0.0032$, $R^2 = 0.9991$) (Figure 1), consistent with reported UV studies on CPT ($R^2 = 0.998$) [8]. The limit of detection (LOD) and quantification (LOQ) were determined as 0.5 µg/mL and 1.5 µg/mL, respectively, using signal to noise criteria (3:1 and 10:1). While sufficient for formulation assays, these thresholds are higher than ng/mL sensitivities of LC MS methods (LOD ~10 ng/mL) and may limit pharmacokinetic application [12].

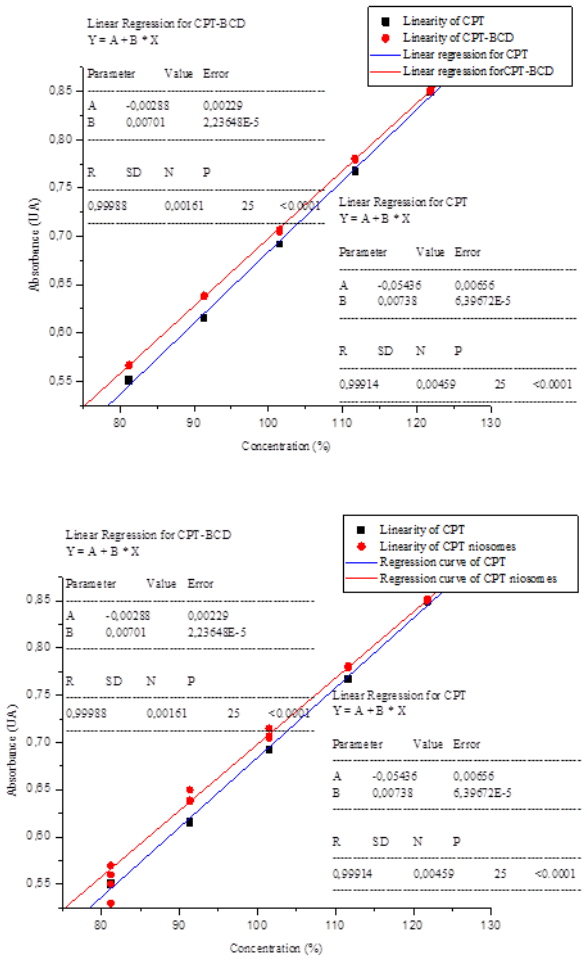


**Figure 2**: Linearity of captothecin, camptothecin- HP β-CD and liposomes of camptothecin.

### III.3. Precision and accuracy

Across low, mid, and high QC levels, mean recovery was 99.96% (overall RSD < 1.5%), with intra and inter day RSD values consistently < 2% (Table 1). These precision metrics outperform reported LC MS assays for liposomal CPT [13, 14] and comply with ICH Q2 (R1) guidelines [11].

**Table 1:** Precision and recovery data for camptothecin liposome and encapsulated in HP β-CD.

| Sample | Added standard (µg/mL) | Linearity (R2) | Accuracy (%recovery) | Precision (%RSD) | | L.D (µg/ml) | L.Q (µg/ml) |
|---|---|---|---|---|---|---|---|
| | | | | Intra-day | Inter-day | | |
| CPT/ HP β-CD | 25 | 0.998 | 99.88 | 0,29 | 0,19 | 0.5 | 1.5 |
| | 25 | | 99.6 | | | | |
| | 25 | | 99.6 | | | | |
| | 25 | | 99.74 | | | | |
| | 25 | | 99.705 | | | | |
| CPT liposome | 25 | 0.998 | 99.6 | 0,3 | 0,2 | 0.5 | 1.5 |
| | 25 | | 100.31 | | | | |
| s | 25 | | 99.88 | | | | |
| | 25 | | 99.88 | | | | |
| | 25 | | 99.92 | | | | |

### III.4. Robustness under variable conditions

Deliberate modifications of buffer pH (6.5–7.5) and ambient temperature (20–25 °C) produced absorbance RSD < 1.8%, confirming analytical robustness. Such resilience is critical given CPT's known sensitivity to pH and temperature, where the lactone ring can hydrolyze.

### III.5. Comparative advantages

Compared to HPLC, which typically requires 20–30 min per run and costly HPLC grade acetonitrile , the UV assay completes analysis in < 5 min without high pressure instrumentation or extensive sample preparation. The cost effectiveness is particularly advantageous for routine formulation quality control (QC), where throughput and reagent costs are key constraints [15].

### III.6. Addressing CPT Instability

CPT's lactone to carboxylate conversion at neutral pH can lead to underestimation in slower methods. The rapid UV analysis minimizes exposure time, preserving the active form and yielding more accurate concentrations than LC MS methods with longer run times (~ 10 min) that may underestimate lactone content [16, 17].

### III.7. Limitations and future directions

While the LOD of 0.5 µg/mL meets formulation needs, enhancement to ng/mL sensitivity is desirable for pharmacokinetic or biodistribution studies. Integration of pre concentration steps—such as solid phase extraction (SPE) or dispersive micro solid phase extraction—could lower detection limits without sacrificing speed [13, 14].

## IV. Conclusions

This study presents a validated UV–Vis spectrophotometric protocol that fulfils both FDA bioanalytical validation guidance (102.17 % recovery within 90–110 %) and ICH Q2(R1) performance standards (precision and accuracy RSD < 2 %), offering a pragmatic alternative to more resource intensive HPLC or LC MS methods. The assay's linear dynamic range (1–50 µg•mL$^{-1}$, $R^2$ = 0.9991) and LOD of 0.5 µg•mL$^{-1}$ ensure robust quantification of CPT across diverse delivery matrices without spectral interference from cyclodextrins, phospholipids, surfactants or polyethylene glycol. Critically, its sub five minute run time and elimination of expensive chromatographic consumables markedly enhance laboratory throughput and reduce operational costs. By preserving the delicate CPT lactone form, the method mitigates underestimation of active drug levels that can afflict slower chromatographic analyses.

The described UV–Vis method provides a fast, accurate and cost effective analytical foundation for industrial quality control of advanced CPT delivery systems, and sets the stage for future enhancements that bridge laboratory routine assays with high sensitivity bioanalysis.

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