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New Validated Method for the Estimation of Capecitabine and Docetaxel Using RP-UPLC

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Authors' contributions

This work was carried out in collaboration between both authors. Author KR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author PRD managed the analyses of the study, managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Study on degradation of Capecitabine and Docetaxel based on a new, validated method using UPLC.
Place and Duration of Study: Department of Chemistry, RVR & JC College of Engineering, Chowdavaram, Guntur, Andhra Pradesh, between February 2021 and August 2021.
Methodology: With a flow rate of 1 mL/min and a wave length of 255 nm, the proposed method successfully separated the target metabolite using a Symmetry C_{18} column (150 mm x 4.6mm,3.5 µm), acetonitrile, and 0.1 percent ortho phosphoric acid (OPA) as the mobile phase. The retention times for Capecitabine and Docetaxel were 1.223 minutes and 1.864 minutes, respectively. The isocratic chromatography procedure took about ten minutes to complete at room temperature.
Results: The analysis was completed in three minutes with a concentration range of 5-75 µg/mL capecitabine and 2-30 µg/mL docetaxel that was honest in linearity. The system's suitability parameters were examined mathematically, and the outcomes fell within acceptable ranges. Stages with regression coefficients of 0.999 were used in the linear analysis. Capecitabine's LOD and LOQ concentrations were 1.5 µg/mL and 5 µg/mL, respectively, while Docetaxel was 0.6

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µg/mL and 2 µg/mL. 98-102% of the drug was recovered, which means the recovery was within acceptable limits.
Conclusion: The approach was found to be suitable for bulk and formulation analysis after the validation results were satisfactory. According to ICH guidelines, the recommended procedure was found to be justified.

Keywords: Anti-cancer drugs; development; validation; RP-UPLC; ICH guidelines.

1. INTRODUCTION

Capecitabine, marketed with a brand name of Xeloda, among others is a chemotherapy [1,2] drug utilized to treat breast cancer [3,4], gastric cancer [5,6] and colorectal cancer [7]. It is also used along with docetaxel for breast cancer. It's swallowed whole. Stomach pain, vomiting, diarrhea, fatigue, and a rash are all common side effects of taking this medication. Blood clotting problems [8], allergic reactions [9], heart problems like cardiomyopathy [10], and low blood cell counts are all serious side effects. People with kidney issues should avoid it. Use during pregnancy has the potential to be harmful to the unborn child. In the body, Capecitabine is transformed into 5-fluorouracil (5-FU), which gives it its action. A member of the fluoropyrimidine drug class, it is related to 5-fluorouracil and tegafur [11].

Chemotherapy drug docetaxel, also known as Taxotere, is used to treat various types of cancer. Breast cancer, head and neck cancer [12,13], stomach cancer, prostate cancer [14], and non-small cell lung cancer [15] are among the more common types of cancer [12,13]. Chemotherapy drugs can be used alone or in combination with this one. A slow injection into a vein is used to deliver the medication to the patient. Hair loss, cytopenia, numbness [16], shortness of breath, vomiting, and muscle pain are all common side effects. Allergies and the development of new cancers are other potentially harmful side effects. People with liver issues [17,18] are more likely to experience side effects [17,18]. If used during pregnancy, the foetus may be harmed. Docetaxel belongs to the taxane [19] family of drugs. It stops cell division by interfering with microtubules [20, 21] normal function. in Fig. 1, Capecitabine and Docetaxel's chemical structures are depicted (https://en.wikipedia.org › wiki › Capecitabine and https://en.wikipedia.org › wiki › Docetaxel.

To date, there have been no UPLC methods for Capecitabine and Docetaxel estimation. Thus, this study aimed to predict Capecitabine and Docetaxel, which is a pharmaceutical component, using RP-UPLC.

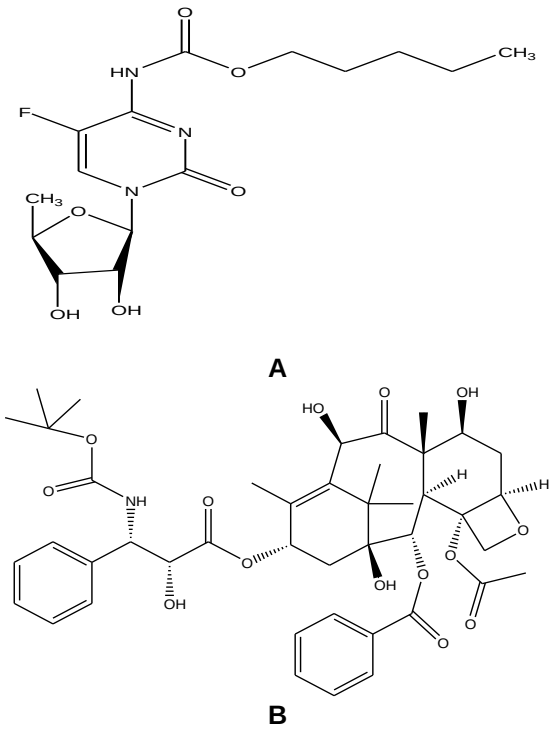


Fig. 1. Chemical structure of (A) Capecitabine and (B) Docetaxel

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Water, ortho phosphoric acid, and acetonitrile were purchased from Merck India Ltd in Mumbai, India for use in the UPLC analysis. Glenmark, Mumbai, provided APIs for Capecitabine and Docetaxel standards.

2.2 Equipment

Waters Acquity model UPLC with quaternary pump, PDA detector with empower 2.0 software was used.

2.3 Chromatographic Conditions

To conduct chromatography using isocratic conditions, an Symmetry C_{18} (150 mm x 4.6 mm, 3.5 µ) column was used at temperature using a Chromatographic conditions separation was administered in isocratic mode at temperature employing Symmetry C_{18} (150 mm x 4.6 mm, 3.5 µm) column. Ortho phosphoric acid (0.1%) and acetonitrile (60:40 v/v) with a flow rate of 1 mL/min were used as a mobile phase in this experiment. Injection volume was 10 µl, and the eluent was found at 255 nm, as the maximum concentration of Capecitabine and Docetaxel were found at this wavelength. So, it was decided to use the wave length of 255 nm.

2.4 Preparation of the Standard Stock Solution

For standard stock solution preparation, add 70 mL of diluents to 50mg of Capecitabine and 20 mg of Docetaxel taken in a 100 mL volumetric flask and sonicate for 10 minutes to fully dissolve the contents and then make up to the mark with diluent.

2.4.1 Preparation of Standard solution

A volume of 5 mL of solution is drawn from the above normal stock solution into a 50mL volumetric flask and diluted up to the level.

3. RESULTS AND DISCUSSION

Method optimization: Different phosphate buffer to acetonitrile ratios in the mobile phase with isocratic mode were tested to optimise the chromatographic conditions. However, the composition of the mobile phase was altered at each trial to improve the resolving power and also to achieve tolerable storage times. As a result, it was decided to use an OPA buffer and acetonitrile solution with isocractic elution of 0.1% each. A variety of stationary phases, such as phenyl and amino C18 phenyl and amino inertsil ODS columns, were tested during method optimization. These tests revealed that peak shapes with a 150 x 4.6mm, 3.5 µm Symmetry C_{18} column were good. To obtain adequate sensitivity, the PDA detector was set at 255 nm. By using above conditions we get retention times of Capecitabine and Docetaxel were about 1.2 and 1.8 min with a tailing factor of 1.12 & 1.04. The number of theoretical plates for Capecitabine and Docetaxel were 4272,3199 which indicate the column's successful output the % RSD for six replicate injections was around 0.58% and 1.23%, the proposed approach suggests that it is extremely precise. The established method was validated in accordance with ICH guidelines.

3.1 Specificity

The placebo test process was used to evaluate the interference between the sample and standard solutions. Because of this, there was no placebo effect on the main peak, as shown in the following graph. The approach is also precise. Blank chromatogram was shown in Fig. 2 (from Empower 2.0 software).

3.2 System Suitability

Stabilization was performed for 60 minutes to encourage a constant bottom line. The system suitability was checked by dispensing six Capecitabine and Docetaxel-branded injections, which each contained 50 µg/mL of Capecitabine and 20 µg/mL of Docetaxel, and assessing the results. To gather the data like plate count and tailing factor all the data, the chromatography software will be utilized (Empower 2.0). Fig. 3 (from Empower 2.0 software) shows Standard chromatogram and Table 1 gives system precision results.

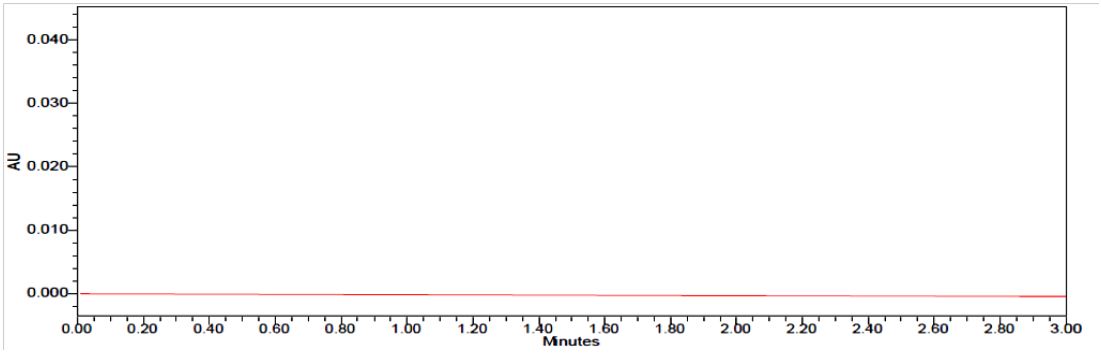


Fig. 2. Chromatogram of blank

Table 1. Results of system suitability

System suitability parameter	Acceptance criteria	Drug name	
		Capecitabine	Docetaxel
USP Plate count	NLT 2000	4272	3199
USP Tailing	NMT 2.0	1.12	1.04
USP Resolution	NLT 2.0	-	3.55
% RSD	NMT 2.0	0.58	1.23

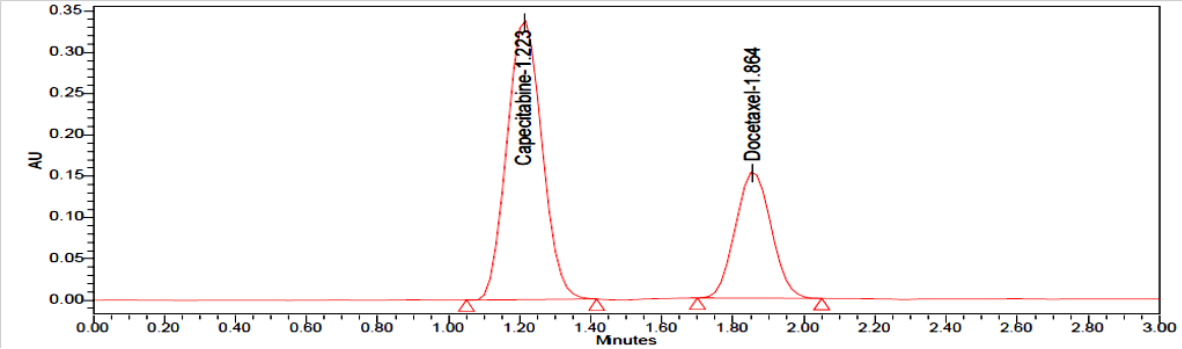


Fig. 3. Chromatogram of standard

3.3 Linearity

The linearity peak area versus different dilutions were evaluated for Capecitabine and Docetaxel at 25, 50, 75, 100, 125 and 150 percent respectively. Linear regression analysis was plotted using peak area versus concentration data. Correlation coefficients of regression, inclination and y-intercept of calibration curves have been determined. From the calibration curve, the correlation coefficients were higher than 0.999 for both the drugs. Linearity results were tabulated in Table 2. The calibration plots (from excel calculation sheet) of Capecitabine and Docetaxel, were shown in Fig. 4. The values of slope, intercept and correlation coefficient were acquired from the linearity calculation sheet.

3.4 Limit of Detection and Quantification

The calibration curve technique was used to determine LOD and LOQ separately. As standard solution concentrations decreased, the RP-UPLC method was used to determine the LOD and LOQ of the compound. There were 1.5 µg/mL and 5 µg/mL LOD and LOQ concentrations of capecitabine with s/n values of 7 and 26, respectively. 0.6 µg/mL and 2 µg/mL of Docetaxel were used as the LOD and LOQ concentrations, respectively and the s/n values were 4, 23.

3.5 Method Precision

The analytical technique's precision is determined by how closely the measurements from multiple homogeneous mixture samplings are spaced. The accuracy of the process of the drugs were calculated by injection of six individual determinations of Capecitabine (50 µg/mL) and Docetaxel (20µg/mL). Method precision results were shown in Table 3 and sample chromatogram was shown in Fig. 5 (from Empower 2.0 software).

3.6 Accuracy

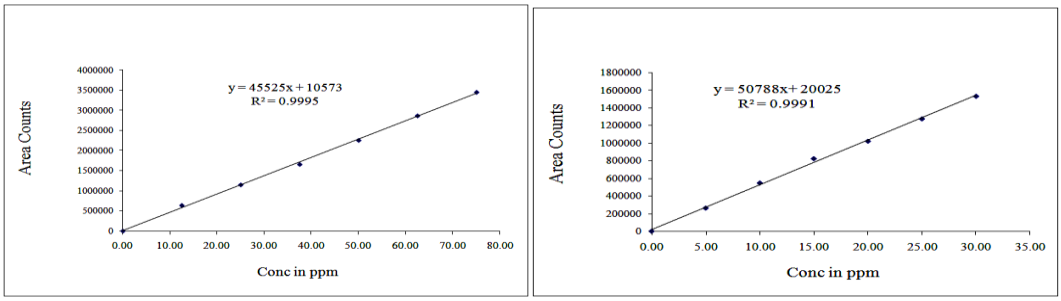
Accuracy was conducted in triplicate by testing the solution of the active pharmaceutical ingredient sampled with known concentrations of drugs at three concentration levels of 50, 100 and 150 percent of each at a specified maximum. For the drugs percentage of recovery was assessed and found to be within the limit. Accuracy results were tabulated in Table 4.

3.7 Ruggedness

Six replicates of the standard solution were analyzed bydifferent researchers and different tools were checked on separate days. The peak regions used to assess the average percent of RSD values have been determined. The findings are shown in the Table 5.

Table 2. Results of linearity

Linearity	Capecitabine		Docetaxel	
	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area
Linearity-1	12.50	638415	5.00	263320
Linearity-2	25.00	1147456	10.00	547956
Linearity-3	37.50	1658742	15.00	824786
Linearity-4	50.00	2256284	20.00	1023654
Linearity-5	62.50	2868745	25.00	1278462
Linearity-6	75.00	3454810	30.00	1534810
Slope	45525.48		50788.66	
Intercept	10573.36		20025.57	
CC	0.9995		0.9991	



A B

Fig. 4. Calibration plots of (A) Capecitabine and (B) Docetaxel

Table 3. Results of Method precision

S. No.	Capecitabine			Docetaxel		
	Conc. (µg/mL)	Area	% Assay	Conc. (µg/mL)	Area	% Assay
1	50	2289315	100.9	20	1061153	101.9
2		2228941	98.2		1052820	101.1
3		2264650	99.8		1022189	98.2
4		2290843	101		1032461	99.1
5		2279315	100.4		1039153	99.8
6		2249421	99.1		1042189	100.1
% RSD	1.10			1.34		

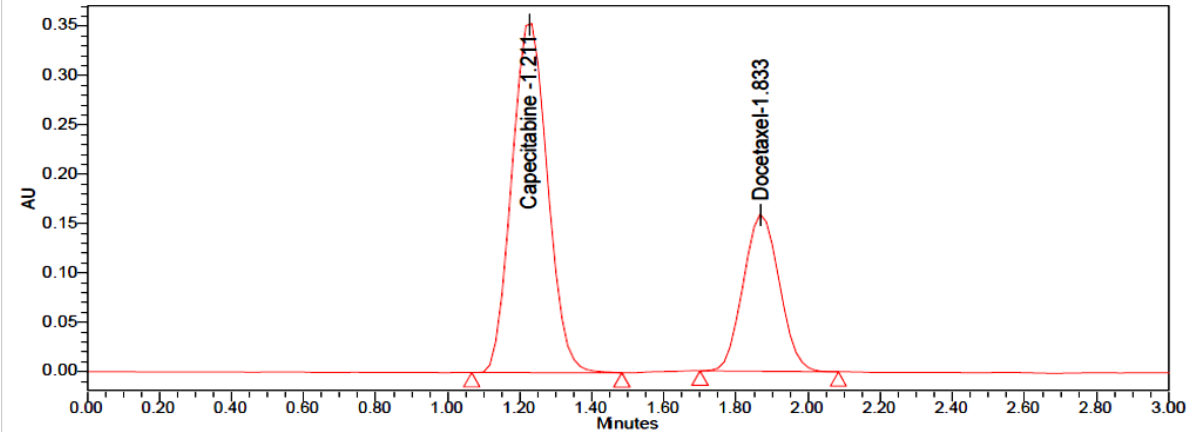


Fig. 5. Chromatogram of sample

Table 4. Results of accuracy

S. No.	% Level	Capecitabine % Recovery	Docetaxel % Recovery
1	50*	100.5	100.9
2	100*	100.5	101.3
3	150*	99.4	99.9

** Results are mean recovery of three sample preparations*

Table 5. Results of intermediate precision

S. No.	Capecitabine			Docetaxel		
	Conc. (µg/mL)	Area	% Assay	Conc. (µg/mL)	Area	% Assay
1	50	2280232	100.5	20	1050853	100.7
2		2291692	101.0		1062148	101.8
3		2276863	100.3		1056443	101.3
4		2263501	99.7		1042918	100.0
5		2236882	98.6		1032645	99.0
6		2257840	99.5		1041448	99.8
%CV	0.85			1.03		

Table 6. Results of robustness

Parameter name	% RSD	
	Capecitabine	Docetaxel
Flow rate (0.8 mL/min)	0.63	0.89
Flow rate (1.2 mL/min)	0.41	0.26
Org Plus (44:56)	0.46	0.54
Org Minus (36:64)	0.33	0.98

Table 7. Results of forced degradation

Degradation condition	Capecitabine		Docetaxel	
	% Assay	% deg	% Assay	% deg
Acid deg	84.8	15.2	87.5	12.5
Alkali deg	85.5	14.5	87.1	12.9
Peroxide deg	83.7	16.3	85.2	14.8
Reduction deg	87.3	12.7	89.3	10.7
Thermal deg	89.8	10.2	92.6	7.4
Hydrolysis deg	91.5	8.5	90.3	9.7

3.8 Robustness

According to RSD's tests, the robustness of the tactic brought in only 2% of RSD. The slightly varied parameters such as flow (±0.2 mL/min) and organic content in the mobile phase (±10 percent) were eliminated in favour of the optimized methods. Robustness results Table 6.

3.9 Forced Degradation

Capecitabine and Docetaxel standard was subjected to various conditions of forced degradation in order to induce partial degradation of the compound. Forced degradation experiments have been performed to establish that the process is acceptable for degradation materials. In addition the studies include information on the condition under which the drug is unstable, such that the steps are also taken during formulation to prevent possible instabilities.

3.9.1 Acid degradation

1 mL of standard stock solution was moved to a volumetric flask of 10 mL. After adding 1 mL of 1N HCl, the solution was allowed to sit for 15 minutes before being transferred to a volumetric flask with a capacity of 10 mL. After 15 minutes, add 1 mL of 1N NaOH to bring the diluent level back up to the desired range.

3.9.2 Alkali degradation

1 mL ofstandard stock solution was moved to a volumetric flask of 10 mL. After adding 1 mL of NaOH, the solution was allowed to sit for 15 min before being transferred to a volumetric flask with a volume of 10 mL. After 15 minutes, add 1 mL of 1N HCl and bring the solution up to the required strength.

3.9.3 Peroxide degradation

Adding 1 mL of 30 percent hydrogen peroxide solution and diluents to 1 mL of standard stock solution produced a volumetric flask with a capacity of 10 mL.

3.9.4 Reduction degradation

For each 10 mL volumetric flask, 1 mL of the standard stock solution was transferred. To this, 1 mL of the 30 percent sodium bi sulphate solution was added, and the solution was diluted to the desired concentration using diluents.

3.9.5 Thermal degradation

For six hours, a standard solution was baked at 105°C in an oven. UPLC system received the final solution and performed analysis.

3.9.6 Hydrolysis degradation

Add 1 mL of HPLC water and dilute with diluents to make up to the mark of 1 mL of standard stock solution in a 10 mL volumetric flask.

Degradation results were shown in Table 7.

4. CONCLUSION

Capecitabine and Docetaxel's quantitative determination can be aided by a simple, selective, validated, and well-defined stability demonstrated by RP-UPLC with isocratic RP-RPLC. The linearity, accuracy, precision, and robustness of Capecitabine and Docetaxel were validated using this method. It was discovered that the RSD values for all parameters were less than 2, indicating that this procedure is reliable and that the results obtained using this procedure are reasonably comparable. With an adequate retention time, all degradation products produced under stress conditions were clearly separated and well resolved, indicating that the proposed method is quick, simple, feasible, and affordable. Under ideal conditions, the new method produced dependable, precise, and accurate results. Because of this, the improved chromatographic method can be used on a regular basis for drug research and pharmaceutical formulations investigations.

CONSENT AND EHICAL APPROVAL

It is not applicable.

DISCLAIMER

The company name used for this research is commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use this company as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Johnstone RW, Ruefli AA, Lowe SW. Apoptosis: A link between cancer genetics and chemotherapy. Cell 2002;108(2):153–64.
2. Gurney H. How to calculate the dose of chemotherapy. British Journal of Cancer. 2002;86(8):1297–302.
3. Song JK, Bae JM. Citrus fruit intake and breast cancer risk: A quantitative systematic review. Journal of Breast Cancer. 2013;16(1):72–6.
4. Runowicz CD, Leach CR, Henry NL, Henry KS, Mackey HT, Cowens-Alvarado RL, et al. american cancer society/american society of clinical oncology breast cancer survivorship care guideline ca. 2016;66(1): 43–73.
5. Orditura M, Galizia G, Sforza V, Gambardella V, Fabozzi A, Laterza MM,

Andreozzi F, Ventriglia J, Savastano B, Mabilia A, Lieto E, Ciardiello F, De Vita F. Treatment of gastric cancer. World Journal of Gastroenterology. 2014;20(7): 1635–49.
6. Wagner AD, Syn NL, Moehler M, Grothe W, Yong WP, Tai BC, Ho J, Unverzagt S. Chemotherapy for advanced gastric cancer. The Cochrane Database of Systematic Reviews. 2017;8:CD004064.
7. Theodoratou E, Timofeeva M, Li X, Meng X, Ioannidis JP. Nature, nurture, and cancer risks: Genetic and nutritional contributions to cancer. Annual Review of Nutrition (Review) 2017;37:293–320.
8. Hunt Beverley J. Bleeding and coagulopathies in critical care. New England Journal of Medicine. 2014;370(9): 847–859.
9. Kay AB. Overview of allergy and allergic diseases: with a view to the future. British Medical Bulletin. 2000;56(4):843–64.
10. Harvey, Pamela A, Leinwand, Leslie A. Cellular mechanisms of cardiomyopathy. The Journal of Cell Biology. 2011;194(3): 355–365.
11. Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB, Schwab M. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydro-pyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clinical Pharmacology and Therapeutics. 2013; 94(6):640–5.
12. Parfenov M, Pedamallu CS, Gehlenborg N, Freeman SS, Danilova L, Bristow CA. Characterization of HPV and host genome interactions in primary head and neck cancers. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(43):15544-9.
13. Liang C, McClean MD, Marsit C, Christensen B, Peters E, Nelson HH, Kelsey KT. A population-based case-control study of marijuana use and head and neck squamous cell carcinoma. Cancer Prevention Research. 2009;2(8): 759–68.
14. Catalona WJ. prostate cancer screening. The Medical Clinics of North America. 2018;102(2):199–214.
15. Nasser Nicola J, Gorenberg, Miguel, Agbarya, Abed. first line immunotherapy for non-small cell lung cancer. Pharmaceuticals. 2020;13(11):373.
16. Moon RE. Hyperbaric oxygen treatment for decompression sickness. Undersea & Hyperbaric Medicine. 2014;41(2):151–157.
17. Blonski W, Siropaides T, Reddy KR. Coagulopathy in liver disease. Current Treatment Options in Gastroenterology. 2007;10(6):464–73.
18. Shibata T, Aburatani H. Exploration of liver cancer genomes. Nature Reviews. Gastro-enterlogy & Hepatology. 2014;11(6):340–9.
19. British national formulary: BNF 69 (69 ed.). British Medical Association. 2015;622.
20. Howard J, Hyman AA. Microtubule polymerases and depolymerases. Current Opinion in Cell Biology. 2007;19(1):31–5.
21. Chaaban S, Brouhard GJ. A microtubule bestiary: structural diversity in tubulin polymers. Molecular Biology of the Cell. 2017;28(22):2924–31.

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