



Research Paper

Development and validation of Cytotec In RP- HPLC

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ARTICLE INFO

ARTICLE HISTORY

Received 08 September 2023

Received in revised form 10 September 2023

Accepted 19 September 2023

Available online 30 September 2023

Key words:

RP- HPLC, cytotec

ABSTRACT

For the first time, researchers have developed and received regulatory approval for a primary, cost-efficient, sensitive, real, effective, and reproducible RP-HPLC method for the quantitative synchronous assessment of Cytotec in their drug measurement form. Partitioning was accomplished with a combination of 0.02M Potassium dihydrogen orthophosphate and 0.03M dipotassium dihydrogen orthophosphate; pH was adjusted to 3.5 with H₃PO₄:Acetonitrile(60:40) as the versatile stage at a stream rate of 1.0mL/min; and phase temperature was maintained at 30°C. UV's rise to fame at 251nm made quantification feasible. I estimated a total of 5.547 minutes for Cytotec's annual maintenance period, and 2.97 minutes for myself. Specificity, linearity, precision, exactness, and power were all passed with flying colors for this method. Since the renovation season of all the active fixes has gone smoothly, it is safe to say that the method is direct. A decent directly courting spanning the fixation range of 0.2-1.20g/mL for Cytotec was confirmed by the adjustment plot data for direct relapse investigations. Limits of detection for Cytotec were thought to be 0.54 g/ml and 0.69 g/ml, respectively. Both 1.65 g/ml and 2.11 g/ml were considered to be the Constraint of Quantitation for Cytotec. In my opinion, the Cytotec RSD price ranges between 0.16 and 0.212. Value restoration was estimated at 99.70% to 99.99%. Consecutive 99% for Cytotec use. The procedure has been tested and shown to be reliable for assessing Cytotec mass and the drug's measurement structure.

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1. INTRODUCTION

HPLC is able to separate macromolecules and ionic species labile natural products, polymeric materials, and a wide variety of other high – molecular weight poly functional group. HPLC is the fastest growing analytical technique for the analysis of the drugs. It's simplicity, high specificity, and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage forms and biological fluids¹.

In this the separation is about 100 times faster than the conventional liquid chromatography due to packing of particles in the range of 3-10µm. Modern LC uses very small particles for packing. The small particle size results in more rapid approach to the distribution

equilibrium and consequently smaller plate height, so that a given length of column includes large number of plates which makes the column efficient and the peak narrow. But close packing of these small particles reduces the flow rate of the mobile phase through the packed bed (the packing said to develop high back pressure) and in order to achieve a reasonable flow rate it is necessary to apply pressure to the mobile phase. So the designation, put forth as high pressure liquid chromatography. Thus HPLC is having advantages of improved resolution, faster separation, improved accuracy, precision and sensitivity².

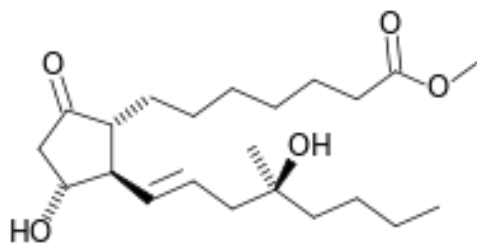


Figure no. 1 Chemical structure of Cytotec

To prevent and treat stomach and duodenal ulcers, to stimulate labor, to induce an early termination, and to treat post pregnancy drainage due to unfortunate constriction of the uterus, cytotec, a designed prostaglandin medication, is utilized. When used to prevent stomach ulcers in NSAID users, it is consumed orally. In early terminations, it is used alone or in combination with mifepristone or methotrexate³. Early termination viability, without outside assistance, is between 66% to 90%. It can be given orally, dissolved in the mouth, or vaginally for job enlistment or early termination. It could also be used rectally for postpartum drainage.

The aim of present study to develop a new fundamental, sensitive, exact, and efficient logical approach for evaluating Cytotec in mass and promoted drug measurements shape is a primary goal of the proposed work.

2. MATERIALS AND METHOD

Instruments

S.No	Instruments And Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector.
2	pH meter	Labindia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Chemicals

S.No	Chemical	Brand names
1	Cytotec (Pure)	Arthrotec, Cytotec, Mifegymiso.
2	Water for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

HPLC Analytical Conditions

The proposed method makes use of a stability C8 (150 x 4.6 mm, 5 m) segment, and it partitions using a mixture of 0.02M Potassium Di Hydrogen Orthophosphate+zero.03M Di Potassium Di Hydrogen Orthophosphate; the pH is adjusted to 3.5 using H3PO4: Acetonitrile (60:forty) as the transportable degree at a circulation rate of 1.0 mL/min; and the segment temperature The indicator's frequency was adjusted to 251 nm. Twenty ml was added to the infusion volume. Testing and cutting-edge making plans were carried out in a solvent mixture of fifty percent methanol and fifty percent water⁴⁻⁵.

Preparation of standard solution

weighed nothing. A standard working dose of 2 mg Cytotec in a 25 ml capacity cup. After adding 10 ml of diluent and sonicating for 10 minutes, the volume of the diluent was increased to its maximum. One milliliter of stock solution was transferred to a ten milliliter volumetric carafe and diluted appropriately.

Preparation of Sample solution

Twenty capsules that were previously weighed and measured become powdered and check out quantitatively as zero. A 25 ml volumetric carafe was filled with water and 2 mg of Misopristol was weighed and transferred into it. Delivery of 10 ml of diluent, a thorough shaking, a 10-minute sonication, and dilution to volume with diluent. Redirect the thought process. Dilute 1 milliliter (ml) of filtrate to 10 milliliters (ml)⁶.

Method validation

For parameters such as framework suitability, linearity and reach, precision (replicability), center precision (roughness), explicitness, exactness, and energy, the developed RP-HPLC method was approved in accordance with international meeting on Harmonization (ICH) guidelines, Approval of Logical Procedures¹⁴⁻¹⁵: Q2(R1).

System suitability

Exams to ensure the method can yield precise and accurate results are what the framework's appropriateness review amounts to. According to USP, all studies evaluating the appropriateness of the framework are complete. The limits of top stage or top vicinity or monotonous infusions, such as the range of hypothetical plates (N), Following variable, aim (Rs), and relative general deviation (RSD), have been considered. A USP36.14-conducted framework reasonableness check involved infusing a standard arrangement several times and documenting the ensuing changes to the lovely top division (purpose), rate relative well-typical deviation for region, maintenance time, stability factor, and hypothetical plates⁷.

Precision

When used to a set of estimates obtained from individual inspection of a similar homogenous example under the recommended conditions, the precision of a perceptive method conveys the closeness of

association (degree of burn up) among the estimates. The precision of the method was evaluated by injecting six different examples. A separate researcher from a comparable research facility evaluated the method's central reliability. Their appearance is not fixed in stone. Calculating the %RSD allowed for an evaluation of the strategy's accuracy and its ability to transition between states⁸.

Linearity

A scientific method is said to be linear if and only if it yields test results that are directly proportional to the fixation (degree) of analyte in the instance. Over the range of 80% to 120% of designated fixation, establishing a development of association of the functioning concepts (combination of every dynamic fixing) leaves the linearity of finder still in the air. The chromatographic framework was injected with those arrangements, and the reaction area was mapped out⁹.

Accuracy

Accuracy to center on the exactness of how diagnostic tests for healing were carried out. The test's reliability is uncertain, but it can be improved with preplanned recovery assessments (the spiking approach) at the 80%, 100%, and 120% levels of focused fixation. There are three layers of protection for each phase of recovery. The from individual chromatograms shows that things haven't yet calmed down. Chromatography was used to analyze samples at various concentrations, and the cost of the service was recouped after the total amount was calculated.

Robustness

We have tweaked the chromatographic conditions by slightly changing the temperature and the flow rate. The impact of temperature range on change price has been analyzed. Infusing a well-known association that had been prepared according to the test method into an HPLC framework at 35°C and 45°C. Changing the movement fee to zero completed the movement charge swap.eight ml/min and one point two ml/min¹⁰.

3. RESULT AND DISCUSSION

The proposed method was validated for the synchronous evaluation of Cytotec by ensuring that it complies with the framework's reasonable limits. Stability C8 (1504.6mm, 5m) yielded a consumable chromatogram for Cytotec. As far as the unevenness factor was concerned, the drug tops were considered even. The original plan for the proposed method is now appealing. Tabulated in are the simplified chromatographic examples. The limit for the ultraviolet spectrum was moved to 251 nm¹¹. There was reliability data in tables 3 and 4. The RSD value of prices has dropped below 2%. The approach was very sensitive, and linearity was shown throughout a wide range of fixation concentrations (0.20-1.20 g/mL for Cytotec). $r^2 = 0.9995$ is used as a limit for Cytotec, and it takes into account the relationship coefficients for male and female analytes.

Cytotec's three-degree accuracy is based on the healing method of spiking convergence of pure medication. The RSD obtained from the accuracy results was less than 2%, and medicine recovery was successfully within the acknowledgment furthest reaches of 98-102%.

Therefore, the newly developed technique is meant to be precise and accurate. By varying the temperature and flow rate and observing no discernible modifications in the chromatograms, it became clear that the method developed is effective. The goal, stability element, and hypothetical plate limits for the stream fee variety and the temperature variety are all contained within the acknowledgment models, demonstrating the method's excellent framework appropriateness under given set of circumstances¹².

Parameter	Chromatographic conditions
Instrument	WATERS 2695-High performance liquid chromatography
Column	SYMMETRY-C8 Column (150×4.6mm,5µm).
Detector	WATERS 2498 UV detector
Diluent	Water:Methanol(50:50%v/v)
Mobile phase	0.02M Potassium Di Hydrogen Orthophosphate+0.03M Di Potassium Hydrogen Orthophosphate pH was adjusted to 3.5 using H3PO4:Acetonitrile(60:40)
Flow rate	1.0ml/min
Detector wavelength	251nm
Run time	10min
Retention time	2.971(Cytotec) 5.547(Mifepristone)
Injection volume	20µl
Mode of separation	Isocratic mode
Column temperature	30°C

Table no.1 Optimized chromatographic conditions and system suitability parameters for proposedHPLC method for Cytotec

Parameters	Cytotec
Retention time	2.971
Plate number	3606.16
Tailing factor	1.09

Table no. 2 Results of System suitability

Injection No.	Area of Cytotec
1	299947
2	300688
3	300840
4	300104
5	301309
6	301550
MEAN	300740
Standard deviation	636.5
%Relative standard deviation	0.212

Table no.3 Results of System precision

Acceptance criteria: Percentage RSD should not be more than 2%.

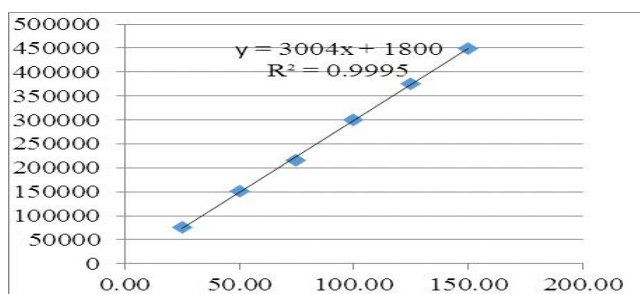


Figure no.1 Results of linearity data

Analyte	Concentration	Average Area	Amount added (mg)	Amount found (mg)	% Recovery
Cytotec	80%	240984	0.16	0.15952	99.70%
	100%	301245	0.2	0.19998	99.99%
	120%	364145	0.24	0.2399	99.96%

Table no. 4 Results of accuracy data

Parameter	Cytotec	
	Retention time	Theoretical plates
Flow rate-(0.8ml)	2.986	5353.03
Flow rate-(1.2ml)	2.741	4230.99
Column Temperature-(25°C)	2.973	4551.88
Column Temperature-(35°C)	2.983	4748.38

Table no.5 Results of Robustness data

Acceptance criteria: Retention time of sample peaks should be comparable with proposed method

4.CONCLUSION

Simple, inexpensive, sensitive, genuine, talented, and reproducible; the RP-HPLC method developed for quantitative synchronous evaluation of Cytotec in mass and drug measurements shape is perfect for its anticipated use¹³. The plan passed all of the tests required for approval in accordance with ICH guidelines, providing convincing data against all of the potential objections. therefore, the proposed method may be used to assess the quantitative guarantee of Cytotec in mass and drug dimension form¹⁴.

5.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

6. ACKNOWLEDGEMENT

We are highly grateful to our Honorable Chairman, Mother Terasa college of Pharmacy, Telangana state for providing the necessary facilities to carry out this research work.

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