International Journal of Newgen Research in Pharmacy & Healthcare
Volume-1, Issue-2, December 2023
www.ijnrph.com
ISSN 2584-0096 https://doi.org/10.61554/ijnrph.v1i2.2023.29

Simultaneous UV Spectrophotometric Method for Estimation of Berberine and Withanolide in Polyherbal Immunity Booster Dosage Form

Chauhan S.,* Tyagi S., Maan P.

Department of Pharmacy, Lloyd Institute of Management and Technology,
Plot No.-11, Knowledge Park-I1, Greater Noida, Uttar Pradesh, 201306, India
Email: shilpivats23@gmail.com

Received 05 July 2023, Accepted for publication 11 Nov 2023, Published 30 Dec 2023

ABSTRACT

The majority of commercial herbal preparations used to improve immunity lack standardization with regard to the active ingredients marker molecules. The objective of present study is the simultaneous estimation of berberine and withanolide in herbal immunity booster tablet using UV spectrophotometric method. The method was validated according to ICH guidelines. The results indicated that berberine and withanolide have maximal absorption wavelength i.e. λmax at 348 nm and 231 nm, respectively. Withanolide and berberine were found to behave linearly over the concentration range of 5–25µg/mL with R2 value of 0.9998 and 0.9999, respectively. Withanolide and berberine have shown % relative standard deviation (RSD) values of 0.07 and 0.09, respectively, in accuracy study. The intraday and interday precision was deemed adequate with a % RSD fewer than 2. Further, the limit of detection (LOD) and limit of quantization (LOQ) value for berberine and withanolide were found to be 0.12 mg/mL and 0.35mg/mL and 0.07, 0.20 mg/mL, respectively. In conclusion, the proposed method was validated in accordance with the International Conference on Harmonization (ICH) guidelines. The developed method can be employed to determine how much berberine and withanolide are present in pharmaceutical preparations.

Keywords: Berberine, Withanolide, UV Spectrophotometer, Simultaneous estimation, Validation.

1. INTRODUCTION

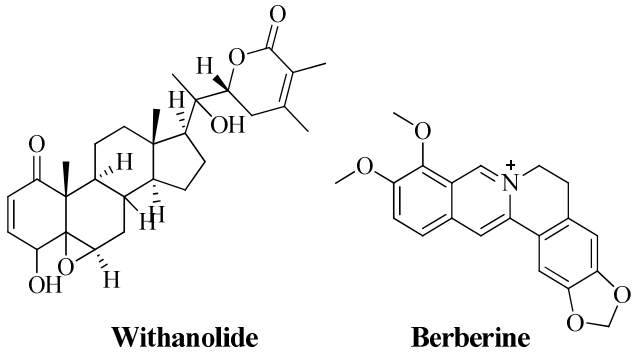
Severe Acute Respiratory Syndrome-COVID-19 (SARS-CoV-2) or is triggered by the coronavirus 2. This disease is exclusive and extraordinary in countless salutations. It has turned healthcare facilities into a battlefield throughout the world. Despite global efforts to stop the pandemic from spreading, this necessitates the use of preventative and therapeutic treatments which that have been clinically proven to be more effective.[1] In light of this, several projects have been made in India to use ayurvedic treatments as a COVID-19 prevention strategy.[2] One or two of the proposed ayurvedic treatments is drinking warm water throughout the day and drinking herbal tea.[3] Since, Ayurveda offers a wide range of immune-stimulating treatments, there are so many immunity booster formulations marketed in India. Coronil tri-herbal formulation is one of them which has incorporated some of the species like Withania somnifera, Ocimum sanctum, and Tinospora cordifolia to increase its effectiveness and productivity.[4]

Withania somnifera (ashwagandha) also called as winter cherry or Indian ginseng, is known to elevate body defense system against diseases, rejuvenate and revitalize the body, as well as to promote mental health. The active component present in this drug are glycowithanolides, alkaloids like ashwagandha, anahygrine, cuscohygrine, topine and steroidal compounds, including ergostane type steroidal lactones, withanolides A–Y, withaferin A, withasomniferin

* Corresponding author: Shilpi Chauhan
E-mail address: shilpivats23@gmail.com

A, withasomnierose A–C, withasomnidienone, withanone. The traditional uses of Withania somnifera include the treatment of neuropsychiatric disorders like anxiety, depression, anxiety, insomnia, and other mental health-related and neurological issues, mild cognitive impairment and schizophrenia.[5] Ocimum sanctum (tulsi) is known as "Mother Medicine of Nature" and suggested in Ayurveda as tonic for the body; mind and spirit also. Its medicinal uses have been found in protection of organs and tissues against chemical stress and physical stress including aiding cough, diarrhea, asthma, fever, arthritis, dysentery, eye diseases, gastric ailments, indigestion, etc. Some of the phytochemicals present in tulsi are eugenol, rosmarinic acid, myretenal, apigenin, β-sitosterol, luteolin, carnosic acid, orintin, and vicenin.[6] Tinospora cordifolia (Guduchi) have innumerable phytoactives, including alkaloids (berberine, choline, tinosporin, anoside, magnoflorine), diterpenoid lactones (furanolactone, clerodane derivatives), glycosides (tinocordiside, tinocordifolioside, cordioside, palmatosides), steroids (β-sitosterol, hydroxyecdysone, giloinsterol), sesquiterpenoid, phenolics, aliphatic compounds (octacosanol, heptacosanol, Nonacosan-15-one dichloromethane) and polysaccharides. It is used in Ayurveda due to its medicinal properties like anti-periodic, anti-diabetic, anti-inflammatory, anti-spasmodic, anti-arthritic, anti-allergic, anti-oxidant, anti-leprotic, anti-stress, hepatoprotective, anti-malarial, anti-neoplastic and immunomodulatory activities.[7]

In the present study, we aimed to perform simultaneous estimation of withanolide and berberine which are the major phytochemicals present in Withania somnifera and Tinospora cordifolia, respectively using UV-visible spectrophotometric method (Chemical structures of withanolide and berberine are given as Fig. 1). There are various studies about the estimation of withanolide and berberine by UV,[8-11] Liquid chromatography (LC),[12] High Performance Liquid chromatography HPLC,[13] HPTLC,[14-15] and other methods[16] individually or in combination with other drugs, but no single study is reported so far about the simultaneous estimation of both of phytoconstituents in herbal formulations.



Withanolide **Berberine**

Fig. 1: Chemical structure of withanolide and berberine.

MATERIALS AND METHODS

Drugs and chemicals

Berberine hydrochloride and withanolide were procured as a free sample from Biomed Ingredients Pvt Ltd, Goa. The Divya Coronil Tablets (Patanjali Ayurved Limited) were purchased from the market. All the solvents and chemicals used were of the AR grade.

Instrumentation

A UV-Visible double beam spectrophotometer (model no. Shimadzu-1800, Japan) with a pair of 1cm two identical quartz cells light path was used to measure absorbance of all the solutions. Glassware (Borosilicate), an ultrasonicator (LOBALife), and an electronic analytical balance (REPTECH) were also employed in this study.

Preparation of standard solution of withanolides

100 mg of withanolide was dissolved in 100 mL solvent having methanol: water in ratio of 50:50 to produce a stock solution of 1000 µg/mL.

Preparation of standard stock solution of berberine

In a volumetric flask, a precisely weighed quantity (10 mg) of each drug was dissolved in sufficient quantity of methanol and then volume was made

up to 10 mL with methanol. 100 ml of methanol were added to 1ml of this mixture in a volumetric flask to make the stock solution with a concentration of 10 µg/mL.

Selection of wavelength

The stock solutions of each drug were scanned in the spectrum mode between 400 nm and 200 nm with a bandwidth of 2 nm against methanol as blank to obtain absorption maxima (λmax). According to the study, withanolide had a well-defined λmax at 231nm (Fig. 2), whereas λmax for berberine was found at 348 nm (Fig. 3).

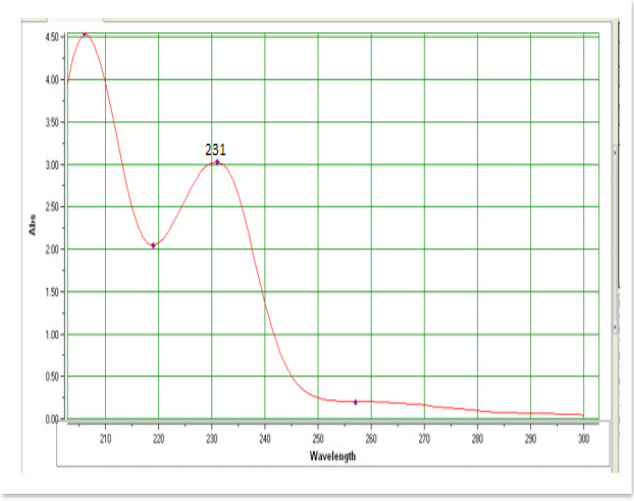


Fig. 2: UV spectra of withanoliode

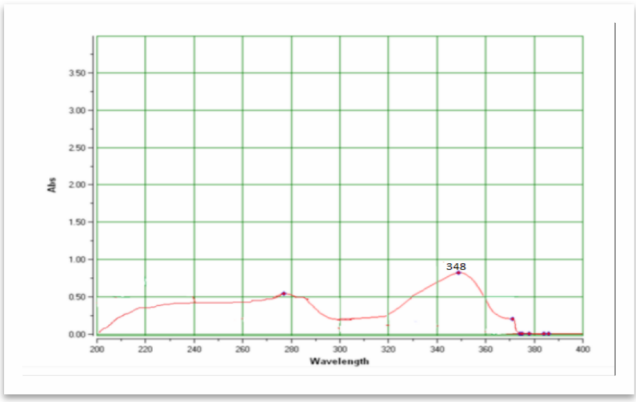


Fig. 3: UV spectra of berberine

Calibration curve of withanolide and berberine

Serial dilutions of concentration range from 2 to 10µg of each drug were prepared with methanol. The absorbance was measured at absorption maxima 231 nm and 348 nm for withanolide and berberine, respectively. A calibration curve was produced for each drug with concentration on the X-axis and absorbance on the Y-axis. A linear regression equation was used to calculate slope (m), intercept (b) and correlation coefficient (R2) (Fig. 4 & 5).

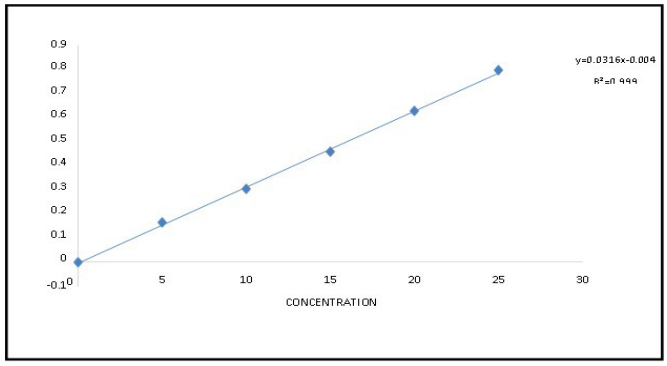


Fig.4: Calibration curve of Withanolide

Estimation of withanolide and berberine

100 mg of the powdered sample of 20 crushed tablets, was mixed in a solvent having 10 mL of methanol and water in ratio of 80:20, and sonicated for 15 minutes. The solution was centrifuged for 5 min. at 5000 rpm before being filtered through a 0.22 m nylon filter. The UV analysis was conducted using the filtered solution.

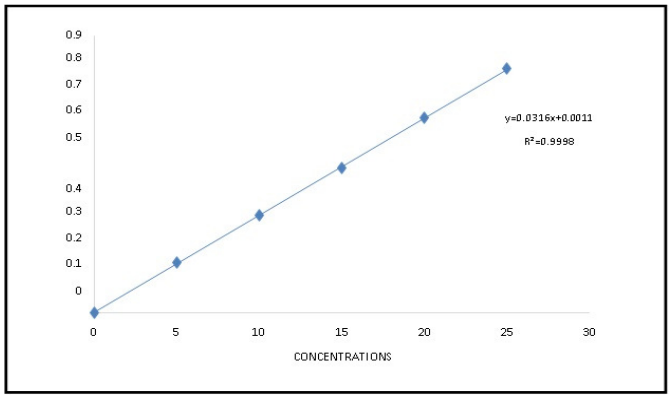


Fig.5: Calibration curve of berberine

Method for Validation[17-18]

Linearity

The capacity of a process to yield test findings directly or through an obvious mathematical

translation that are proportionate to analyte concentration within a certain range is known as linearity. For linearity, five different concentrations range from 5-25 µg/mL of each drug were prepared with methanol. The absorption of each concentration was analyzed in triplicates. The concentration on x-axis and absorbance on y-axis was plotted. The relation between concentration and absorbance was given by correlation coefficient (R2) of linear regression equation (y=mx+b), where m=slope and b=intercept.

Accuracy

Accuracy is defined as the degree of agreement between the mean values of an experimental process with true value. In this method, the accuracy was determined by calculating analyte recovery by the standard addition method at 3 different levels (80%, 100% and 120%). Three replicates of the each concentration solution were prepared for each level and obtained % recovery for each concentration.

Precision

Both intra-day and inter-day studies were conducted for precision. Each experiment was performed with triplicate samples of each drug. The intraday precision was determined by measuring the absorbance at three different times (0 hr, 3 hr, 6 hr) on the same day for each drug. The interday precision was determined by measuring the absorbance at three different days (3th day, 5th day, 7th day) over a period of one week for each drug. Standard deviation (SD) and relative standard deviation (RSD) were calculated for each analysis.

Sensitivity

The ICH guidance includes several techniques, such as visual inspection, signal to noise ratios, and analyzing the slope and standard deviation of the response, for determining the detection limit. The signal-to-noise ratio is derived by comparing measured signals from samples with known low analyte concentrations with those of a blank. When the detection limit depends on the standard deviation and slope of the response, the equation (1) was used to calculate it:

Limit of detection (LOD)= 3:3σ/S Equation (1)

Where σ is the standard deviation of the response

S is the slope of the calibration curve

Based on 10 independent measurements of sample blanks, the limit of detection is typically calculated as the analyte concentration corresponding to the sample blank plus three sample standard deviations.

The ICH guideline lists numerous methods for figuring out the quantization limit, including a method based on eye inspection, the signal-to-noise ratio, response standard deviation, and a method based on slope. The equation (2) is used to determine the quantization limit when it is dependent on the response's standard deviation and slope:

LOQ=10σ/S Equation (2)

Where S denotes the calibration curve's slope and σ denotes the response's standard deviation.

Five, six, or ten standard deviations from the blank mean are commonly chosen as the cut off for quantization. The threshold of determination is another name for it on occasion.[13]

RESULTS AND DISCUSSION

The present study is a simple, accurate, precise, and rapid UV spectrophotometric method for simultaneous analysis of withanolides and berberine in herbal formulation. Moreover, the present method was validated according to ICH guidelines.[18] The developed method was used for quantitative determination of withanolide and berberine present in herbal formulation.

Linearity

For linearity, five different concentrations i.e. 5, 10, 15, 20, 25 µg/mL of each drug were prepared with methanol. There is excellent linearity in a range of 5-25µg/mL of each drug. The observations for linearity were compiled as Table 1.

Table 1: Optical parameters of withanolide and berberine

Parameters	Withanolide	Berberine
Wavelength λ_{max}	231 nm	348 nm
Range	5-25	5-25
Slope (m)	0.0316	0.0316
Intercept (C)	-0.004	0.0011
Correlation coefficient (r^2)	0.999	0.9998
Precision (%RSD) Intraday Precision Interday Precision	%RSD= 0.509 %RSD=1.438	%RSD= 1.763 %RSD=1.499
LOD	0.12	0.07
LOQ	0.35	0.20

Accuracy

For testing of accuracy of sample, recovery experiments were executed by calculating standard deviation at three levels: 80%, 100% and 120%. The approach used was considered to be valid because a high percentage of recovery was attained. For berberine and withanolide, the results showed that the standard deviation and percent relative standard deviation (% RSD) were 0.729, 0.944, 0.07, and 0.09 respectively, which demonstrated accuracy. The recovery data is given in Table 2.

Table 2: Accuracy data of drugs

Total Conc	Sample conc. (ppm)	Std. conc. (ppm)	Withanolides		Berberine	
			Sample conc. difference (ppm)	% Recovery	Sample conc. difference (ppm)	% Recovery
80%	12	10	12.10127	100.84	12.03785	100.32
	12	10	12.17722	101.48	12.06940	100.58
	12	10	11.93671	99.47	12.11672	100.97
100 %	15	10	15.09810	100.65	15.15457	101.03
	15	10	15.25949	101.73	14.86120	99.07
	15	10	14.86392	99.09	15.14826	100.99
120 %	18	10	17.98734	99.93	17.86435	99.25
	18	10	18.27215	101.51	18.11672	100.65
	18	10	18.02215	100.12	18.08517	100.47
SD			0.944		0.729	
RSD			0.09		0.07	

Precision

Tables 1 provided the intraday and interday precision data for withanolide and berberine. Withanolide and berberine both had RSDs lesser than 2%.

CONCLUSION

All the validated parameters showed that the developed method is simple and accurate method for simultaneous determination of withanolide and berberine in herbal formulations by UV spectroscopy. The proposed method is precise and simple method for quantitative determination of withanolide and berberine in crude drugs. Moreover, the validation of the studied method was carried out in accordance with ICH guidelines.

Conflicts of Interest: Authors declare no conflict of interest.

Acknowledgement: The authors would like to thank the Lloyd Institute of Management and Technology, for their assistance and for providing the necessary resources for my research.

REFERENCES

1. Yan Y, Du X, Lai L, Ren Z, Li H. Prevalence of depressive and anxiety symptoms among Chinese older adults during the COVID-19 pandemic: A systematic review and meta-analysis. J Geriatr Psychiatry Neurol. 2022 Mar; 35(2): 182-195.
2. Vellingiri B, Jayaramayya K, Iyer M, Narayanasamy A, Govindasamy V, Giridharan B, et al. COVID-19: A promising cure for the

global panic. Sci Total Environ. 2020 Jul 10; 725: 138277.
3. Gautam S, Gautam A, Chhetri S, Bhattarai U. Immunity against COVID-19: Potential role of Ayush Kwath. J Ayurveda Integr Med. 2022 Jan-Mar; 13(1): 100350.
4. Balkrishna A, Tomer M, Verma S, Joshi M, Sharma P Srivastava, et al. Phytometabolite profiling of Coronil, a herbal medicine for COVID-19, its identification by mass-spectroscopy and quality validation on liquid chromatographic platforms. J Sep Sci. 2021 Sept 12; 44(22): 4064–81.
5. Subbaraju GV, Vanisree M, Rao CV, Sivaramakrishna C, Sridhar P, Jayaprakasam B, et al. Ashwagandhanolide, a bioactive dimeric thiowithanolide isolated from the roots of *Withania somnifera*. J Nat Prod. 2006 Dec; 69(12): 1790-2.
6. Cohen MM. Tulsi - *Ocimum sanctum*: A herb for all reasons. J Ayurveda Integr Med. 2014 Oct-Dec; 5(4): 251-9.
7. Saha S, Ghosh S. *Tinospora cordifolia*: One plant, many roles. Anc Sci Life. 2012 Apr; 31(4): 151-9.
8. Ajage RK, Kasture VS. Validated UV spectroscopic method for the estimation of three marker compounds in marketed polyherbal ayurvedic formulation. Der Pharm. Lett. 2014, 6 (3): 160-166.
9. 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. Pharm. Educ. Res. 2022; 56(3): 873-80.
10. Joshi HR, Kanaki N. Quantitative analysis of berberine in an ayurvedic formulation-rasayana churna by UV spectrophotometry. J. Pharm. Sci. Bio-Sci. Res. 2013; 3: 32–34.
11. Karthikeyan R, Babu CS, Babu PS. Quantitative analysis of berberine in homeopathic formulation containing *Berberis vulgaris* L. by UV. Electronic J Biosci. 2014; 2(1): 91–98.
12. Koshy R, Anand MS, Murali B, Brunelle SL. Determination of withanolides in *Withania somnifera* by liquid chromatography: Single-laboratory validation, first action 2015.17. *J. AOAC Int.* 2016 Nov 1; 99 (6): 1444–58.
13. Manwar J, Mahadik RK, Paradkar A, Takle SP, Lohidasan S, Patil SV. Determination of withanolides from the roots and herbal formulation of *Withania somnifera* by HPLC using DAD and ELSD detector. Der Pharmacia Sinica. 2012; 3(1): 41.
14. Sharma V, Gupta A, Bhandari P, Gupta RC, Singh B. A validated and densitometric HPTLC method for the quantification of withaferin-A and withanolide-A in different plant parts of two morphotypes of *Withania somnifera*. Chromatographia. 2007; 66: 801-804.
15. Ahamad J, Kaskoos AR, Amin S, Mir SR.. Development and validation of an HPTLC method for estimation of berberine in *Berberis aristata*. Res. J. Phytochem. 2021; 15(2): 58-65.
16. Pasrija A, Singh R, Katiyar CK. 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. Int J Ayurveda Res. 2010 Oct; 1(4): 243-6.
17. Belouafa S, Habti F, Benhar S, Belafkih B, Tayane S, Hamdouch S, et al. Statistical tools and approaches to validate analytical methods: Methodology and practical examples. Int. J. Metrol. Qual. Eng. 2017; 8: 9.
18. Validation of analytical procedures; text and methodology, proceeding of the International conference on Haromonization (ICH) Geneva, 2005.