Method Development and Validation of Gemcitabine and Irinotecan by RP-HPLC in Pharmaceutical Formulation

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A Simple, Precise, Specific, Accurate, Selective and Rapid Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method with UV detection was developed and validated for analysis of Gemcitabine HCl, Irinotecan at 275nm and 220nm. Chromatography was performed with mobile phase containing a mixture of acetonitrile and phosphate buffer in the ratio of 60:40, v/v with flow rate 1.2 mL/min and 1 mL/min. The calibration graph of Irinotecan and Gemcitabine was found to be linear over the range of 20-150 μg/mL with correlation coefficient of 1.000. Sensitivity, accuracy, range, precision, robustness, ruggedness, stability, specificity, limit of detection, limit of quantification and system suitability parameters were validated for the developed method. The developed method was successfully applied to estimate the amount of Irinotecan and Gemcitabine in pharmaceutical formulations.

Keywords: Gemcitabine HCl, Irinotecan, Method development, RP-HPLC, validation.

INTRODUCTION

Irinotecan is used as an anti-neoplastic agent, chemically known as (14'-Bipiperidine)-1-carboxylic acid, 4,11-diethyl-3,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1H-pyran-[3,4,6,7]-indolizino[1,2-b]quinolin-9-y1ester. Monohydrochloride, trihydrate, which acts as potent inhibitor of topoisomerase-I, a nuclease enzyme that plays a critical role in DNA replication and transition.

Gemcitabine HCl is chemically is a nucleoside analogue that exhibits antitumor activity. Gemcitabine HCl is 4-amino-1-(2-deoxy-2, 2-difluoro-β-β-erythropentofuranosyl) pyrimidine-2(1H)-one Hydrochloride. And used as an anti-tumour agent. It is an analogue of nucleoside. It is metabolized in vivo by two active metabolites, Gemcitabine-diphosphate (dFdCDP) and gemcitabine triphosphate (dFdCTP). It is a radiation sensitising agent.

In this paper we describe a simple, precise, sensitive, rapid and accurate method and sensitive RP-HPLC is used for the development and validation of gemcitabine and Irinotecan in pharmaceutical formulation.

MATERIALS AND METHODS

Working standards of Irinotecan and gemcitabine were obtained from well reputed research laboratory. HPLC grade methanol, sodium acetate, mannitol, methanol (HPLC grade), mono basic sodium phosphate (AR grade), Milli Q water, Gemcitabine HCl working standard were proceed from market method was carried out on RP-HPLC method. (Agilent 1100 series (binary) with pre packed, column (Zorbax Rx C8, 4.6mmx280mm, 5μ or equivalent) using filter and degassed mixture of monobasic sodium phosphate and phosphoric acid: methanol as mobile phase.

Chromatographic Condition

For Gemcitabine: Mobile phase was monobasic sodium phosphate: methanol. Flow rate 1.2 mL/min, detection wavelength 275nm, injection volume 20 μl, column used, Zorbax Rx C8, 4.6mm x 250mm.5μ or equivalent column temperature 25°C ± 2°C.

For Irinotecan: Mobile phase buffer: Acetonitrile: methanol (600:200:200 v/v) respectively. Flow rate 1 mL/min, detection wavelength 220 nm, in volume 10μl column used, water symmetry shields RP C18, 4.6mm×250nm, 5μ.

Preparation of Solutions

Standard preparation: About 20 gm. of gemcitabine HCL, into 200 mL of volumetric flask, dissolve and dilute volume with water, about 50 mg of irinotecan hydrochloride trihydrate to 50 mL volumetric flask, and add 30 mL of methanol to it then sonicate to dissolve and diluted to volumetric with methanol, and mix properly, pipette out 2 mL of above solution in 25 mL of volumetric flask and dilute to volume with methanol.

Method Development: Working standard of various concentrations was prepared by taking aliquots of standard solution and dilutes to get required concentrations for calibration plot and which was injected.

Procedure: Standard preparation of sample and reference were separately injected, and chromatographed

System Suitability: System suitability was assessed by five replicate analysis of Irinotecan and gemcitabine.Standard solutions at a volume of 10μl and the chromatogram were obtained. The system suitability parameters such as tailing factor, and reproducibility (%RSD of analyte retention time) were calculated from the chromatogram.

Parameters Acceptance criteria: Tailing factor should not be more than 2. % RSD should not be more than 2%.

METHOD VALIDATION

Linearity for Gemcitabine: Linearity was demonstrated by analysing different concentration of active compound peak...
areas were recorded for all the peaks and calibration plot was constructed by plotting average areas of gemcitabine on y axis, and concentration level on x axis and concentration level on x-axis. And calculated the correlation coefficient \( r = 1.000 \).

**Linearity of Detector Response**

For **Irinotecan:** Prepare series of solutions of Irinotecan hydrochloride trihydrate samples at 25%, 50%, 75%, 100%, 125%, and 150%, of the assay target concentration. Inject each concentration plot calibration curve by taking concentration of Irinotecan hydrochloride trihydrate on x-axis, and average area of Irinotecan hydrochloride trihydrate on y-axis and calculate the correlation coefficient. Irinotecan hydrochloride trihydrate (0.9999). It should not be less than 0.99.

**Linearity graph of Irinotecan**

\[ y = 48194x + 24984 \]
\[ R^2 = 0.999 \]

**Accuracy**

For **Irinotecan:** Inject standard and sample solution twice into HPCL. Calculate the % assay content at each level and calculate the recovery at each sample concentration. (102.5).

For **gemcitabine:** Inject the standard solution accuracy 40%, 80%, 100%, 120% and 160%. calculate the amount added and amount found for gemcitabine HCL and calculate the individual recovery and mean recovery values.

**Ruggedness**

For **gemcitabine:** Inject the standard and sample solution twice into HPCL and measure the area, for all 6 injections, calculate the % RSD for each injection. The %RSD for the area of the five replicate injections was found to be within the specified limits.

**Precison**

For **Gemcitabine:** Injected the standard and sample solutions twice into the HPLC and measure the area for all five injections. The %RSD for the area of the five replicate injections was found to be within the specified limits.

**Acceptance criteria**

The assay of the Gemcitabine sample should be not less than 97% and not more than 103%.

For **Irinotecan:** Inject the standard and sample solution twice in HPLC and calculate the assay content in 6 sample solutions. (97%- 103%).

**Robustness**

**Procedure for Irinotecan:** Inject standard and sample solutions twice into HPLC and calculate the assay content of the 6 sample solutions. Acceptance criteria the assay of the Irinotecan hydrochloride trihydrate sample should be not less than 97.5% and not more than 101.5%

<table>
<thead>
<tr>
<th>System Suitability Parameters</th>
<th>Flow rate in ml/min</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSD for Irinotecan hydrochloride trihydrate of 5 replicate injections.</td>
<td>0.1 0.1 0.2</td>
<td>Not more than 2.0%</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.5 1.5 1.4</td>
<td>Not more than 2.0</td>
</tr>
</tbody>
</table>

**Procedure for Gemcitabine:** Injected the above prepared standard and sample solutions twice into the HPLC and measure the average area of the standard and sample solutions are considered for the calculation of assay of sample.

<table>
<thead>
<tr>
<th>System suitability parameters</th>
<th>Column oven temperature</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSD for Gemcitabine peak area of 5 replicate injections</td>
<td>0.1 0.1 0.1</td>
<td>Not more than 1.0%</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.2 1.2 1.2</td>
<td>Not more than 1.5</td>
</tr>
<tr>
<td>Resolution between Gemcitabine &amp; α- anomer</td>
<td>15.4 15.0 14.6</td>
<td>Not less than 8.0</td>
</tr>
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</table>

**Acceptance criteria**

The method is considered robust for the above variations, if all the system suitability acceptance criteria values should meets the acceptance criteria set forth in the test method.

**RESULTS AND DISCUSSION**

For gemcitabine linearity value was found to be 0.999 and for Irinotecan -0.999, for gemcitabine accuracy was 97%, and for Irinotecan – 99.3%. Flow rate for gemcitabine was 1.2 ml/min, and for Irinotecan 1.0 ml/min. Retention time for gemcitabine was 11.78 min, and for Irinotecan 5.647 min. the system suitability parameters i.e.; %RSD, tailing, reduction and impurity %LOD and %LOQ meets the predefined acceptance criteria. From all the above parameters combined with the
simplicity and case of operation ensures that the determination of gemcitabine and irinotecan in formulation. Acetonitrile (55:45 v/v) have been considered from accuracy standard.

CONCLUSION

The proposed method was found to be simple, sensitive, rapid and economical for the determination of Gemcitabine and irinotecan in formulation (injection). The developed method was also checked for the performance characteristics and has also been validated. The assay by HPLC method adopted for Gemcitabine HCl USP, irinotecan hydrochloride trihydrate USP was found to be precise, linear and accurate. It was also proved to be robust. Sample solution and mobile phase stability was found to be stable upto 48hrs. Hence the method was easily and conveniently adopted for the routine analysis.

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