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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF URIDINE TRI ACETATE BY RP-HPLC METHOD IN BULK AND PHARMACETICAL DOSAGE FORMS

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ABSTRACT

A new method was established for estimation of Uridine Triacetate by RP- HPLC method. The retention times were found to be 2.425mins. The % purity of Uridine Triacetate was found to be 99.94%.The system suitability parameters for Uridine Triacetate such as theoretical plates and tailing factor were found to be 4187, 1.5. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Uridine Triacetate was found in concentration range of 20µg-100µg and correlation coefficient (r^2) was found to be 0.999, % recovery was found to be 99.95%, %RSD for repeatability was 0.2, % RSD for intermediate precision was 0.1. The precision study was precision, robustness and repeatabilty.LOD value was3.06 and LOQ value was 10.14.

Key words: Uridine Triacetate, RP- HPLC method, Pharmaceutical dosage form.

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INTRODUCTION

Analytical chemistry is the study of the separation, identification, and quantification of the chemical components of natural and artificial materials. It deals with methods for determining the chemical composition of samples of matter.

- A Qualitative Method yields information about the identity of atomic or molecular species or the functional groups in the sample.
- A Quantitative Method, in contrast, provides numerical information as to the relative amount of one or more of these components (1, 2).

Pharmaceutical analysis may be defined as a process or a sequence of processes to identify and/or quantify a substance or drug, the components of a pharmaceutical solution or mixture or the determination of the structures of chemical compounds used in the formulation of pharmaceutical product (2). Analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formula for the calculation, etc. Chromatography is a technique in which the components of a mixture are separated based on the rates at which they are carried through a stationary phase by a gaseous or liquid mobile phase. Chromatography involves two phases chosen such that the components of the sample

have differing affinities in each phase and a sample (or sample extract) being dissolved in a mobile phase (which may be a gas, a liquid or a supercritical fluid). The mobile phase is then forced through the stationary phase. A component with high affinity towards the stationary phase will take longer to travel through it than a component with low affinity towards the stationary phase and high affinity towards the mobile phase. As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase (3). High-performance liquid chromatography also known as High-pressure or High price or High speed liquid chromatography, HPLC) is a form of column chromatography used frequently in analytical chemistry and biochemistry to identify, separate, and quantify compounds. It is a powerful tool in analysis. It is basically an improved form of column chromatography which has been optimized to provide rapid high resolution separations. Early LC used gravity fed open tubular columns with particles 100s of microns in size; the human eye was used for a detector and separations often took hours or even days to develop. HPLC is probably the most universal type of analytical procedure. In addition HPLC also ranks as one of the most sensitive analytical procedures and is unique in that it easily copes with multi-component mixtures. Its application areas include quality control, process control, forensic analysis, environmental monitoring and clinical testing. It has achieved this position as a result of the constant evolution of the equipment used in LC to provide higher and higher efficiencies at faster and faster analysis times with a constant incorporation of new highly selective column packings.

Uridine Triacetate -[(2R,3R,4R,5R)-3,4-bis(acetyloxy)-5-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1- yl)oxolan-2-yl]methyl acetate (Fig-1).

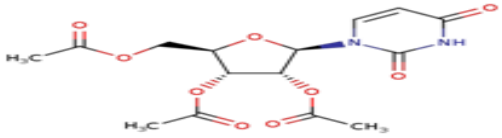

Fig-1 Structure of Uridine Triacetate

Uridine triacetate is a synthetic uridine pro-drug that is converted to uridine in vivo. When used for the treatment or prevention of toxicity associated with fluorouracil and other antimetabolites, uridine triacetate is utilized for its ability to compete with 5-fluorouracil (5-FU) metabolites for incorporation into the genetic material of non-cancerous cells. It reduces toxicity and cell-death associated with two cytotoxic intermediates: 5-fluoro-2'-deoxyuridine-5'-monophosphate (FDUMP) and 5-fluorouridine triphosphate (FUTP). By pre-administering with uridine (as the prodrug uridine triacetate), higher doses of 5-FU can be given allowing for improved efficacy and a reduction in toxic side effects [A18578] such as neutropenia, mucositis, diarrhea, and hand–foot syndrome. Uridine triacetate is also used for replacement therapy in the treatment of hereditary orotic aciduria, also known as uridine monophosphate synthase (UMPS) deficiency. As a result of UMPS deficiency, patients experience a systemic deficiency of pyrimidine nucleotides, accounting for most symptoms of the disease. Additionally, orotic acid from the de novo pyrimidine pathway that cannot be converted to UMP is excreted in the urine, accounting for the common name of the disorder, orotic aciduria. Furthermore, orotic acid crystals in the urine can cause episodes of obstructive uropathy. When administered as the prodrug uridine triacetate, uridine can be used by essentially all cells to make uridine nucleotides, which compensates for the genetic deficiency in synthesis in patients with hereditary orotic aciduria. The solubility of uridine triacetate in aqueous media is 7.7mg/ml ,and is independent of pH. Uridine triacetate is administered orally.Uridine triacetate delivers 4-fold to 6- fold more uridine into the systemic circulation compared to equimolar doses of uridine itself.circulating uridine is taken up into cells via specific nucleoside transpoters and crosses the blood brain barrier .uridine can be excreted by the kidneys but is also metabolised by normal pyrimidine

catabolic pathways present in most tissues.the half life ranges from approxmately 2 to 2.5 hours (4).

Literature review reveals that there is no analytical method reported for the analysis of Uridine Triacetate by estimation by RP-HPLC. Spectrophotometer, HPLC and HPTLC are the reported analytical methods for compounds either individually or in combination with other dosage form. Hence, it was felt that, there is a need of new analytical method development for the estimation of Uridine Triacetate in pharmaceutical dosage form. Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the analysis of Uridine Triacetate. The developed method will be validated according to ICH guidelines.

MATERIALS AND METHODS (5-7)

Preparation of mobile phase

Mix a mixture of 800 ml Methanol (80%) and 20 ml of Water (20%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents preparation

Mobile phase was used as the diluent.

Preparation of the individual Uridine Triacetate standard preparation

10 mg of Uridine Triacetate working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the Uridine Triacetate standard and sample solution Sample solution preparation

10 mg of Uridine Triacetate tablet powder was accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

10 mg Uridine Triacetate working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

10µL of the blank, standard and sample was injected into the chromatographic system and areas for the Uridine Triacetate the peak was used for calculating the % assay by using the standard formula.

RESULTS AND DISCUSSION

The present investigation reported in the thesis was aimed to develop a new method development and validation for the estimation of Uridine TA by RP-HPLC method. Literature reveals that there are no analytical methods reported for the estimation Uridine TA by RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the estimation of Uridine TA in pharmaceutical dosage form.

Method Development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The spectrums are shown in Fig-2.

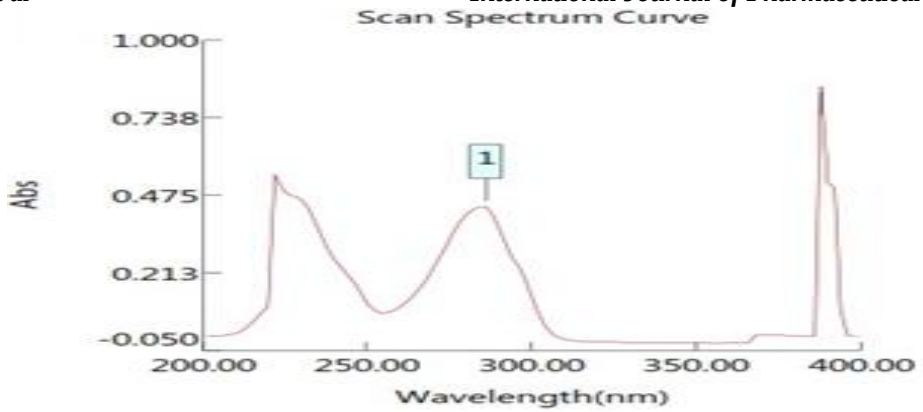

Fig-2 Spectrum showing overlapping spectrum of Uridine TA

The chromatographic method development for the estimation of Uridine TA were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method was selected for the separation and quantification of Uridine TA in API and pharmaceutical dosage form by RP-HPLC method.

Assay calculation for Uridine TA

The assay study was performed for the Uridine TA. Each three injections of sample and standard were injected into chromatographic system. The results are tabulated in table-1.

Table-1 Assay value of Uridine TA

S. No	Name	Area
1	Uridine TA	698557
2	Uridine TA	699824
3	Uridine TA	693170
Mean		697184
Std. Dev.		3533.2
% RSD		0.51

The retention time of Uridine TA was found to be 2.425mins.The system suitability parameters for Uridine TA such as theoretical plates and tailing factor were found to be 4159.0, 1.5.The % purity of Uridine TA in pharmaceutical dosage form was found to be 98.94%.

The linearity study was performed for the concentration of 20-100 ppm Uridine TA. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The results are tabulated in Table-2. Calibration graph for Uridine TA was shown in Fig-3.

Table-2 Linearity Results for Uridine TA

S. No	Concentration (µg/ml)	Area
1	20	264840
2	40	491415
3	60	677620
4	80	873311
5	100	1048958
Correlation coefficient		0.999

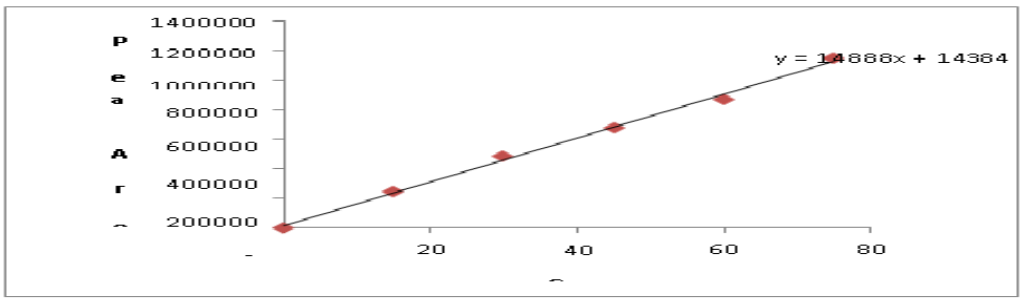

Fig-3 Showing calibration graph for Uridine TA

The linearity study was performed for concentration range of 20µg-100µg Uridine TA and the correlation coefficient was found to be 0.999 (NLT 0.999).

The accuracy study was performed for 50%, 100% and 150 % for Uridine TA. Each level was injected in triplicate into chromatographic system. Thearea of eac h level was used for calculation of % recovery (table-3).

Table-3 Showing accuracy results for Uridine TA

%Concentration (at specification level)	Average Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	728287	5	4.96	99.91%	99.95%
100%	1378202	10	9.98	99.18%	
150%	2115480	15	15.02	99.60%	

The accuracy study was performed for % recovery of Uridine TA. The % recovery was found to be 99.95% (NLT 98% and NMT 102%). The LOD was performed for Uridine TA was found to be 3.04. The LOQ was performed for Uridine TA was found to be 10.14.

CONCLUSION

A new method was established for estimation of Uridine Triacetate by RP- HPLC method. The chromatographic conditions were successfully developed for the separation of Uridine Triacetate by using YMC C18 column (4.6×150mm) 5µ, flow rate was 1.0 ml/min, mobile phase ratio was Water: meoH (20:80% v/v), detection wavelength was 274nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.425mins. The % purity of Uridine Triacetate was found to be 99.94%.The system suitability parameters for Uridine Triacetate such as theoretical plates and tailing factor were found to be 4187, 1.5. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Uridine Triacetate was found in concentration range of 20µg-100µg and correlation coefficient (r^2) was found to be 0.999, % recovery was found to be 99.95%, %RSD for repeatability was 0.2, % RSD for intermediate precision was 0.1. The precision study was precision, robustness and repeatabilty.LOD value was3.06 and LOQ value was 10.14. Hence the suggested RP-HPLC method can be used for routine analysis of Uridine Triacetate in API and Pharmaceutical dosage form.

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