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Stability Indicating UPLC Method for Quantifying Assay of Capecitabine

Pawar Geetanjali Raosaheb[1], Dr. Sourobhi Datta[2]

[1]Research Scholar, Monad University, Hapur

[2]Professor, Monad University, Hapur

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Address for Correspondence: Pawar Geetanjali Raosaheb

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Abstract

Capecitabine, a prodrug of 5-fluorouracil, is widely used in the treatment of various cancers, including colorectal, breast, and gastric cancers. Ensuring the stability and accurate quantification of capecitabine is crucial for its efficacy and safety in clinical practice. In this study, a stability indicating Ultra Performance Liquid Chromatography (UPLC) method was developed and validated for the quantitative determination of capecitabine in pharmaceutical formulations. The UPLC method utilized a reverse-phase C18 column with a mobile phase consisting of acetonitrile and 0.1% formic acid in water, delivered in an isocratic mode at a flow rate of 0.4 mL/min. Detection was performed at a wavelength of 220 nm. The stability indicating capability of the method was demonstrated through forced degradation studies under various stress conditions, including acidic, basic, oxidative, thermal, and photolytic stress. Capecitabine remained stable under all stress conditions, with no significant degradation observed. Additionally, the method showed good selectivity, specificity, precision, accuracy, and robustness in the quantification of capecitabine. Overall, the developed UPLC method provides a reliable and sensitive approach for the quantitative determination of capecitabine in pharmaceutical formulations. Its stability indicating nature ensures the accurate assessment of capecitabine stability and potency, thereby facilitating quality control and assurance in the production and use of capecitabine-based medications.

Keywords: Clinical, Pharmaceutical, Acetonitrile, Formic Acid, Quantification.

Introduction

Capecitabine is a chemotherapeutic drug that is taken orally and is used to treat malignancies of the colon and breast that have spread to other parts of the body. Upon conversion to 5-fluorouracil in the tumour, the prodrug capecitabine blocks DNA synthesis and decreases tumour development. A solid with an off-white colour, it is insoluble in water but soluble in acetonitrile, alcohol, and pentyl l-(5-deoxy-b-Dribofuranosyl)-5-fluoro-l, 2-dihydro-2-oxo-4-pyrimidinecarbamate (with a molecular weight of 395.34 g/mol).

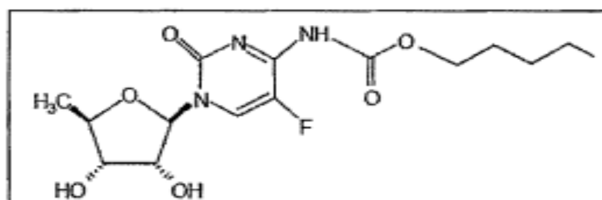
Development of a fast LC method for the determination of assay of Capecitabine:

Creative Organics of Bangalore provided the capecitabine API with a purity of 99.9% and its impurities with a purity of >99.0%. The Merck Company supplied the acetonitrile and methanol, while Qualigens supplied the glacial acetic acid. The Millipore Milli-Q plus water purification system is used to create water of very high purity.

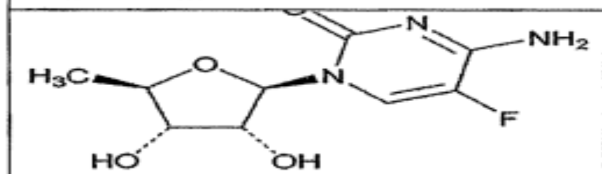
High-performance liquid chromatography:

A photodiode array detector (PDA), auto sampler, column oven, and quaternary gradient pump were all components of an Agilent high-performance liquid chromatography system (USA) used for the study. The data for the chromatography was collected using the Empower 2 programme.

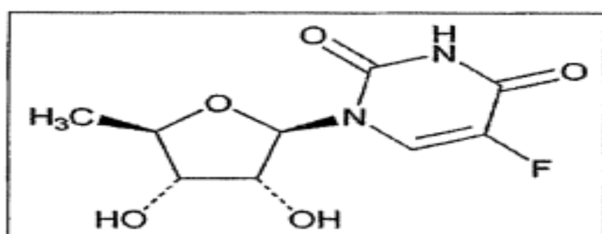
Chemical structure of Capecitabine and its impurities:



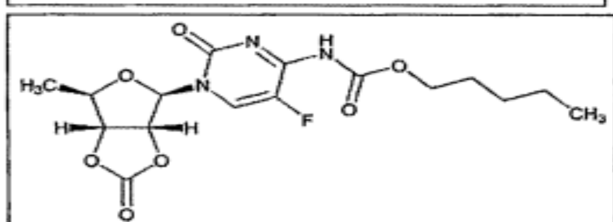
Compound name : Capecitabine
Chemical name : 5'-Deoxy-5-fluoro-N4-pentyloxycarbonyl-cytidine
Molecular formula : $C_{15}H_{22}FN_3O_6$
Molecular weight : 359.34 g/mol



Impurity name : Capecitabine related compound A
Chemical name : 5'-Deoxy-5-fluorocytidine
Molecular formula : $C_9H_{12}FN_3O_4$
Molecular weight : 245.21 g/mol



Impurity name : Capecitabine related compound B
Chemical name : 5'-Deoxy-5-fluorouridine.
Molecular formula : $C_9H_{11}FN_2O_5$
Molecular weight : 246.19 g/mol



Impurity name : Capecitabine Related Compound C
Chemical name : 2',3'-*O*-Carbonyl-5'-deoxy-5-fluoro-N_4-(pentyloxycarbonyl) Cytidine
Molecular formula : $C_{16}H_{20}FN_3O_7$
Molecular weight : 385.34 g/mol.

Ultra performance liquid chromatography:

For the analysis, a binary gradient pump, auto sampler, column oven, and photodiode array detector (PDA) were all parts of a Waters Acquity UPLC system (Waters, USA). In this investigation, chromatographic data was collected using Empower 2 software. The analysis was conducted using a Millipore Milli Q Plus water purification system, and a Metier balance.

Column: POROSHELL C18 column of dimension 4.6 X 50 mm, 2.7 um

METHOD DEVELOPMENT AND OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

A basic mixture for the mobile phase A mixture of acetonitrile, methanol, and 0.1% acetic acid in water made up mobile phase-A. The proportions of these three components were 11:2:7. For the separation of Capecitabine and its related compounds, the mobile phase-B was used as follows: 0.1% acetic acid in water, acetonitrile, and methanol at a ratio of 4:1:15. The isocratic technique was first used, however the impurity resolution was not achieved. Then, after 6.0 minutes of using the basic gradient approach, we saw that all the components were clearly separated. Capecitabine may be somewhat ionised, since its pKa value is 5.41, which is within ±1.5 units of pKa. If the chemical is outside of this range, it is ionised or it is not. For this investigation, we used an Agilent high strength POROSHELL Cl8 column with the following specifications: 50 mm length, 4.6 mm diameter, and 2.7 pm particle size minimum. The pH range in which this column is useful is 2 to 8.

The chemicals' solubility and compatibility with the mobile phase determine the diluent selection. The sample diluent for the analysis was chosen to be a mixture of water, acetonitrile, and methanol in the proportion of 11:2:7. By keeping the diluent's composition near to the mobile phase's starting gradient composition in the gradient of the technique, interferences, base line drift, and upset are eliminated.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Below are the ideal chromatographic conditions for the recently created procedure. A mixture of acetonitrile, methanol, and 0.1% acetic acid in water made up mobile phase-A. The proportions of these three components were 11:2:7. There was a 4:1:15 ratio of acetonitrile, methanol, and mobile phase-B, which consisted of 0.1% acetic acid in water. The mobile phase was ultrasonically degassed and filtered through a 0.2 pm PTFE filter before use. In a ratio of 11:2:7, the sample diluent consisted of water, acetonitrile, and methanol. The solvent composition of the weak needle wash and seal wash was 8 parts Milli-Q water to 2 parts acetonitrile. The strong needle wash was made using a mixture of 2 parts Milli-Q water and 8 parts acetonitrile. We used a flow rate of 0.70 mL min'1 and a linear gradient condition to conduct the analysis. Table 4.1 details the mobile phase's gradient composition. There has a total running duration of six minutes.

Fast, powerful, and agile the analysis was conducted using a POROSHELL Cl8 column with the following dimensions: 50 mm length, 4.6 mm diameter, and 2.7 pm particle size. The temperature of the sample compartment was kept at ambient, whereas the temperature of the column was fixed at 30°C. At a wavelength of 250 nm, chromatograms were formed. The needle was overfilled with an injection volume of 1 opL in a partial loop. Twenty points per second was the detection sampling rate. There was a 1.2 nm resolution in the spectrum. The filter time constant was set at auto/sec and the mode was remained at usual.

Table 3.1: Gradient program

Time (minute)	Solution A	Solution B
0.00	100	40
4.00	100	40
4.80	45	65
5.20	45	65
6.00	100	40

ASSAY STANDARD PREPARATION (DUPLICATE):

Standard preparation 1 and standard preparation 2 were made by accurately weighing and quantitatively transferring 30.0 mg of Capecitabine reference standard into a 50 ml standard volumetric, dissolved flask. Then, the flask was filled to volume with diluent, which is composed of water, acetonitrile, and methanol in a ratio of 11:2:7. Finally, the flask was filtered through a 0.22um syringe to obtain a concentration of 0.60 mg/ml.

Assay sample preparation (duplicate):

Similarly, using a precise scale, transfer 30.0 mg of Capecitabine into a 50 ml standard volumetric flask. Dissolve and dilute with diluent until the flask is filled to the top. Filter the mixture through a 0.22um syringe to get a concentration of 0.60 mg/ml. Both preparations are labelled.

Table 3.2: Retention time and relative retention time of impurity peaks

Name of the Analyte	~ RT (min)	RRT
Related comp. A*	0.77	0.22
Related comp. B*	0.84	0.24
Related comp. C*	3.89	1.13
Capeeitabine*	3.45	1.00

Determine the percentage of Capeeitabine in the sample by using the following formula.

$$\% \text{ Assay (on dried basis)} = \frac{Au}{As} \times \frac{Ws \times f}{50} \times \frac{50}{Wu} \times \frac{(100 - Ms)}{(100 - Mu)} \times 100$$

Here, Au represents the mean peak area of Capeeitabine in the chromatogram of the collected sample, The standard chromatograms show the mean peak area of Capeeitabine, as Wu denotes the sample weight in milligrammes, whereas Ws denotes the weight of the Capeeitabine reference standard in milligrammes. In this context, f stands for the standard's potency factor, Ms for the sample's water content, and Mu for the standard's water content.

Degradation results:

An initial drug concentration of 0.60 mg/ml was used for all stress breakdown experiments, including acid, base, and peroxide. A literature review is used to choose the deterioration conditions. The following tables display the findings.

3.10.1 Degradation in acidic solution:

Using a 250 ml round-bottom flask, 30 mg of sample was added to 5 ml of the degradation agent, which is a 1M HC1 solution. The flask was then heated for 2 hours to conduct the acid degradation analysis. After cooling, neutralise with 5 ml of a 1M NaOH solution. Transfer to a 50 ml volumetric flask, add diluent until the flask is filled too full. Filter through a 0.22um syringe to get a concentration of 0.6 mg/ml.

Table 3.8: Acid degradation results

S. No	Name	Retention Time (min)	Purity Angle	Purity Threshold
1	Capecitabine Rel. com. A	0.78	0.56	0.84
2	Unknown	2.85	30.14	45.58
3	Capecitabine	3.61	0.184	0.405

3.10.2 Degradation in basic solution:

We heated a 250 ml round-bottom flask containing 30 mg of material for 2 hours before adding 5 ml of a 1M NaOH solution, which acts as a degradation agent. In order to get a concentration of 0.6 mg/ml, the mixture was chilled and neutralised with 5 ml of a 1M HC1 solution. It was then transferred to a 50 ml volumetric flask, diluted to volume with diluent, and filtered through a 0.22 um syringe. The chromatogram that resulted from injecting this solution into the UPLC system is shown below figure 3.8.

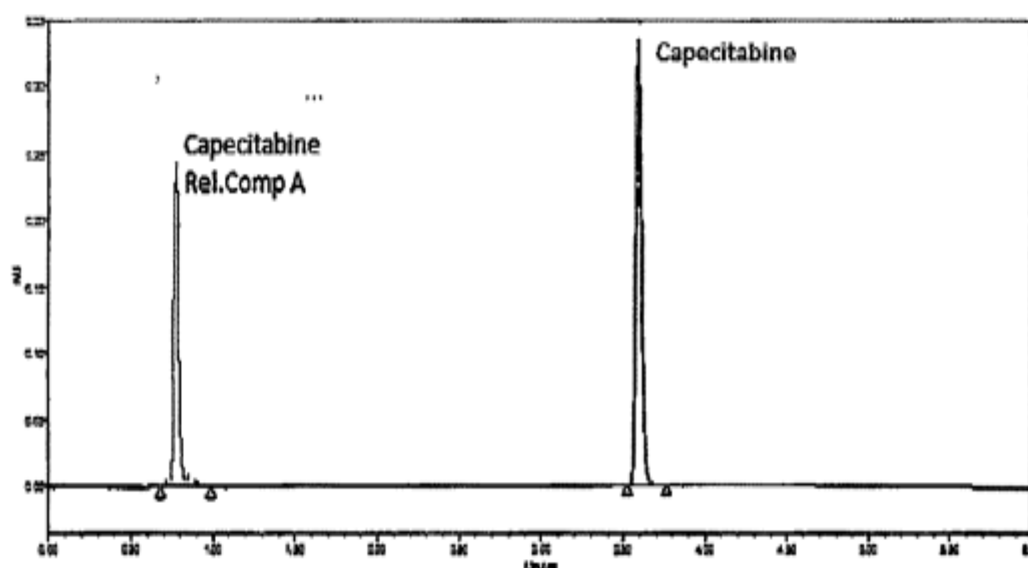


Fig: 3.8: CAPECITABINE BASE DEGRADATION CHROMATOGRAM

Table 3.9: Base degradation results

S. No	Name	Retention Time (min)	Purity Angle	Purity Threshold
1	Capecitabine Rel. com. A	0.76	0..96	1.26
2	Capecitabine	3.60	0.061	0.293

3.10.3 Degradation in peroxide solution:

The peroxide degradation analysis was conducted by placing 30 mg of the sample into a 250 ml round bottom flask, adding 5 ml of a 10% H2O2 solution as the degradation agent, and then heating the mixture for 2 hours. The solution was thereafter chilled and transferred to a 50 ml standard volumetric flask. It was then diluted to volume using diluent and filtered through a 0.22um syringe to achieve a concentration of 0.6 mg/ml. Figure 3.9 shows the resultant chromatogram, which was obtained by injecting this solution into a UPLC chromatographic machine.

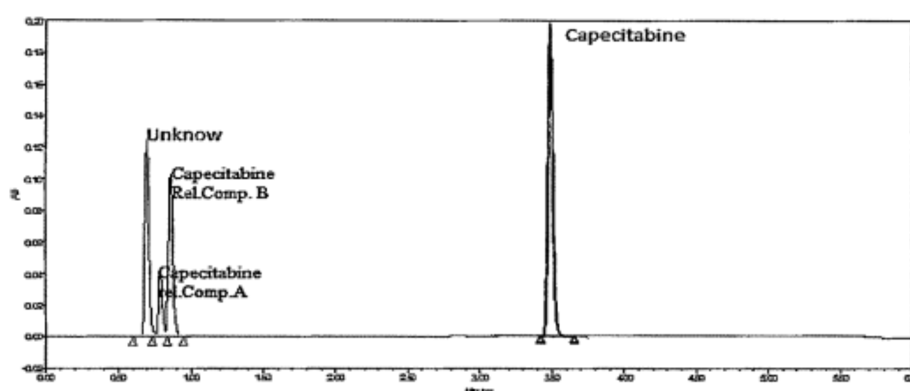


Fig: 3.9: CAPECITABINE PEROXIDE DEGRADATION CHROMATOGRAM

Table 3.10: Peroxide degradation results

S. No	Name	Retention Time (min)	Purity Angle	Purity Threshold
1	Unknown	0.56	3.15	5.18
2.	Capecitabine Rel. Com. A	0.76	10.47	15.16
3	Capecitabine Rel. Com. B	0.85	6.85	11.78
4.	Capecitabine	3.56	2.58	8.18

All things considered, the findings show that the RP-UPLC technique that was created is stable, which means that it has one.

Comparison study of chromatographic techniques:

Through the injection of a system suitability solution, data comparing the chromatographic performances of HPLC and UPLC have been collected. It was determined that 10 jil and 1 pi, respectively, were the injection volumes for HPLC and UPLC. Compared to the traditional HPLC approach using 5-micron columns, the elution time of all medicinal substances in the UPLC method was shown to be five times shorter. If we had used a 2.7-micron column instead, the analysis time would have been four to five times shorter than using the HPLC technique, according to van Deemter curves. When compared to HPLC, the resolution achieved by UPLC for all of the medicinal substances included in this investigation was noticeably superior. Because to its superior resolving power and gradient separation

efficiency, UPLC has a greater peak capacity than HPLC. Table-3.11 displays the performance metrics for both systems.

In these ideal circumstances, there was no tailing and good resolution between the analyte peak and the peaks of the contaminants. All of the peaks had tailing factors less than 2.0. While the HPLC approach yielded a nominal retention time of 18.31 minutes at a flow rate of 1.0 mL/min for Capecitabine, the novel UPLC method yielded a value of 3.45 millimetres at a flow rate of 0.70 mL/min. Figure 3.10 and figure 3.11 show the typical chromatograms produced from the final HPLC and UPLC technique settings, respectively.

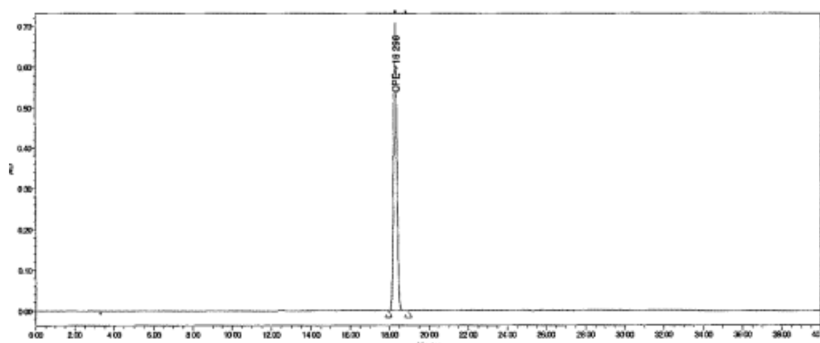


Figure 3.10: Capecitabine assay sample HPLC chromatogram

To further comprehend the elution and run duration of the HPLC procedure, figure 3.10 l provides an HPLC chromatogram of Capecitabine.

Table 3.11 Gradient program of HPLC method

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	100	100
5	100	100
20	49	51
30	49	51
31	100	0
40	100	0

3.11.2 Optimized Chromatographic Conditions for Determination of Assay of Capecitabine by UPLC

Table 3.12 Gradient program of UPLC method

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.00	100	100
4.00	100	100
4.80	45	55
5.20	45	55
6.00	100	0

Figure 3.11 and Figure 3.12 illustrate the UPLC chromatograms for the assay preparation and spiking sample preparation of Capecitabine, respectively. These chromatograms explain the elution of contaminants and the run duration of the newly designed UPLC technique.

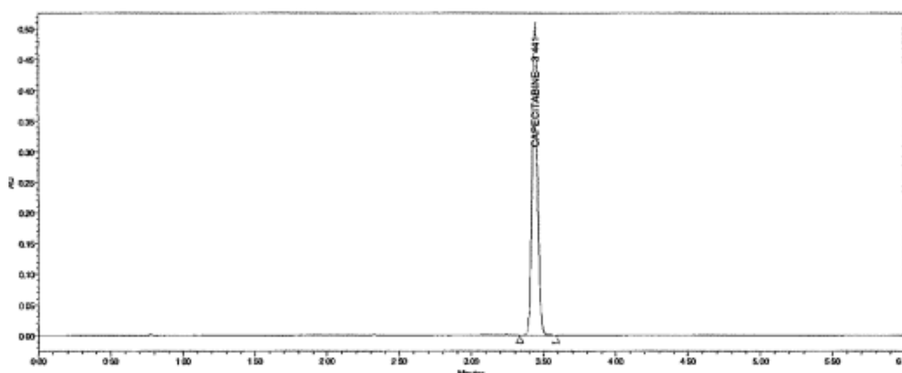


Figure 3.11: Capecitabine assay sample chromatogram by UPLC

Table 3.13: Comparison of system suitability data by UPLC and HPLC

Name of drug component	Retention Time(min)		USP Resolution		USP Tailing		USP Plate Count	
	UPLC	HPLC	HPLC	UPLC	HPLC	UPLC	HPLC	UPLC
Capecitabine Rel. comp.A	0.77	3.15	*NA	*NA	1.4	1.0	6156	9169
Capecitabine Rel. comp.B	0.84	3.49	2.0	3.0	1.3	1.0	6310	9001
Capecitabine	3.45	18.31	62.0	65.4	1.1	1.1	30045	37547
Capecitabine Rel. comp.C	3.89	20.20	6.2	8.2	1.2	1.1	45237	53365

***NA= as it is first peak**

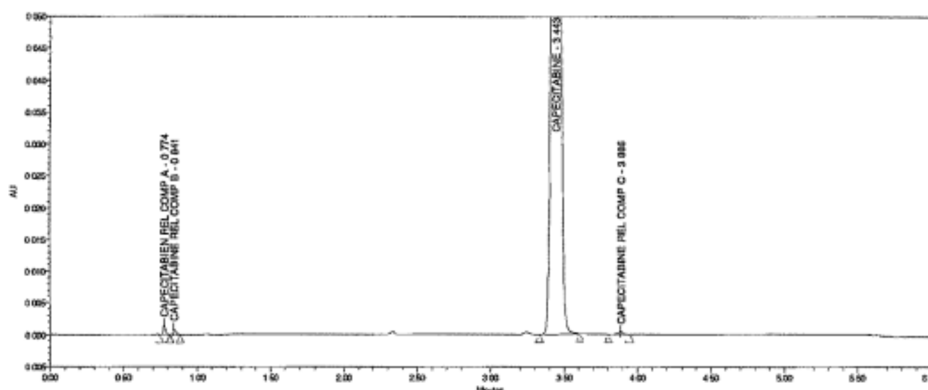


Figure 12 Capecitabine UPLC Spiked sample chromtogram

3.12. Comparative analysis:

We used both the old HPLC technique and the new UPLC method for the experiment to choose three batches in a row. The findings may be seen in table 3.14, which compares the HPLC technique to the newly created method.

Table 3.14 comparative assay results

Component	Batch no. 01		Batch no. 02		Batch no. 03	
	HPLC	UPLC	HPLC	UPLC	HPLC	UPLC
%Assay	99.4	99.6	99.6	99.8	99.5	99.7

In order to quantify Capecitabine, a novel stability-indicating UPLC technique was developed and validated. The samples were subjected to stress testing according to the degradation parameters specified by the ICH. Quick, exact, accurate, and selective is the UPLC technique that was created for the determination of Capecitabine's test. All of the

method validation parameters were satisfied, indicating full method validation. Since the peaks of the breakdown products did not interfere with the primary drug peak, this approach was seen as being "specific" to the bulk active medicinal component. Therefore, Capecitabine bulk drug samples from ordinary manufacture and samples under stability may be assayed using the suggested approach.

Conclusion

The development and validation of a stability indicating Ultra Performance Liquid Chromatography (UPLC) method for quantifying the assay of capecitabine have been successfully achieved. The method demonstrated excellent linearity, sensitivity, selectivity, precision, accuracy, and robustness, making it suitable for routine analysis of capecitabine in pharmaceutical formulations. The stability indicating capability of the method was confirmed through forced degradation studies, where capecitabine remained stable under various stress conditions, including acidic, basic, oxidative, thermal, and photolytic stress. This indicates that the developed UPLC method is capable of accurately quantifying capecitabine while simultaneously detecting and separating its degradation products.

The method offers several advantages, including rapid analysis time, high sensitivity, and the ability to analyze complex matrices. Furthermore, its stability indicating nature ensures the reliable assessment of capecitabine stability and potency, contributing to quality control and assurance in the production and use of capecitabine-based medications. Overall, the developed UPLC method represents a valuable analytical tool for pharmaceutical companies, regulatory agencies, and research laboratories involved in the analysis, formulation, and quality control of capecitabine-containing products. It provides a robust and reliable approach for quantifying the assay of capecitabine, thereby ensuring the safety and efficacy of this important chemotherapeutic agent in clinical practice.

References

1. Irena Kasagić-Vujanović et.al “Quality by Design oriented development of hydrophilic interaction liquid chromatography method for the analysis of amitriptyline and its impurities” Journal of Pharmaceutical and Biomedical Analysis Volume 173, 5 September 2019, Pages 86-95
2. Useni Reddy MALLU et.al “Rapid Stability Indicating HPLC Method for the Analysis of Leflunomide and Its Related Impurities in Bulk Drug and Formulations” Turk J Pharm Sci 2019;16(4):457-65 DOI: 10.4274/tjps.galenos.2018.34635
3. Mannemala, Sai & Krishnan, Nagarajan. (2015). Development and validation of a generic liquid chromatographic method for the simultaneous determination of five commonly used antimalarial drugs: Application to pharmaceutical formulations and human plasma: Liquid Chromatography. Journal of separation science. 38. 10.1002/jssc.201401465.
4. Gałuszka, Agnieszka & Migaszewski, Zdzisław & Namieśnik, Jacek. (2013). The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. TrAC Trends in Analytical Chemistry. 50. 10.1016/j.trac.2013.04.010.
5. Jeremie, Mbinze & Yemoa, Achille & Lebrun, Pierre & Sacré, Pierre-Yves & Habyalimana, Vedaste & Kalenda, Nicodème & Bigot, André & Atindehou, Eugène & Hubert, Philippe & Marini, R.D. & Marini. (2015). Fighting Poor Quality Medicines: Development, Transfer and Validation of Generic HPLC Methods for Analyzing Two WHO Recommended Antimalarial Tablets. American Journal of Analytical Chemistry. 6. 127-144. 10.4236/ajac.2015.62012.
6. Amigo, José & Babamoradi, Hamid & Elcoroaristizabal, Saioa. (2015). Hyperspectral Image Analysis. A tutorial. Analytica Chimica Acta. X. X. 10.1016/j.aca.2015.09.030.
7. Rodionova, Oxana & Titova, Anna & Pomerantsev, Alexey. (2016). Discriminant analysis is an inappropriate method of authentication. TrAC Trends in Analytical Chemistry. 78. 10.1016/j.trac.2016.01.010.
8. Degardin, Klara & Guillemain, Aurélie & Guerreiro, Nicole & Roggo, Yves. (2016). Near Infrared spectroscopy for counterfeit detection using a large database of pharmaceutical tablets. Journal of

Pharmaceutical and Biomedical Analysis. 128. 10.1016/j.jpba.2016.05.004.

9. Mazivila, Sarmento & Páscoa, Ricardo & Castro, Rafael & Ribeiro, David & Santos, João. (2020). Detection of melamine and sucrose as adulterants in milk powder using near-infrared spectroscopy with DD-SIMCA as one-class classifier and MCR-ALS as a means to provide pure profiles of milk and of both adulterants with forensic evidence: A short communication. Talanta. 216. 10.1016/j.talanta.2020.120937.
10. Deidda, Riccardo & Sacré, Pierre-Yves & Clavaud, Matthieu & Coic, Laureen & Avohou, Hermane & Hubert, Philippe & Ziemons, Eric. (2019). Vibrational spectroscopy in analysis of pharmaceuticals: Critical review of innovative portable and handheld NIR and Raman spectrophotometers. TrAC Trends in Analytical Chemistry. 114. 10.1016/j.trac.2019.02.035.
11. Yabre, Moussa & Ferey, Ludivine & Sakira, Abdoul & Bonmatin, Camille & Fauré, Clotilde & Some, Touridomon & Gaudin, Karen. (2020). Green Analytical Methods of Antimalarial Artemether-Lumefantrine Analysis for Falsification Detection Using a Low-Cost Handled NIR Spectrometer with DD-SIMCA and Drug Quantification by HPLC. Molecules. 25. 3397. 10.3390/molecules25153397.
12. Fadhel, Sahar & Khalil, Shaymaa. (2018). Simultaneous Estimation of Chlorpromazine hydrochloride and Carvedilol in Bulk and Pharmaceutical Dosage Forms Using HPLC. 5-9.
13. Hameed, Eman & El-Naby, Zaitona & Gindy, Alaa & Zaitone, Sawsan & Alshaman, Reem & Saraya, Roshdy & Khairy, Gasser. (2022). Two New HPLC Methods, Assessed by GAPI, for Simultaneous Determination of Four Antipsychotics in Pharmaceutical Formulations: A Comparative Study. Separations. 9. 220. 10.3390/separations9080220.
14. Kumar, Lalit & Reddy, M. Sreenivasa & Managuli, Renuka & Pai, K.. (2015). Full Factorial Design for Optimization, Development and Validation of Hplc Method to Determine Valsartan in Nanoparticles. Saudi Pharmaceutical Journal. 23. 549-555. 10.1016/j.jsps.2015.02.001.
15. Managuli, Renuka & Kumar, Lalit & Chonkar, Ankita & Shirodkar, Rupesh & Lewis, Shaila & Koteshwara, Kunnatur & Reddy, Meka & Mutalik, Srinivas. (2016). Development and Validation of a Stability-Indicating RP-HPLC Method by a Statistical Optimization Process for the Quantification of Asenapine Maleate in Lipidic Nanoformulations. Journal of Chromatographic Science. 54. bmw062. 10.1093/chromsci/bmw062.