IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2022 Vol.:24, Issue:2

Development and Validation of UV-Spectrophotometric Method for Estimation of Benzonatate in Bulk and Soft Gelatin Capsule Dosage Form

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Shivani R. Sawarkar, Santosh V. Gandhi*

Department of Pharmaceutical Quality Assurance, AISSMS College of Pharmacy, Kennedy Road, Near RTO, Pune-411001, India.

Submitted: 21 April 2022
Accepted: 27 April 2022
Published: 30 May 2022

Keywords: Words: Benzonatate, Capsule, COVID-19, UV spectrophotometer, Linearity, Precision, ICH Q2 R1 guideline.

ABSTRACT

Older than 10 years of age. Recently, In COVID-19, Benzonatate capsules have been used frequently to ease cough in many patients. Chemically, Benzonatate 2,5,8,11,14,17,20,23,26-nonaoxaoctacosan-28-yl p- (butyl amino) benzoate is clear, pale yellow, viscous liquid, freely soluble in chloroform, in alcohol, and benzene. A simple UV-spectrophotometric method was developed for the estimation of Benzonatate in the bulk and pharmaceutical dosage forms. The λmax was found to be 308 nm in methanol. Pure drug concentrations were prepared in the range of 5-30 µg/ml and the linear regression analysis data of Absorbance vs Concentration showed a good linear relationship with an R^2 value of 0.990. The method was validated according to International Conference on Harmonization (ICH Q2 R1) guidelines concerning linearity, range, precision, accuracy and robustness, the limit of detection, and the limit of quantitation. The limit of detection and the limit of quantitation were found to be 0.388 and 1.176 µg/ml, respectively. Recoveries were found to be in the range of 99.88 % to 100.71 % and % RSD less than 2 % which indicate that the developed method was accurate, precise, rapid, and suitable for the analysis of commercial samples.

INTRODUCTION:

The process of validation of the analytical method is adopted to confirm that the employed analytical procedure for a specific test meets the intended requirements. Results from the method validation can be considered to judge the quality, reliability as well consistency of analytical results.

The main parameters used for validation purposes are linearity and range, accuracy and precision, recovery, limit of detection, and limit ofquantitation[1]. Benzonatate is the only non-narcotic antitussive available as a prescription drug across the globe. Benzonatate was approved by the FDA in 1958 under the market name Tessalon Perles[2]. Benzonatate is a non-opioid drug that suppresses transmission of the cough reflex at the level of the medulla where the afferent impulse is transmitted to the motor nerves. Its concentration can be extrapolated based on the concentration of its metabolite, 4- (butyl amino) benzoic acid (BBA)[3]. A non-narcotic antitussive agent, Benzonatate is 2, 5, 8, 11,14,17,20,23,26-nonaoxaoctacosan-28-yl p-(butyl amino) benzoate, with a molecular weight of 603.75 (Fig. 1). The appearance of the drug is a clear, pale yellow, viscous liquid with slight hygroscopic nature. The drug has a pKa of 3.03 and is freely soluble in water, methanol, acetone, ethanol, and tetrahydrofuran[4]. In Covid 19, therapies for lower respiratory tract symptoms were reported and are mostly cured by benzonatate (93.8%)[5].

The literature survey revealed few analytical and bioanalytical methods reported for estimation of benzonatate which include HPLC- MS/MS[3], LC-MS[4], HPLC[6,] and Spectrophotometric[7] either in bulk or in combination. Reported methods were not much cost-effective in terms of time and solvent consumption. No analytical method thus far reported for the estimation of benzonatate using methanol as solvent. The present work was carried out with the view of establishing a simple, rapid, accurate, economic, precise, and robust UV method for estimation of Benzonatate in bulk and capsule dosage form, using methanol as the solvent.

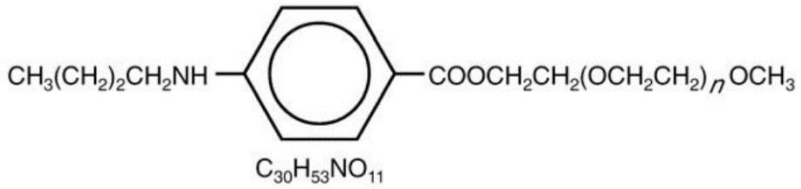

Fig 1: Structure of benzonatate

MATERIAL AND METHODS:

Chemicals and Equipment:

A standard sample of Benzonatate was received as a generous gift sample from Strides Pharma Science Limited, Bangalore (India). The marketed Benzonatate capsules containing 100 mg of Benzonatate, manufactured by Gelnova Laboratories (Navi Mumbai, India) were purchased from the market. All other chemicals used were of analytical grade. A double beam UV/Vis spectrophotometer (Jasco) with a single monochromator, model UV-730 having a spectral bandwidth of 1 nm was used to record spectrums.

Preparation of Standard Solution:

100 mg of Pure Benzonatate was weighed and transferred in a 100 ml volumetric flask by adding solvent methanol and volume made up to 100 ml to give a solution having 1000 µg/ml concentration. From the stock solution, a 10 ml pipette was out and transferred to a 100 ml volumetric flask with methanol giving a 100 µg/ml concentration solution. From the working standard solution, further dilutions were made with methanol to get range of solutions having concentration 5-30 µg/ml.

Wavelength Selection:

The solution of benzonatate (10 µg/ml) was scanned in the wavelength region of 200-400 nm (Fig. 2) and the λmax was found to be 308 nm.

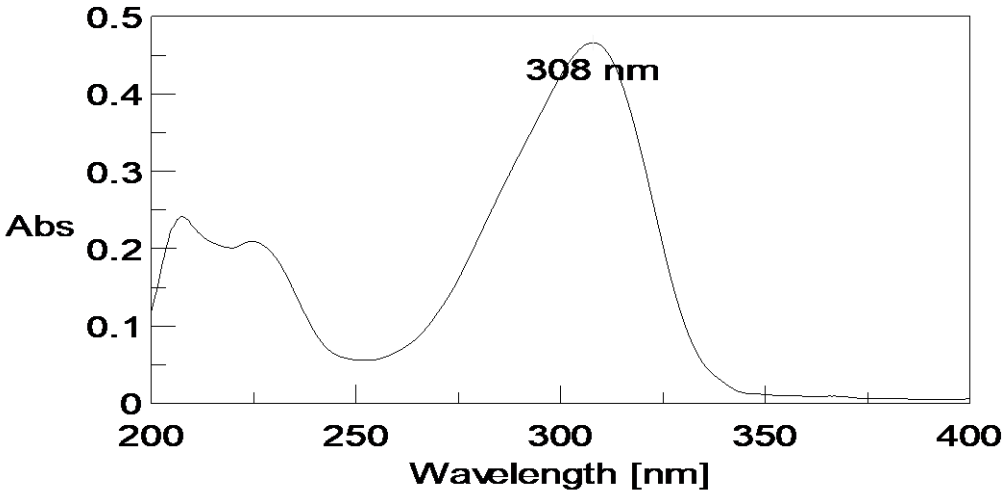

Fig 2: UV Spectrum of pure Benzonatate of 10 µg/ml

Sample Solution (Marketed Formulation Analysis):

Marketed capsules of Benzonatate containing 100 mg drug per capsule were taken and pierced. Capsule content equivalent to 100 mg of Benzonatate was transferred into the 100 ml volumetric flask. The prepared solution was found to be a clear having the strength 1000 µg/ml. From this stock solution 10 ml of solution was taken and transferred to 100 ml volumetric flask to get 100 µg/ml solution. From this solution, 1 ml pipette out and diluted to 10 ml with methanol which gives 10 µg/ml solution which was analysed at 308 nm.

Method validation[8]

The method was developed and validated according to ICH Q2 (R1) guidelines for validation of analytical procedures to determine linearity, accuracy, precision, ruggedness, and robustness.

Linearity

The linearity of the method was determined in the concentration range of 5-30 µg/ml for Benzonatate. The absorbance of each solution was recorded at 308 nm. The calibration curve was plotted and correlation co- efficient with regression line equation for Benzonatate was determined.

Accuracy (% Recovery)

Accuracy (% Recovery) was evaluated at three different concentration levels equivalent to 50, 100 and 150% of the target concentration of active ingredient, by adding a known amount of standard and sample solutions in a same volumetric flask and calculating the % recovery for each concentration.

Limit of detection (LOD) and limit of quantification (LOQ):

ICH defines the limit of detection of an analytical method as the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value whereas the limit of quantitation of an analytical procedure is the lowest amount of analyte in a sample, which can be determined quantitively with suitable precision and accuracy. LOD and LOQ were calculated by using the following formula: LOD =3.3×σ/S and LOQ = 10×σ/S, where, σ is the standard deviation of y-intercepts of the regression line, and S is the slope of the calibration curve.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was determined as Intra-day and Inter-day. The experiment was performed three times in a day for intra-day precision and on 3 different days for inter-day precision at three different concentrations levels and results were reported as % RSD.

Robustness:

The robustness was carried out to evaluate the influence of a small but deliberate variation in the spectrometric condition for the determination of Benzonatate in bulk and capsule dosage form. The Robustness data for variations in wavelength of detection (308±1nm) and the absorbance value was estimated.

RESULTS AND DISCUSSION:

The linearity of Benzonatate was found to be in the range of 5-30 µg/ml with linear correlation coefficient of 0.990 (Fig 3). The % RSD was found to be in the range of 0.418-

0.608 for intra-day precision and 0.362-0.598 for inter-day precision. Accuracy of the method was checked by the recovery studies at three different levels i.e., 50%, 100% and 150 % and % Recovery was found to be close to 100 percent. The sensitivity of the method was assessed by determining the LOD and LOQ. The LOD and LOQ for Benzonatate were found to be 0.388 and 1.176 µg/ml, respectively. The Assay was performed and the result found to 101.23 ± 0.749. Method robustness was tested by measuring the absorbance (± 1 nm) at 307, 308, 309 nm and the method was found to be robust having % RSD within limits. The linearity of the calibration curve showed that the proposed method could be successfully used for analysing pharmaceutical dosage form without any interference from common excipients. The results of validation parameters are presented in Table 1 to Table 5 in detail.

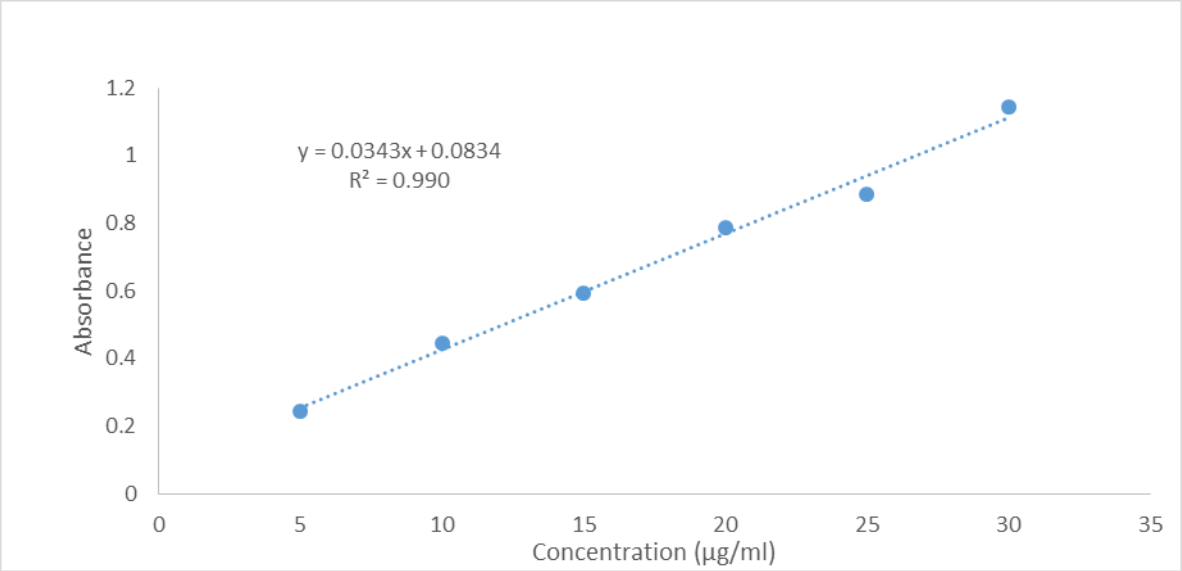

Fig 3: Calibration Curve Plot of Standard Benzonatate

Table 1: Quantitative spectrophotometric method parameters of UV.

Parameters	**Results**
λ_{max} (nm)	308 nm
Beer's Lambert's law limits (µg/ml)	5-30
Regression equation	0.0343x+0.0834
Slope	0.0343
Intercept	0.0834
Correlation coefficient (R^2	0.990

Table 2: Results of intra-day and inter-day precision

Concentration (µg/ml)	Intraday Precision			Interday precision		
	Absorbance	SD	%RSD	Absorbance	SD	%RSD
10	0.428	0.421	0.418	0.427	0.367	0.362
10	0.431			0.426		
10	0.429			0.429		
20	0.784	0.591	0.583	0.783	0.530	0.524
20	0.776			0.775		
20	0.775			0.776		
30	1.120	0.616	0.608	1.119	0.611	0.598
30	1.135			1.124		
30	1.124			1.134		

Table 3: Results of recovery studies

Amount in marketed formulation (µg/ml)	Amount of API added (µg/ml)	Absorbance	Amount recovered	% Recovery	Avg	SD	%RSD
10	5	0.599	15.09	100.19	99.88	0.32	0.32
10	5	0.598	15.00	100.01			
10	5	0.595	14.91	99.43			
10	10	0.763	19.80	99.03	98.15	0.85	0.87
10	10	0.749	19.39	96.99			
10	10	0.759	19.68	98.44			
10	15	0.941	25.00	100.03	100.71	0.27	0.27
10	15	0.947	25.10	100.71			
10	15	0.944	25.09	100.39			

Table 4: Robustness results at three different wavelengths

Wavelength	%RSD*
307	0.60
308	0.70
309	0.87

*Average of three experiments

Table 5: Assay of Benzonatate capsule formulation

Concentration (µg/ml)	Amount Recovered (µg/ml)	Percent Recovered (%)	Mean ± RSD
10	10.02	100.262	101.23 ± 0.749
10	10.07	100.758	
10	10.10	100.997	
10	10.24	102.449	
10	10.17	101.663	
10	10.16	101.662	

CONCLUSION:

The proposed method quantitatively evaluated in terms of linearity, accuracy, precision, assay and robustness. All these factors lead to the conclusion that the proposed UV-Spectrophotometric method is simple, accurate, precise, sensitive and cost-effective.

Acknowledgment:

The author thankful to Strides Pharma Bangalore, India for providing gift sample of Benzonatate sample. The authors are also thankful to the Department of Pharmaceutical Quality Assurance, AISSMS College of Pharmacy Pune, Maharashtra, India for encouragement and providing laboratory facilities.

REFERENCES:

1. Ravisankar P, Navya CN, Pravallika D, Sri DN. A review on step-by-step analytical method validation. IOSR J Pharm. 2015; 5(10):7-19.
2. Katakam LN, Dongala T. Quality by design with design of experiments approach for the development of a stability-indicating LC method for benzonatate and its impurities in liquid oral dosage form. Separation science plus. 2020; 3(7):276-285.
3. Man J, Jiao F, Wang Y, Gu Y, Ding L, Shu C. Determination of benzonatate and its metabolite in human plasma by HPLC–MS/MS: a preliminary pharmacokinetic study in healthy Chinese volunteers after oral administration of benzonatate soft capsule. Journal of pharmaceutical and biomedical analysis. 2019; 173:134-143.
4. Katakam LNR, Ettaboina SK, Marisetti VM. Development and validation of LC-MS method for the determination of heptaethylene glycol monomethyl ether in benzonatate bulk drugs. Biomed. Chromatography. 2021; 35(7), e5096: 1-7
5. O'Keefe JB, Newsom LC, Taylor TH. A Survey of Provider-Reported Use and Perceived Effectiveness of Medications for Symptom Management in Telemedicine and Outpatient Visits for Mild COVID-19. Infectious diseases and therapy. 2021; 10 (2):839-851.
6. Kishk SM, Salama I, Mostafa S, E-Sadek M. Stability-indicating chromatographic method for the determination of benzonatate, diphenhydramine, guaifenesin, and phenylephrine. Journal of liquid chromatography & related technologies. 2014; 37(5):726-747.
7. Darwish HW, Metwally FH, Bayoumi A. Simultaneous spectrophotometric determination of diphenhydramine, benzonatate, guaifenesin and phenylephrine in their quaternary mixture using partial least squares with and without genetic algorithm as a powerful variable selection procedure. Digest Journal of Nanomaterials & Biostructures. 2014 ; 9(4): 1359-1372
8. https://www.ich.org