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"A NOVEL ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF IRINOTECAN HCL TRIHYDRATE USP IN BULK AND PHARMACEUTICAL INJECTION DOSAGE FORM BY USING AREA UNDER CURVE UV-SPECTROPHOTOMETRIC METHOD"

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ABSTACT:

A novel, simple Area under curve UV-spectrophotometric method was developed and validated for the estimation of Irinotecan HCl Trihydrate USP in bulk and pharmaceutical Injection dosage form. The developed analytical method employs the measurement of the AUC at an absorption maximum (λ_{max}) within the range of **216-226nm** by using **99% AR grade methanol** as a solvent. And obeys the beer's law in the concentration range of **10-50µg/ml**.

The method exhibits excellent linearity, reproducibility, and precision in the concentration range of **10-50µg/ml** with a high correlation coefficient**(r^2) of 0.9999** as described by the regression equation **Y= 0.193X +0.001**.

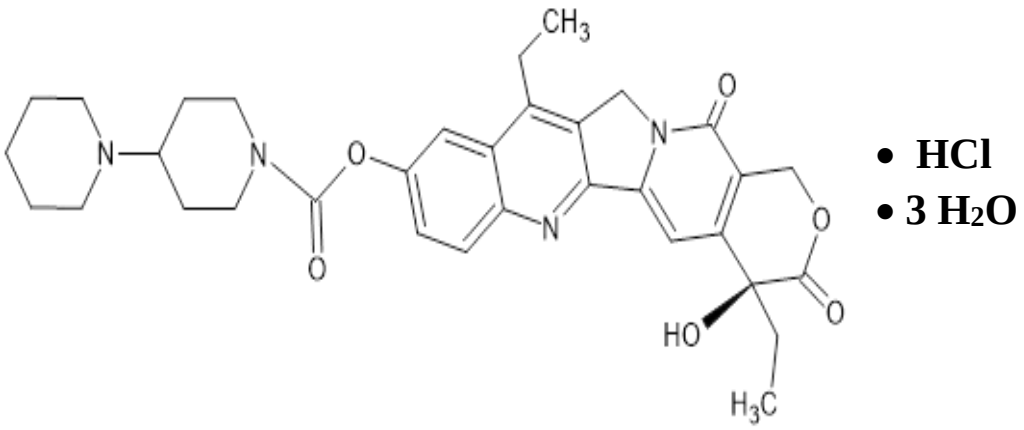
The method accuracy is expressed interms of % recovery. The % recovery of Irinotecan HCl was found to be in the range of **99.46 – 99.88%,** these obtained range values indicates the developed method is accurate. The LOQ and LOD for estimation of Irinotecan HCl was found to be **0.170µg/ml and 0.518µg/ml** respectively. The %RSD values were found to be **< 2%.** The present developed Area under curve UV-spectrophotometric method was accomplishing the validation parameters according to ICH guidliencess for Specificity, Range, linearity, accuracy, precision, LOD, LOQ and Ruggerdness. The developed method is simple, highly accurate, novel and sucessfully applied for the analytical method development of Irinotecan HCl trihydrate USP in bulk and pharmaceutical Injection dosage form.

KEY WORDS: Irinotecan HCl, UV-Spectrophotometric, USP, AUC, ICH-guidlience.

INTRODUCTION:

Irinotecan is a semi-synthetic compound derived from the natural alkaloid camptothecin, which is extracted from the Chinese plant Camptotheca acuminata **[1,2].** Marketed under the brand name Camptosar **[1,2],** it serves as a derivative of camptothecin **[1,3]** (CPT). Irinotecan is a crystalline solid compound with a pale-yellow color **[1]** and exhibits solubility in solvents like organic solvents DMSO, DMF and also polar solvents such as water, methanol**[1].**It is a chemotherapeutic agent**[4],** which has the potency to inhibit the action of topoisomerase -I **[1,4].** Irinotecan preventing the resealing of DNA strands by binding to the topoisomerase I-DNA complex **[3,4].** It is primarily used as first-line drug choice for treatment of colorectal cancer **[1,4].** The most common side effects associated with Irinotecan include severe diarrhea, profound immune system suppression, and a consequential significant reduction in white blood cell(WBC) count in the blood **[4].**

- **Chemical structure [1,2]**



- **HCl**
- **3 H_2O**

Fig -1: Chemical structure of Irinotecan Hcl Trihydrate USP

- **Drug: IRINOTECAN HYDROCHLORIDE TRIHYDRATE USP (IHT) or (IRN)** [2]
- **Chemical name: (S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo- 1H-pyrano [3',4':6,7]-indolizino [1,2-b] quinoline-9-yl-[1,4'bipiperdine]-1'-carboxylate, monohydrochloride, trihydrate** [1,4]
- **Synonym:** Camptosar [1,2].
- **Molecular formula:** $C_{33}H_{38}N_4O_6.HCl.3H_2O$ [1,2]
- **Molecular weight:** 677.17[1]
- **Chemical class:** Chemotherapeutic agent [4], anti-neoplastic agent [1,5].

- **Origin/source of Irinotecan HCl** [1,2]

Fig-2: Camptotheca acuminta plant

- **Origin:** Irinotecan is a semisynthetic compound derived from the natural alkaloid camptothecin, which is obtained from the Chinese plant Camptotheca acuminata [1,2].
- **Chemical Properties:** Irinotecan (IRN) is a pale yellow to crystalline solid compound. It is soluble in organic solvents DMSO, DMF and also Polar solvents such distil. Water and methanol [1].
- **Derivative of Camptothecin:** Irinotecan is a derivative of camptothecin (CPT), which is another natural compound with anti-cancer properties [1,3].
- **Mechanism of action:** [1,4,3]
  - IRN potentially inhibits the "Topoisomerase-I"
  - IRN prevents relegation of the DNA strand by binding to topoisomerase -I DNA complex

↓

- The formation of this ternary complex interferes with the moving replica fork, which induces replication arrest and lethal double stranded breaks in DNA

↓

- Finally, DNA damage and apoptosis occurs.

- **Category:**
  - Used as first-line drug choice for the treatment of the colorectal cancer [1,3,6].
  - Used for the salvage theraphy of breast cancer in Europe [6].
  - It was approved for use in breast cancer treatment in Japan [6].

- **Adverse Effects:** Some of the most significant adverse effects of Irinotecan include:
  - **Severe Diarrhea [4]:** This is a common and potentially serious side effect of Irinotecan. Patients receiving this drug may experience diarrhea, which can be severe and may require medical intervention.
  - **Suppression of the Immune System [4]:** Irinotecan can lead to a significant suppression of the immune system, making patients more susceptible to infections.
  - **Low White Blood Cell (WBC) Count [4]:** The immune system suppression caused by Irinotecan can lead to a dramatic reduction in white blood cell count (WBC) in the blood. This condition, known as neutropenia, increases the risk of infections.

IRN HCl is officially listed in IP-2007, USP and BNF, which provides guidance on the HPLC method for estimation of IRN HCl in Injection formulations. Various analytical techniques have been reported for assessing IRN HCl, including HPLC methods, LC-MS methods, HPTLC methods, visible spectrophotometric methods, and a cyclic volumetric method.

A comprehensive literature review survey of various analytical methods employed for the estimation of Irinotecan HCl. These methods include the Visible Spectrophotometric method (Balaram et al., 2011), RP-HPLC method (Saini et al., 2009), and HPLC method (Shende et al., 2009; Satyanarayana et al., 2009; Venkateswara Rao et al., 2007; B. Mohammed Ishaq et al., 2010; Gogineni Ratna Prasad et al., 2011).

Furthermore, related substance estimation within Irinotecan HCl has primarily has been conducted in human plasma, as evidenced by studies carried out by Iman Barilero et al. (1995), Iman Barilero et al. (1992), Wei Zhang et al. (2009), and Owens et al. (2008). Sushama Talegaonkar et al. (2011) have contributed to the field by developing stability-indicating studies of Irinotecan HCl using HPTLC, while Mohammad Ali et, al. (2010) has introduced a method for the simultaneous estimation of Irinotecan HCl and SN-38 via RP-HPLC.

Smitha Sharma et al. (2012) have conducted research on the development and validation of a spectrophotometric method and TLC densitometric determination for IRN HCl in pharmaceutical dosage forms.

However, no evidence has been reported for the estimation of Irinotecan HCl Trihydrate USP using the Area under the Curve UV-Spectrophotometric method.

"Therefore, the primary objective of the present study was to establish a simple and novel analytical method for the validation and estimation of Irinotecan HCl Trihydrate USP in both bulk and pharmaceutical Injection dosage forms, utilizing the Area under the Curve UV-Spectrophotometric Method".

MATERIALS AND METHOD:

Instrument:

UV/VIS Spectrophotometer, Shimadzu, Model No. UV-1800, is a double beam high speed scanning spectrophotometer with a single monochromator having a 1200 grooves/ mm concave grating, used for all spectral measurements.

All wights are taken in analytical balance.

Chemicals:

Irinotecan Hydrochloride Trihydrate USP API drug was obtained as a gift sample from Laurus Laboratories Ltd, Visakhapatnam, Andhra Pradesh, India and its pharmaceutical dosage form Irinotecan Hydrochloride Injection labelled claim 20mg -5ml vial (20*5 =100mg) under the brand name Irinotel from Net-Meds pharmacy manufactured by Fresenius kabi Oncology Ltd. All chemicals used were AR grade from SD fine Chem, Merck, Fischer scientific, Mumbai, India.

Solvent:

99% AR grade methanol

METHOD:

Area under curve (AUC) spectrophotometric determination of Irinotecan hydrochloride Trihydrate USP using 99% methanol.

Method Development:

Solvent selection:

In order to select suitable solvent for determination of Irinotecan Hydrochloride Trihydrate USP, various solvents were selected for the solubility studies and it was found that Irinotecan Hydrochloride Trihydrate USP was completely soluble in organic solvents such as DMSO, DMF and also soluble in polar solvents like distil. water, and 99% methanol.

99% AR grade methanol selected as solvent for our novel Area under curve UV- spectrophotometric analytical method development.

Reason:

- We have checked the solubility of the IRN in different organic solvents such as DMSO, DMF, Acetonitrile and also aqueous buffer PBS, polar solvents such as distill water, 99% methanol and ethanol.
- We have observed and concluded that the based-on literature review survey distil. water already used for the method development. The other organic solvents such as DMSO and DMF both are aprotic solvents

(acts as both acidic and basic) possibility to majority of drugs dissolve this fact impact on UV- absorbance interference, so we have not selected for the method development.

- Ethanol is a weak UV radiation signal absorbance due to this reason we have not used for the method development.
- 99% AR grade methanol shows good compatability with Irinotecan HCl and also shows instrumental suitability.

Hence finaly we have selected as 99% AR grade methanol selected as solvent for our novel Area under curve UV-spectrophotometric analytical method development.

Selection of Analytical wavelength:

Appropriate dilutions were prepared for drug Irinotecan HCl Trihydrate USP from the standard stock solution and the solution was scanned in the wavelength range of 200-400 nm. The absorption spectra thus obtained was showing the absorption maxima at 221nm and Area under curve in absorption spectra were measured between the wavelength range of 216-226nm which illustrated in Figure 3.

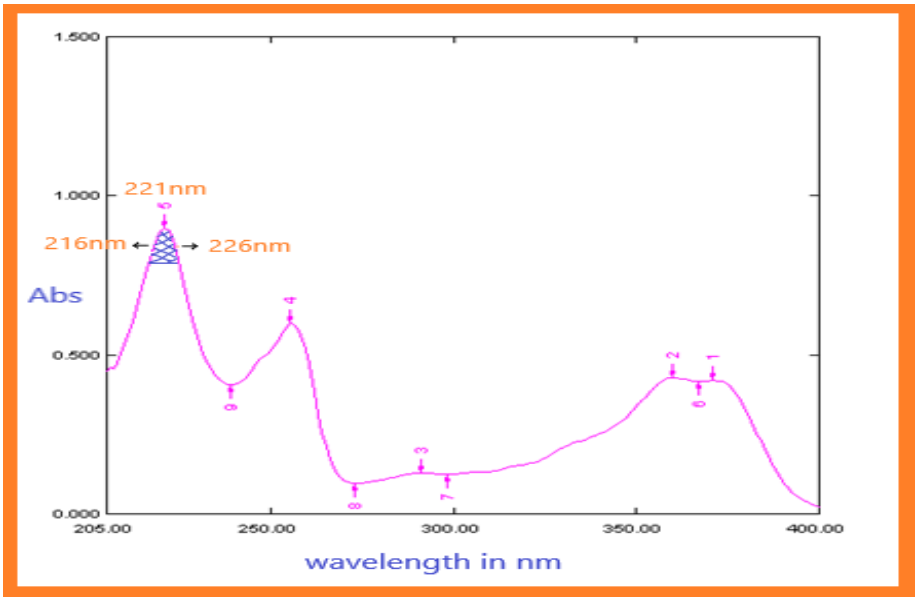


Fig -3: Typical zero order spectra of Irinotecan HCl showing AUC from 221nm-226nm.

Preparation of Standard stock solution:

Standard stock solution was prepared by dissolving accurately weighed 100 mg of Irinotecan HCl Trihydrate USP API in 99% methanol and the volume was made up to 100 ml with 99% methanol in 100 ml volumetric flask (Stock solution -I, 1000 µg/ml). 10 ml of stock solution-I was diluted to 100 ml with 99% methanol (Stock solution-II, 100

µg/ml). 1, 2, 3, 4, 5 ml of stock solution-II was taken into individual 10 ml volumetric flask and add 99% methanol up to mark, this gives 10, 20, 30, 40 and 50 µg/ml concentration.

Dilutions:

Weigh 100 mg IRN API → 100 ml (99% methanol) → 1000 µg/ml → Stock solution-I

↓

Pipette out 10 ml → 100 ml (99% methanol) → 100 µg/ml → Stock solution-II

↓ pipette out

- 1 ml → into 10 ml (volumetric flask) → 10 µg/ml
- 2 ml → into 10 ml (volumetric flask) → 20 µg/ml
- 3 ml → into 10 ml (volumetric flask) → 30 µg/ml
- 4 ml → into 10 ml (volumetric flask) → 40 µg/ml
- 5 ml → into 10 ml (volumetric flask) → 50 µg/ml

Sample preparation of Irinotecan Injection IP:

The commercially available Irinotel I.P. Injection 20mg labelled claimed 5 ml vials contains 100 mg (20mg*5ml =100mg) of Irinotecan hydrochloride. From this 5 ml were transfer into 100 ml of volumetric flask then it was diluted with 99% methanol and the volume was made up to the mark 100 ml with 99% methanol in 100 ml volumetric flask (Stock solution -I, 1000 µg/ml).

10 ml of stock solution-I was diluted to 100 ml with 99% methanol (Stock solution-II, 100 µg/ml). **1.5**, **3**, **4.5** ml of stock solution-II was taken into individual 10 ml volumetric flask and add 99% methanol up to mark, this gives **15**, **30** and **45 µg/ml** concentration.

Dilutions:

Take 5 ml of Irinotel Injection IP →100 ml (99%methanol) →1000 µg/ml→ Stock solution-I

(1ml=20mg,5ml*20 =100mg)

↓pipette out

Pipette out 10 ml → 100 ml (99% methanol) → 100 µg/ml → Stock solution-II

↓ pipette out

- 1.5 ml → into 10 ml (volumetric flask) → 15 µg/ml
- 3 ml → into 10 ml (volumetric flask) → 30 µg/ml
- 4.5 ml → into 10 ml (volumetric flask) → 45 µg/ml

Method validation:

The novel analytical method development for estimation of Irinotecan HCl validated according to ICH guidliencess.

RESULT AND DISCUSSION:

Linearity:

Serial dilutions of the working standard solution were carried out using 99% methanol, resulting in a concentration range of 10-50µg/ml. Subsequently, a calibration curve for Irinotecan HCl trihydrate USP was established by measuring the absorbance within the wavelength range of 216-226nm.The corresponding absorbance values are detailed in Table -1, and the calibration graph is visually depicted in Fig- 4. Statistical parameters including the slope, intercept, coefficient of correlation, and Sandel's sensitivity were determined and are presented in Table -2.

Table - 1: Results of Mean AUC Absorbance values for Calibration curve of Irinotecan HCl at 216-226nm.

Sl.no.	Concentration (µg/ml)	Mean AUC Abs. Value
1.	10	0.190
2.	20	0.385
3.	30	0.575
4.	40	0.776
5.	50	0.960

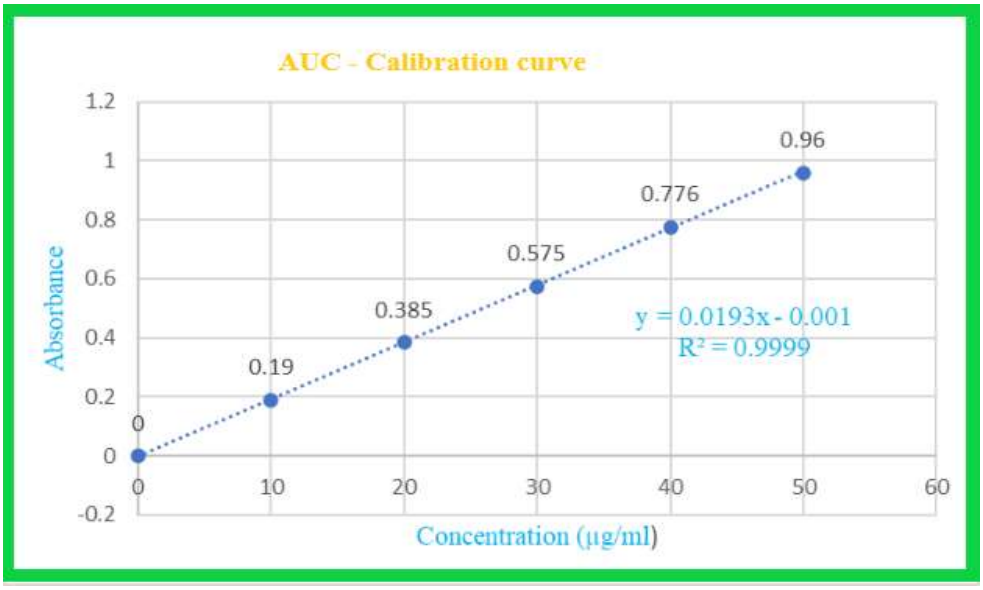


Fig-4: Calibration Curve for Irinotecan HCl by AUC Spectroscopic method at 216-226 nm

Sr. No.	Regression Parameters	Irinotecan HCl
1	Concentration Range	10-50 µg/ml
2	Absorption Maxima	216-226 nm
3	Regression Equation	Y= 0.0193x+0.001
4	Slope (m)	0.0193
5	Intercept (c)	0.001
6	Correlation coefficient (r^2)	0.9999
7	Sandell's Sensitivity	0.0521 µg/cm^2

Table – 02: Optimum conditions, optical characteristics and statistical data of regression equation for Irinotecan HCl at 216-226 nm by using AUC spectroscopy.

Precision:

The precision method was carried out by two methods intra-day and inter-day precision studies. Intra-day precision was carried out by analyzing solutions with concentrations of 10,20,30,40 and 50 µg/ml 6 times on the same day every 4hrs. Meanwhile, inter-day precision also carried out by analyzing solutions with the same concentrations on 6 consecutive days. The results of the precision studies are tabulated in Table - 3.

Table-3: Precision study data of Irinotecan HCl at 216-226 nm by Area under curve UV- Spectroscopy

Concentration (µg/ml)	Intra-day mean AUC Absorbance ± SD	% RSD	Inter-day mean AUC Absorbance ± SD	% RSD
10	0.199 ± 0.0011	0.551%	0.200 ± 0.0013	0.670%
20	0.394 ± 0.0043	0.093%	0.401 ± 0.0023	0.573%
30	0.588 ± 0.0017	0.288%	0.600 ± 0.0012	0.20%
40	0.779 ± 0.0012	0.153%	0.796 ± 0.0035	0.439%
50	0.904 ± 0.0020	0.221%	0.914 ± 0.0055	0.603%

***Average of six- determinations**

Accuracy:

We conducted recovery studies at three different spiked levels i. e, 50%, 100%, and 150% to assess the accuracy of the proposed method. The formulation concentration remained constant, while we systematically varied the pure drug (API) concentration. The results are represented in Table - 4.

Table-4: Recovery (Accuracy) study data of Irinotecan HCl at 216-226nm by AUC UV- spectroscopy.

Sr. No.	Spiked levels	Amount of sample (µg/ml) Irinotel Injection	Amount of standard (µg/ml) IRN bulk	Amount recovered (µg/ml)	% Recovery ± SD*	% RSD
1	50%	30	15	44.95	99.8± 0.244	0.244%
2	100%	30	30	59.68	99.4± 0.159	0.160%
3	150%	30	45	74.76	99.6± 0.347	0.347%

***Average of six- determinations**

Ruggerdness:

Ruggedness was carried out by different analysts, and the %RSD values were found to be less than 2%. The obtained results are represented in Table -5.

Table -5: Ruggedness results of Irinotecan HCl at 216-226nm by Area under curve UV- Spectroscopy.

Analysts	Analyst-1	Analyst-2
Mean Absorbance	0.554	0.566
Standard deviation*	0.004	0.005
% RSD	0.740%	0.954%

*Average of six- determination

Limit of Detection and Limit of Quantification:

The method's Limit of Detection (LOD) and Limit of Quantitation (LOQ) for Irinotecan HCl Trihydrate USP were determined based on the standard deviation of the response and the slope of the linearity curve. **The calculated values for LOD and LOQ were found to be 0.170µg/ml and 0.518µg/ml, respectively.**

CONCLUSION:

The present newly developed Area under Curve UV-Spectrophotometric method was accomplishing validation parameters a/c to ICH guidliencess, including Specificity, Range, Linearity, Accuracy, Precision, LOD, LOQ, and Ruggedness.

Finally concluded that present developed Area under curve UV-Spectrophotometric, analytical method is Innovative, simple, cost-effective compared to other methods, and offers high accuracy and precision for routine analysis. It is well-suited for efficient analysis of numerous samples in a short time and has been effectively applied in the Novel analytical method development and validation of Irinotecan HCl Trihydrate USP in both bulk and Irinotecan HCl Injection dosage form.

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