ORIGINAL ARTICLE

Development and Validation of a New Stability Indicating Reversed-phase High-Performance Liquid Chromatography Method for the Simultaneous Estimation of Tegafur and Uracil in Capsule Dosage Form

Sreenivasa Charan Archakam[1], Sridhar Chenchugari[2], Chandrasekhar Kothapalli Banoth[3]

[1]Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Anantapuramu, Andhra Pradesh, India, [2]Department of Pharmaceutical Analysis, Sri Padmavathi School of Pharmacy, Tirupati, Andhra Pradesh, India, [3]Department of Chemistry, Jawaharlal Nehru Technological University, Anantapuramu, Andhra Pradesh, India

Abstract

Aim: The aim of this study is to develop a new stability indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Tegafur (TEG) and Uracil (URA) in capsule dosage form. **Materials and Methods:** Chromatography was carried out on a C18 Phenomenex column (250 mm × 4.6 mm i.d., 5 µm particle size) with an isocratic mobile phase composed of acetate buffer (pH 4) and methanol in the proportion of 70:30 (v/v). The compounds were eluted at a steady flow rate of 1.0 mL/min with a total run time of 10 min. The detection was carried out by ultraviolet-detector at 280 nm. The developed method was validated as per the ICH guidelines, and stability studies were performed. **Results and Discussion:** TEG retention time was about 5.2 min, and URA was 2.9 min. A calibration curve was obtained in the range of 25–125 µg/mL for TEG and 20–100 µg/mL for URA. Specificity experiments revealed the absence of interference from excipients. The mean assay for TEG was found to be 101.4% (w/w) and for URA was 102.8% (w/w). The method was also evaluated for robustness and ruggedness, and the results obtained were satisfactory. Forced degradation studies were performed for TEG and URA in acidic and alkaline, thermal, and oxidative conditions, and no substantial degradation was observed in all the conditions. **Conclusion:** It is concluded that the developed stability indicating RP-HPLC method was specific, precise, accurate, sensitive, and robust for the simultaneous estimation of TEG and URA in capsules.

Key words: Reversed-phase high-performance liquid chromatography, stability indicating assay, Tegafur, Uracil, validation

INTRODUCTION

Tegafur (TEG) [Figure 1], a prodrug to fluorouracil, is used in combination with uracil (URA) [Figure 2] for the treatment of malignant growth of colon, stomach cancer, bowel cancer, and several other precancerous and cancerous growth conditions.[1] TEG is known to be a potential thymidylate synthase inhibitor. The URA causes higher amounts of 5-Fluorouracil to stay inside the cells and kill them. An extensive literature review revealed that no stability indicating analytical method was reported for the simultaneous estimation of TEG and URA. However, analytical methods for individual drugs and the selected drugs with other drug combinations were reported. Most of the reported methods were pharmacokinetic and bioanalytical methods which were employed in clinical

Address for correspondence:
Sreenivasa Charan Archakam, Research Scholar, Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Anantapur, Anantapuramu - 515 002, Andhra Pradesh, India.
Phone: +91-9490844432.
E-mail: charan4ma@gmail.com

Received: 17-07-2018
Revised: 09-08-2018
Accepted: 16-08-2018

trials of drug discovery.[2-15] Thus, there is a scope for the development of a new stability indicating analytical method for the simultaneous estimation of TEG and URA and validate the developed method as per ICH guidelines. Forced degradation studies were carried to study the effect of various environments on the stability of the drugs.

MATERIALS AND METHODS

Chemicals and reagents

TEG and URA were purchased from TCI Chemicals Pvt. Ltd., Japan. TEG and URA capsules were available in the market as LUPORAL Capsules (LUPIN Ltd., India). All other AR grade chemicals and high-performance liquid chromatography (HPLC) grade solvents were purchased from Merck Pvt. Ltd., Mumbai.

Equipment

The chromatography was performed on Shimadzu Prominence LC system with LC-20AT pump, rheodyne injector, and SPD-20A ultraviolet (UV)-visible detector. The data handling system used was Lab solutions. Calibrated analytical balance (Shimadzu-AY220) and pH meter (Digisun) was also used in the study.

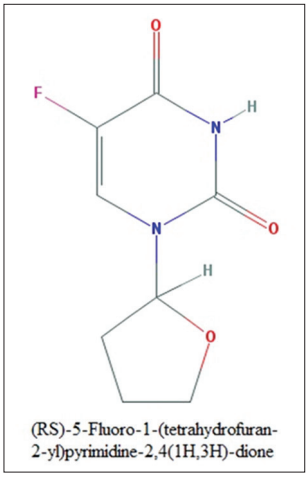

Figure 1: Chemical structure of tegafur

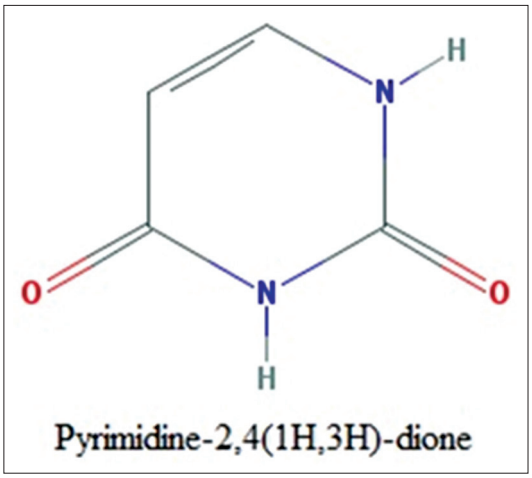

Figure 2: Chemical structure of uracil

Chromatographic separation conditions

Chromatography was carried out on a C18 Phenomenex column (250 mm × 4.6 mm i.d., 5 µm particle size) with an isocratic mobile phase composed of acetate buffer (pH 4) and methanol in the proportion of 70:30 (v/v), with a steady flow rate of 1.0 mL/min with a run time of 10 min. The detection was carried out by UV-detector at 280 nm. The temperature conditions were maintained at ambient conditions.

Preparation of stock solutions of TEG and URA

Stock solutions of TEG and URA were prepared by transferring 25 mg individually into 25 mL of volumetric flasks and dissolving in 0.1N sodium hydroxide. Finally, volume was made up to the mark with 0.1 N sodium hydroxide to give a solution containing 1000 µg/mL.

Method validation

For linearity, the concentration range 25–125 µg/mL was established for TEG and the concentration range 20–100 µg/mL was established for URA. 20 µL of each of the prepared stock solutions was injected into the chromatographic system connected to a column, and the average peak area in each of the cases was noted. By plotting concentrations on X-axis and corresponding peak area on Y-axis, the linearity was determined. The precision of the method was demonstrated through two parameters which are injection reproducibility (system precision) and the method precision. Accuracy was performed in three levels of concentration, i.e. 50%, 100%, and 150% of label claim by standard addition technique. The robustness of a method is evaluated by varying method parameters such as flow rate (±0.2 mL/min), wavelength (±2 nm), analyst-to-analyst variation, and determining the effect (if any) on the results of the method.

Assay of capsule dosage form

20 capsules were taken and accurately weighed 0.174 g of fine powder of capsule and transferred into a 50 mL clean, dry volumetric flask, then add 0.1 N sodium hydroxide and kept for sonication for 10 min to dissolve, and then make up the volume with diluent and then filtered through 0.45 µ membrane filter. The test solution was prepared to get a final concentration of 25 µg/mL (TEG) and 56 µg/mL (URA). Triplicate solutions of prepared solutions of 20 µL were injected into the reversed-phase HPLC (RP-HPLC) system, and the chromatographic parameters were analyzed.

Forced degradation studies

Forced degradation studies were performed for TEG and URA in acidic hydrolysis, basic hydrolysis, thermal, and oxidative conditions. Hydrolysis under acidic conditions was performed by exposing the drug solution to 0.1N HCl at 60°C for 6 h and then cooled, neutralized with 0.1 N NaOH, and the volume was made up to 10 mL with diluent and filtered. Hydrolysis under alkaline conditions was performed by exposing the drug solution to 0.1N NaOH at 60°C for 6 h and then cooled, neutralized with 0.1 N HCl, and the volume was made up to 10 mL with diluent and filtered. Thermal degradation was carried by exposing the drug solution to 60°C for 24 h and then cooled, and volume was made up to 10 mL with diluent and filtered. Oxidative stress was studied by exposing the drug solution to 0.3% v/v H_2O_2 for

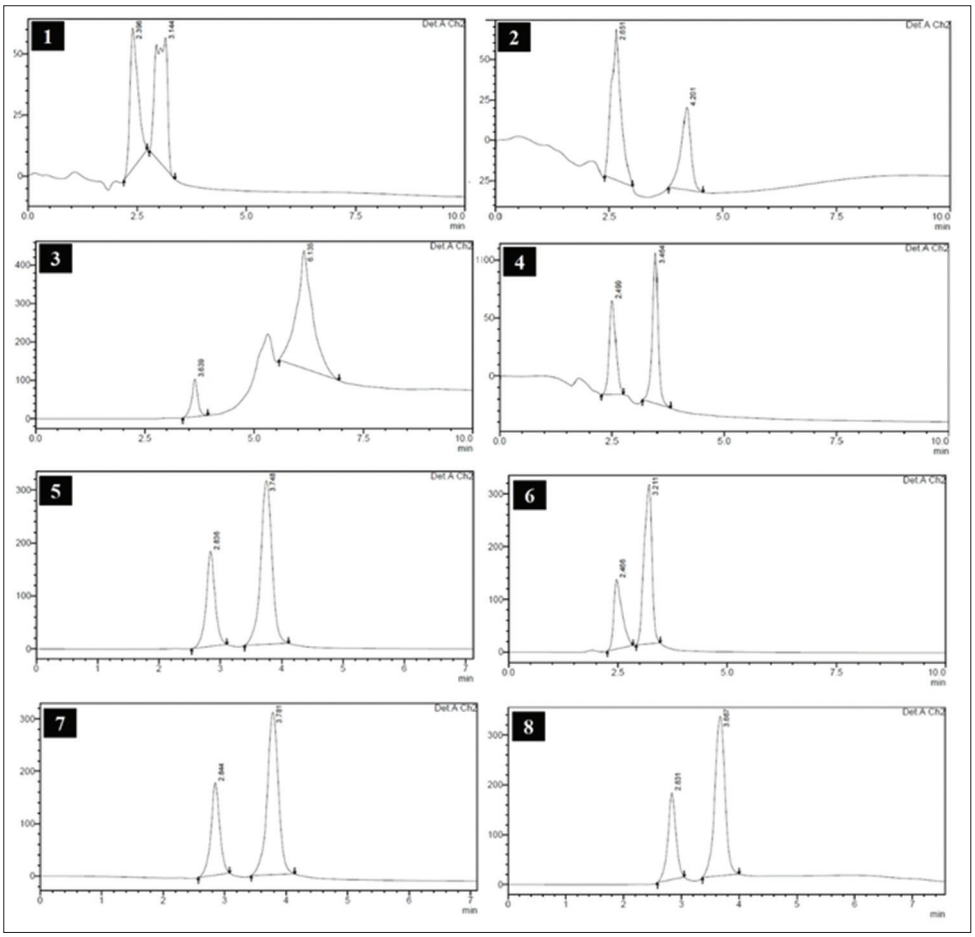

Figure 3: (1-8) Trial chromatograms during method development

Table 1: HPLC trails performed during method development

Trials	Mobile phase	Observations
1.	Phosphate buffer pH - 6.8: Acetonitrile (50:50)	No complete separation of peaks. Less theoretical plate count
2.	Phosphate buffer pH - 6.8: Methanol (70:30)	Baseline disturbances. Less theoretical plate count
3.	Phosphate buffer pH - 5: Acetonitrile (50:50)	Baseline disturbances. Interference of ghost peaks
4.	Phosphate buffer pH - 5: Acetonitrile (60:40)	Low resolution. Baseline disturbances
5.	Acetate buffer pH - 4: Methanol (50:50)	Low resolution and less theoretical plate count
6.	Acetate buffer pH - 4: Acetonitrile (50:50)	Low resolution and less theoretical plate count
7.	Acetate buffer pH - 4: Methanol (55:45)	Low resolution and less theoretical plate count
8.	Acetate buffer pH - 4: Methanol (60:40)	Less theoretical plate count

HPLC: High-performance liquid chromatography

15 min. All the respective solutions were injected (20 µL) into HPLC system, and their chromatographic parameters were analyzed.

RESULTS AND DISCUSSION

RP-HPLC method development and optimization

The initial conditions for the chromatography were designed based on the nature of sample, literature, availability, and cost parameters. Several trials were performed by altering mobile phase and keeping the column, flow rate, and other conditions without changing. The result chromatograms [Figure 3] of the trials are presented in Table 1. The final optimized conditions for the separation were optimized, and the conditions are shown in Table 2.

A simple RP-HPLC method was developed for the simultaneous estimation of TEG and URA using a C18 phenomenex column (250 mm × 4.6 mm i.d., 5 µm particle size) with an isocratic mobile phase composed of acetate buffer (pH 4) and methanol in the proportion of 70:30 (v/v) with a steady flow rate of 1.0 mL/min with a run time of 10 min. The detection was carried out by UV-detector at 280 nm. TEG retention time was about 5.2 min and URA was 2.9 min which was confirmed by injecting individual drug solutions under the same conditions.

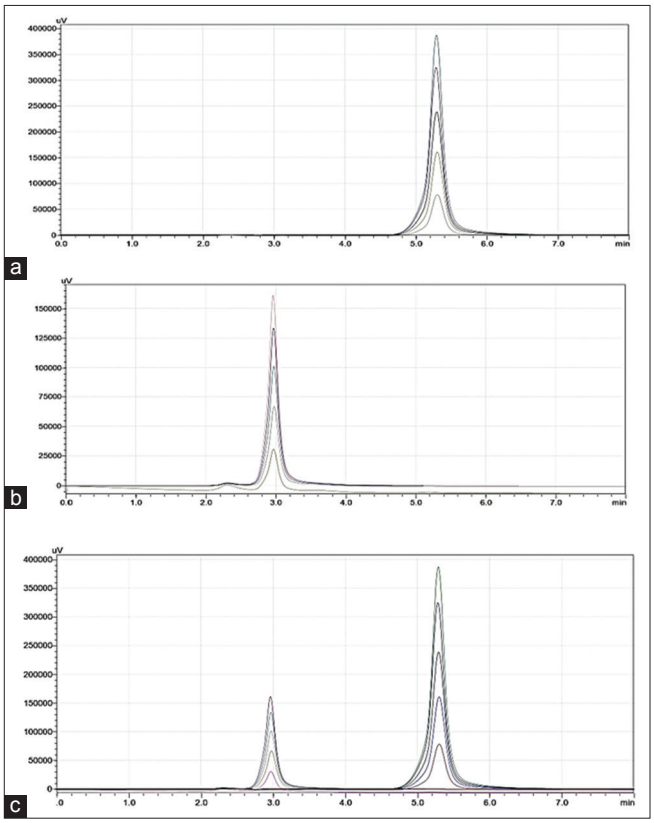

Figure 4: Optimized chromatograms: (a) Linearity chromatograms of tegafur (TEG) (b) linearity chromatograms of uracil (URA) (c) optimized method chromatograms for TEG and URA

Method validation

The developed method was validated as per the ICH guidelines[16,17] for various parameters such as linearity, range, precision, accuracy, and robustness [Figure 4]. A calibration curve [Tables 3 and 4] was obtained in the range of 25–125 µg/mL for TEG and 20–100 µg/mL for URA [Figures 5 and 6]. Both calibration curves showed a good correlation of >0.999. Specificity experiments revealed the absence of interference from excipients. The precision was done for

Table 2 : Optimized chromatographic conditions

Parameter	Optimized conditions
Column	Phenomenex C18 5 µ (250 mm×4.6 mm)
Mobile phase	Acetate buffer pH 4-methanol (70:30); isocratic conditions
Flow rate	1.0 mL/min
Injection volume	20 µL
Temperature	Ambient temperature
Detection wavelength	UV-Visible detection at 280 nm
Runtime	10 min
Retention time for TEG	5.294±0.011 min
Retention time for URA	2.974±0.006 min

UV: Ultraviolet, TEG: Tegafur, URA: Uracil

Table 3: Linearity data of TEF

Concentration (µg/mL)	Peak area of TEG
25	1070098
50	2220290
75	3358442
100	4420979
125	5382209

TEG: Tegafur, URA: Uracil

Table 4: Linearity data of URA

Concentration (µg/mL)	Peak area of URA
20	361052
40	678334
60	1047877
80	1352974
100	1666935

TEG: Tegafur, URA: Uracil

both system [Table 5] and method [Table 6] precision. All the results were acceptable with % relative standard deviation values <2. Accuracy was performed based on percentage recovery studies [Table 7]. TEG showed a mean percentage recovery of 99.69% and URA showed mean percentage recovery of 100.82%. The method was also evaluated for robustness and ruggedness, and the results obtained were satisfactory [Figure 7].

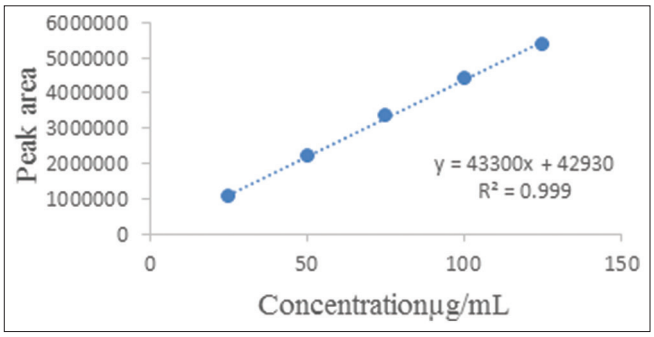

Figure 5: Linearity curve of tegafur

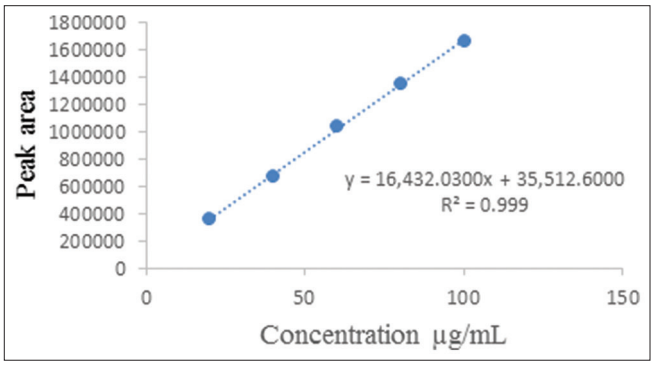

Figure 6: Linearity curve of uracil

ASSAY RESULTS

The mean assay for TEG was found to be 101.41% (w/w) and for URA was 102.82% (w/w) [Table 8].

Forced degradation studies

Forced degradation studies were performed for TEG and URA at various conditions such as acidic hydrolysis and basic hydrolysis, thermal, and oxidative conditions, and the percentage degradation values were calculated. All the chromatograms showed no considerable degradation of the drugs at various stress conditions.

CONCLUSIONS

The developed stability indicating RP-HPLC method was specific, precise, accurate, sensitive, and robust for the simultaneous estimation of TEG and URA in capsules. The proposed method can be used for the simultaneous analysis of the two drugs by various pharmaceutical laboratories and research institutions.

Table 5: System precision data of TEG and URA

Injection No.	TEG		URA	
	Peak Area	Retention time (min)	Peak Area	Retention time (min)
1	1168809	5.297	970920	2.978
2	1204278	5.306	1007132	2.983
3	1169445	5.292	1002866	2.967
Mean±SD	1180844±20296.93	-	993639.3±19790.8	-
%RSD	1.71	-	1.99	-

RSD: Relative standard deviation, SD: Standard deviation, TEG: Tegafur, URA: Uracil

Table 6: Method Precision data of TEG and URA

Injection No.	TEG		URA	
	Peak area	Retention time (min)	Peak area	Retention time (min)
1	950135	5.520	1003680	2.999
2	951850	5.523	998350	3.002
3	991848	5.525	998335	3.002
Mean±SD	964611±23603.52	-	1000122±3081.616	-
%RSD	0.269	-	0.308	-

RSD: Relative standard deviation, SD: Standard deviation, TEG: Tegafur, URA: Uracil

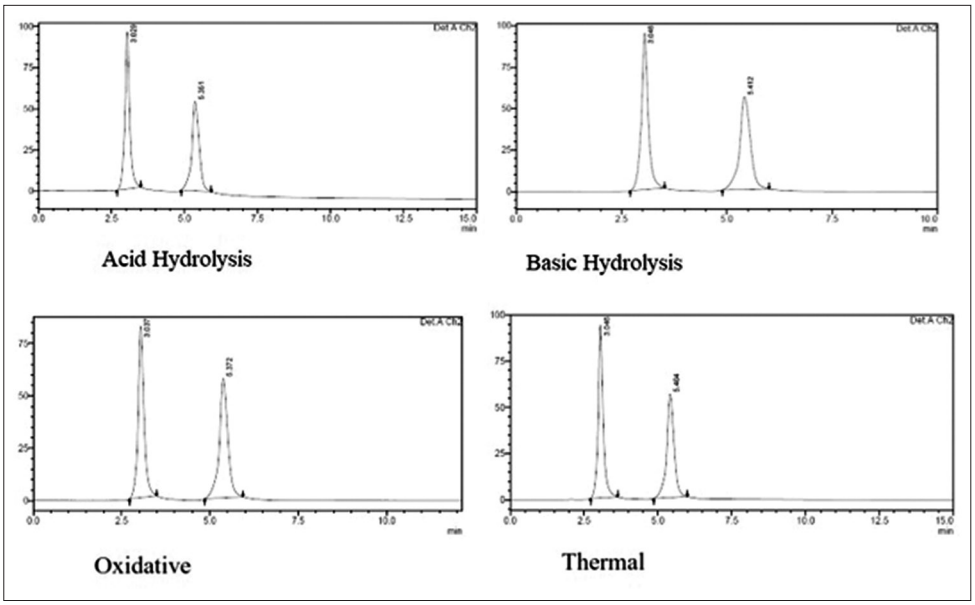

Figure 7: Chromatograms of forced degradation studies

Table 7: Accuracy (percentage recovery) of TEG and URA

TEG			URA		
% Level	Sample peak area	% recovery±SD	% level	Sample peak area	% recovery±SD
50	1599879	97.08±0.33	50	1453739	102.46±0.50
100	2289678	104.0±0.5	100	1795767	95.6±0.8
150	2648976	98.0±0.4	150	2452195	104.4±0.65

SD: Standard deviation, TEG: Tegafur, URA: Uracil

Table 8: Assay of capsule dosage form

Peak areas	TEG	URA
% Assay	101.41% (w/w)	102.82% (w/w)

TEG: Tegafur, URA: Uracil

ACKNOWLEDGMENT

The authors would like to thank Dr. D. Ranganayakulu, Principal, and Smt. P. Sulochana, Correspondent, Sri Padmavathi Educational Institutions, Tiruchanoor, Tirupati, for providing research facilities to carry out this work.

REFERENCES

1. Etienne-Grimaldi M, François E, Cardot J, Renée N, Douillard J, Gamelin E. A clinical pharmacokinetic analysis of tegafur-uracil (UFT) plus leucovorin given in a new twice-daily oral administration schedule. Clin Pharmacokinet 2007;46:953-63.
2. Breda M, Barattè S. A review of analytical methods for the determination of 5-fluorouracil in biological matrices. Anal Bioanal Chem 2010;397:1191-201.
3. Maring JG, Schouten L, Greijdanus B, De Vries EG, Uges DR. A simple and sensitive fully validated HPLC-UV method for the determination of 5-fluorouracil and its metabolite 5, 6-dihydrofluorouracil in plasma. Ther Drug Monit 2005;27:25-30.
4. Alsarra IA, Alarifi MN. Validated liquid chromatographic determination of 5-fluorouracil in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2004;804:435-9.
5. Zufia L, Aldaz A, Castellanos C, Giraldez J. Determination of 5-fluorouracil and its prodrug tegafur in plasma and tissue by high-performance liquid chromatography in a single injection: Validation for application in clinical pharmacokinetic studies. Ther Drug Monit 2003;25:221-8.
6. Chu D, Gua J, Liu W, Fawcett JP, Dong Q. Sensitive liquid chromatographic assay for the simultaneous determination of 5-fluorouracil and its prodrug, tegafur, in beagle dog plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2003;795:377-82.
7. Casale F, Canapaio R, Muntoni E, Serpe L, Zara GP, Della Pepa C, *et al*. Simultaneous HPLC determination of 5-fluorouracil and its metabolites in plasma of cancer patients. Biomed Chromatogr 2002;16:446-52.
8. Loos W, De Bruijn P, Van Zlen L, Verweij J, Nooter K,

Stoter G, *et al*. Determination of 5-fluorouracil in microvolumes of human plasma by solvent extraction and high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1999;735:293-7.
9. Tonano JS, Vermes A, Guchelaar HJ. Simultaneous determination of flucytosine and fluorouracil in human plasma by high performance liquid chromatography. Biomed Chromatogr 2001;15:89-94.
10. Escoriaza J, Aldaz A, Calvo E, Giraldez J. Simple and sensitive determination of 5-fluorouracil in plasma by high-performance liquid chromatography: Application to clinical pharmacokinetic studies. J Chromatogr B Biomed Sci Appl 1999;736:97-102.
11. Jung M, Berger G, Pohlen U, Pauser S, Reszka R, Buhr HJ. Simultaneous determination of 5-fluorouracil and its active metabolites in serum and tissue by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1997;702:193-202.
12. Gamelin E, Boisdron-Celle M, Turacant A, Larra F, Allain P, Robert J. Rapid and sensitive high-performance liquid chromatographic analysis of halogenopyrimidines in plasma. J Chromatogr B Biomed Sci Appl 1997;695:409-16.
13. Joulia JM, Pinguet F, Grosse PY, Astre C, Bressolle F. Determination of 5-fluorouracil and its main metabolites in plasma by high-performance liquid chromatography: Application to a pharmacokinetic study. J Chromatogr B Biomed Sci Appl 1997;692:427-35.
14. Wattanatorn W, McLeod HK, Cassidy J, Kendle KE. High-performance liquid chromatographic assay of 5-fluorouracil in human erythrocytes, plasma and whole blood. J Chromatogr B Biomed Sci Appl 1997;692:233-7.
15. Coe RA, Earl RA, Johnson TC, Lee JW. Determination of 5-fluorouracil in human plasma by a simple and sensitive reversed-phase HPLC method. J Pharm Biomed Anal 1996;14:1733-41.
16. ICH Harmonized Tripartite Guidelines. Validation of Analytical Procedures: Methodology (Q2B). Geneva: ICH Harmonized Tripartite Guidelines; 1996.
17. International Conference on Harmonization of Technical Requirement for the Registration of Pharmaceutical for Human Use: Validation of Analytical Procedures, Text and methodology- Q2(R1); 2005.

Source of Support: Nil. **Conflict of Interest:** None declared.