Original Article

Development and Validation of UV-spectrophotometric Method for Estimation of Berberine Hydrochloride in Marketed Formulation and Poly Lactic Co-glycolic Acid Nanoparticles

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ABSTRACT

Aim: The objective of present investigation is to develop and validate UV-Spectrophotometric method for estimation of Berberine hydrochloride in marketed formulation and Poly lactic co glycolic acid nanoparticles. **Materials and Methods:** Methanol was used for the development of UV-Spectrophotometric method. Berberine hydrochloride was detected using a 422nm wavelength. According to ICH requirements, the developed method was validated in terms of, selectivity, linear range, precision, robustness, ruggedness, and reproducibility. The developed method was further successfully used to estimate berberine hydrochloride in marketed formulations and poly lactic co glycolic acid nanoparticles. **Results:** Berberine hydrochloride has a maximum absorption wavelength of 422nm. Beer's law was followed at concentrations ranging from10-50μg/ml. The limit of detection and limit of quantification were found to 2.81μg/ml and 8.54μg/ml respectively. The precision and repeatability scores were all within acceptable limits. Berberine hydrochloride recovery in marketed formulations was found to be between 100-105%. The precision and repeatability values were within a 2% tolerance range. Berberine hydrochloride was found to have a purity of 92.27 %. **Conclusion:** The method was found to be easy, environmentally friendly, repeatable, and cost-effective, and it can be used for Berberine hydrochloride analysis on a regular basis.
Keywords: Berberine HCl, Beer's law, Methanol, Water, Validation, Poly lactic co-glycolic acid nanoparticles.

INTRODUCTION

Berberine hydrochloride is an isoquinoline alkaloid found in a variety of medicinal plants, mainly in the Berberis genus and the Berberidaceae family. *Berberis vulgaris*, goldenseal, goldthread, Oregon grape, rosid dicot genus and turmeric, Guduchi, and other plants contain this chemical component.[1,2]

Berberine alkaloids have a wide range of pharmacological activities, including bactericide, antiviral, blood pressure reducing, hypoglycaemic, medicine, and tumour metastatic effects.[3,4] Glyco-X 500 capsules, Bio-Berberine capsules, Berbeshine tablet, and other formulations with Glyco-X 500 capsules, Bio-Berberine capsules, and Berbeshine tablet, for example. In some malignant cells, it inhibits cell proliferation while encouraging programmed cell death.

Biodegradable and biocompatible polymers are supported by natural and artificial components in nanoparticles for drug delivery applications. Poly lactic co glycolic acid nanoparticles have also demonstrated their efficacy as drug delivery vehicles for a variety of medicinal treatments.[5]

Submission Date: 19-07-2021;
Revision Date: 29-11-2021;
Accepted Date: 14-04-2022.

DOI: 10.5530/ijper.56.3.140
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Different researchers employ only a few UV-Spectrophotometric techniques to examine berberine hydrochloride in commercial formulations, nanoparticles, and other food products.[6-16] The reported methods have their own limitation such as use of costly and hazardous solvent and also reported method have not fully validated the results. The reported methods have their own set of limitations, such as the use of a pricey and hazardous solvent, and the results have not been completely confirmed.

As a result, a UV-Spectrophotometric method for estimating Berberine hydrochloride in marketed formulations must be developed and standardized.

MATERIALS AND METHODS

Instrumentation

For analytical method development of berberine hydrochloride, Shimadzu UV-1900 with scientific laboratory solutions software system and Shimadzu UV-1800 with UV Probe software system were utilized.

Drug Sample

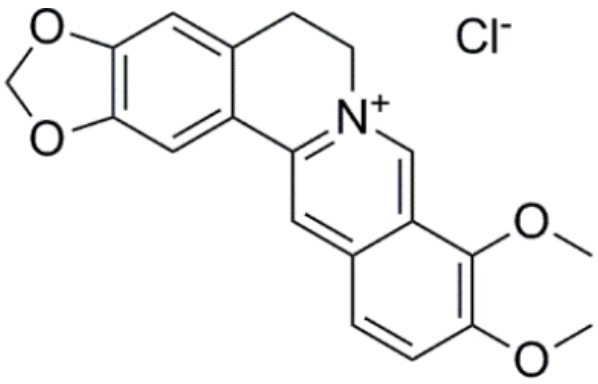
Berberine hydrochloride was provided as a free sample by Biomed Ingredients Pvt Ltd in Goa, and the marked formulation was obtained from the market.

Reagents and Chemicals

Methanol was bought from Fisher Scientific, Mumbai.

Selection of Wavelength

Berberine hydrochloride was employed throughout the study since it is soluble in methanol. Berberine hydrochloride 10 µg/ml of working standard solution was scanned in the UV – Spectrophotometer between 800 nm and 400 nm, with the highest absorption at 422 nm (Figure 1).



16,17-dimethoxy-5,7-dioxa-1azoniapentacyclol[11.8.0.02,10,04,8,015,20]henicosa-1(13),2,4(8),9,14,16,18,20 - octaene ;chloride.

Figure 1: Structure of Berberine Hydrochloride.

Preparation of stock solution

A precisely weighed 10mg of berberine hydrochloride was dissolved in methanol in a clean and dried 10ml volumetric flask, and the volume was calculated using the same. This was considered a standard stock solution with a 1000µg/ml concentration. Further dilutions were made using this standard stock solution.

Preparation of calibration curve

Serial dilutions of 10, 20, 30, 40, and 50 µg/ml were made from the standard stock solution. The absorbance of the solution was measured at 422nm, and a standardization curve was produced with concentration on the X-axis and absorbance on the Y-axis, and a linear regression equation was calculated.

Method development and validation[17-19]

In methanol, berberine hydrochloride was shown to be soluble. As a result, this solvent was utilized to determine the detection wavelength and standard dealing concentration. The International Conference on Harmonization (ICH) has issued validation guidelines for analytical techniques, which characterize this method as characteristic performance verified through laboratory research. The developed technique was validated in accordance with ICH recommendations.

Specificity and selectivity

Berberine hydrochloride has the highest absorbance at 422nm, indicating that the procedure is selective. And because the spectra of the solvent revealed no absorbance at the wavelength of berberine hydrochloride, 422nm, this approach was determined to be selective.

Linearity

Linearity was tested in the 10 - 50 µg/ml range. After accurately weighing 10 mg of Berberine hydrochloride into a clean and dried 10 ml volumetric flask, the volume was brought up to the mark using methanol as the solvent. Pipette 1 mL of the above-mentioned solution into a 10 mL volumetric flask, which was then filled with methanol to make the volume. Dilutions of this solution were prepared to assess the linearity. The detection and quantification limits were found to be 2.81 µg/ml and 8.54 µg/ml, respectively.

Precision

To determine system precision, three replicates of a solution containing 10 µg/ml, 30 µg/ml, and 50 µg/ml of berberine hydrochloride were produced, with the absorbance of each solution measured at 422nm and the percentage Relative Standard Deviation (%RSD) was calculated.

The precision of the method was determined by performing a sample assay under the conditions of the tests. Intraday precision and Interday precision are two types of precision. In Intraday, three replicates of a solution having concentrations of 10µg/ml, 30µg/ml, and 50µg/ml of Berberine hydrochloride were evaluated for intraday precision, and percent RSD was calculated at completely distinct time intervals on the same day. In Interday, three replicates of a solution comprising concentrations of 10 µg/ml, 30 µg/ml, and 50 µg/ml of berberine hydrochloride were evaluated for Interday precision, and percent RSD was calculated on three successive days.

Ruggedness

Ruggedness was determined by using a comparable planned procedure on a separate instrument and checking the reproducibility with multiple analysts.

Robustness

Robustness is done by doing the sonication for 10min and by varying the wavelength.

Accuracy

Accuracy was confirmed by doing recovery experiments in which the percent mean recovery of the sample was calculated using a standardization approach at three distinct levels: 50%, 100%, and 150 percent of the sample solutions. Any dilutions are made from the above solution. Three replicates of the concentration solution were prepared for each level, and a recovery study was conducted.

Analysis of marketed formulation

Berberine Hydrochloride in marketed formulations was determined using the established method.

Poly lactic co glycolic acid (PLGA) Nanoparticle preparation and characterization

Sonication for 20 min with poly lactic co glycolic acid polymer, surfactants and drug (20 mg). The particle size, PDI, and entrapment efficiency of the developed poly lactic co glycolic acid nanoparticles were tested. The developed UV technique is used to determine the amount of berberine present in poly lactic co glycolic acid nanoparticles. Centrifugation nano formulation 15000 rpm for 3 min was used to measure entrapment efficiency.

RESULTS AND DISCUSSION

Method development

Using the UV-1800 equipment and methanol as a solvent, a UV-spectrophotometric technique was devised. The details of the method established are shown in Table 1.

Table 1: Developed method parameters

S. No.	Method	Spectrometric
1	Instrument	UV
2	Model	1800
3	Make	Shimadzu
4	Software	UV Probe
5	Drug	Berberine Hydrochloride
6	λ max	422nm
7	Solvent	Methanol

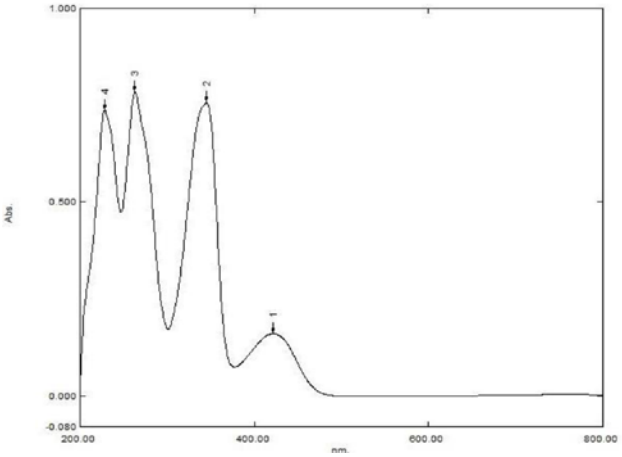


Figure 2: UV spectra of Berberine Hydrochloride.

Method validation

According to ICH requirements, the developed method was validated in terms of specificity, selectivity, linear range, precision, robustness, ruggedness, and reproducibility.

Specificity and Selectivity

Berberine hydrochloride has the highest absorbance at 422nm, indicating that the technique is specific and selective (Figure 2).

Linearity

Dilutions are made for the linearity range of 10-50µg/ml, as specified in the aforementioned technique. Figure 3 depicts the linearity graph, Table 2 depicts the linearity and range, and Figure 4 depicts the calibration curve.

System precision

As stated in the procedure, three replicates of a solution containing 10µg/ml, 30µg/ml, and 50µg/ml of berberine hydrochloride were created and the absorbance of each solution was measured at 422nm to determine system precision. The percent RSD was calculated, and it was found to be less than 2%. (Table 3).

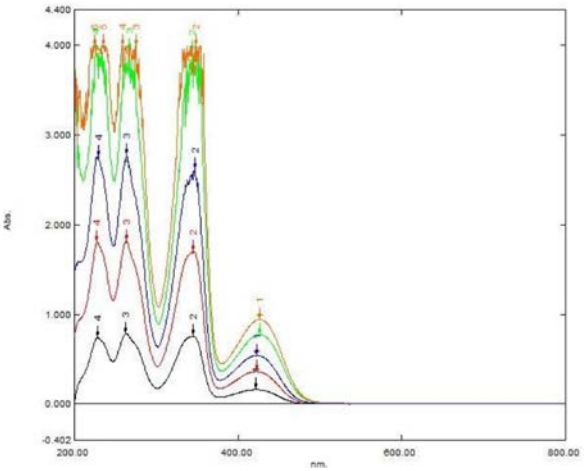


Figure 3: Linearity graph of Berberine Hydrochloride.

Table 2: Linearity and range data of Berberine Hydrochloride.

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.157
2	20	0.311
3	30	0.474
4	40	0.645
5	50	0.813

r^2 = 0.9996
Slope = 0.016
RSD = 0.014
LOD = 2.81
LOQ = 8.54
r^2 = linear regression
RSD=Relative standard deviation
LOD=Limit of detection
LOQ=Limit of quantitation

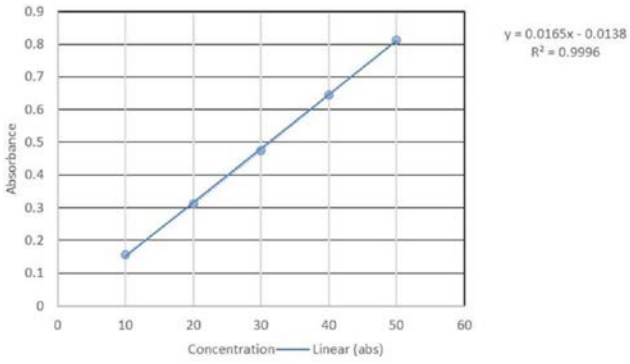


Figure 4: Linearity of Berberine Hydrochloride.

Intraday precision

For intraday precision three replicates of solution containing concentration 10µg/ml, 30µg/ml, 50µg/ml of berberine hydrochloride analyzed and %RSD was calculated at different time intervals on same day and %RSD was found to be less than 2% (Table 4).

Table 3: System precision data of Berberine hydrochloride.

Concentration (µg/ml)	Absorbance* (nm)	Standard Deviation	% Relative Standard Deviation
10	0.16	0.0006	0.36
30	0.53	0.0012	0.22
50	0.78	0.0038	0.49

* = Average absorbance of three replicates (*n=3*)

Table 4: Intraday precision data of Berberine Hydrochloride.

Concentration (µg/ml)	Absorbance* (nm)		Standard Deviation	% Relative Standard Deviation
10	Abs 1hr	0.13	0.0020	1.55
	Abs 4hr	0.12	0.0021	1.67
30	Abs 1hr	0.56	0.0038	0.67
	Abs 4hr	0.57	0.0025	0.44
50	Abs 1hr	0.64	0.0021	0.33
	Abs 4hr	0.78	0.0052	0.67

* = Average absorbance of three replicates (*n=3*)

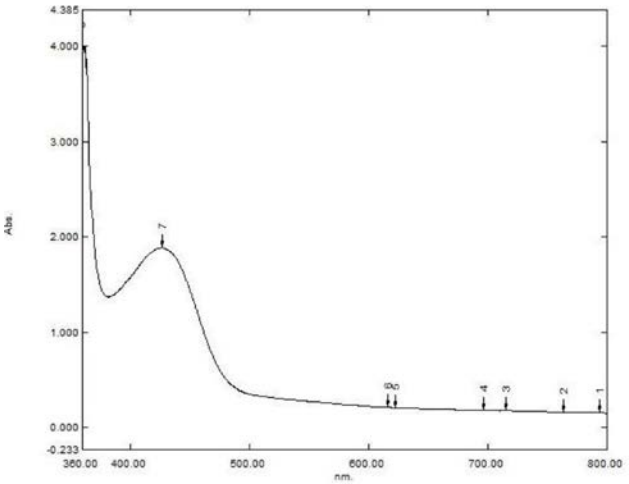


Figure 5: Marketed formulation UV.

Interday precision

Three replicates of a solution comprising concentrations of 10µg/ml, 30µg/ml, and 50µg/ml of berberine hydrochloride were evaluated for Interday precision, and percent RSD was calculated on three consecutive days. Furthermore, the %RSD was determined to be less than 2%. (Table 5).

Ruggedness

Ruggedness was determined by repeating the suggested method on two different instruments (UV-1800 and

UV-1900) and having independent analysts check the repeatability, which revealed a percent RSD of less than 2%, indicating that the method developed is ruggedness (Table 6,7).

Table 5: Interday precision data of Berberine hydrochloride.

Concentration (µg/ml)	Absorbance* (nm)		Standard Deviation	% Relative Standard Deviation
10	Day 1	0.16	0.0017	1.12
	Day 2	0.16	0.0006	0.36
	Day 3	0.12	0.0023	1.95
30	Day 1	0.52	0.0006	0.11
	Day 2	0.53	0.0015	0.29
	Day 3	0.46	0.0006	0.12
50	Day 1	0.78	0.0006	0.07
	Day 2	0.72	0.0021	0.29
	Day 3	0.68	0.0012	0.17

* = Average absorbance of three replicates (n=3)

Table 6: Ruggedness data of Berberine Hydrochloride.

Concentration (µg/ml)	Absorbance* (nm)			Standard Deviation	% Relative Standard Deviation
10	Change in wavelength	421	0.16	0.0010	0.63
		423	0.16	0.0006	0.37
30	Change in wavelength	421	0.50	0.0021	0.41
		423	0.50	0.0025	0.50
50	Change in wavelength	421	0.78	0.0040	0.52
		423	0.78	0.0035	0.45

* = Average absorbance of three replicates (*n=3*)

Accuracy and Recovery

The accuracy of the sample was tested by executing recovery experiments in which the percent mean recovery of the sample was calculated using a standardization approach at three distinct levels: 50%, 100%, and 150% of the sample solutions were created. The amount of medication in the sample was consistent with the formulation's label claim. The percent assay resulted in a result of 92.27%. And the percent recovery ranges from 100 to 112% (Table 8), the data of Characterization of PLGA nanoparticles and estimation of Berberine in marked formulation were presented in Table 9.

The data of comparison between previously published UV methods and newly developed method was presented in Table 10.

Table 7: Robustness data of Berberine Hydrochloride.

Concentration	Absorbance*		Standard Deviation	% Relative Standard Deviation
10 µg/ ml	Analyst 1 UV-1800	0.15	0.0015	1.00
	Analyst 2 UV-1900	0.17	0.0006	0.33
30 µg/ ml	Analyst 1 UV-1800	0.51	0.0012	0.23
	Analyst 2 UV-1900	0.52	0.0012	0.22
50 µg/ ml	Analyst 1 UV-1800	0.71	0.0012	0.16
	Analyst 2 UV-1900	0.63	0.0006	0.09

* = Average absorbance of three replicates (*n=3*)

Table 8: Recovery data of Berberine Hydrochloride.

Total concentration	Standard concentration (µg/ ml)	Sample concentration (µg/ ml)	Absorbance (206nm)		Concentration (µg/ ml)	Sample concentration difference (µg/ ml)	% Recov- ery
			Standard	Sample			
10 µg/ ml (50%)	5	5	0.161	0.163	10.12	5.12	102.4
	5	5	0.161	0.164	10.18	5.18	103.6
	5	5	0.161	0.163	10.12	5.12	102.4
20 µg/ ml (100%)	5	15	0.334	0.339	20.29	15.29	101.93
	5	15	0.334	0.341	20.41	15.41	102.73
	5	15	0.334	0.339	20.29	15.29	101.93
30 µg/ ml (150%)	5	25	0.508	0.508	30	25	100
	5	25	0.508	0.509	30.05	25.05	100.2
	5	25	0.508	0.510	30.11	25.11	100.44

* = Average absorbance of three replicates (*n=3*)

Table 9: Characterization of PLGA nanoparticles and estimation of Berberine in marked formulation.

PLGA Nanoparticles	Particle Size (nm)	PDI	Entrapment Efficiency (%)
Blank PLGA Nanoparticles	216.3	0.29	_
Berberine Loaded PLGA Nanoparticles	311.0	0.444	90%

Table 10: Comparison between previously published UV methods.[18-22]

S. No.	Developed UV method	Wavelength (nm)	Limitation	Reference
1	Validated HPLC-UV method for the determination of berberine in raw herb *Daruharidra (Berberis aristata* DC), its extract, and in commercially marketed Ayurvedic dosage forms.	346 nm	Good linear relationship, reliability, simplicity, reproducibility, and speed	Ali M, Sharma SK. Heterocyclic constituents from *B. lyceum roots. Indian J Chem.* 1996; 6:127–30.
2	Quantitative analysis of berberine in homeopathic formulation containing *berberis vulgaris* L. by UV.	428nm	Simple, rapid, precise, accurate	Anonymous, Bheshaj Samhita (Ayurvedic Pharmacopeia), (1966). Swasthya Mantralaya, Gujarat, Ahmadabad, 574
3	Development and validation of first order derivative spectroscopic method for simultaneous estimation of mangiferin and berberine HCl in bulk and synthetic mixture	257 nm (λ_{max} of mf) and 265 nm (λ_{max} of berberine)	Precise, fast	Chawla R: Evidence based herbal drug standardization approach in coping with challenges of holistic management of diabetes: a Dreadful lifestyle disorder of 21st JDMD 2013; 12-35.
4	Solvent effect on the UV/Vis absorption and fluorescence spectroscopic properties of berberine	421–431 nm	Constant	M. L. Freile, F. Giannini, G. Pucci, A. Sturniolo, L. Rodero, O. Pucci, V. Balzaretti and R. D. Enriz, Antimicrobial activity of aqueous extracts and of berberine isolated from Berberis heterophylla, Fitoterapia, 2003, 74, 702–705.
5	Development and Validation of UV spectrophotometric method for the estimation of Curcumin in Bulk Drug and Pharmaceutical Dosage Forms	421nm	A rapid, simple, selective, precise	Ahuja S, Scypinsk S. 2001. Handbook of modern pharmaceutical analysis. 5th ed., London: Academic Press. p 345-442.

CONCLUSION

It may be stated that the developed method for estimating berberine hydrochloride in marketed formulations was simple, sensitive, precise, and consistent. The marketed sample evaluated had no excipients that interfered with the analysis, demonstrating the specificity of the methods for this formulation. In comparison to previous methods, this newly developed UV methodology allows for straightforward berberine hydrochloride quantification. The validation of the established technique was carried out in accordance with ICH guidelines.

Author Contributions

SMT main worker, developed and performed the study, data analysis, wrote and drafted the manuscript. BK guided with nanoparticle preparation and its characterization and revision manuscript. SS guided with UV validation and revision manuscript. KP, BK and SS reviewed literature, data analysis, and were responsible for revision of manuscript. KP supervised the study, provided inputs, reviewed drafts, and finalized the manuscript

ACKNOWLEDGEMENT

The authors are grateful to the Principal of KLE College of Pharmacy, Belagavi for providing the necessary resources for this study. Authors also appreciate Biomed Ingredients, Goa, for providing gift samples.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

UV: Ultraviolet; **BBR HCI:** Berberine Hydrochloride; **PLGA:** Poly Lactic Co-Glycolic Acid; **ICH:** International Conference on Harmonization; **LOD:** Limit of detection; **LOQ:** Limit of Quantification; **RSD:** Relative standard deviation; **PDI:** Poly Dispersity Index; **EE:** Entrapment Efficiency; **RPM:** Revolution Per Minute; **HPLC:** High Performance Liquid Chromatography.

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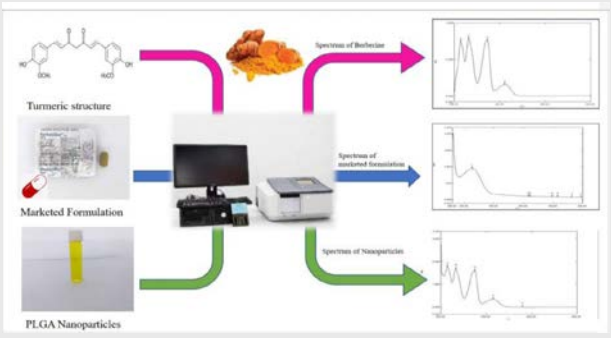
PICTORIAL ABSTRACT



SUMMARY

For berberine hydrochloride, a UV technique was successfully developed and verified. Berberine hydrochloride Poly lactic co-glycolic acid nanoparticles was successfully evaluated using the proposed UV technique. The applicability of the developed method was also found in berberine hydrochloride and marketed available items.

About Authors

Ms. Snehal Tavade Completed her M. Pharmacy (Pharmacognosy and Phytochemistry) from KLE College of Pharmacy, KAHER, Belagavi and currently working as research trainee in ICMR-NITM, Belagavi. Her area of interest includes preparation and characterization of Nano formulations of herbal extracts and their constituents. Her area of interest also covers gene set enrichment analysis of compounds modulated molecular pathway identification, compound-protein-pathway network analysis, molecular docking and dynamics studies. Further, she is interested in evaluating herbal extract nanoparticles for in vivo evaluation.

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Cite this article: Tavade S, Patil K, Kurangi B, Suryawanshi S. Development and Validation of UV-spectrophotometric Method for Estimation of Berberine Hydrochloride in Marketed Formulation and Poly Lactic Co-Glycolic Acid Nanoparticles. Indian J of Pharmaceutical Education and Research. 2022;56(3):873-80.