RJC

RASĀYAN *J. Chem.*
Vol. 15 | No. 4 |2893-2900| October - December | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

DEVELOPMENT OF METHOD AND VALIDATION FOR ASSAY IN IRINOTECAN HYDROCHLORIDE INJECTION BY APPLYING STABILITY INDICATING HPLC METHODOLOGY BY HPLC

Ch. V. Kameswara Rao[1], K. Ramakrishna[1], V. D. N. Kumar Abbaraju✉, Umamaheswara Rao Tadikamalla[3] and Dunna Rahul[4]

[1]Department of Chemistry, GITAM School of Science, GITAM (Deemed to be University), Visakhapatnam-530017, India

[2]Department of Environmental Science, GITAM School of Science, GITAM (Deemed to be University), Visakhapatnam-530017, India

[3]Department of Chemistry, Hindu College, Guntur, Andhra Pradesh-522002, India

[4]Department of Chemistry, J K C College, Guntur, Andhra Pradesh-522002, India

✉Corresponding Author: vabbaraj@gitam.edu

ABSTRACT

A validated HPLC technique for determining Irinotecan Hydrochloride (IRN) in pharmaceutical formulations was developed. For this chromatographic investigation, isocratic elution at a flow rate of 1.0 ml/min was utilized on Zorbax C18 150mm 4.6 mm, 5µ, or similar. The mobile phase is made up of 45 volumes of methanol and 55 volumes of buffer solution. The UV detection wavelength was 220 nm, and a sample of 10.0 µl was injected. The run time for Sample, Unmarked, Placebo, System Suitability and Sensitivity solutions is approximately 12 minutes, and 60 minutes for diluted Regular. The approximate retention time for IRN was determined to be 3.8 minutes. The percentage R.S.D. IRN was determined. Irinotecan's mean percentage recovery is found to be within the specified limit. The approach was validated in accordance with ICH recommendations. As a result, the suggested HPLC approach may be successfully used for routine formulation quality control examination. The devised approach is simple and superior to the methods published in the literature.

Keywords: RP-HPLC, Refractive Index Detector, IRN, Rate of Flow, ICH Guidelines, USP Reference.

RASĀYAN *J. Chem.*, Vol. 15, No.4, 2022

INTRODUCTION

Irinotecan Hydrochloride (IRN) has the chemical formula $C_{33}H_{38}N_4O_6.HCl.3H_2O$. This drug is often sold as IRN under the brand name Camptosar and is used to treat cancer. It is used in the colon as a single dosage in conjunction with fluorouracil.[1] IRN is represented in Fig.-1. IRN is often classified as a topoisomerase IRN inhibitor.[2] In today's world, this therapy is both very effective and extremely safe, and it is critical to human health.[3] This medication is often derived from a Chinese tree known as Camptothecaacuminata.[4] The most serious negative consequences of IRI are the high severity of sickness, particularly diarrhea, and the maximal inhibition of the immune system.[5] IRN may have broad molecular activity through gap complexes in topoisomerase-1-*DNA* positioned as guanine +1 in the *DNA* sequence.[6] Smita Sharma presented a simultaneous estimate of IRN.HCl. The wavelength is 247 nanometers.[7] The linearity signal ranges from 2.0µg/ml to10.0µg/ml. A 9.2v:5v:0.9v:0.8v combination of toluene, ethyl acetate, methanol, or carbon tetrachloride is employed as the mobile phase. 317nm is the wavelength. IRN linear regression analysis in the 200 ng/band-1200 ng/band strength range. The LOD is 36 ng/spot while the LOQ is 57 ng/spot. LOD is 36ng/spot and LOQ is 57ng/spot. P. Sunil Reddy validated the results in a water symmetry shield RP-18 (250mm x 4.6m) 5µm column with an isocratic mobile phase containing 0.02M potassium di-hydrogen ortho-phosphate.[8] The pH is adjusted to 3.5 using ortho-phosphoric acid 60v, methanol 20v, and acetonitrile 20v. The flow rate is 1.00 mL/min. The temperature is 25 degrees Celsius. The wavelength is 220nm. The injection volume is 100µl. The calibration curve is linear from 0.024µg/mL to 0.143µg/mL, with a correlation value of 0.997. The DL

http://doi.org/10.31788/RJC.2022.1547057

and QL are both 0.008µg/ml and 0.023µg/ml. In order to evaluate their samples, M.V. Kumudhavalli employed an Inertsil ODS 250 X 4.6 mm and 5µ particle size column. Acetonitrile at concentrations of 40 v/v and 60 v/v is utilized as the mobile phase.[9] The pH of the buffer is 3.2. It flows at a rate of 1 ml per minute. The runtime is thirty seconds. It has a wavelength of 222 nm. The IRI eluted time is 2.1 minutes. Up to120 µg/ml the calibration curve is linear. It is 0.9999 for the correlation coefficient. LOQ is 2.0ng/ml, whereas LOD is 0.8ng/ml. % RSD is 0.5%. The evaluation is 98.2–100%. Retention time is shorter than 121 seconds. Murali Balaram V. employed acetonitrile as a 25v/v buffer and phosphate as a 75v/v buffer. The pH is set to 2.5, and the wavelength is 225 nm.[10] Rt lasts 5.82 minutes. Finally, after carefully examining all of the available literature, the author has concluded that regular examination of the IRN injectable dosage form should effectively employ speedy, specific, exact, and accurate methods.
Mallikarjuna Rao Pichika proposed the HPLC process with the help of a diode array to quantify predominant gingerols (4-, 6-, 8-, and 10-) as well as shogaols (6-, 8-, and 10-) in supercritical carbon dioxide extract of Halia bara.[11] Various circumstances used by the authors are ODS Genesis column, 25°C is column temperature, gradient elution consists of acetonitrile along with aqueous formic acid which is in aqueous form; 282 nm is the wavelength, 1 mL/min is flow rate, 20 µL is injection volume; and 38 min as run-time. S. M. Sinaga proposed that average % recoveries are 100.16% and 99.71% to amlodipine besylate as well as valsartan to 5/80 mg tablet dosage form also 100.29% and 99.64% to amlodipine besylate and valsartan to 10/160 mg tablet dosage form.[12] The authors subtracted the absorption of amlodipine besylate at 247.2 nm.

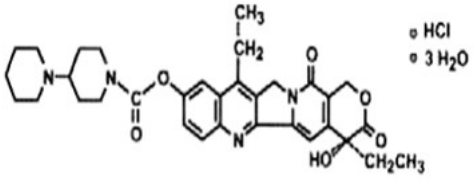

Fig.-1: Structure of Irinotecan Hydrochloride (IRN)

EXPERIMENTAL

In this challenge, the LC 20AT pump, UV-Visible detector with adjustable wavelength program, and Rheodyne injector are employed. Zorbax C18 150mm x 4.6mm, 5µ or equivalent. The mobile phase containing the gas was separated using the Loba ultrasonic bath sonicator. The common balance is used to measure the medications. The reference sample of IRN is obtained from the local market. Water is collected and utilized, and the Methanol is AR grade. To make the buffer solution, carefully pour 1.00mL of trifluoroacetic acid into 1000.00mL of distilled water. These constituents are carefully combined. Following that, it is filtered using a 0.45µm filter paper. 55 volumes of buffer solution and 45 volumes of Methanol were transferred to prepare the mobile phase.

Preparation of Solutions

IRN Regular Stock is prepared by transferring the desired amount of IRN Trihydrate, i.e., around 5 mg, into a 5.00 mL standard flask, allowing it to dissolve, and then marking the volume with the appropriate analyte. 1.00 mL of the solution, as mentioned earlier, was transferred into a 10.00 mL ordinary flask labelled to volume with the proper analyte. This whole preparation yields 0.10 mg/ml IRN. To make the sample solution (20mg/ml), take around 1.00ml of sample and transfer it to a 100.00ml ordinary flask. Transferred 5 mL of the above solution to a 10 mL normal flask and diluted with water. Placebo is made by taking around 1 mL of a placebo, transferring it to a 100 mL normal flask, diluting it to an appropriate quantity with a suitable diluent, and thoroughly blending it. Transfer 5 mL of the above solution to a 10 mL normal flask and dilute with water. One blank injection, six regular chromatography injections, and a system appropriateness parameters check.

Method Development

A UV spectrophotometer was used to detect a 100ppm solution of IRN for wavelength detection. Methanol's spectra were captured independently. At the apex of the 220 nm wavelength, IRN spectra were apparent. On a Zorbax C18, 150 mm, 4.6 mm, 5µ Column, or a similar, the stationary and mobile

phase separation requirements and peak appearances are defined. Trifluoroacetic acid is typically blended by adding precisely 1.00mL into 1000.00mL of distilled water, which is then added in the specified ratio. Compared to other peaks, the chromatographic peak observed is the best; it is almost free from tailing and has shown to be the most effective of all combinations. A flow rate of 0.50-1.50 mL/min is used to obtain the best separation and track the reaction's progress. According to the reaction rate of flow observation, 1.0 mL/min is suitable for efficient Analyte separation.

Validation of Proposed Method and Requirements

System Suitability

To determine whether the system is functioning correctly, highly exact and precise system suitability parameters are defined. Inject one Blank (as one injection) and five standard solutions (as five injections) into chromatography and record various chromatograms. Using the data shown below, it is determined that the suggested approach is better suited for method validation. The acquired results are summarized in Table-1.

Table-1: System Suitability Results

	Regular Solution	IRN Results
Tailing factor	Irinotecan	1.2
% RSD	Irinotecan	0.4

Specificity

This experiment was carried out by injecting a blank, placebo, ordinary solution, Impurity-A, Impurity-C, sample solution, and spiked sample solution (Sample + Impurity) into a chromatographic apparatus and recording varied retention periods. There should be no interference since the peaks produced for Blank (diluent), Placebo, Impurity-A, and Impurity-C should not interact. Finally, it is decided that there is no blockage owing to Blank (Diluent), Placebo, Impurity-A, Impurity-C, or regular at the retention time peak of IRN using the acquired data. The outcome obtained is summarized in Table-2. The Regular solution chromatogram and sample solution chromatogram were represented in Fig.-2(a) and 2(b).

Table-2: Specificity Results

Solution		Retention Time
Blank (Diluent)		--
Regular solution	IRN	3.9
Placebo Preparation		-
Sample preparation	IRN	3.8
Impurity-A		8.5
Impurity-C		2.5
Spiked sample preparation	IRN	3.9
	Impurity-A	8.5
	Impurity-C	2.5

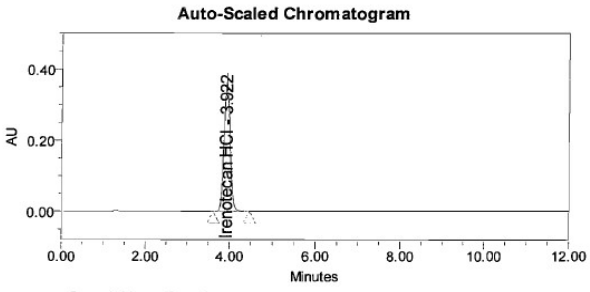

Fig.-2(a): Results for Regular Solution Chromatogram

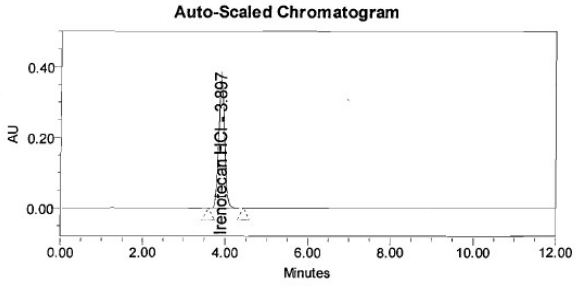

Fig.-2(b): Results for Sample Solution Chromatogram

Stressed Condition Studies

To finalize stability information, establish shelf life, or identify any non-persistent compounds that cannot be mixed utilizing IRN injection peak, stress condition research must be done. In this scenario, the sample is formed by taking around 1 mL of a sample, transferring it to a 100 mL ordinary flask, diluting to an appropriate quantity with a suitable diluent, and complete mixing. Transfer 5 mL of the above solution to

a 10 mL normal flask and dilute with water. Placebo is made by taking around 1 mL of Placebo, transferring it to a 100 mL ordinary flask, diluting it to an appropriate quantity with a suitable diluent, and thoroughly mixing. Additionally, transfer 5 ml of the above solution into a 10 ml flask and top it off with a diluent. An Acid Stressed specimen (1.0N HCl), an Alkali Stressed sample (1.0N NaOH), 3.0% w/v H_2O_2 Stressed sample, a Neutral Stressed sample, UV light exposed sample, Photostability and sunlight exposed sample, a Thermal Stressed (Dry heat) sample, an Alkali Stressed sample, a Thermal Stressed (Dry heat) sample, a Thermal Stressed (Dry heat) sample (1.0N NaOH), After 8 hours, the material was determined to be deteriorating in alkali. The IRN peak degrades gradually in various solutions such as peroxide, neutral, and acidic environments. Regardless, different unknown impurities, known impurities, and degrading impurity peaks are separated from the IRN peak. Empower program confirms that IRN peaks are purer. As a result, the assay technique is regarded as a more specific and stable indication. The outcome obtained is represented in Table-3.

Table-3: Stress Condition Results

Condition	Purity Angle	Purity Threshold	% Assay
Sample as such	0.113	0.235	99.1
1N HCl	0.112	0.235	95.4
0.1N HCl	0.112	0.235	97.0
1N NaOH (15mins@25°C)	1.173	1.276	97.5
0.1 N NaOH (15mins@25°C)	0.593	0.656	99.1
3.0%w/v H_20_2	0.112	0.234	95.0
Neutral	0.112	0.235	99.7
UV Light	0.110	0.235	97.4
Sun Light	0.112	0.235	97.3
Thermal	0.111	0.235	96.9

Precision

System Precision

The retention time (RT) and area for six determinations were calculated, as well as the percentage RSD. Regular preparation RT% RSD and IRN peak response were recorded. The acquired results show that the RT and peak responses are identical, which RSD corroborates (less than 1.0% and less than 2.0%, respectively). As a result, it might be concluded that the system's accuracy achieves the exactness of method validation. The relative regular deviation (%RSD) of RT for IRN, obtained after six injections of the diluted standard solution, equals NMT 1.0. NMT 2.0 is the relative regular deviation (%RSD) for IRN obtained from 6 injections of diluted regular solution. Finally, retention time and area responses were found to be consistent. These are demonstrated using relative regular deviation. As a result, it is completed since the SP parameters fulfill the validation criterion. Table-4 summarizes the outcomes collected.

Table-4: System Precision Results

Irinotecan Hydrochloride		
Sample set No.	Retention Time (Min)	Area Response
1	3.826	3730047
2	3.829	3719978
3	3.830	3698794
4	3.834	3700045
5	3.832	3699853
6	3.839	3700671
Mean	3.832	3708231
RSD	0.1%	0.4%

Method Precision

Analysed IRN injection samples were given six times to the same group. Six determinations' Irinotecan assay values underwent NMT 2.0. The findings are listed in Table-5.

Table-5: Method Precision Results

Assay for Irinotecan Hydrochloride	
Sample Set No.	% of Assay
1	100.7
2	102.7
3	100.7
4	100.8
5	100.5
6	100.8
Mean	101.0
RSD	0.8%

Intermediate Precision

We used this method to examine an Irinotecan HCl injection sample for six reduplications and determined the IRN % test. The identical procedure is done several times with various tools and columns on multiple days. Calculated intermediate precision values and assay percent against method precision for 12 determinations (Method Precision and Intermediate Precision). NMT 2.0 is the computed %RSD for the test for six determinations. NMT 2.0 strength is the %RSD determined for the test for six determinations. The results are shown in Table-6.

Table-6: Comparison of Results of precision

Sample Set No.	% of Assay
1	100.7
2	102.7
3	100.7
4	100.8
5	100.5
6	100.8
7	101.2
8	101.4
9	98.4
10	98.7
11	96.9
12	99.9
Mean	100.2
%RSD of 12 Determinations	1.5

Comparison between Method and Intermediate Precision

Stability in analytical Solution

This is computed stability by injecting a specific range of routine sample preparation daily at room temperature (25°C). The percentage difference between normal and IRN is within ± 2.0. The standard solution remains at 25°C for 32 hours (the percentage difference is -1.3). At 25°C, 20mg/ml sample solutions are stable for 31 hours (a percentage difference of 1.0). The results at different temperatures are summarized in Table-7.

Table-7: Solution, Sample Stability for Regular at 25° C

Regular Solution			Sample Solution 20mg/ml		
Time (Hrs.)	Area	% Difference	Time (Hrs.)	Area	% Difference
Initial	3730047	-	Initial	3716267	-
2	3694394	-1.0	13	3733443	0.5
13	3695711	-0.9	15	3732576	0.4
15	3689023	-1.1	16	3731271	0.4
17	3690150	-1.1	18	3741985	0.7
19	3689403	-1.1	20	3751153	0.9

21	3685894	-1.2	21	3740513	0.7
23	3688799	-1.1	23	3757636	1.1
25	3689189	-1.1	25	3766875	1.4
27	3686739	-1.2	26	3746104	0.8
29	3683711	-1.2	28	3750535	0.9
31	3685879	-1.2	29	3751212	0.9
32	3680859	-1.3	31	3753802	1.0

Linearity

The linearity of IRN is tested with a working concentration between 50% and 150% and spans a minimum of five ranges from 80% to 120%. IRN is used to perform linearity. The area response to each range was computed, and the slope, intercept, and correlation coefficient. Passing the solution through the chromatographic apparatus six times yielded the tested intercept for statistical equivalence to zero. Prepare a graph with PPM on the X-axis and area response on the Y-axis. Various known impurity solutions with differing ranges are computed for this method. This also gives the information needed for spiked samples. Both the correlation coefficient and the R square are 1.000. The intercepts should be within the ± 5.0 response limit at 100%. Precision is 5% at lower and higher ranges. RSD is equal to NMT 5.0. A straight line was constructed between the 50% range and the 150% specification limit based on a statistical analysis of IRN linearity data. The correlation and regression coefficients are both close to 0.998. The residual indicates that the collected measurements were incoherently dispersed to zero, and the P-value was calculated. The P-value is more significant than 0.9. The basis lies between the minimum and maximum boundaries of the 95% confidence range, giving the value produced for intercept a high degree of certainty. With a response area of 100%, the intercept is within ±2. The coefficient correlation and coefficient regression values are calculated using these samples and are shown in Table-8. The related graphs are obtained by this investigation are shown in Fig.-3.

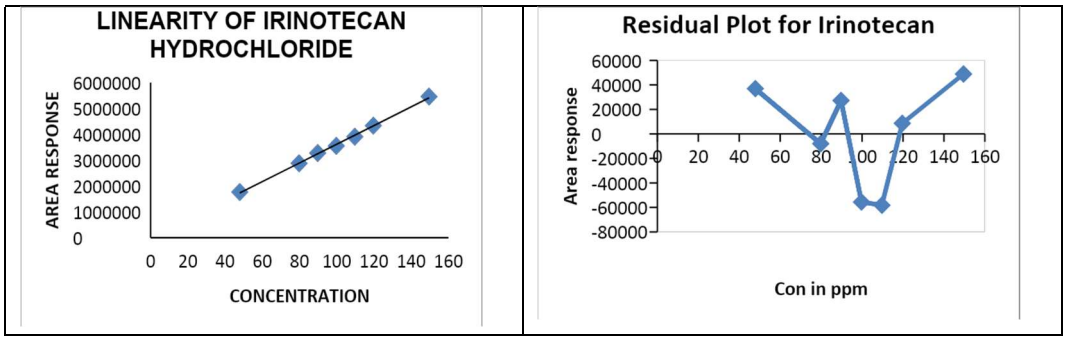

Fig.-3: Linearity and Residual Plot for IRN

Table:-8: Preparation of Linearity Ranges

S. No	Levels	Linearity Standard Stock solution	Made up the volume in mL (With diluent)
1	50%	0.24	5
2	80%	0.40	5
3	90%	0.45	5
4	100%	0.50	5
5	110%	0.55	5
6	120%	0.60	5
7	150%	0.75	5

Accuracy

Single and mean recovery for known impurities ranges between 50% and 150%. Individual and mean recovery for each range for IRN is between 80.0% and 120.0%. The accuracy results are shown in Table-9.

Table-9: Precision at Lower and Higher Ranges

S. No	Lower Level	Higher Level
1	1763499	5438018
2	1764768	5513951
3	1763189	5430558
4	1762548	5436811
5	1763127	5463217
6	1769151	5441844
Mean	1764380	5454067
RSD	0.1%	0.6%

Range

For all accuracy range determinations, the %RSD attained is NMT 2.0. The correlation and regression coefficients for the linearity and accuracy range parameters are NLT 0.998. The linearity and accuracy range results are shown in Table-10. Authors are concluded that the method's operating range is between 50% and 150% of the IRN target strength.

Table-10: Recovery Ranges

Set	Levels (About)	Area Response	mg added	mg Added (Actual)	mg recovered	% recovery	Mean % Recovery	% RSD
1	50 %	1663441	2.517	2.2890	2.3305	101.8	101.0	0.7
2	50 %	1646328	2.522	2.2935	2.3065	100.6		
3	50 %	1644025	2.515	2.2871	2.3033	100.7		
1	100 %	3310112	5.007	4.5534	4.6375	101.8	101.2	0.7
2	100 %	3293428	5.007	4.5534	4.6141	101.3		
3	100 %	3297855	5.059	4.6007	4.6203	100.4		
1	150 %	4990692	7.573	6.8869	6.9920	101.5	101.6	0.2
2	150 %	4977459	7.544	6.8605	6.9734	101.6		
3	150 %	4968448	7.521	6.8396	6.9608	101.8		
% RSD for 3x3 levels						0.6		

Robustness

The temperature of the column is reduced to roughly 5°C. For all situations, system suitability specifications were followed. Total impurities and the IRN peak were subtracted from a sample spiked with contaminants. They concluded from the data that the procedure is resistant to the slight fluctuations conceivable in this method. The results from the robustness are summarized in Table-11.

Table-11: Results for Robustness

Evidence Perception		Tailing Factor	%RSD
Original Condition		1.1	0.3
Flow rate Change	-0.20ml/min	1.2	0.0
	+0.20 ml/min	1.2	0.1
Temperature	-5°C	1.2	0.2
	+5°C	1.1	0.0
Wavelength	-5nm	1.2	0.4
	+5nm	1.2	0.1
Organic ratio	-2.0%	1.2	0.2
	+2.0%	1.2	0.7

RESULTS AND DISCUSSION

Numerous RP-HPLC parameters have been upgraded, and various mobile phase combinations have been confirmed and tested. The mobile phase configurations are used to measure adequate segregation with good peak symmetry. It is handled as a mobile phase by transferring 1 mL of trifluoroacetic acid into 1000 mL of WFI. The 1.0 mL/min flow rate produced a higher resolution and peak shape than the other mixes. The column used in this measurement is Zorbax (150 x 4.6) mm, 5µ, or proportionate. In stressed conditions, the peaks of Blank (diluent), Placebo, Impurity-A, and Impurity-C may not interfere with IRN

Peak and each other. Blank (diluent), Placebo, and known impurities peaks do not interfere with IRN Peak. According to the empowering software, degradation products are well separated from IRN Peak, and each other purity angle is less than the peak threshold. For system suitability, the tailing factor is about 2.0. The following outcomes: The %RSD of IRN peak retention duration calculated from 6 repeated injections of the normal solution may not equal the maximum value of 2.0. The %RSD of IRN peak retention time calculated from a total of six injections of the diluted solution may not be 1.0. NMT 2.0 is the %RSD area of the IRN peak response determined after six injections of the diluted regular solution. The %RSD of the assay is 0.8 for six determinations. The %RSD for a total of 12 measurements (Method Precision & Intermediate Precision) is NMT 2.0 which is defined as a 1.5% difference in sample solution obtained between the initial and after the specified period is 1.0 at 25 °C. The estimated HPLC method of related substances in drug product IRN injection is validated as per ICH guidelines. The proposed process is found to be specific. The technique is also indicated, as evidenced by stress conditions.

CONCLUSION

According to ICH requirements, the estimated HPLC method of associated compounds in drug product IRN injection is verified. The proposed procedure has been determined to be specific. The approach is also shown by stressful circumstances.

ACKNOWLEDGEMENT

The authors are grateful to GITAM University's administration for providing essential lab facilities for this research.

REFERENCES

1. Irinotecan Hydrochloride, The American Society of Health-System Pharmacists, Archived from the original on 22 December 2016, Retrieved 8 December 2016.
2. British National Formulary: BNF 69 (69 ed.), British Medical Association, 2015, p. 624.
3. World Health Organization (2019), World Health Organization Model List of Essential Medicines: 21st list 2019, Geneva: World Health Organization.
4. M. Heinrich, E. M. Williamson, S. Gibbons, J. Barnes, Fundamentals of Pharmacognosy and Phytotherapy E-Book, Elsevier Health Sciences, 2nd edition**,** 2012
5. M. Kotaka, R. Xu, K. Muro, *Chinese Journal of Cancer*, **35**, 1(2016), https://doi:10.1186/s40880-016-0166-3
6. Y. Pommier, Drugging Topoisomerases: Lessons and Challenges, *ACS Chemical Biology*, **8**, 1(2013), http://doi:10.1021/cb300648v
7. S. Sharma, M. C. Sharma, *Arabian Journal of Chemistry*, **9**, 2(2016), https://doi.org/10.1016/j.arabjc.2012.02.012
8. R. P. Sunil, B. K. Sudhakar, N. Kumar, *Revista Cubana de Farmacia*, **47**, 1(2013).
9. M. V. Kumudhavalli, C. Prakash, B. S. Venkateshwarlu, M. Kumar, *Research Journal of Pharmacy and Technoogy,* **12**, 12(2019), https://doi.org/10.5958/0974-360X.2019.01046.1
10. V. Murali Balaram, J. Venkateswara Rao, S. Ramakrishna, G. Sankar Ganesh, T. Balamurali Krishna, *E-Journal of Chemistry*, **4**, 1(2007), https://doi.org/10.1155/2007/597409
11. Shiming Zhang, Kit-Kay Mak, Puvaneswari Marappan, Madhu Katyayani Balijepalli, Gun-Hean Chong and Mallikarjuna Rao Pichika, *Rasayan Journal of Chemistry*, **15,** 1(2022), http://dx.doi.org/10.31788/RJC.2022.1516682
12. S. M. Sinaga, N.N. Pertiwi and Muchlisyam, *Rasayan Journal of Chemistry*, **15,** 2(2022), http://dx.doi.org/10.31788/RJC.2022.1526611

[RJC-7057/2022]