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## RP-HPLC method development and validation for the simultaneous estimation of Irinotecan hydrochloride and Capecitabine in Active Pharmaceutical Ingredients (APIs)

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### ABSTRACT

The combination of Irinotecan Hydrochloride (IRI) and Capecitabine (CAP) is indicated for the treatment of cancer. IRI and CAP were developed by an simultaneous simple reverse phase liquid chromatographic method and were subsequently validated from their APIs. The proposed method was based upon the separation of these two chemical agents using Agilent 1200 series HPLC with Qualisil gold C18 (250 × 4.6 mm, 5 $\mu$ ) column and it was maintained at ambient temperature. The effective chromatogram was obtained using the mobile phase of methanol: water (60:40 (% v/v)) and the pH was adjusted to 3 with orthophosphoric acid at the flow rate of 1.0 ml/min. The column effluents were detected using Photo Diode array detector at wavelength of 340 nm. The proposed reverse phase liquid chromatographic method was validated as per ICH Q2 (R1) guidelines. Based upon the optimized parameters, these drugs were effectively separated and the retention time was found at 4.08 min. for IRI and 7.8 min. for CAP with resolution of 7.6. There is no interference with the impurities or degradation products. The calibration plots were found to be linear over the concentration range of 4-24  $\mu$ g/ml and 40-240  $\mu$ g/ml respectively. The LOD and LOQ of IRI were found to be 0.12  $\mu$ g/ml and 0.373  $\mu$ g/ml while LOD and LOQ of CAP were found to be 0.254  $\mu$ g/ml and 0.771  $\mu$ g/ml respectively. The mean percent recovery of triplicate analysis of IRI and CAP were found to be 100.58% and 100.03% respectively. In conclusion, the developed method can be used in the quality control laboratories for the determination of IRI and CAP in APIs.



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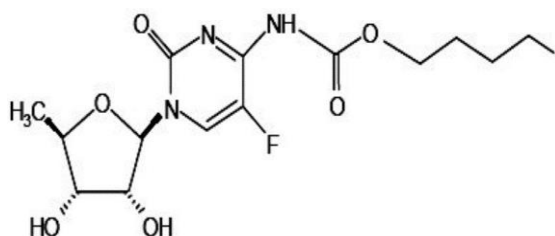
### INTRODUCTION

Irinotecan Hydrochloride (IRI) (Molecular formula: C<sub>33</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub> and Molecular Weight: 586.678 g/mol), a prodrug and chemically it is known as (S)-10-[4-(-piperidino) piperidino carbonyloxoyl]-4, 7-diethyl-4-hydroxy-1H-pyrano [3, 4: 6, 7] indolizino [1, 2-b] diethyl 13, 14[4H, 12H]-dione (Fig. 1). IRI is biologically active by utilizing the enzyme of carboxylesterase and it forms the active metabolite of 7-ethyl-10-hydroxy-camptothecin (SN-38) (PubChem Open Chemistry database). In the treatment of small cell lung cancer, colon and

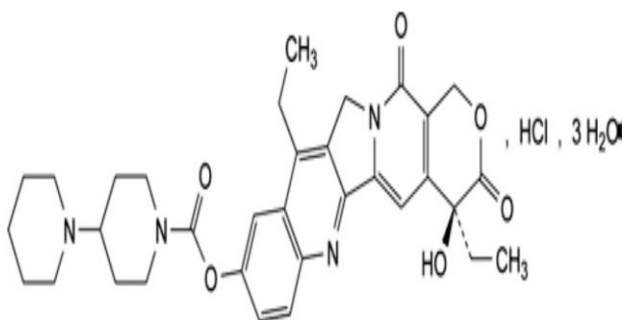
in advanced pancreatic cancer IRI was employed. (British national formulary 2015). IRI is yellow to pale yellow crystalline powder in nature. API IRI showed highest solubility in methanol, followed by 96 % ethanol (sparingly Soluble) and least solubility in water (slightly soluble).

Capecitabine (CAP) (Molecular formula: C<sub>15</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>6</sub> and Molecular weight: 359.35 g/mol), a prodrug and chemically it is known as pentyl N-{1-[(2R, 3R, 4S, 5R)-3, 4-dihydroxy-5-methyloxolan-2-yl]-5-fluoro-2-oxo-1, 2-dihydropyrimidin-4-yl} carbamate (Fig. 2). It is a deoxycytidine derivative and it is indicated to treat breast cancer, esophageal cancer, gastric cancer and colorectal cancer (Cancer Research).

The combination of these IRI and CAP agents are used to treat advanced bowel cancer (colorectal cancer) (Kerr DJ, 2002). As per the literature survey, methods estimated for both of these drugs are available individually but to my knowledge, there is no simultaneous method have been published for this combination. Based upon literature survey reported for IRI it was found to be Spectroscopy (Kuna M, 2012), HPLC (Ali M, 2010; Tariq M, 2015; Navneet K, 2012)) UPLC and LC-MS (Pallavi K, 2016) while as for CAP it was UV (Ramesh G, 2015; Jothieswari D, 2014) HPLC (Farkouh A, 2010; Narendra D, 2013; Sreevatsav ASK, 2013) LC/MS (Montange D, 2010; Licea-Perez H, 2009).



**Figure 1: Chemical structure of Capecitabine Hydrochloride**



**Figure 2: Chemical structure of Irinotecan**

A suitable analytical method needs to be developed on this combination for future usage. Hence, the present study was aimed to develop a simultaneous RP-HPLC method and to carry out validation as per ICH guidelines for the selected anticancer

drugs IRI and CAP which is economic, simple, accurate and precise.

## MATERIALS AND METHODS

### Instrumentation

The analysis was performed on a liquid chromatographic system of Agilent LC 1200 series with PDA detector. The data was processed using EZ Chrome software.

### Chemicals & Reagents

Reference standards of IRI and CAP were obtained as gift samples from Dr Reddy's laboratory and Hetero labs, Hyderabad, India. The HPLC grade of methanol and water were procured from Merck India Ltd, Mumbai, India. Analytical grade reagents and chemicals were used in the entire study.

### Chromatographic Conditions

The chromatographic conditions for the development include a stationary phase [Qualisigold C18 (250 mm X 4.6 mm, 5 μm) column which was maintained at ambient temperature. Methanol: Water in the ratio of (60:40 v/v) used as mobile phase, Orthophosphoric acid (OPA) at a flow rate of 1 mL.min<sup>-1</sup> was used for adjusting the pH to 3.0. Nylon 0.45 μ membrane was used for the mobile phase filtration and degassed before performing the analysis. 340 nm wavelength was used for monitoring the separation IRI and CAP with an injection volume of 20 μL.

### Preparation of standard IRI and CAP solutions

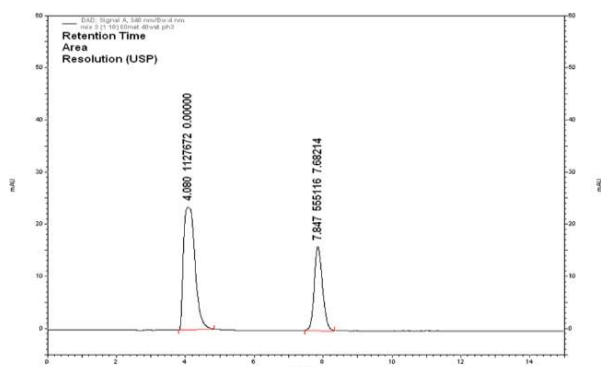
In dried 100 ml clean standard volumetric flasks about 100 mg each of IRI and CAP standards were weighed and transferred into it. Methanol was used to make up the volume to the mark. Further using the above stock solutions, IRI (10 μg/ml) and CAP (100 μg/ml) concentrations were prepared.

## RESULTS

### Method Development and Optimisation

A simultaneous RP-HPLC method was developed for the two anti-neoplastic agents and which can be conveniently employed for routine quality control testing in APIs. For the method development, various trials were conducted in the chromatographic system for the estimation of IRI and CAP in their APIs. The various columns were tried finally, Qualisigold C18 (250 mm X 4.6 mm, 5 μm) was chosen in view of excellent peak symmetry, better reproducibility and high-resolution power. Various mobile phase compositions & pH were tried and finally the ratio of methanol: water (60:40 v/v) was selected, orthophosphoric acid (OPA) was used to adjust the pH to 3.0. The flow rate of 1.0 mL.min<sup>-1</sup> was found to be ideal to resolve the peaks with an injection volume of 20 μL. 23±1°C was used for

maintaining the column temperature. The drug elements of IRI and CAP were monitored at 340 nm and a good response was observed. IRI and CAP were retained at 4.080 min and 7.847 min respectively (Fig. 3).



**Figure 3: Typical chromatogram of IRI and CAP with detection wavelength at 340 nm**

### Method Validation

The parameters of the validation were performed in accordance with ICH Q2 guidelines 22.

### System Suitability

**Table 1: Summary of the System Suitability of IRI and CAP**

S. No.	Parameter	IRI	CAP
1.	Retention time	4.08 min.	7.8 min.
2.	Resolution	7.6	-
3.	Peak area*	803074±7662.30	37201866±738.34
4.	Tailing factor	1.25	1.18
5.	No. of theoretical plates	3645	5124

\*Average of 6 determinations

**Table 2: Summary of the validation data of IRI and CAP**

S. No.	Parameter	IRI	CAP
<b>Linearity</b>			
1.	Linearity Range	4-24 µg/mL	40-240 µg/mL
2.	Regression Equation	y=75221x+56764	y=4226.5x-59002
3.	Slope	75221	4226.5
4.	Intercept	56764	59002
5.	Coefficient of Correlation (R <sup>2</sup> )	0.9996	0.9993
<b>Precision</b>			
6.	Intra-day Precision (10 µg/mL)*	0.74 %	0.84 %
7.	Inter-day Precision (100 µg/mL)*	1.02 %	0.75 %
<b>Accuracy</b>			
8.	**Spiked concentrations of 80 %, 100 % and 120 %	100.59 %	100.03 %
<b>LOD</b>			
9.	Calculated based on 3:1	0.12	0.25
<b>LOQ</b>			
10.	Calculated based on 10:1	0.37	0.77
<b>Robustness</b>			
11.	Flow rate (± 0.1 mL/min)	0.14 %	0.26 %
12.	Mobile phase composition (± 10%)	0.32 %	0.56 %
13.	pH (± 0.2)	0.23 %	0.45 %
14.	Wavelength (± 5 nm)	0.05 %	0.15 %

\*Average of 6 determinations, \*\*Average of 3 determinations

The system suitability parameters were measured to check the system performance, the resolution, asymmetry and number of theoretical plates were measured and the results were tabulated in Table 1.

### Specificity

By comparing the acquired chromatograms from drug standards and placebo solution the specificity of the method was assessed. No excipient peaks were co-eluted and it indicates that the followed method is selective and specific.

### Linearity

The linearity range was confirmed over the range of 4-24 µg/ml for IRI and 40-240 µg/ml for CAP. R<sup>2</sup> value of IRI and CAP was found to be 0.9996 and 0.9993 respectively. It depicts the good linearity of the method for IRI and CAP. The results of IRI and CAP are summarised in Table 2.

### LOD and LOQ

By injecting a series of dilutions with known concentrations the LOD and LOQ for IRI and CAP were

determined at a signal to noise ratio 3:1 and 10:1 respectively. The LOD and LOQ for IRI were 0.12 µg/mL and 0.37 µg/mL, and for CAP 0.25 µg/mL and 0.77 µg/mL respectively.

### Precision

The precision of the method was determined by studying intra-day and inter-day variation. Intra-day precision was done with one concentration of six replicate injections. By analysing the same concentration for three different days Inter-day precision was determined. The results of IRI and CAP were summarised in Table 2 and the percentage RSD values were found to be less than 2%.

### Accuracy

Recovery assays were carried out for IRI and CAP by spiking their solutions with known amounts of both standards and the results were summarised in Table 2. It was found to be significant under specification limits.

### Robustness

The experimental parameters were altered and the method was evaluated which include the flow rate, the mobile phase composition, the wavelength and column temperature. The results of robustness of IRI and CAP were summarised in Table 2.

### CONCLUSION

New simultaneous RP- HPLC method had been developed for estimation of IRI and CAP in bulk formulation. Symmetric peak shapes, good resolution, and reasonable retention times were obtained for both the drugs. All the method validation parameters showed the satisfactory results with good recoveries. Hence, the present developed method can be used for the simultaneous estimation of IRI and CAP in quality control studies of API for routine drug analysis.

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