Research Article

Preparation, Estimation and Validation of the parameters of the standard curve of Ibuprofen by comparative study

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Abstract

Objective: The objective of present study was to study and validation of different parameters for Ibuprofen. **Material and methods:** UV spectrophotometric method for the comparative quantitative determination of Ibuprofen was developed for the validation of the prepared standard curve of the drug in two different conc. of same solvent (HCl). The parameters like linearity, accuracy, precision, limit of detection, limit of quantization, range, Robustness & Ruggedness were studied according to International Conference on Harmonization guidelines (ICH). The absorbance values were measured at 222 nm. **Results and conclusion:** Results of the analysis were validated statistically and also by recovery studies. The proposed method can also be used for the reliable quantization of Ibuprofen in pharmaceutical formulation. In the UV spectroscopic method linearity over the concentration range of (100-600) ug/ml with a correlation coefficient 0.9972 (Solvent is 0.1N HCl) and 0.9937 (Solvent is 1N HCl) for (50-100) ug/ml were found out.

Keyword: Ibuprofen, ICH, Range, Concentration, LOD

Introduction

Ibuprofen is a drug which exists in the non-steroidal anti-inflammatory drug (NSAID) class that is used for treating pain, fever, and inflammation. This includes painful menstrual periods, migraines, and rheumatoid arthritis ("Ibuprofen". The American Society of Health-System Pharmacists. Retrieved 2016-10-12). About 60% of people improve with any given NSAID, and it is recommended that if one does not work then another should be tried (London: British Medical Assn. 2014. pp. 686–688). It may also be used to close a patent ductus arteriosus in a premature baby.

Formula: $C_{13}H_{18}O_2$

Molar mass: 206.29 g/mol

Density: 1.03 g/ml g/cm^3

Melting point: 75 to 78 °C (167 to 172 °F)

Boiling point: 157 °C (315 °F).

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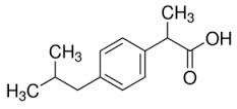


Figure 1. Structure of Ibuprofen.

Material and methods

Materials

The drug that was taken during the experiment was Ibuprofen, and the solvents that were selected are 0.1 N HCl and 1N HCl.

Apparatus

Double beam UV-Vis Spectrophotometer, SA 165, Array spectrophotometer was used for measuring the absorbance of the solutions in the present work.

Preparation of stock solutions of Ibuprofen

Ibuprofen was dried overnight in desiccators as 100 mg was dissolved in 100 ml of 0.1N HCl and 1N HCl to prepare a stock solution of concentration 1000 ug/ml. Working standard of Ibuprofen was prepared by diluting the stock solutions.

Selection of analytical wavelength

UV Spectra of pure drug solution was prepared by using 10 µg/ml of dilute solution in a 1cm cuvette and scanned from

200 to 400 nm in a double beam UV-Spectrophotometer. One wave length was selected where molecule produced maximum & proper absorbance (within range of Beer Lamberts law).

Preparation of standard solution

Standard Ibuprofen solutions were prepared several times by using the working standard solution.

Estimation of Validation Parameters (ICH Topic Q2B, "Validation of Analytical procedures": Methodology, 6th Nov 1996b).

Specificity

Specificity generally refers to a method that produces a response for a single analyte only. *Selectivity* refers to a method which provides responses for a number of chemical entities that may/may not be distinguished from each other. If each response is distinguished from all other responses, then the method is said to be selective. Use of the term 'specificity' is appropriate for microbiological assay, radio-immunoassay etc. methods rather than selectivity. Use of the selectivity is appropriate for the methods based on techniques such as HPLC, GC, CE, etc., than specificity.

Linearity

The linearity of an analytical procedure is such which is having the ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. In order to determine the quantity of any analyte present in unknown sample, some kind of relationship (mathematical/empirical) between concentration and response was mandatory. Response should be directly proportion to the concentration.

Acceptance criteria: The correlation coefficient should be less than 1.

Accuracy

The accuracy of an analytical procedure express closeness of agreement between the values, which is accepted either as a conventional true value or an accepted reference value and the value found as test value.

Range

The range of an analytical procedure is the interval between the upper and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which it can be proved that the analytical procedure has a suitable level of accuracy and linearity. The range of an analytical procedure is the concentration interval within which acceptable accuracy and linearity were obtained.

Acceptance criteria: Linearity and Recovery are required to be shown.

Limit of Detection and Limit of Quantification

LOD: Lowest amount of analyte in a sample which can be detected but not necessarily quantitated, under the stated experimental conditions (LOD).

LOQ: Lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy (LOQ).

Table 1. Different approaches suggested by ICH, USP & EP

Approach parameters	LOD	LOQ
Visual observation	Minimum detection level	Minimum quantifiable level
Signal to Noise Ratio	3:1	10:1
SD of response (σ) and slope (S)	{3.3x σ}/s	{10.0x σ}/s
RSD Criteria	Concentration at which RSD<33.0%	Concentration at which RSD<10.0

Procedure: SD of response (σ) and Slope(S)

Linearity curve was prepared with a series of working standard solutions of ibuprofen at different concentrations. Similarly LOD and LOQ were estimated for ibuprofen. The absorbance values were measured at 222 nm. Five absorbance values provided the standard deviation (σ) of and the slope (S) will be obtained from the standard curve of ibuprofen. LOD and LOQ of ibuprofen at a specific wavelength were obtained from the following equations:

$$LOD = \frac{3.3\sigma}{S} \quad \text{and} \quad LOQ = \frac{10\sigma}{S}$$

Precision

Precision is the measurement of how close the data values to each other for a number of measurements under the same analytical conditions. Precision may be considered at two levels according to ICH.

a. Repeatability

System Precision

Precision under same operative conditions (within a laboratory over a short period of time using the same analyst with the same equipment) was determined.

Acceptance criteria: % RSD should be in between 98%-102%.

Method Precision

Six preparations were prepared individually using single batches of standard solutions each containing Ibuprofen as

per test method and each solution were measured at specific 222 nm.

Acceptance criteria: % RSD should be in between 98%-102%.

b. Reproducibility

Precision between laboratories/intermediate precision can be considered during the standardization of a procedure before it is submitted to the pharmacopoeia. A simple logic behind this parameter was some degree of inconsistency (occurrence of random error) was allowed for every analytical measurement. But, the extent depends on steps involved (weighing, dilution etc.), technique used in other expected variables (stability) and intended use of the procedure.

Ruggedness (intermediate precision)

It is the precision under different laboratory conditions (within-laboratory variation, as on different days, or with different analysts, or equipments within the same laboratory). This experiment is done to see whether the test results are coming under the acceptance criteria or not.

Acceptance criteria: % RSD should be in between 98%-102%.

Robustness

Here the closeness of the values are seen in small changes of different parameters like solvent, temperature, pH etc. Here the mean, SD, % RSD is calculated.

Acceptance criteria: % RSD should be in between 98%-102%.

Results and discussion

Determination of analytical wavelength from UV-spectra

The spectrum was obtained from standard solution of pure Ibuprofen (10µg/ml), the spectrum is provided as follows:

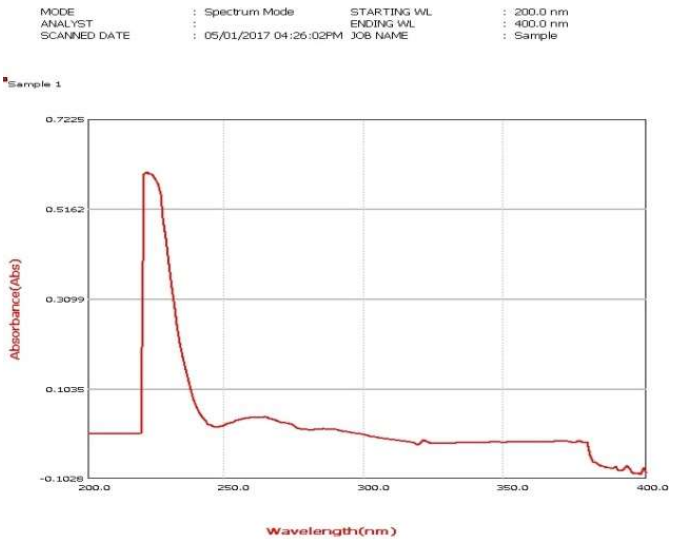


Figure 2. UV Spectra for pure Ibuprofen

Ibuprofen showed maximum peak at 222 nm (solvent: 0.1N HCl/ 1N HCl) so the analytical wavelength was selected at 222 nm.

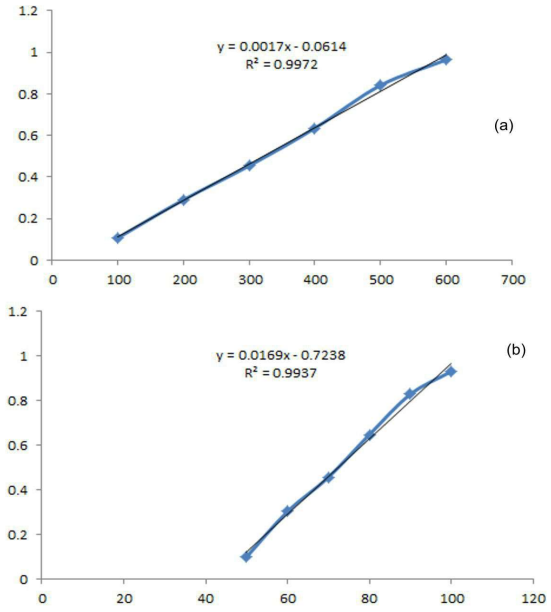


Figure 3. Standard Curve of pure Ibuprofen: (a) (SOLVENT- 0.01 N HCl) (b) (SOLVENT- 1 N HCl)

Results for determination of validation parameters

Specificity (Solvent- 0.01 N HCl)

Since this procedure determines one drug in a solution therefore this method alone is sufficient to prove the specificity. For proving the specificity spectrum scan was done and Ibuprofen gave a specific maximum absorbance at 222 nm.

Specificity (Solvent- 1 N HCl)

Since this procedure determines one drug in a solution therefore this method alone is sufficient to prove the specificity. For proving the specificity spectrum scan was done and Ibuprofen gave a specific maximum absorbance at 222 nm.

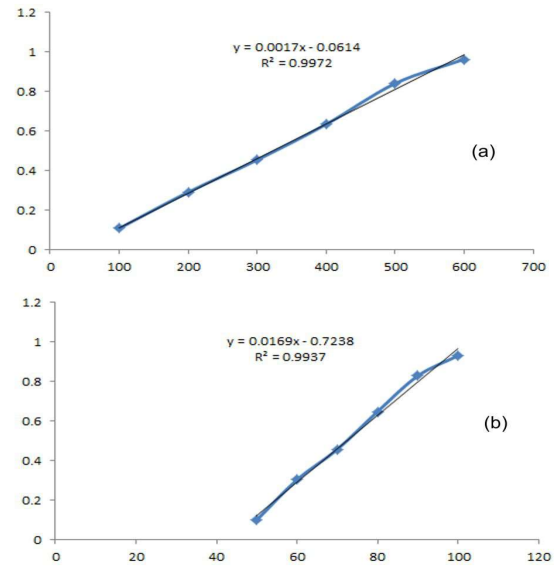


Figure 4 Linearity Curve**: (a)** (Solvent- 0.01 N HCl) (b) (Solvent- 1 N HCl)

Linearity (Solvent- 0.01 N Hcl)

The linearity found in between the above stated range as the correlation coefficient value is less than 1.

Linearity (Solvent- 1 N HCl)

The linearity found in between the above stated range as the correlation coefficient value is less than 1.

Accuracy (Solvent- 0.01 N HCl)

Observations of test of Accuracy

Acceptance Criteria: Recovery should be within 98% to 102%.

Table 2(a). Results of accuracy calculation

Concentration (500ug/ml)	Absorbance	Mean	Concentration	% Recovery
100	0.0576	0.0574	118.4333	118.4333
	0.0632			
	0.0515			
200	0.2105	0.2079	268.9	134.45
	0.2091			
	0.2041			
300	0.2911	0.291167	352.1667	117.3889
	0.2941			
	0.2883			
400	0.3321	0.3292	390.2	97.55
	0.3429			
	0.3126			
500	0.4398	0.4426	503.5667	100.7133
	0.4282			
	0.4597			
600	0.7997	0.757	818	136.3333
	0.7676			
	0.7037			

The recovery results indicating that the test method has an acceptable level of accuracy for the assay with respect to Ibuprofen 500µg/ml but not for the other concentrations.

(Solvent- 1 N HCl)

For 1N HCl: The recovery results indicating that the test method has an acceptable level of accuracy for the assay with respect to Ibuprofen 90µg/ml but not for the other concentrations.

Range (Solvent- 0.01 N HCl)

From the linearity and accuracy experiments the range of the analytical method was found to be between 100ug/ml to 600 ug/ml concentration for Ibuprofen.

For 1N HCl: From the linearity and accuracy experiments the range of the analytical method was found to be between 50ug/ml to 100 ug/ml concentration for Ibuprofen.

Table 2(b): Results of accuracy calculation

Conc. (90ug/ml)	Absorbance	Mean	Conc.	% Recovery
50	0.0192	0.0153	46.1458	92.2917
	0.0175			
	0.0093			
60	0.4015	0.3739	68.5583	114.2639
	0.3612			
	0.3591			
70	0.4744	0.4760	74.9354	107.0506
	0.4732			
	0.4803			
80	0.6914	0.6580	86.3125	107.8906
	0.6362			
	0.6464			
90	0.7479	0.7403	91.4583	101.6204
	0.7622			
	0.7109			
100	0.5042	0.5091	77.0063	77.0063
	0.4995			
	0.5236			

LOD & LOQ (Solvent- 0.01 N HCl)

Table 3. LOD & LOQ Calculation: (a) Solvent- 0.01 N HCl (b) Solvent- 1N HCl

Solvent- 0.01 N HCl		Solvent- 1N HCl	
Conc.	**Absorbance**	**Conc.**	**Absorbance**
100	0.076	50	0.2025
200	0.2746	60	0.227
300	0.364	70	0.4029
400	0.5107	80	0.5748
500	0.5144	90	0.8121
600	0.6104	100	0.9821
Mean	0.3917	Mean	0.5336
SD	0.1955	SD	0.3166
Slope	925.2	Slope	58.12
LOD	0.0007	LOD	0.0180
LOQ	0.0021	LOQ	0.0545

For 0.01 N HCl:

The LOD found was: 0.0007 µg/ml

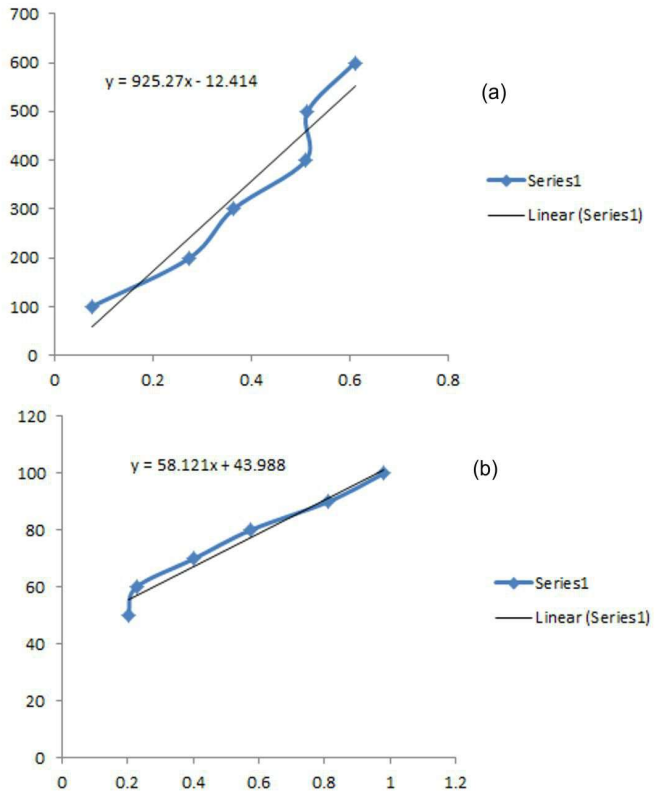
The LOQ found was: 0.0021 µg/ml.

For 1N HCl:

The LOD found was: 0.0180 µg/ml

The LOQ found was: 0.0545 µg/ml.

Figure 5. LOD Curve: (a) Solvent- 0.01 N HCl (b) Solvent- 1N Hcl



Precision (Solvent- 0.01 N HCl)

System precision, conc.: 500µg/ml

Table 4. System Precision Calculation

Repeat	Abs
1	0.8256
2	0.8477
3	0.8324
4	0.8561
5	0.8606
6	0.8427
Mean	0.8442
SD	0.0135
% RSD	1.5983

The system precision found within the acceptance criteria.

Method precision, conc.: 500µg/ml

The method precision found within the acceptance criteria.

Thus process is precised as per system & method precision.

For 1N Hcl:

Table 5. Method Precision Calculation

Prep	Abs
1	0.8152
2	0.8275
3	0.8319
4	0.8554
5	0.8175
6	0.8388
Mean	0.8311
SD	0.0148
% RSD	1.7857

Table 6. Method & System Precision Calculation

System Precision		**Method Precision**	
Conc.	**90ug/ml**	**Conc.**	**90 ug/ml**
Repeat	Abs	Prep	Abs
1	0.8694	1	0.7932
2	0.8459	2	0.8035
3	0.8562	3	0.7863
4	0.8861	4	0.7677
5	0.8622	5	0.7761
6	0.8692	6	0.7733
Mean	0.8648	Mean	0.7834
SD	0.0137	SD	0.0135
% RSD	1.5788	% RSD	1.7224

Ruggedness (Solvent- 0.01 N HCl)

With the range of conc. for six days*:*

Table 7. Ruggedness Calculation (Day Wise)

Days	Abs. (500ug/ml)
1	0.8138
2	0.8294
3	0.8224
4	0.8123
5	0.8083
6	0.8197
Mean	0.8177
SD	0.0077
% RSD	0.9405

It is found from the above experiment that only 500 µg/ml values are day wise rugged.

With different analysts, conc.: 500µg/ml.

Table 8. Ruggedness Calculation (Analyst Wise)

Analyst	Abs (500 µg/ml)
1	0.8185
2	0.8378
3	0.8503
4	0.8275
5	0.8038
6	0.8686
Mean	0.8344
SD	0.0231
% RSD	2.7698

It is found from the above experiment that the method is analyst wise rugged.

For 1N HCl:

Table 9. Ruggedness Calculation (Day Wise).

Days	Conc. (90 ug/ml)
1	0.7894
2	0.7916
3	0.7932
4	0.7767
5	0.8026
6	0.7889
Mean	0.7904
SD	0.0084
% RSD	1.0571

It is found from the above experiment that only 90 µg/ml values are day wise rugged.

Table 10. Ruggedness Calculation (Analyst Wise)

Conc.	90 ug/ml
analyst	abs
1	0.8458
2	0.8154
3	0.8559
4	0.8489
5	0.8171
6	0.8124
Mean	0.8326
SD	0.0196
% RSD	2.3579

It is found from the experiment that the method is analyst wise is not rugged (table 10).

Robustness (Solvent- 0.01 N HCl)

With different temperature, Conc.: 500µg/ml

Table 11. Robustness Calculation (With Different Temperature)

0.01N HCl		1N HCl	
Temp(^{0}C)	Abs (500µg/ml)	Temp(^{0}C)	Abs (90 ug/ml)
5	0.8038	5	0.7665
10	0.8859	15	0.7872
15	0.8667	30	0.7193
25	0.867	45	0.7718
35	0.8562	60	0.7326
45	0.8707	Mean	0.7555
55	0.8901	SD	0.0284
Mean	0.8701	% RSD	3.7593
SD	0.0124		
%RSD	1.4247		

It is found from the above experiment that for 0.01 N HCl the method is robust in between a temperature range of 5-55°C and for 1N HCl the above experiment is not robust temperature wise.

Table 12. Result summary

Conc.(0.1N) HCl (Solvent)	Conc.(1N) HCl (solvent)
The linearity found in between the above stated range as the correlation coefficient value is less than 1 (0.9972).	The linearity found in between the above stated range as the correlation coefficient value is less than 1 (0.9937).
500 µg/ml conc. is recovered properly, so is accurate.	90 µg/ml conc. is recovered properly, so is accurate.
LOD found was 0.0007 ug/ml.	LOD found was 0.0180µg/ml.
LOQ found was 0.0021µg/ml.	LOQ found was 0.0545 µg/ml.
Process is precised as per system & method.	Process is precised as per system & method.
Process is day wise rugged but not analyst wise.	Process is day wise rugged but not analyst wise.
Temperature wise the process is robust.	Temperature wise the process is not robust but lab's wise it is robust.

Conclusion

So from the above result it can be concluded that while the concentration is changed of the solvent (0.1N HCl & 1N HCl) the value of the statistical parameter is varied like the concentration in linearity curve, in LOD & LOQ value also

in robustness calculation although all the parameters are statistically validated individually.

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