International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.6, pp 274-280, 2017

UV-Visible Spectrophotometry Method Validation for Analysis of Nefopam HCl in Poly-3-Hydroxybutyrate and Poly-ε-Caprolactone Microspheres

Neelam Sharma[1, 2]*, Sandeep Arora[1], Jitender Madan[3]

[1]Chitkara College of Pharmacy, Chandigarh-Patiala National Highway, Patiala, Punjab, India
[2]Department of Research, Innovation and Consultancy, IKG Punjab Technical University, Jalandhar-Kapurthala Highway, Kapurthala, Punjab, India
[3]Chandigarh College of Pharmacy, Landran, Kharar, Sahibzada Ajit Singh Nagar, Punjab, India

Abstract : UV-visible spectrophotometry analytical method was validated for detection and quantitative analysis of nefopam hydrochloride(NPH) in microspheres synthesized using poly-3-hydroxybutyrate and poly-ε-caprolactone. UV absorption maximum (λ_{max}) of NPH in phosphate buffer (pH 7.4) was found 266 nm. Limit of detection (LOD) and limit of quantification (LOQ) of NPH were found 16.75 and 50.77 µg/ml, respectively. Repeatability, intermediate precision and specificity of developed analytical method wereestablished(RSD< 2%). The analytical method was found robust at different wavelengths (± 6) and temperatures (± 20 ^{o}C) (RSD < 2%). It was concluded that developed and validated UV spectrophotometry analytical method can be employed for routine quantitative analysis of NPH in microspheres synthesized using poly-3-hydroxybutyrate and poly-ε-caprolactone.
Keywords: Nefopam Hydrochloride, Poly-3-Hydroxybutyrate and Poly-ε-Caprolactone, Limit of Detection, Limit of Quantification, Intermediate Precision.

Introduction

Spectroscopy is domain of science dealing with the analysis of interaction between electromagnetic radiation and matter. UV-Visible spectrophotometry is most widely applied method in pharmaceutical analysis for analysis of specific compound in precise and specific manner. In qualitative analysis, it quantifies the amount of ultraviolet or visible radiation absorbed by material in solution to determine the quantity of material. Quantitative spectrophotometric analysis is based upon Beer -Lambert law. Beer-Lambert law states that when beam of parallel monochromatic light is passed through a transparent cell containing a solution of an absorbing substance, intensity of light may decreases. Mathematically, Beer- Lambert law is indicated as:

$$A = a.b.c \qquad \text{Eq. (1)}$$

Where, 'A' is absorbance, 'a' is absorptivity or extinction coefficient, 'b' is path length of radiation through sample (cm), 'c' is concentration of solute in solution [1-3].

Analytical method validation is the process to demonstrate that analytical procedure employed for specific test is suitable for its intended use and support identity, strength, quality, purity and potency of drug

substances and drug products. Nefopam hydrochloride (NPH) is a potent non-opioid centrally acting analgesic of benzoxazocine class [4]. In this investigation, efforts were made to develop a simple and economic UV-visible spectrophotometric approach for detection and quantitative analysis of NPH in microspheressynthesized using poly-3-hydroxybutyrate and poly-ε-caprolactone. Developed analytical method was validated for appropriate system parameters as per guidelines of international conference on harmonization (ICH) to establish sensitivity (limit of detection and limit of quantification), repeatability, intermediate precision, specificity and robustness [5, 6].

Experimental

Preparation of Stock and Working Standard Solution

100 mg of NPH was accurately weighed and dissolved in 50 ml of phosphate buffer pH 7.4 in a volumetric flask to produce 2000 µg/ml solution. 25 ml solution was withdrawn and diluted to 50 ml with phosphate buffer, pH 7.4 to give standard stock solution having concentration of 1000 µg/ml. From stock solution, 2.5 ml solution was transferred into 10 ml volumetric flask and volume was made up to mark with phosphate buffer, pH 7.4 to produce 250 µg/ml working standard solutions.

Determination of Absorption Maxima (λ_{max}) of NPH

A working standard solutions of NPH (250 µg/ml) was prepared using phosphate buffer, pH 7.4 and 8 ml of solution was diluted to 10 ml with phosphate buffer, pH 7.4 to obtain 200 µg/ml reference solution. An UV spectroscopic scanning (200-400 nm)was carried out with reference solution to determine λ_{max} for detection of NPH using phosphate buffer, pH 7.4 as blank.

Calibration Curve of NPH

From 1000 µg/ml standard stock solution, aliquots (*i.e.* 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml, 3.5 ml and 4 ml) were transferred into 10 ml volumetric flask and volume was adjusted with phosphate buffer, pH 7.4 to make dilutions having 50-400 µg/ml concentration and analyzed for absorbance [7, 8].

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of NPH was evaluated from the slope (S) of calibration curve and standard deviation of y-intercept of the regression equation using following equations:

$$\text{LOD} = \frac{3.3\sigma}{S} \quad \text{Eq. (2)}$$

$$\text{LOQ} = \frac{10\sigma}{S} \quad \text{Eq. (3)}$$

The LOD is lowest amount of analyte which can be detected in sample, but not necessarily quantities as an exact value while LOQ is minimum quantity that can be quantified by the instrument[9].

Repeatability (intra-day precision)

The different concentrations of nefopam hydrochloride *i.e.* 150, 200 and 250 µg/ml were analyzed at three different times within a day. The % RSD should be less than 2% for acceptable repeatability [10, 11].

Intermediate Precision

Intermediate precision articulates within-laboratories variations: different days (inter-day), different analysts and different equipment. The different concentrations of nefopam hydrochloride *i.e.* 150, 200 and 250 µg/ml were analyzed (% RSD limit: < 2%) on three different days (inter-day precision) [10, 11]. 200 µg/ml NPH

solutions were analyzed by different equipments (Systronics AU-2701, Ahmedabad, India; Systronics 2202, Ahmedabad, India) and analysts in six trials. Absorbance's were subjected to calculation for mean, standard deviation, % relative standard deviation (% RSD limit: < 2%), standard error and % coefficient of variation [5].

Specificity

30 mg of NPH was mixed with 100% (30 mg), 200% (60 mg), 300% (90 mg), 400% (120 mg) and 500% (150 mg) of excipients (polyhydroxybutyrate and polycaprolactone) and analyzed for % recovery of NPH. The accepted limits of % recovery and RSD for validating specificity are 98% - 102% and < 2%, respectively [11].

Robustness

Robustness of UV spectrophotometry analytical method was determined by analyzing the 250 µg/ml NPH solutions at different temperatures *i.e.* 25±20°C and wavelengths (λ_{max}) *i.e.* 260 ± 6 nm. % RSD acceptance limit is < 2% [5].

UV Spectrophotometry Method Validation

Absorption Maxima (λ_{max}) of NPH

Fig. 1 depicts UV scan of solution of NPH solution in phosphate buffer acquired using UV spectroscopy at λ_{max} of 266 nm.

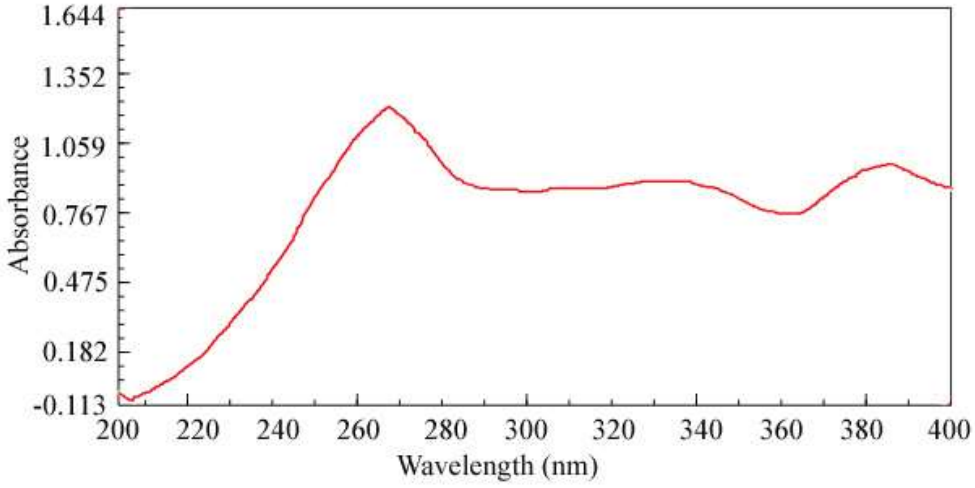

Fig. 1 UV scans of NPH solution in phosphate buffer, pH 7.4.

Calibration Curve of NPH

Calibration curve of NPH was acquired using UV spectrophotometric technique by plotting a graph between concentrations of NPH *vs.* absorbance value obtained at 266 nm (Fig. 2). Statistical analysis of calibration curve of NPH was performed by curve linear regression. Regression coefficient and *P* value was found 0.9976 and 0.008694 ($P^* < 0.05$), respectively, which illustrated goodness of fit as well as statistical significance of proposed method (Table 1).

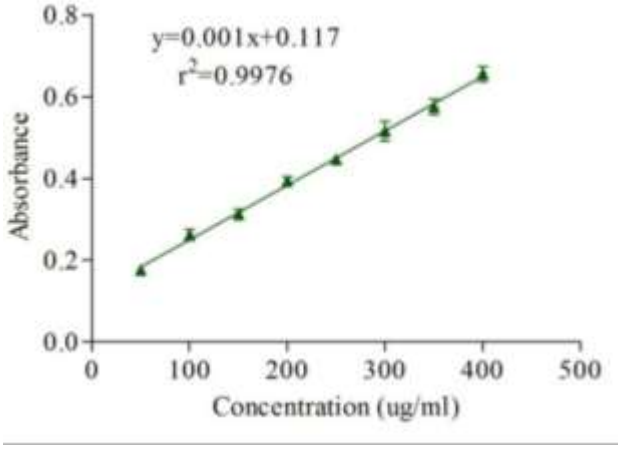

Fig. 2 Calibration curve of NPH in phosphate buffer, pH 7.4 using UV spectroscopy.

Table 1 Linear regression statistical data of calibration curve for NPH.

Parameter	**Value**
Best-fit values	
Slope	0.001334 ± 0.00002683
Y-intercept when X=0.0	0.1171 ± 0.006774
X-intercept when Y=0.0	-87.79
1/slope	749.9
95% Confidence intervals	
Slope	0.001268 to 0.001399
Y-intercept when X=0.0	0.1005 to 0.1336
X-intercept when Y=0.0	-105.0 to -72.11
Goodness of fit	
R square	0.9976
P value	0.008694

Analytical Method Validation Parameters[9, 12]

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ of NPH in phosphate buffer, pH 7.4 were found 16.75 and 50.77 µg/ml, respectively

Repeatability

The % RSD for absorbance values of 150, 200 and 250 µg/ml NPH solutions at three different time periods within a day was found to be 1.14, 0.64 and 0.67 % (< 2%), which validated repeatability of analytical method (Table 2) [11, 13, 14].

Intermediate Precision

The % RSD for absorbance values of 150, 200 and 250 µg/ml NPH solutions on three different days was found 0.79, 0.51 and 0.56 % (< 2%), which validated inter-day precision of analytical method (Table 2)[10, 11, 14].

Table 2 Repeatability and inter-day precision determined for three different concentrations of nefopam hydrochloride (*n* = 3).

Conc. (µg/ml)	Repeatability		Inter-day precision	
	Absorbance measured	**% RSD**	**Absorbance measured**	**% RSD**
150	0.315	1.14	0.3167	0.79
200	0.3937	0.64	0.395	0.51
250	0.446	0.67	0.4457	0.56

% RSD of absorbance values of sample solutions analyzed by analyst-1, analyst-2, equipment-1 and equipment-2 was found 0.46250%, 0.55647%, 0.33063% and 0.48463%, respectively. The % RSD values were < 2% which indicated intermediate precision of developed analytical method (Table 3) [5].

Table 3 Intermediate precision data of UV spectrophotometry analytical method.

Condition	Trials	Absorbance	Mean	SD	% RSD	Std. error	% CV
Analyst-1	1	0.4430	0.4467	0.002066	0.46250	0.0008433	0.46
	2	0.4480					
	3	0.4470					
	4	0.4490					
	5	0.4460					
	6	0.4470					
Analyst-2	1	0.4480	0.4462	0.002483	0.55647	0.001014	0.56
	2	0.4460					
	3	0.4420					
	4	0.4470					
	5	0.4450					
	6	0.4490					
Equipment-1	1	0.4450	0.4452	0.001472	0.33063	0.0006009	0.33
	2	0.4470					
	3	0.4460					
	4	0.4430					
	5	0.4460					
	6	0.4440					
Equipment-2	1	0.4430	0.4457	0.002160	0.48463	0.0008819	0.48
	2	0.4490					
	3	0.4440					
	4	0.4450					
	5	0.4460					
	6	0.4470					

Specificity

Specificity of UV spectrophotometry analytical method was determined by analyzing NPH in presence and absence of excipients (poly-3-hydroxybutyrate and poly-ε-caprolactone). Mean recovery of NPH was found 99.93 % which was within accepted limit (98 % - 102 %). The % RSD was found 0.8644 % (< 2 %) which validated specificity of analytical method (Table 4) [11, 15].

Table 4 Specificity data of UV spectrophotometry analytical procedure.

PHB:PCL* (1:1)	NPH input (mg)	NPH recovered (mg)	NPH Recovered (%)	Mean Recovered	Statistical analysis
100 %	30	30.2	100.67	99.93 %	Mean = 99.93 SD= 0.8638 % RSD = 0.8644 Std. error = 0.386 % CV = 0.86%
200 %	30	30.3	101.00		
300 %	30	29.8	99.33		
400 %	30	29.9	99.67		
500 %	30	29.7	99.00		

*PHB: Poly-3-hydroxybutyrate; PCL: Poly-ε-caprolactone

Robustness

% RSD of absorbance values of sample solutions analyzed at different wavelengths and temperatures was found 0.3424% and 0.5634%, respectively. The % RSD values were < 2% which indicated that proposed analytical method remained unaffected by small but deliberate variations in method parameters and provided an indication of its reliability during normal usage (Table 5) [5, 14].Validation parameters of spectrophotometric method are shown in Table 6.

Table 5 Robustness studies of UV spectrophotometry analytical method.

Condition	Parameter	Absorbance	Mean	SD	% RSD	Std. error	% CV
Change in Wavelength	260 nm	0.448	0.4463	0.00152	0.3424	0.0008819	0.34
	266 nm	0.445					
	272 nm	0.446					
Change in temperature	5 ^{o}C	0.447	0.4467	0.00251	0.5634	0.001453	0.56
	25^{o}C	0.449					
	45 ^{o}C	0.444					

Table 6 UV spectrophotometry analytical method validation parameters.

Parameter	Result
λ_{max} (nm)	266
Regression equation (y= mx + c)	y = 0.001x + 0.117
Regression coefficient (r^2)	0.9976
Limit of detection (LOD)	16.75 µg/ml
Limit of quantitation (LOQ)	50.77 µg /ml
Repeatability indicated by % RSDfor NPH (150, 200 and 250 µg/ml)	1.14, 0.64 and 0.67 %
Intermediate precision	
% RSD for NPH, 150, 200 and 250 µg/ml (Inter-day)	0.79, 0.51 and 0.56 %
Indicated by % RSD (analyst-I, analyst-2)	0.46250, 0.55647 %
Indicated by % RSD (equipment-1, equipment-2)	0.330, 0.484 %
Specificity indicated by % recovery	99.93 %
Robustness indicated by % RSD (λ_{max}, ± 6 nm)	0.3424 %
Robustness indicated by % RSD (Temp. ± 20^{o}C)	0.5634 %

Conclusions

UV absorption maximum (λ_{max}) of NPH in phosphate buffer (pH 7.4) was found 266 nm. LOD and LOQ of NPH were found 16.75 and 50.77 µg/ml, respectively. The repeatability and intermediate precision of developed analytical method was validated through % RSD(< 2%). The % RSD was found 0.8644 % (< 2 %) on analyzing NPH in presence and absence of poly-3-hydroxybutyrate and poly-ε-caprolactone which validated specificity of analytical method. The % RSD values were found < 2% when sample solutions were analyzed at different wavelengths (± 6) and temperatures (± 20 ^{o}C) which validated the robustness of method.It was

concluded that developed and validated UV spectrophotometry analytical method can be employed for routine analysis and quantitative evaluation of NPH in microspheres synthesized using poly-3-hydroxybutyrate and poly-ε-caprolactone.

Acknowledgement

The support for providing access to ScienceDirect and anti-plagiarism software from department of Research, Innovation and Consultancy, IKG Punjab Technical University, Jalandhar, is greatly acknowledged. The authors also wish to gratitude Chitkara University for providing facilities for this research.

Conflict of Interests

The authors report no conflicts of interest in this work.

References

1. Willard-Hobart H, Merritt, JLL, Dean, JA. Instrumental Methods of Analysis, fifth ed. Von Nostrand, University of Michigan, 1974.
2. Chatwal GR, Anand S. Instrumental Methods of Chemical Analysis, fifth ed. Himalaya Publishing House, New Delhi, 2002.
3. Davidson AG. Ultraviolet-visible absorption spectrophotometry, In: Beckett AH, Stenlake JB. Practical Pharmaceutical chemistry, fourth ed. CBS Publishers and distributors, New Delhi, 2002, 275-278.
4. Kim KH, AbdiS. Rediscovery of Nefopam for the Treatment of Neuropathic Pain. Korean J. Pain, 2014, 27(2): 103-111.
5. Dey S, Kalyani K, Samyuktha B, Sahoo SK, Mohapatra S, Murthy PN, Kumar D. Development and Validation of a UV-Vis Spectrophotometric Method for the Estimation and Degradation Monitoring of Cefadroxil in Bulk and Pharmaceutical Dosage Forms. Int. J. Chem. Res., 2010, 1(1): 29-34.
6. ICH Harmonized Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology, ICH Q2 (R1), (2005).
7. Nagisetty P, Shanta Kumar SM, Kumar PR. Analytical Method Development and Validation of Anti-HIV Drug AbacavirSulphate. J. App. Pharm. Sci., 2012, 2(1): 85-89.
8. Singh S, Sharma N, Singla YP, Arora S. Development and Validation of UV-Spectrophotometric Method for Quantitative Estimation of Nefopam Hydrochloride in Polymethacrylate Nanospheres. Int. J Pharm. Pharm. Sci., 2016, 8(1): 414-419.
9. Khoshneviszadeh R, Bazzaz BSF, Housaindokht MR, Ebrahim-Habibi A, Rajabi O. UV Spectrophotometric Determination and Validation of Hydroquinone in Liposome. Iranian J Pharm. Res., 2015, 14(2): 473-478.
10. Choudhari VP, Parekar SR, Chate SG, Bharande PD, Kuchekar BS. Development and Validation of UV Visible Spectrophotometric Baseline Manipulation Methodology for Simultaneous Analysis of Drotraverine and Etoricoxibin Pharmaceutical Dosage Forms. Pharm. Methods, 2011, 2(4): 247-252.
11. Malequea M, Hasan MR, Hossen F, Safi S. Development and Validation of a Simple UV Spectrophotometric Method for the Determination of Levofloxacin both in Bulk and Marketed Dosage Formulations. J. Pharm. Anal., 2012, 2(6): 454-457.
12. Bhowmick AA, Khandelwal KR, Mhaske DV, Khadke S. Analytical Method Development and Validation for Piracetam as Bulk and in Pharmaceutical Formulation. Int. J. PharmTech Res. 2010, 2(1): 201-204.
13. Aneesh TP, Rajasekaran A. Method Development and Validation for the Estimation of Sildosin in Bulk and Pharmaceutical Dosage forms Using UV-VIS Spectrophotometry. Asian. J. Pharm. Clin. Res., 2012, 5(4): 150-152.
14. Rathod BH, Rani SS, Kartheek N, Kumar AA. UV Spectrophotometric Method Development and Validation for the Quantitative Estimation of Indinavir Sulphate in Capsules. Int. J. Pharm. Pharm. Sci., 2014, 6(6): 598-601.
15. Singh AV, Nath LK, Pani NR. Development and Validation of Analytical Method for the Estimation of Lamivudine in Rabbit Plasma. J. Pharm. Anal., 2011, 1(4): 251-257.

*****