International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.1, pp 307-311, Jan-Mar 2010

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CAPECITABINE IN CAPSULES

K. Ravi Kumar[1]*, CH. M.M.Prasada Rao[3], CH. Babu Rao[1], K.B.Chandra sekhar[2], P. Gangi Reddy[3]

[1]Department of Pharmaceutical Chemistry, Siddhartha Institute of Pharmaceutical sciences, Jonnalagadda, Narsaraopet(M), Guntur Dist,AndhraPradesh,India522601.

[2]Department of Pharmaceutical Sciences, JNTU, Anantapur, Andhra pradesh, India.

[3]Department of Pharmaceutical Chemistry, st.ann's college of pharmacy, nayunipalli, vetapalem,chirala, Andhra Pradesh, India.

***Corres. author: victory2ravi@yahoo.co.in, chennuprasad@gmail.com**

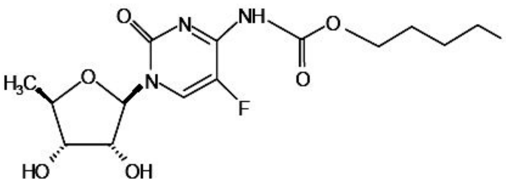
Abstract: A simple, rapid and selective RP-HPLC method has been developed for quantification Capecitabine from bulk drug and pharmaceutical formulations using a mobile phase consisting mixture of Buffer and acetonitrile in the ratio (80:40) (v/v) at the flow rate of 1.2 ml/min. An Hypersil BDS C_8, 250, 5µm, was used as stationary phase. The retention time for Capecitabine was 15min. Linearity was observed in the concentration range of 40 to 60 µg/ml, with good linearity response greater than 0.997. The mean % recovery obtained is 100.1%. The proposed method is precise, accurate, selective and rapid for the determination of Capecitabine in capsules.

Key words: Capecitabine, RP-HPLC Method.

Introduction

Capecitabine CAP [N4-pentoxycarbonyl- 5_-deoxy-5-fluorocytidine] is an anticancer prodrug of 5-fluorouracil (5-FU) that was designed to undergo preferential conversion to 5-FU within tumors[1-3]. 5-FU has also been widely used as an anticancer agent in the chemotherapy of solid tumors but its efficacy is limited by dihydropyrimidine dehydrogenase catalyzed formation of dihydro-5-fluorouracil. Since it lacks selectivity toward tumor cells, 5-FU also exhibits significant toxicity[1,4,5]. Prodrugs of 5-FU have been developed to improve efficacy and to reduce side-effect toxicity[1,4]. For example, tegafur [5-fluoro-1-[(*RS*)-tetrahydrofuran-2-yl]-pyrimidine-2,4-(1H, 3H)-dione] maintains an effective 5-FU concentration over a longer period while doxifluridine [5_deoxy-5-fluorouridine] achieves some selectivity toward tumors[6,7]. However, both prodrugs still show adverse effects, such as diarrhea after oral and intravenous administration[7,9].

The structure of Capecitabine:



Capecitabine was developed to reduce such adverse effects while improving the selectivity toward tumors[3,10,34]. Literature survey revealed several methods based on techniquesviz. HPLC- UV[12-22], HPLC- MS[23,24], LC- MS[25,26],LC- MS –MS[27-30], GC-MS[31] and MS- MS[32,33] for its determination in human plasma and pharmaceutical dosage forms. This paper presents simple, rapid, reproducible and economical method for Capecitabine by RP- HPLC in capsule dosage form.

Experimental

Apparatus and Chromatographic Conditions:

The analysis was performed by using HPLC (Shimadzu), Column used is of Hypersil BDS $C_{8,}$250 5µm column, with a flow rate of 1.2 ml/min. The mobile phase consists of Buffer: Acetonitrile as 80:40, the injection volume is 20µL and the photo diode array detection was at 240 nm.

Reagents and solutions:

Gift sample of Capecitabine was kindly supplied by the Dr. Reddy's laboratories, Hyderabad. Acetonitrile and buffer used were of HPLC and milli-Q grade respectively. Optimized chromatographic conditions are listed in [Table -1]

Standard Preparation:

100mg of Capecitabine is weighed accurately into a clean 100ml volumetric flask, dissolved and made up to the mark with mobile phase. 3ml is diluted to 25ml to attain a concentration of 12 µg /ml.

Sample preparation:

5ml of 1mg/ml Capecitabine standard solution was diluted in a 25ml volumetric flask and made up to volume, to get the concentration of 20 µg /ml of Capecitabine.

Method Validation:

Linearity:

Several aliquots of standard solutions of Capecitabine was taken in different 10ml volumetric flasks and diluted up to the mark with mobile phase such that the final concentration of CAP is 40-60µg/ml. Evaluation of drug was performed with PDA detector at 240 nm, peak area recorded for all the peaks. The slope and intercept value for calibration curve was $y = 152882x - 414517(R^2 = 0.9985)$. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated above regression graph was shown at Fig: 2.

Assay:

50 µl of standard and sample solutions were injected into an injector of RP-HPLC, from the peak area of CAP amount of drug in sample were computed. The values are given in Table: 3.

Limit of Detection and Limit of Quantification:

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Capecitabine found to be 0.088mcg/ml. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 0.26 µg /ml for Capecitabine.

Ruggedness and Robustness:

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC and Agilent HPLC by different operators using different columns of similar types. The %RSD values with two different instruments Shimadzu HPLC and Agilent HPLC, analysts and columns were 0.5- 0.5, 0.6- 0.5 and 0.4-0.3% respectively.

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as change in mobile phase, flow rate and column temperature. With the change in mobile phase composition 90, 100, 110% of acetonitrile organic phase, the %RSD values are 0.2, 0.4 and 0.2% respectively. In second with the change in flow rate 0.8, 1.0, 1.2ml/min, the %RSD values are 0.1, 0.2 and 0.1% respectively. In final with the change in column temperature 28^o, 30^o, 32^oC, the %RSD values are 0.2, 0.2 and 0.3% respectively. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, is rugged and robust. The robustness limit for mobile phase variation, flow rate variation, and temperature variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

Recovery Studies:

To study the accuracy and reproducibility of the proposed method recovery experiments were carried out. A fixed amount of pre-analyzed sample was taken and standard drug was added at 80, 100 and 120% levels. Each level was repeated three times. The contents of Capecitabine per tablet found by proposed method are shown in Table: 3, the lower values of %RSD of assay indicate the method is accurate. The mean recoveries were in range of 100.1% which shows that there is no interference from excipients.

Results and discussion

The RP-HPLC procedure was optimized with a view to develop accurate and stable assay method with the pure drug CAP tablet dosage form. A Hypersil BDS C_8, 250, 5µm column in isocratic mode, with mobile phase Buffer: Acetonitrile (80:40). The flow rate was 1.2ml/min and identical components were measured with PDA Detector at 240nm. Linearity was assessed by plotting concentration vs. Area which is shown in Fig: 2 with the linear in range of 40 - 60 µg/ml for CAP with correlation coefficient 0.9985 with good linearity response greater than 0.998. The % recovery was found to be with in limits of the acceptance criteria with mean recovery of 100.1%. The %RSD for intra-day and Inter-day precision is shown in Table: 4. The sensitivity of method LOD and LOQ is shown in Table: 2. Typical chromatogram of the sample is shown in Fig: 1. The assay procedures were repeated for Five times and the percentage of individual drugs and the results were found to give 99.41% which is shown in Table: 3.

Fig 1:Chromatogram of standard

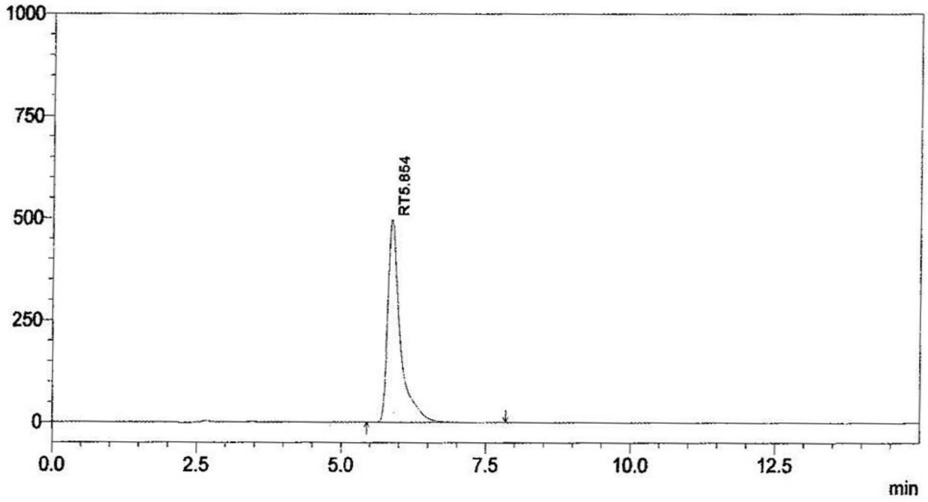
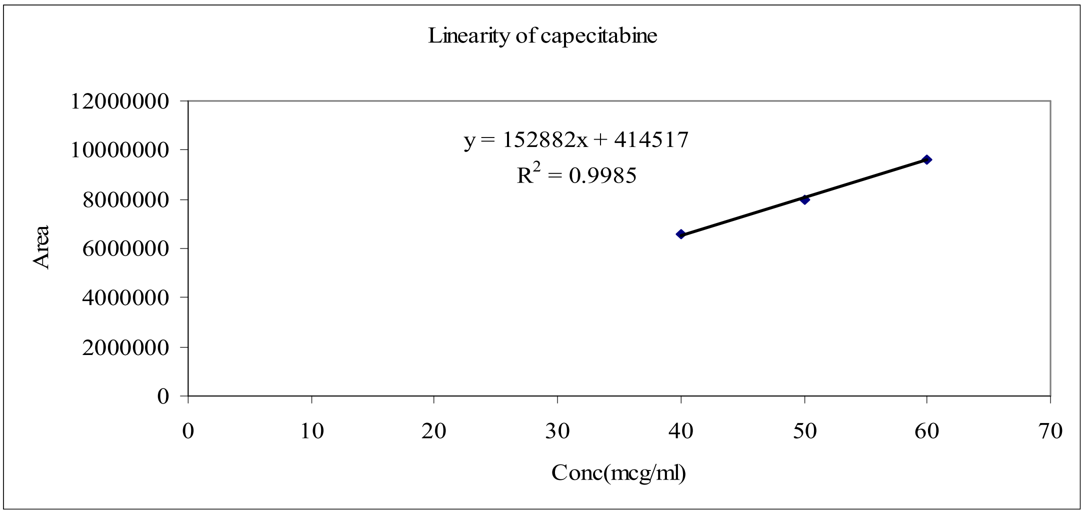


Fig 2: Linearity of Capecitabine:



Regression analysis of the calibration curve for Capecitabine showed a linear relationship between the concentration and peak area with correlation coefficients higher than 0.998 in all the curves assayed.

Conclusion

A convenient and rapid RP- HPLC method has been developed for estimation of Capecitabine in capsule dosage form. The assay provides a linear response across a wide range of concentrations. Low intra- and inter-day % RSD coupled with excellent recoveries. The proposed method is simple, fast, accurate and precise for the simultaneous quantification of Capecitabine in dosage form, bulk drugs as well as for routine analysis in quality control.

Table 1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Shimadzu with 2487 PDA)
Column	Hypersil BDS C_8, 250, 5µm
Mobile Phase*	Buffer: ACN (80:40).
Flow rate	1.2 ml/min
Detection	240 nm
Injection volume	20ml
column Temperature	Ambient

Table 2: System suitability parameters

Parameter	Capecitabine
Calibration range (µg/ml)	40-60
Theoretical plates	3889
Resolution	-
Tailing factor	1.8
LOD (µg/ml)	0.088
LOQ (µg/ml)	0.26

Table 3: Analysis of formulation and recovery studies

Drug	Label claim (mg/ml)	Estimation		Recovery	
		Mg/capsule	% label claim	Amount added µg/ml	% Recovery
Capecitabine	500	500	99.41	80	99.96
				100	100.31
				120	100.32

Table 4: Validation Parameters

Parameters	CAP
Linearity range	40 – 60 µg/ml
Correlation coefficient	0.998
Slope	15288
Y Intercept	41451
Recovery(n=3)	100.19 %
Intra-day(n=6)(% RSD)	0.6
Inter-day(n=6) (% RSD)	1.58%

%RSD= % Relative standard deviation

References

1. M. Malet-Martiono, R. Martino, Oncologist., 2002, 7, 288.
2. F. Desmoulin, V. Gilard, M. Malet-Martino, R. Martino, Drug Metab. Dispos., 2002, 30, 1221.
3. I.R. Judson, P.J. Beale, J.M. Trigo, W. Aherne, T. Crompton, D. Jones, E. Bush, B. Reigner., Invest. New Drugs., 1999, 17, 49.
4. A.H. Braun, W. Achterrath, H. Wilke, U. Vanhoefer, A. Harstrick, P. Preusser., Cancer., 2004, 100, 1558.
5. M.R. Mader, M. M¨uller, G.G. Steger, Gen., Pharmacol., 1998, 31, 661.
6. T. Moriwaki, I. Hyodo, T. Nishina, K. Hirao, T. Tsuzuki, S. Hidaka, T. Kajiwara, S. Endo, J. Nasu, S. Hirasaki, T. Masumoto, A. Kurita., Cancer Chemother. Pharmacol., 2005, 56, 138.
7. Y. Ohta, K. Sueki, K. Ketta, K. Takemoto, H. Ishitsuka, Y. Yagi., Gann., 1980, 71, 190.
8. Y. Ninomiya, M. Miwa, H. Eda, H. Sahara, K. Fujimoto, M. Ishida, I. Umeda, K. Yokose, H. Ishitsuku, Jpn. J., Cancer Res., 1990, 81, 188.
9. T. Tabata, M. Katoh, S. Tokudome, M. Hosakawa, K. Chiba, M. Nakajima, T. Yokoi., Drug Metab. Dispos., 2004, 32, 762.
10. T. Ishikawa, F. Sekiguchi, F. Yu, N. Sawada, H. Ishitsuka., Cancer Res., 1998, 58 685.
11. P. Compagnon, L. Thiberville, N. Moore, C. Thuillez, C. Lacroix., J. Chromatogr. B., 1996, 677, 383.
12. E. Gamelin, M. Boisdron-Celle, A. Turcant, F. Larra, P. Allain, J. Robert., J. Chromatogr. B., 1997, 695, 416.
13. W.E. Wung, S.B. Howell., Clin. Chem., 1980, 26, 1708.
14. A.R. Buckpitt, M.R. Boyd., Anal. Biochem., 1980, 106, 437.
15. F.P. LaCreta, W.M. Williams., J. Chromatogr., 1987, 414, 201.
16. W. Wattanatorn, H.L. McLeod, J. Cassidy, K.E. Kendle., J. Chromatogr. B., 1997, 692, 237.
17. M.J. Del Nozal, J.L. Bernal, A. Pampliega, P. Marinero, M. Pozuelo., J. Chromatogr. B., 1994, 656, 405.
18. M.J. Moore, P. Bunting, S. Yuan, J.J. Thiessen., Ther. Drug Monit., 1993, 15, 399.
19. N. Christophidis, G. Mihaly, F. Vajda, W. Louis., Clin. Chem., 1979, 25, 86.
20. L.J. Schaaf, D.G. Ferry, C.T. Hung, D.G. Perrier, I.R. Edwards., J. Chromatogr., 1985, 342, 313.
21. J. Escoriaza, A. Aldaz, E. Calvo, J. Giráldez., J. Chromatogr. B., 1999,736, 102.
22. L. Zuf´ıa, A. Aldaz, C. Castellanos, J. Giráldez., Ther. Drug Monit., 2003, 25, 228.
23. B. Reigner, J. Verweij, L. Dirix, J. Cassidy, C. Twelves, D. Allman,E. Weidekamm, B. Roos, L. Banken, M. Utoh, B. Osterwalder., Clin.Cancer Res., 1998, 4, 941.
24. L. Zufia, A. Aldaz, J. Giraldez., J. Chromatogr. B., 2004, 809, 51.
25. S.M. Guichard, I. Mayer, D.I. Jodrell., J. Chromatogr. B., 2005, 826, 232.
26. Y. Xu, J. Grem., J. Chromatogr. B., 2003, 783, 273.
27. Y. Xu, J.L. Grem., J. Chromatogr. B Anal. Technol. Biomed. Life Sci., 2003, 783, 273.
28. C. Siethoff, M. Orth, A. Ortling, E. Brendel, W. Wagner-Redeker., J. Mass Spectrom., 2004, 39, 884.
29. B. Reigner, J. Verweij, L. Dirix, J. Cassidy, C. Twelves, D. Allman, E. Weidekamm, B. M. Utoh, B. Osterwalder., Clin.Cancer Res., 1998, 4, 309.
30. Roos, L. Banken, M. Utoh, B. Osterwalder., Clin. Cancer Res., 1998, 4, 941.
31. B. Reigner, S. Clive, J. Cassidy, D. Jodrell, R. Schulz, T. Goggin, L. Banken, B. Roos, M. Utoh, T. Mulligan, E. Weidekamm., Cancer Chemother. Pharmacol., 1999, 43, 315
32. B. Reigner, S. Clive, J. Cassidy, D. Jodrell, R. Schulz, T. Goggin, L. Banken, B. Roos, M. Utoh, T. Mulligan, E. Weidekamm., Cancer Chemother. Pharmacol., 1999, 43, 309.
33. C. Siethoff, M. Orth, A. Ortling, E. Brendel, W. Wagner-Redeker., J. Mass Spectrom., 2004, 39, 884.
34. Mugunthu R. Dhananjeyan, Jidong Liu, Crystal Bykowski, Jill A. Trendel, Howard Ando, Paul W. Erhardt., J. Chromatogr: B., 2007, 1138, 101–108.

*****