RESEARCH ARTICLE

UPLC-MS/MS Method Development and Validation of Tegafur, Gimeracil, and Oteracil in Rat Plasma and its Application for Pharmacokinetic Study

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ABSTRACT

A successful combination of Tegafur, Gimeracil, and Oteracil is used to treat stomach cancer in adults. Once converted to 5-fluoro uracil, the active form of Tegafur inhibits the manufacture and function of DNA and RNA, hence preventing the proliferation of cancer cells. Consequently, a new, sensitive, and cost-effective bioanalytical approach for the detection of these medications performed by liquid chromatography-mass spectrometry on rat plasma is needed. Isocratic mode was employed at room temperature to achieve separation on a C18 column (150x4.6mmx3.5µm). A mobile phase composed of ACN and 0.1% formic acid was used in the 40:60 ratio with a flow rate of 1mL/min. An analysis was conducted on Tegafur, Gimeracil, and Oteracil using a 6-minute retention time and the retention times of them were found to be 2.027, 3.176, and 4.015 respectively. The R^2 values were found to be 0.9999 showing the method linear. The method developed was found to be accurate and precise as per ICH guidelines.

Keywords: Cancer, Tegafur, Gimeracil, Oteracil, ACN, Formic acid, ICH guidelines.

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INTRODUCTION

A variety of malignancies, including advanced gastric and colorectal tumours can be treated with Tegafur, a prodrug of Fluorouracil (5-FU), an antineoplastic medication. Combined with Gimeracil and Oteracil, or with fluorouracil as tegafur-uracil, this pyrimidine derivative is an active chemotherapeutic drug. Antineoplastic treatment with Gimeracil augments chemotherapy by raising the concentration and activity of the primary active ingredients. Gimeracil is part of the commercially marketed product "Teysuno" along with [DB03209] and [DB09256]. For Teysuno to work, it relies on [DB09256], a pro-drug of [DB00544] (5-FU), a cytotoxic anti-metabolite medication that targets cancer cells that divide quickly. As an auxiliary component of anticancer treatment, Oteracil lessens the severity of chemotherapy's harmful side effects. "Teysuno" is a commercially available medicine that combines Oteracil with Gimeracil and Tegafur. [1-19].

Drug profile of Tegafur

How does tegafur work?

To drive DNA and purine synthesis in cells, the conversion of 2'-deoxyuridylate (dUMP) to 2'-deoxythymidylate (dTMP) is absolutely necessary. A DNA synthesis enzyme known as thymidylate synthase converts deoxyribonucleotide monophosphate (dUMP) to deoxyribonucleotide triphosphate (dTMP), a building block for thymidine triphosphate (TPP).

Drug Profile of Gimeracil

MOA of gimeracil

The primary function of Gimeracil within Teysuno is to inhibit the hydrolysis of 5-fluorouracil, allowing for the maintenance of sufficient concentrations to exert a long-lasting impact on cancer cells. It works by inhibiting 5-FU breakdown through the reversible inhibition of the enzyme dihydropyridine dehydrogenase (DPD).

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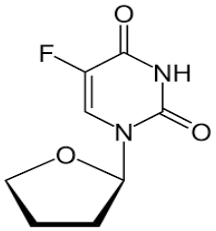


Figure 1: Structure of tegafur

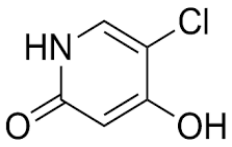


Figure 2: Structure of gimeracil

Drug profile of Oteracil

MOA of oteracil

As a component of Teysuno, Oteracil primarily lessens gastrointestinal toxicity by decreasing 5-FU activity inside normal gastrointestinal mucosa 1. It prevents 5-FU generation by inhibiting the enzyme orotate phosphoribosyl transferase (OPRT). [20]

From the literature, it is evident that there isn't a single bio analytical estimation using rat plasma for the combination of Tegafur, Gimeracil and Oteracil. There are various methods like HPLC, UPLC, LC-MS for the above-mentioned drugs both in combination and as single drug. A simple SPE method was developed on LC-MS/MS employing a low volume of 100 µL plasma without usage of high volumes of chemicals with a shorter chromatographic runtime of 6.0 minutes using Chlorambucil as internal standard. Among the reported literature methods, the present method involves less retention time and Solid Phase extraction which is modern and less reported method. The proposed analytical method also satisfies the scientists in terms of simplicity, sensitivity, runtime, time consumption, sample volume and efficient extraction procedure. [21-33].

Materials used

The following medications were purchased from Zydus Cadila in Ahmadabad, India: Tegafur, Gimeracil, Oteracil, and Chlorambucil. The following materials were procured from

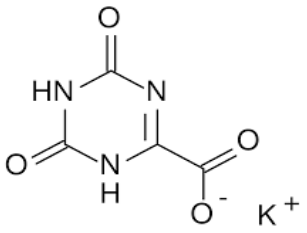


Figure 3: Structure of oteracil

Merck (India) Ltd.: acetonitrile (LCMS Grade, 99.99 purity), and formic acid, Worli and Mumbai in India. The rest of the equipment used in the experiment was either commercially available or of AR quality.

For the Best Chromatographic Results:

A Waters 2695 HPLC system was employed with an Inertsil ODS column measuring 150 mm x 4.6 mm and having a particle size of 3.5Î1⁄4m. The LC-MS chromatographic conditions were optimized by running the system for 7 minutes at room temperature. A mobile phase comprising 40% acetonitrile and 60% formic acid was utilized, with a flow rate of 1 ml/min. Table 2 describes the optimised chromatographic conditions.

Optimized Method's Chromatogram:

Regular method for preparing solutions:

To prepare the Tegafur parent stock solution, measure 5 mg of the Tegafur working standard and transfer it to a 100 ml volumetric flask that has been diluted with diluent. Use diluent to further dilute 0.8 ml to 10 ml. To make the parent stock solution of Gimeracil, first measure 5 mg of the drug and transfer it to a 100 ml volumetric flask that has been diluted with diluent until it is full volume. Using diluent, 2.32 ml was further diluted to 100 ml. The Oteracil parent stock solution is prepared by weighing 9 mg of the Oteracil working standard and then adding the diluent to a 100 ml volumetric flask until it reaches the desired concentration. This was further diluted with diluent from 0.35 ml to 10 ml. To 10 ml volumetric add 2 ml each of Tegafur, Gimeracil, and Oteracil parent stock solutions and dilute to desired volume. This will make the standard stock solution. To prepare the internal standard stock solution (800ng/ml), weigh 5 mg of chlorambucil and transfer it to a 100 ml volumetric flask that has been diluted with diluent until it reaches capacity. Diluted with diluent from 0.8 ml to 10 ml.

Preparation of plasma sample solution

A working solution containing 500 µL of internal standard (IS) was spiked into 200 µL portions of rat plasma specimens for

Table 1: Drug profile

Parameters	*Tegafur*	*Gimeracil*	*Oteracil*
IUPAC Name	(RS)-5-Fluoro-1-(tetrahydrofuran-2-yl) pyrimidine-2,4 (1H,3H)-dione	5-chloro-4-hydroxy-1H-pyridin-2-one	Potassium 6-hydroxy-4-oxo-4,5-dihydro-1,3,5-triazine-2-carboxylate
Molecular Weight	200.16g/mol	145.54 g/mol	195.175 g/mol
Chemical Formula	$C_8H_9FN_2O_3$	$C_5H_4ClNO_2$	$C_4H_2KN_3O_4$

Table 2: Optimised chromatographic method

Parameters of LC		*Parameters of MS*	
HPLC	*Waters Alliance e-2695*	*MS*	*Sciex QTRAP 5500*
Isocratic mode	ACN: 0.1% Formic acid 40:60 v/v	Ionization source	Drying gas: Nitrogen Drying flow rate: 5 mL/min Pressure: 55 psi
	Flow level: 1 mL/min		Source temperature: 550°C
	Injection volume: 7 µL		Capillary voltage: 5500V
Waters symmetry C_{18}	150mm length	Collision cell gas	Nitrogen with high purity
	4.6 mm ID	Mode	MRM
	3.5 µm PS		
Specimens	Tegafur	Tegafur Multiple reaction monitoring transitions	m/z-201.05 m/z-70.12 CE– 15V
	Gimeracil	Gimeracil Multiple reaction monitoring transitions	m/z-145.99 m/z- 46.59 CE - 15V
	Oteracil	Oteracil Multiple reaction monitoring transitions	m/z-195.96 m/z-60.89 CE – 15V
IS	Chlorambucil	Chlorambucil Multiple reaction monitoring transitions	m/z- 305.21 m/z- 106.32 CE-15V

sample preparation. The specimens were centrifuged at 4000 rpm for 20 minutes after 300 µL of acetonitrile was combined in a vortex. The next step was to separate, collect, and filter a solution that had been handled with the supernatant using a 0.45 nylon syringe filter. The resulting sample was then introduced into the LCMS system.

Method Validation

The proposed approach was used to confirm the bioanalytical procedure in accordance with USFDA recommendations.

RESULTS AND DISCUSSIONS

The bioanalytical approach was developed by selecting the ESI mode that produced the strongest reaction relative to the APCI mode. Using the MRM mode, the ions of Tegafur, Gimeracil, and Oteracil have been quantified. At m/z 201.0599, 145.9935, 195.9684, the ion pair scan of the medicines resulted in [M+H] + at m/z 174.5735, 136.3095; 112.6587, 75.0941; and 153.1539, 121.1686. Internal standard scans for chlorambucil at m/z 305.2127 and 254.1163 are also similar. In contrast to an ion negative mode, Tegafur, Gimeracil, and Oteracil have a positive ion response mode. Figure 4 (A-D) shows the mass spectra of the drugs and Internal Standard.

Specificity and Selectivity:

Plasma samples from six different types of rat specimens were tested for blockage from unknown materials by using Tegafur, Gimeracil, Oteracil, and IS. Figure 5,6 shows chromatogram of Blank plasma and Standard.

Validation and Bioanalytical Process

Recovery and matrix effect

The quality measures with medicine and IS recoveries were evaluated at three distinct concentrations: lower, medium, and higher. A recovery is considered to have occurred when the sample's reactions are comparable to those of a tidy normal solution. When comparing analytical results with the amount of analyte used, the effectiveness of the extraction process becomes clear. Below you can see the chromatograms of the LQC, MQC, and HQC recoveries, both extracted and un-extracted.

Linearity, Precision, Consistency:

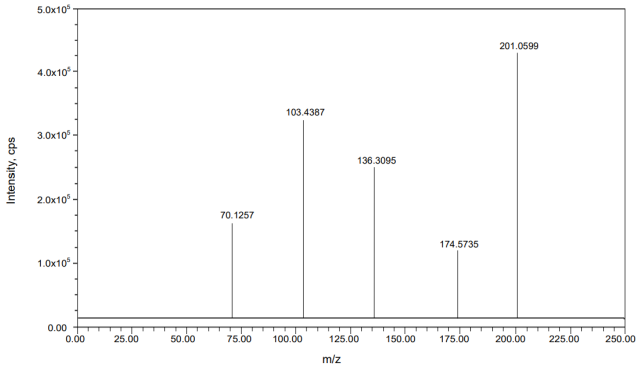
There is a linear representation of the Tegafur, Gimeracil, and Oteracil medication concentrations (20–400 ng/mL, 5.8–116 ng/mL, and 16–315 ng/mL, respectively). The average coefficient was 0.999. To quantify the samples, the ratio of the analyte peak region to the internal standard peak region was utilized. Table 3 and 4 shows the results of linearity, precision.

Integrity of dilution and carry over

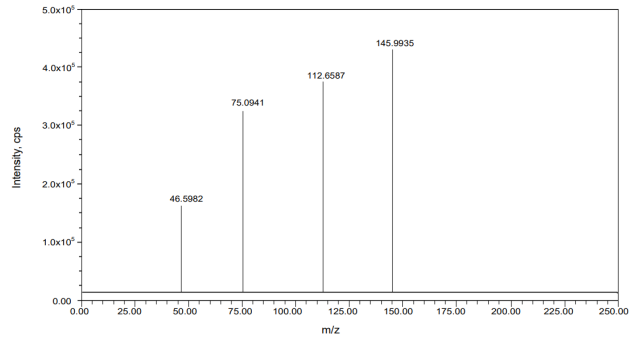
An analyte matrix fixation over the ULOQC should be shown by diluting this sample with a blank matrix to demonstrate

Table 3: Linearity data

Parameters	*Tegafur*	*Gimeracil*	*Oteracil*
Range (ng/mL)	0-400	0-116	0-315
R^2	0.99997	0.99997	0.99998
Slope	0.0051	0.0051	0.0051
Y- intercept	0.00108	0.00088	0.00119



A



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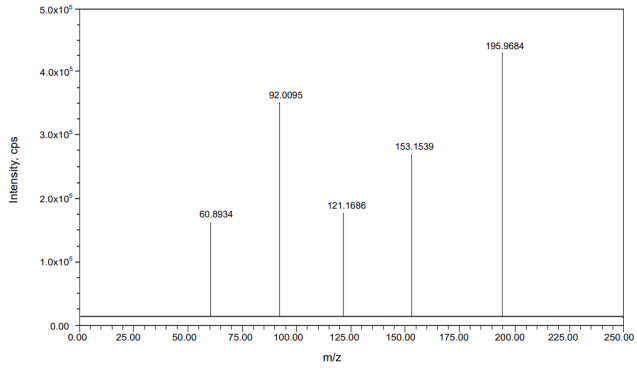

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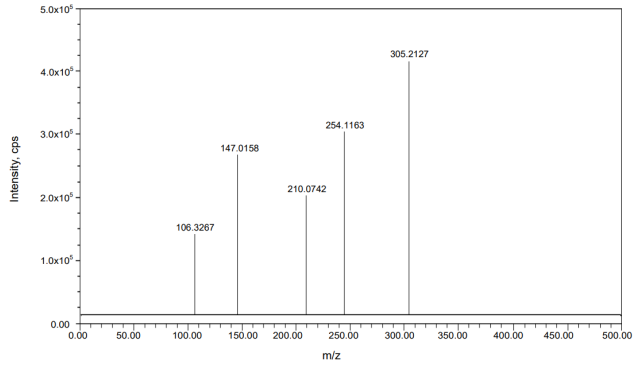

D

Figure 4: Mass Spectra's of (A) Tegafur (B) Gimeracil (C) Oteracil and (D) Chlorambucil (IS)

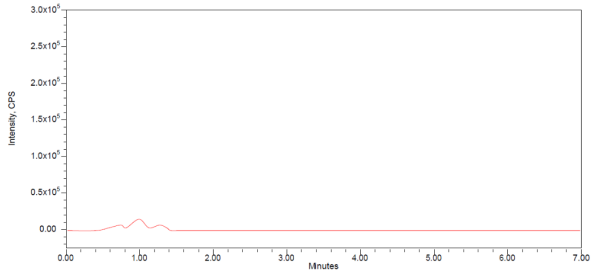

Figure 5: Chromatogram of blank plasma

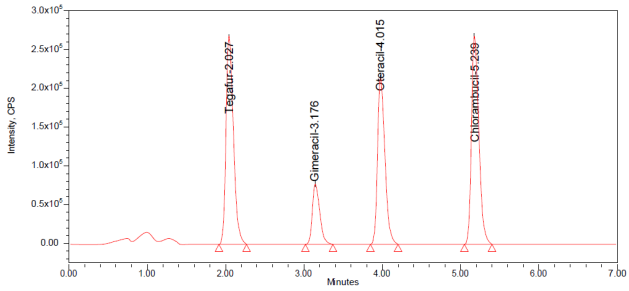

Figure 6: Chromatogram of Standard

dilution integrity. At ULOQC (400 ng/mL, 116 ng/mL, 315 ng/mL for Tegafur, Gimeracil and Oteracil), dilution integrity was tested. Carryover error is a kind of system mistake that may have an impact on the measured value of a sample. A 10 µL system blank injection of 0.1 percent Formic acid and Acetonitrile was performed using the flow injection method on a water Z-spray triple quadrupole mass detector (60:40). We may infer that this method had no impact on the accuracy or precision of the proposed technique.

Stability studies

An overnight room temperature storage of a stock solution of Tegafur, Gimeracil, and Oteracil was used to ascertain the stability of the compound on the benchtop. After 24 hours of room temperature retention in an Autosampler, a stock solution shows dependable stability behaviour in this test. For one day, the stock solution was maintained at a temperature of (28±5) °C in order to test its freeze-thaw stability. The stability of the moist extract was tested by storing the stock solution at 2-8°C for 18 hours, whereas the stability of the dry extract was tested by storing the stock at (-20±3) °C for 18 hours. Medications exhibited short-term stability during 7 days at 53 °C, whereas the stock solution exhibited long-term stability for 28 days at (-20±3) °C when introduced into the UPLC. Check the stability of both a recently made stock solution and one that was made more than 24 hours ago. A percent change of 1.42,1.81,1.74 for the drugs indicates that the solutions are stable for at least 24 hours. Under varied circumstances at room temperature, the drugs were stable in plasma. We tested plasma specimens spiked with Tegafur, Gimeracil, and Oteracil at various concentrations and discovered that further freezing and thawing had no impact on their stability. As far as long-term stability is concerned, the drugs remained effective for a whole day while stored at a capability temperature of -20°C.

Table 4: Precision and accuracy outcomes of Tegafur, Gimeracil, Oteracil in rat plasma

Matrix	*Concentration range of specimen*	*Tegafur*			*Gimeracil*			*Oteracil*		
		Accuracy	*RSD of Precision (%)*		*Accuracy*	*RSD of Precision (%)*		*Accuracy*	*RSD of Precision (%)*	
			Intraday	*Interday*		*Intraday*	*Interday*		*Intraday*	*Interday*
Plasma	LLOQC	0.95	2.59	2.89	0.96	4.49	2.57	0.96	2.94	1.68
	LQC	0.95	0.62	0.74	0.96	3.06	3.36	0.97	0.68	0.91
	MQC	0.98	0.54	0.28	0.98	0.95	1.09	0.99	0.57	0.38
	HQC	0.98	0.18	0.25	0.98	0.59	0.93	0.97	0.37	0.26

DISCUSSION

The improved approach demonstrated exceptional liquid chromatography (LC) separation and mass transitions by utilizing a (50:50v/v) combination of acetonitrile and 0.1% formic acid, which was necessary to meet the volatility requirement in mass spectrometry analysis. A column with dimensions of 150mmx4.6mm and a particle size of 3.5 µ, namely the Inertsil ODS, was utilized for optimal column efficiency at a temperature of 30°C. Concentration is necessary because of the need for volatility in mass spectrometry analysis. A column with dimensions of 150mmx4.6mm and a particle size of 3.5 was employed for optimal column efficiency. The column utilized was an Inertsil ODS and was maintained at a temperature of 30°C. The linear regression model calculates the best calibration curve that fits the connection between the chromatographic response and concentration. The intra- and inter-day data for Tegafur, Gimeracil, and Oteracil values exhibit a level of precision and accuracy that falls within the acceptable range. The extraction yield was good, constant, exact, and reproducible using the improved extraction process. The injector carry-over test showed no carry-over for the extracted blank samples, followed by the extraction of upper and lower limit of quality control samples. Experiments conducted on the freeze-thaw stability of substances subjected to freezing at a temperature of -30°C and subsequent thawing three times have shown that acceptable ranges fall between 85% and 115%. The Auto sampler's sample stability was tested at a temperature of 20°C for a duration of seventy hours. The accuracy of the samples, measured as a percentage, fell within the range of 85% to 115% for both the Low-quality control and High-quality control concentration levels. The accuracy was judged to be within the range of 85% to 115%. The long-term stability was assessed for a duration of twenty-eight days and compared to the starting concentrations of the Lower Quality Control and High-Quality Control. The accuracy was judged to fall within the range of 85 to 115%.

Pharmacokinetic Study

Administering 0.33 mg/kg Tegafur, 0.097 mg/kg Gimeracil, and 0.26 mg/kg Oteracil orally to rats allowed researchers to obtain mean plasma concentration-time profiles, which were then used to determine the pharmacokinetic parameters of these three drugs (Fig.9, 10, 11). There are clear distinctions between Tegafur, Gimeracil, and Oteracil in pharmacokinetic studies. We took bodily fluid samples from the rats at various intervals following drug administration, including0.5,1,1.5,2,4,5,10, and 15 hours. Injecting the prepared test sample into the chromatographic apparatus and recording the results followed. Oral administration of Tegafur, Gimeracil, and Oteracil resulted in the following concentrations at Cmax (173.6, 13.3 and 135.4 ng/ml), half-life (t1/2) as determined by 0.693/ Kelquotient, Kel (the apparent first request terminal rate constant), and Tmax (2, 1.5 and 2 hours). The acceptable range was determined by the AUC0-24 and AUC0-∞ values, which were 2458, 86, and 1860 ng-hr/mL respectively. Figure 8-10 shows the recovery plot of the drugs.

CONCLUSION

Plasma analysis of Tegafur, Gimeracil, and Oteracil using a novel MS/HPLC method in rats. The pharmacokinetic behaviour of Tegafur, Gimeracil, and Oteracil was demonstrated by their rapid absorption from the rat body following intravenous administration. This method is easy to replicate, works well, and takes very little time. This technique has the potential to be useful in pharmacokinetic studies as well as in accurately and efficiently measuring analyte concentrations in body fluids across an excellent linear concentration range. For the record, we must conduct these inquiries without delay.

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