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Development and Validation of a RP-HPLC Method for the Determination of Capecitabine and its Impurities in Pharmaceutical Dosage Form

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Abstract

The analysis of established HPLC technique for the separation and quantification of Capecitabine and its impurities are described. Samples are analysed by reverse phase (RP-HPLC) using stationary phase Inertsil ODS-3V (250 x 4.6mm, 5µm) column and the movable segment consisted of two channels. Channel A: 20mM Ammonium Formate buffer, Methanol and Acetonitrile in the proportion of (75:25:5 %volume/volume/volume) and Channel B: 20mM methanol, ammonium acetate buffer and Acetonitrile in the proportion of (80:15:5 %volume/volume/volume). The run velocity is 1.0 mL/min, the column oven was preserved at 40°C and sampler cooler oven was preserved 5°C, infused 10µL and wavelength fixed at 250nm UV-detection. The established HPLC technique was authenticated with admiration to specificity, precision, linearity, accuracy, LOD, LOQ and solution stability. Corroboration study compared as stated by ICH instruction.

Keywords: Capecitabine, Assessment of related substances, LOQ, Forced Degradation and Liquid chromatography.

Introduction

Capecitabine has the chemical name pentyl (1-(3,4-dihydroxy-5-methyl-tetrahydrofuran-2-yl)-5-fluoro-2-oxo-1Hpyrimidin- 4-yl) amino methanoate (1). It is a white to almost white solid with a molecular formula of $C_{15}H_{22}FN_3O_6$ and a molecular weight of 359.3. Capecitabine tablets contain NLT 93.0% and NMT 105.0% of the labeled amount of Capecitabine (2). Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. Capecitabine is a prodrug, that is enzymatically converted to 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue. The activation of Capecitabine follows a pathway with three enzymatic steps and two intermediary metabolites, 5'-deoxy-5-fluorocytidine (5'-DFCR) and 5'-deoxy-5- fluorouridine (5'-DFUR), to form 5-fluorouracil (3).

The final step is the tumor-activated conversion to 5-FU by TP, predominantly in malignant cells. This site-specific action may result in higher tumor site concentrations and lower systemic toxicity than that seen with intravenous use of 5-FU (4). Capecitabine, sold under the brand name Xeloda among others, is a anticancer medication used to treat breast cancer, gastric cancer and colorectal cancer (5). For breast cancer it is often used together

with docetaxel. It is taken by mouth (6).

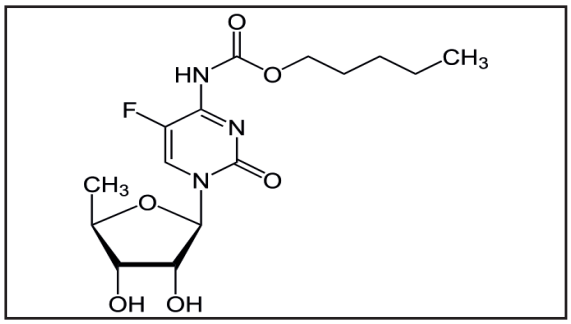
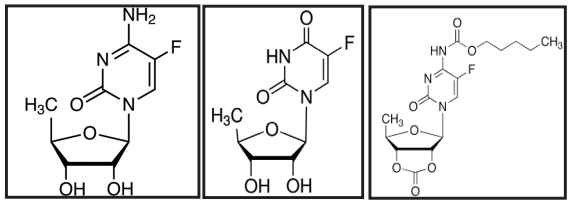


Figure: 1 Chemical structure of Capecitabine



Capecitabine Impurity-A Capecitabine Impurity-B Capecitabine Impurity-C

Figure: 2 Chemical structures Capecitabine impurities

An all-embracing literature assessment was approved and established a few high-performance liquid chromatographic (HPLC) techniques for the determination of Capecitabine in drug substance and formulations (7-12) were reported for the impurity profile study of Capecitabine. Stability indicating and simultaneous determination methods (8-22) in human plasma and formulations were also reported. One UV spectrophotometric method (23) were also developed for the determination of Capecitabine.

The main objective of the proposed method is to develop a steadiness representative HPLC technique and authenticated by means of ICH (24) and USP corroboration instructions for the inference of Capecitabineand its impurities in pharmaceutical dosage forms (Solid).

Materials and Methods

Reagents and chemicals

Ammonium Formate, Methanol, Acetonitrile was procured from Merck. Water (Milli-Q). All chemicals were of an analytical grade and used as received. Impurities are procured from SynZeal Research Private Limited, Ahmedabad, Gujarat. Capecitabine Tablets (CAPNAT) was procured from local market.

Instrumentation

Chromatographic partition was achieved by using an waters alliance e2695, Empower^3 software using an Inertsil ODS 3V (250 x 4.6mm, 5µm) and the movable segment consisted of two channels. Channel A: 20mM Ammonium Formate buffer, methanol and Acetonitrile in the proportion of (75:25:5 %volume/volume/volume) and Channel B: 20mM methanol, ammonium acetate buffer and Acetonitrile in the proportion of (80:15:5 %volume/volume/volume). The run velocity is 1.0 mL/min, the column oven was preserved at 40°C and sampler cooler oven was preserved 5°C, infused 10µL and wavelength fixed at 250nm UV-detection. The sprint instance was 45 minutes.

Preparation of solutions

Preparation of 0.02 m ammonium formate solution

Accurately weighed and transferred 1.2 g of Ammonium formate in to 1000 mL of water and mixed well. Filter through 0.45 µm membrane filter and sonicate to degas.

Preparation of mobile phase a

Mixed 700 mL of 0.02M Ammonium formate, 250 mL of methanol and 50 mL of acetonitrile in the ratio 70:25:5 (%v/v/v). Filter through 0.45 µm membrane filter and sonicate to degas.

Preparation of mobile phase b

Mixed 800 mL of Methanol, 150 mL of Ammonium formate and 50 mL of acetonitrile in the ratio 80:15:5 %v/v/v. Filter through 0.45 µm membrane filter and sonicate to degas.

Preparation of diluent

Mixed 250 mL of Methanol, 700 mL of water and 50 mL of acetonitrile in the ratio 70:25:5 %v/v/v. Filter through 0.45 µm membrane filter and sonicate to degas.

Preparation of standard stock solution

Weighed accurately 30.0 mg of the Capecitabine working standard in to a 50 mL volumetric flask, sonicate for 5 minutes to dissolve, and dilute to volume with diluent and mixed well.

Preparation of standard solution

Transferred 1.0 mL of the above standard stock solution into a 100 mL volumetric flask, and made up to volume with diluent and mixed well. Pipette out 5.0 mL of the above standard solution into a 50 mL volumetric flask, and made up to volume with diluent and mixed well. (The concentration of the solution is about 0.6 µg/ mL of Capecitabine).

Preparation of Placebo solution

Weighed accurately 8.21 mg of Capecitabine placebo powder, transferred into 50 mL volumetric flask, added 20 mL of diluent, sonicate for 15 minutes with intermediate shaking to dissolve and cool to room temperature, then diluted to the volume with diluent, mixed well and filtered the solution through 0.45 µm PVDF membrane filter.

Preparation of sample solution

Weighed 10 tablets, taken average weight, and crushed into fine powder. Weighed accurately 38.97 mg of Capecitabine sample powder, transferred into 50 mL volumetric flask, added 20 mL of diluent, sonicated for 15 minutes with intermediate shaking to dissolve and cool to room temperature, then diluted to the volume with diluent, mixed well and filtered the solution through 0.45 µm PVDF membrane filter. (The concentration of the solution is about 0.6 mg/ mL of Capecitabine

Method development

Method optimization parameters

A sympathetic of the character of API (functionality, acidity, or basicity), the synthetic procedure, related impurities, the possible degradation pathways and their degradation products are needed for successful method development in reverse-phase HPLC. In addition, successful method development should result a robust, simple and time efficient method that is capable of being utilized in manufacturing setting.

Selection of wavelength

The sensitivity of the HPLC method depends upon the selection of detection wavelength. An ideal wavelength is one that gives good response for related substances and the drugs to be detected. The wavelength for measurement was selected as 250 nm from the absorption spectrum.

Selection of stationary phase

Proper selection of the stationary phase depends up on the nature of the sample and chemical profile. The drug selected for the present study was polar compound and could be separated either by normal phase chromatography or reverse phase chromatography. From literature survey, it was found that different C18 columns could be appropriately used for the separation of related substances for Capecitabine.

Selection of mobile phase

Different mobile phases are employed to develop a suitable LC method for the quantitative determination of impurities in Capecitabine, different mobile phase composition were tried to get good peak shapes and selectivity for the impurities present in Capecitabine.

Results and Discussion

Specificity

Specificity was demonstrated by infused

blank solution, placebo solution, standard solution, sample solution, spiked sample and creature impurities as well as scrutinized as stated by the test technique. It was scrutinized that identified impurities are not co eluting with apiece additional and foremost analyte crest.

Table: 1 Impurity interference data (Specificity results)

S. No	Sample	Retention time (min)	Blank	Placebo
1	Blank	ND	NA	NA
2	Placebo	ND	NA	NA
3	Standard solution	24.916	No	No
4	Sample solution	24.913	No	No
5	Spiked sample solution	24.915	No	No
6	Impurity-A	3.787	No	No
7	Impurity-B	5.452	No	No
8	Impurity-C	22.529	No	No

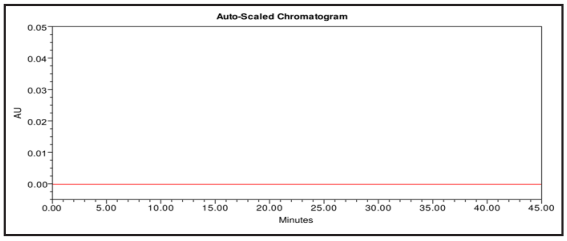


Figure: 3 typical chromatogram of blank

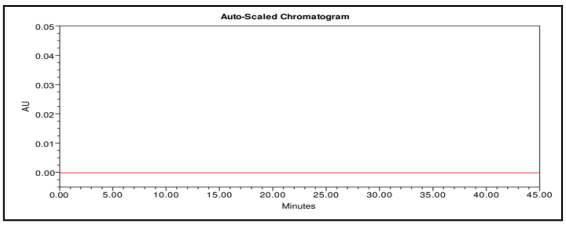


Figure: 4 typical chromatogram of placebo

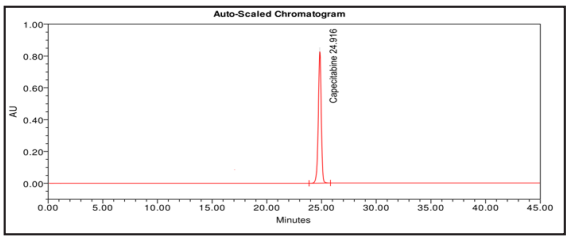


Figure: 5 typical chromatogram of standard

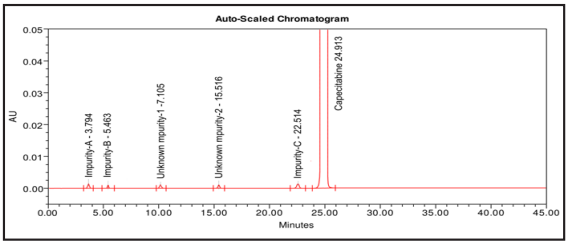


Figure: 6 typical chromatogram sample

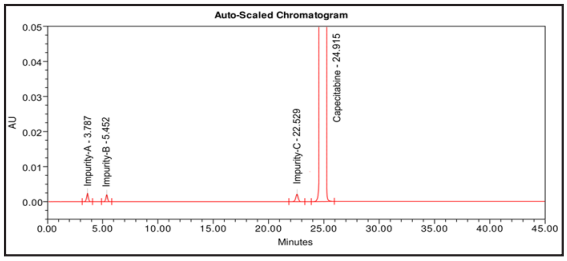


Figure: 7 typical chromatogram spiked Sample

Forced degradation study

Sample solutions and placebo solutions were exposed to the following stress conditions to degradation. Stressed and unstressed samples were injected into the HPLC system with photo diode array detector. All degrading peaks were resolved from Capecitabine peak in the chromatograms of all samples and placebo did not show any interference at the retention time of Capecitabine and impurities.

Table: 2 Forced Degradation results

S. No	Stress Condition	Imp- A	Imp- B	Imp- C	Maximum Unknown impurity	Net Degradation (%)
1	Unstressed Sample	0.148	0.028	0.007	ND	0.22
2	Acid stress sample (1N HCl/5mL/60°C/1hr)	0.160	44.58	ND	1.38	46.1
3	Base stress sample (1N NaOH/5mL/60°C/1hr)	3.36	13.88	ND	1.18	20.3
4	Peroxide stress sample (30%H2O2/5mL//1.0hr@ RT)	0.87	0.13	ND	ND	1.1
5	Humidity Stress sample (90%RH/24hrs)	0.151	0.032	0.01	ND	0.25
6	Thermal Stress sample (60°C/24hrs)	0.155	0.039	0.02	ND	0.31

Capecitabine was sensitive to stress condition like acid and alkali. The results proved that the developed method has good selectivity and specificity. Hence it is suitable for determination of impurities in Capecitabineliquid dosage form.

Precision system exactitude

System exactitude was exhibited by organized standard solution as stated by the test technique and infused for six times keen on HPLC system. The preservation instance and vicinity rejoinder of analyte crest were recorded.

Table: 3 System precision data for standard

S.No.	Area response
1	754636
2	758050
3	750943
4	747413
5	753537
6	744376
Average	751493
STDV	4993.0
% RSD	0.7

The %RSD of crest vicinity for Capecitabine standard was established 0.7% which is underneath 5.0% designates that the system gives precise result.

Method exactitude

Table 4: Results of method precision

S.No.	Sample Details	Impurity (%)		
		Imp-A	Imp-B	Imp-C
1	Method Precision Spiked Prep-1	1.062	1.101	0.534
2	Method Precision Spiked Prep-2	1.064	1.059	0.538
3	Method Precision Spiked Prep-3	1.059	1.068	0.519
4	Method Precision Spiked Prep-4	1.055	1.069	0.521
5	Method Precision Spiked Prep-5	1.046	1.068	0.529
6	Method Precision Spiked Prep-6	1.065	1.068	0.541
Avg.	1.059	1.072	0.530	
STD.	0.0071	0.0146	0.0090	
%RSD	0.67	1.36	1.69	

Method exactitude was revealed by organized six samples by spiking of impurities at designed level and analyzed as stated by the test technique.

The consequences were well inside the limits. From the above consequences, it is concluded that technique is precise.

Limit of quantitation (LOQ)

A solution containing 0.1522 µg/mL of Capecitabine, 1.944 µg/mL of Impurity-A, 2.034 µg/mL of impurity-B, 0.732 µg/mL impurity-C was injected six times. The %RSD areas of each impurity and standard were calculated.

Table 5: LOQ for Capecitabine and impurities

S.No.	Imp-A	Imp-B	Imp-C	Capecit abine
1	29945	21654	24974	30994
2	30753	20745	23994	31452
3	28925	20087	24015	30078
4	29452	19997	25003	29974
5	31008	20001	24984	30125
6	28874	21632	24651	30746
AVG	29826	20686	24604	30562
SD	908.6908	792.2333	482.0227	597.2516
%RSD	3.05	3.83	1.96	1.95

The limit of quantitation values obtained for each impurity and Capecitabine are within the acceptance criteria.

Linearity

The linearity of detector rejoinder for Capecitabine and its impurities was demonstrated by preparing solutions over the range of LOQ level to 150 % level of target concentration level. A plot of concentration vs. area response of peak was done. The correlation co-efficient between concentration and area response was evaluated.

Table: 6 Linearity solution preparation for impurity-A

S.No.	Linearity Level	Concentration in ppm	Area response
1	LOQ	1.944	30757
2	50	3.24	51545
3	75	4.86	75999
4	100	6.48	102524
5	125	8.1	127455
6	150	9.72	155789
Correlation coefficient (r2)			0.9996
Slope			15969.3573
Intercept			-730.4347
%Y-Intercept			-0.71

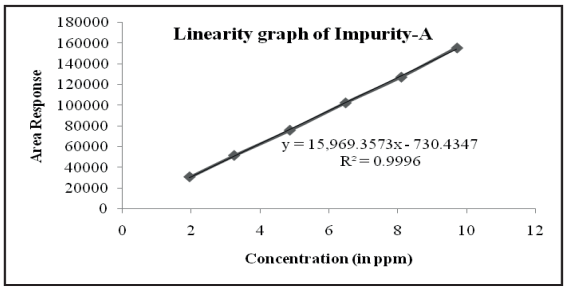


Figure: 8 Linearity graph of impurity-A

Table: 7 Linearity solution preparation for impurity-B

S.No.	Linearity Level	Concentration in ppm	Area response
1	LOQ	2.034	20145
2	50	3.39	33997
3	75	5.085	49874
4	100	6.78	66109
5	125	8.475	83743
6	150	10.17	99058
Correlation coefficient (r2)			0.9997
Slope			9716.0933
Intercept			631.3169
%Y-Intercept			0.95

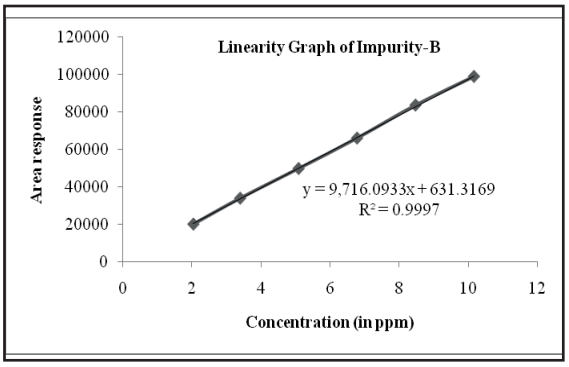


Figure: 9 Linearity graph of impurity-B

Table: 8 Linearity solution preparation for impurity-C

S.No.	Linearity Level	Concentration in ppm	Area response
1	LOQ	0.732	25545
2	50	1.525	50099
3	75	2.257	75590
4	100	3.05	102120
5	125	3.782	127905
6	150	4.575	155187
Correlation coefficient (r2)			0.9997
Slope			33909.6232
Intercept			-571.5186
%Y-Intercept			-0.56

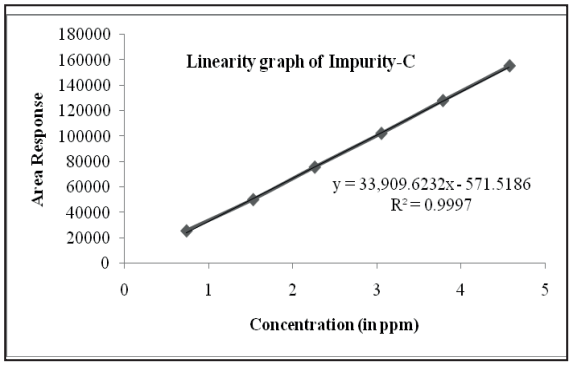


Figure: 10 Linearity graph of impurity-C

Table: 10 Linearity solution preparation for Capecitabine

S.No.	Linearity Level	Concentration in ppm	Area response
1	LOQ	0.15215	31109
2	50	0.3043	63742
3	75	0.45645	95158
4	100	0.6086	125480
5	125	0.73032	150062
6	150	0.9129	191928
Correlation coefficient (r2)			0.9995
Slope			209047.7694
Intercept			-683.1094
%Y-Intercept			-0.54

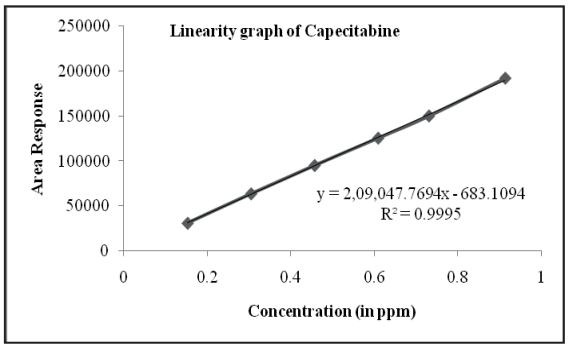


Figure: 11 Linearity graph of Capecitabine

The linearity results for Capecitabine and all the impurities in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.

Accuracy

Recovery of Capecitabine impurities in Capecitabine solid dosage formulation was performed. The sample was taken and varying amounts of Capecitabine impurities spiking at LOQ level to 150 % of specification level were added to the flasks.

Table: 11 Accuracy study of Capecitabine

S.No.	Theoretical (%)	% Mean Recovery		
		Impurity-A	Impurity-B	Impurity-C
1	LOQ	107.7	103.1	94.0
2	50	104.5	101.8	90.2
3	100	100.2	101.5	98.2
4	150	99.7	103.2	99.9

Accuracy at LOQ level to 150% level for impurity-A, impurity-B and impurity-C is meeting the acceptance criteria. From the above results, it is concluded that method is accurate.

Solution stability

Stability of solutions such as standard solution and sample solutions was established at various conditions such as bench top and in refrigerator (2-8°C). The response of these was compared with respect initial standard solution and spiked sample solution.

Solution stability parameter was established, standard solution were stable upto 48 hrs on bench top in refrigerator and sample solutions were stable upto 24 Hours on bench top and 36 hrs in refrigerator condition.

Discussion

An uncomplicated, fiscal, accurate and precise HPLC technique was productively urbanized. In this technique it was carried out by using stationary phase Inertsil ODS-3V (250 x 4.6mm, 5µm) column and the movable segment consisted of two channels. Channel A: 20mM Ammonium Formate buffer, Methanol and Acetonitrile in the proportion of (75:25:5 %volume/volume/volume) and Channel B: 20mM methanol, ammonium acetate buffer and Acetonitrile in the proportion of (80:15:5 %volume/volume/volume). The run velocity is 1.0 mL/min, the column oven was preserved at 40°C and sampler cooler oven was preserved 5°C, infused 10µL and wavelength fixed at 250nm UV-detection. The consequences

obtained were accurate and reproducible. The technique urbanized was statistically authenticated in terms of selectivity, accuracy, linearity, precision and stability of solution.

For selectivity, the chromatograms were recorded for standard, sample and spiked sample solutions of Capecitabine and its related substances. Selectivity studies reveal that the peaks are well separated from each other. Therefore the method is selective for the determination of related substances in Capecitabine. There is no interference of blank and placebo at Capecitabine and impurities peaks. The elution order and the retention times of impurities and Capecitabine obtained from individual standard preparations and mixed standard preparations are comparable.

For precision studies six replicate injections were performed. %RSD was determined from the peak areas of Capecitabine and its impurities. The acceptance limit should be not more than 5.0, and the results were found to be within the acceptance limits.

The limit of quantitation (LOQ) for 0.1522 µg/mL of Capecitabine, 1.944 µg/mL of Impurity-A, 2.034 µg/mL of impurity-B, 0.732 µg/mL impurity-C respectively.

The linearity results for Capecitabine and all the impurities in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.

The accuracy studies were shown as % recovery for Capecitabine and its impurities at specification level. The limit of % recovered shown is in the range of LOQ and 150% and the results obtained were found to be within the limits. Hence the method was found to be accurate.

Solution stability parameter was established, standard solution were stable upto 48 hrs on bench top in refrigerator and sample solutions were stable upto 24 Hours on bench top and 36 hrs in refrigerator condition.

Conclusion

The new-fangled HPLC method developed and validated for determination of Capecitabine pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of drug in its solid dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control.

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Conflict of interests

The authors claim that there is no conflict of interest.

References

1. The Merck Index, 6281, 13th edition. Merck research Laboratories. Merck & Co., Inc., USA. 2001, pp 1154.
2. United States Pharmacopoeia 32-National Formulary 27. 2009, pp 2148-2149.
3. Guichard SM, Mayer I, Jodrell DI. Simultaneous determination of Capecitabine and its metabolites by HPLC and mass spectrometry for preclinical and clinical studies J Chromatogr B 2005; 826:232-7.
4. Vermes A, Guchelaar HJ, Dankert J. Flucytosine: a review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. J Antimicrob Chemother 2000;46:171-9.
5. British national formulary : BNF 69 (69 ed.). British Medical Association. 2015. pp. 585,

588. ISBN 9780857111562.

6. "Capecitabine". The American Society of Health-System Pharmacists. Archived from the original on 15 April 2016. Retrieved 8 December 2016.

7. Ravisankar P, Devala Rao G, Naveen Kumar M. An Improved RP-HPLC method for the quantitative determination of Capecitabine in bulk and pharmaceutical tablet dosage form. Der Pharmacia Lettre.5(3);2013: 249-260.

8. Narendra Devanaboyina, Y. Sai Kishore, P. Pushpalatha; Development and Validation of New RP-HPLC method for analysis of Capecitabine in pharmaceutical dosage form. International Journal of science inventions Today.2(1);2013:21-30.

9. K. Srinivasu, J Venkateswara Rao, N. Appala Raju and K. Mukkanti. RP-HPLC method for the estimation of Capecitabine in tablet dosage forms. Asian Journal of Chemistry 22(4);2010: 3255-3259.

10. G. Chaitanya, G. Venkata Ramana, A.K.M. Pawar RP-HPLC method development and validation of Capecitabine in bulk drug and formulation. International Journal of Pharmacy and analytical Research. 5 (1) :2016: 190-198.

11. Vijayasanthi S, Boovizhikannan Thangabalan, Ramalingam Kalaichelvi, Gummadi Gayatri, Kannan Vadivel, A Vijayalakshmi. RP-HPLC estimation of Capecitabine in pure and pharmaceutical dosage form. Journal of Pharmacy Research.5(5); 2012:2834-2836.

12. Hassanlou S, Rajabi M, Shahrasbi AA, Afshar M. Development and validation of an ecofriendly HPLC-UV method for determination of Capecitabine in human plasma: Application to pharmacokinetic studies. S Afr J Chem 2016; 69: 174-9.

13. Berard M, Demarchi M, Muret P, Piédoux S, Kantelip JP, Royer B. An APCI LC-MS/MS method for routine determination of Capecitabine and its metabolites in human plasma. Journal of mass spectrometry. 45(6);2010:670-677

14. Deenen MJ, Rosing H, Hillebrand MJ, Schellens JH, Beijnen JH .Quantitative determination of Capecitabine and its six metabolites in human plasma using liquid chromatography coupled to electrospray tandem mass spectrometry. Journal of Chromatography B Analyt Technol Biomedical Life Sciences. 913-914; 2013 : 30-40.

15. Deng P, Ji C, Dai X, Zhong D, Ding L, Chen X. Simultaneous determination of Capecitabine and its three nucleoside metabolites in human plasma by high performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2015; 989: 71-79.

16. Piorkowska E, Kaza M, Fitatiuk J, Szlaska I, Pawiński T, Rudzki PJ. Rapid and simplified HPLC-UV method with online wavelengths switching for determination of Capecitabine in human plasma. Pharmazie 2014; 69: 500-5.

17. Montange, Berard M, Demarchi M, Muret P, Piedoux S, Kantelip JP, Royer B. An APCI LC-MS/MS method for routine determination of Capecitabine and its metabolites in human plasma. J Mass Spectrom 2010; 45: 670-7.

18. Vainchtein LD, Rosing H, Schellens JHM, Beijnen JH. A new, validated HPLC-MS/MS method for the simultaneous determination of the anti-cancer agent Capecitabine and its metabolites: 5′-deoxy-5- fluorocytidine, 5′-deoxy-5- fluorouridine, 5-fluorouracil and 5-fluorodihydrouracil, in human plasma. Biomed Chromatogr 2010; 24: 374-86.

19. Svobaite R, Solassol I, Pin uet F, Mazard T,

Ivanauskas L, Ychou M, Ressolle FMM. A liquid chromatography-mass spectrometry method for the simultaneous determination of Capecitabine, 5′-deoxy-5-fluorocytidine, 5′-deoxy-5- fluorouridine, 5-fluorouracil, and 5-fluorodihydrouracil in human plasma. J Liq Chromatogr Relat Technol 2010; 33: 1705-19.

20. Licea-Perez H, Wang S, Bowen C. Development of a sensitive and selective LC-MS/MS method for the determination of α-fluoro-β-alanine, 5-fluorouracil and Capecitabine in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2009; 877: 1040-6.

21. Singhal P, Shah PA, Shah JV, Sharma P, Shrivastav PS. Determination of Capecitabine-an anticancer drug in dried blood spot by LC-ESI-MS/MS. Int J Pharm Pharm Sci 2015; 7: 238-45.

22. Guichard SM, Mayer I, Jodrell DI. Simultaneous determination of Capecitabine and its metabolites by HPLC and mass spectrometry for preclinical and clinical studies. J Chromatogr B Analyt Technol Biomed Life Sci 2005; 826: 232-7.

23. Kishore M, Jayaprakash M, Vijayabhaskarareddy T, Spectrophotometric determination of Capecitabine in Pharmaceutical Formulations. International Journal of Chem Tech Research. 3(1); 2011: 63-69.

24. International Conference on Harmonization (2005), Validation of Analytical Procedure: Text and Methodology, Q2 (R1).