ISSN: **2229-7359** Vol. 11 No. 5S, 2025

https://www.theaspd.com/ijes.php

Optimizing Uv Spectrophotometry: A Reliable Method For 2-Hydroxybenzoic Acid Analysis In Face Serum

Sushmita Hiremath¹, Onkar Jadhav², Anuj Shinde³, Shubham Jadhav⁴, Shriya Hiremath¹, Nadeem Shaik¹, Akshata Hebballi¹, Snehal Tavade^{5*}

ABSTRACT

Objectives: The objective of present work is to develop and standardize UV-Spectrophotometric method for the estimation of 2-Hydroxybenzoic Acid in marketed formulation.

Materials and Methods: Ultara Voilet-Spectrophotometric method was developed using Methanol as solvent. The developed method was standardized in terms of validation parameters such as specificity, selectivity, linear range, precision, robustness, ruggedness and reproducibility as per ICH (International Council for Harmonisation) guidelines. Newly developed and standardized method was successfully applied for estimation of 2-Hydroxybenzoic Acid in marketed formulation.

Results: 2-Hydroxybenzoic Acid exhibits λ max at 300nm and beer's law was obeyed in the concentration range of 10 to $50\mu g/ml$ and limit of quantification is found to be $\mu g/ml$. The limit of detection found to be $1.20\mu 25$ Recovery of 2-Hydroxybenzoic Acid in marketed formulation was observed in the range of 90-110%. All the precision and repeatability results were within acceptance range less than 2%. Assay of 2-Hydroxybenzoic Acid was found to be 78.25%

Conclusion: The method was found to be simple, accurate, environment friendly, reproducible and can be used for routine estimation analysis of 2-Hydroxybenzoic Acid in marketed formulation.

Keywords: Beer's law, Method development, 2-Hydroxybenzoic Acid, UV-Spectrophotometer, Validation

INTRODUCTION

2-Hydroxybenzoic Acid (SA), a β-hydroxy acid (C₇H₆O₃), is a cornerstone in dermatology for its keratolytic, anti-inflammatory, and antimicrobial properties, widely used in acne, psoriasis, and wart treatments(1–3). Its efficacy in topical formulations depends on maintaining precise concentrations, as deviations can compromise therapeutic outcomes or trigger adverse effects(4,5). Consequently, robust analytical methods for quantifying SA in marketed products are critical to ensure quality, stability, and regulatory compliance(6)

While high-performance liquid chromatography (HPLC) and gas chromatography (GC) remain gold standards for SA quantification due to their sensitivity, these techniques are cost-prohibitive, time-consuming, and require specialized infrastructure, limiting their utility in routine quality control (QC)(7). UV spectrophotometry, a simpler and economical alternative, has been explored for SA analysis but faces challenges. For instance, due to significant excipient interference in creams, it highlighted spectral overlaps in formulations containing parabens(8). Furthermore, existing UV methods often lack validation for critical parameters like robustness, ruggedness, and matrix effects. Recent studies also emphasize the need for eco-friendly, ICH-compliant UV methods tailored to complex formulations like gels and emulsions(9,10).

¹Department of Pharmaceutical Chemistry, KLE College of Pharmacy, Hubballi, Karnataka. 580031

²Department of Pharmaceutics, Rani Chennamma College of Pharmacy, Belagavi. Karnataka. 590010

³Department of Pharmaceutics, Nootan College of Pharmacy, Kavathemahankal. Maharastra- 416405

⁴Department of Pharmaceutical Chemistry, Shivraj College of Pharmacy, Gadhinglaj. Maharastra-416502

⁵Department of Pharmacognosy, Krishna Institute of Pharmacy, Karad. Maharastra- 415539

ISSN: **2229-7359** Vol. 11 No. 5S, 2025

https://www.theaspd.com/ijes.php

Despite advances, there is no validated UV spectrophotometric method for SA that simultaneously addresses excipient interference, adheres to green chemistry principles, and complies with ICH Q2(R1) guidelines for diverse marketed formulations. This study bridges this gap by asking: Can a simple, cost-effective, and eco-friendly UV spectrophotometric method be optimized and validated for accurate SA quantification in commercial topical products while overcoming matrix interference?

This work aims to provide manufacturers and regulators with an accessible QC tool to ensure batch-to-batch consistency, patient safety, and compliance with global pharmacopeial standards. By reducing reliance on costly instrumentation and minimizing solvent waste, the method aligns with sustainable analytical trends

Figure 1: (2- hydroxybenzoic acid)

MATERIALS AND METHOD

Instrumentation: UV-Spectrophotometer of Shimadzu UV-1900 with Lab Solutions software and Shimadzu UV-1900 with UV Probe software were used for quality control of 2-Hydroxybenzoic Acid Calibrated weighing balance was used for weighing.

Drug Sample: 2-Hydroxybenzoic Acid (API) was synthesized and marketed formulation is purchased from market.

Reagents and Chemicals: Methanol and other chemicals used for the experiment were obtained from the store house of KLE College of Pharmacy, Hubballi.

Selection of Wavelength: Methanol was selected throughout the study because 2-Hydroxybenzoic Acid is soluble in methanol. 2-Hydroxybenzoic Acid 20 μ g/ml working standard solution was scanned in between 400nm to 200nm and exhibited maximum absorption at 300nm in UV-Spectrophotometer.

Preparation of stock solution: An accurately weighed 10mg of 2-Hydroxybenzoic Acid was taken in clean and dried 10ml volumetric flask and dissolved in methanol then volume is made using the same. This was considered as standard stock solution .This having concentration of $1000\mu g/ml$ Standard stock solution was used for making further dilutions.

Preparation of calibration curve: From the standard stock solution, serial dilutions containing concentrations of 10-50 μ g/ml were prepared. The concentrations solutions were analyzed for 3 sets and the absorbance were measured at, 212nm, 232nm, 300nm. Linearity curve was plotted as Concentration on x-axis and Absorbance on y-axis and linear regression equation was calculated.

Method development and validation: 2-Hydroxybenzoic Acid was found to be soluble in methanol. Therefore, this solvent was used for the determination of detection wavelength and working concentration of standard. International Conference on Harmonization (ICH) has provided guidelines i.e. Q2 (R1) for validation of analytical method which defines this process as characteristic performance that is established by

ISSN: **2229-7359** Vol. 11 No. 5S, 2025

https://www.theaspd.com/ijes.php

laboratory studies. Developed method was validated according to the ICH guidelines for the validation of analytical procedures in order to prove the suitability of method using method parameters.

Specificity and selectivity: 2-Hydroxybenzoic Acid selectively showed maximum absorbance at 300nm hence the method is found to be selective. And spectrum of solvent showed no absorbance at wavelength of 2-Hydroxybenzoic Acid i.e. 300nm hence this method is found to be specific

Linearity: Linearity was examined in the range of $10-50\mu$ g/ml. Accurately weighed 10mg of 2-Hydroxybenzoic Acid is transferred into a clean and dried 10ml of volumetric flask and then the volume is made upto the mark using Methanol as solvent. From the above standard solution 1ml is pipette out and transferred into the 10ml of volumetric flask and the volume is made using methanol. From this solution further dilutions are made to examine the linearity.

LOD and LOQ: Limit of detection is concentration at which analyte in the test sample is detected(11). Limit of quantification is the concentration at which analyte in the test sample is quantified. By using the following formula LOD and LOQ are calculated.

$$LOD = \frac{3.3xstandard\ deviation\ of\ regression}{slope}$$

$$LOQ = \frac{10 x standard deviation of regression}{slope}$$

Precision: In order to determine system precision g/ml, μ three replicates of solution containing 10 g/ml ,30 μ g/ml and 50 μ g/ml of 2-Hydroxybenzoic Acid were prepared and absorbance of each solution was measured at 212nm,232nm,300nm and %RSD (Relative Standard Deviation) was calculated. Method Precision was determined by performing assay of sample under the tests of

- 1) Intraday Precision
- 2) Interday Precision.

For Intraday Precision three replicates of solution containing concentration of $10\mu g/ml$, $30\mu g/ml$, $50\mu g/ml$ of 2-Hydroxybenzoic Acid was analyzed and %RSD was calculated at different time intervals on the same day.

For Interday Precision three replicates of solution containing concentration 2-Hydroxybenzoic Acid was analyzed $10\mu g/ml$, $30\mu g/ml$, $50\mu g/ml$ and %RSD was calculated on three consecutive days

Ruggedness: Ruggedness was determined by performing the same proposed method on different instrument and which was carried out by different analyst to check the reproducibility.

Robustness: Methanol is used as solvent because 2-Hydroxybenzoic Acid is soluble in methanol. Maximum absorbance of 2-Hydroxybenzoic Acid is found at 300nm. Robustness is done by doing the sonication for 30min and by changing the wavelength.

Accuracy: Accuracy was determined by performing recovery experiments in which determination of % mean recovery of sample by standardization method at three different levels 50%, 100% and 150% of the sample solutions were prepared. 10mg of accurately weighed 2-Hydroxybenzoic Acid is transferred into the clean 10ml of volumetric flask and the volume is made up to the mark using methanol as solvent because 2-Hydroxybenzoic Acid is soluble in methanol. From this above solution further dilutions are made. At each level three replicates of concentration solution was prepared and recovery study was carried out.

ISSN: **2229-7359** Vol. 11 No. 5S, 2025

https://www.theaspd.com/ijes.php

Analysis of marketed formulation: The validated method was applied for the determination of salicylic in marketed formulation. 0.01gm of 2-Hydroxybenzoic Acid serum was weighed. The amount of drug in sample was in good agreement with the label claim of the formulation. Percent assay was found to be 93.96%.

RESULTS AND DISCUSSION: Method development: UV-spectrophotometric method was developed by using UV-1900 instrument using methanol as solvent. Maximum absorbance of 2-Hydroxybenzoic Acid was found at 212nm,232nm,300nm and details of method developed were presented in Table 1.

Table 1: Developed method parameters.

S no	Parameter	Specifications
1	Method	Spectrometric
2	Instrument	UV
3	Model	1900
4	Make	Shimadzu
5	Software	UV probe
6	Drug	2-Hydroxybenzoic Acid
7	$\lambda_{ m max}$	300nm
8	Solvent	Methanol

Method validation:

Developed method was standardized in terms of validation parameters such as specificity, selectivity, linear range, precision, robustness, ruggedness and reproducibility as per ICH guidelines.

Specificity and Selectivity: 2-Hydroxybenzoic Acid showed maximum absorbance at 300nm and solvent i.e. methanol showed no absorbance at 300nm. Hence this results that the method is found to be specific and selective

Linearity: As mentioned in the above method dilutions are made for the linearity range i.e. 10-50µg/ml. The linearity graph is given in Figure 4,the linearity and range is given in Table 2 and the calibration curve is .

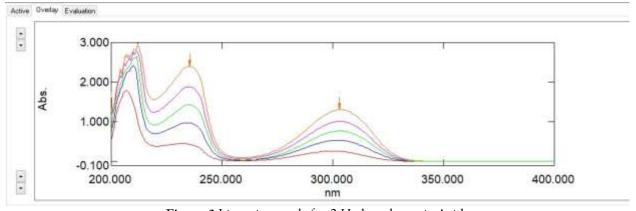


Figure 2-Linearity graph for 2-Hydroxybenzoic Acid

https://www.theaspd.com/ijes.php

Table 2-Linearity and range data of 2-Hydroxybenzoic Acid (212nm)

Sr. no	Concentration	Absorbance
1	10μg	0.910
2	20μg	1.753
3	30μg	2.303
4	40μg	2.680
5	50μg	2.913
	$r^2 =$	0.945
	Slope=	0.0493
	Standard error=	0.224981
	LOD=	15.15181
	LOQ=	45.91457

Figure 3-linearity graph of 2-Hydroxybenzoic Acid(212nm)

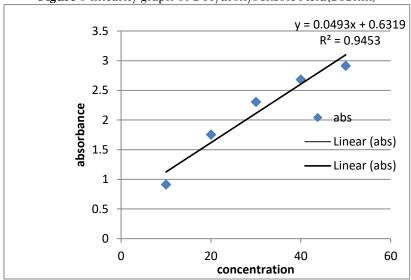


Table 3 - Linearity and range data of 2-Hydroxybenzoic Acid (232nm)

S	Concentration	Absorbance
no		
1	10µg	0.459
2	20μg	0.964
3	30 μg	1.372
4	40 µg	1.817
5	50 μg	2.3
	$r^2=$	0.999
	Slope=	0.045
Stan	dard error=	0.026323
	LOD=	1.930353
	LOQ=	5.849554

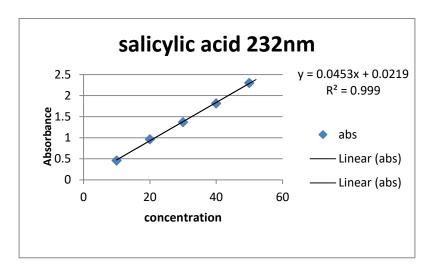


Figure 4 - linearity graph of 2-Hydroxybenzoic Acid(232nm)

Table 4 - Linearity and range data of 2-Hydroxybenzoic Acid (300nm)

S NO	Concentration	Absorbance
1	10 μg	0.264
2	20 μg	0.511
3	30 μg	0.747
4	40 μg	0.981
5	50 μg	1.26
	$r^2 =$	0.999
	Slope=	0.024
Standar	d error=	0.014245
	LOD=	71.958752
	LOQ=	5.935611

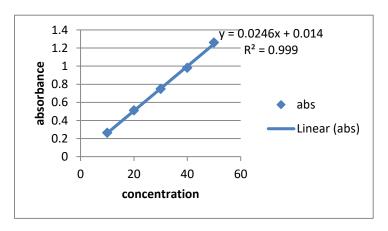


Figure 5- Graph of 2-Hydroxybenzoic Acid(300nm)

ISSN: **2229-7359** Vol. 11 No. 5S, 2025

https://www.theaspd.com/ijes.php

Precision:

System precision: As mentioned in the method in order to determine system precision three replicates of solution containing $10\mu g/ml$, $30\mu g/ml$, $50\mu g/ml$, of 2-Hydroxybenzoic Acid were prepared and absorbance of each solution was measured at 212nm.232nm,300nm The %RSD was calculated and it found to be less than 2%

Table 5- System precision data of 2-Hydroxybenzoic Acid (212nm)

Concentration	Absorbance*	Standard deviation	% relative standard deviation
10 μg	0.912	0.002	0.104
30 μg	2.304	0.001	0.06
50 μg	2.916	0.001	0.05

^{* =} Average absorbance of three replicates

Intraday precision: For intraday precision three replicates of solution containing concentration $10\mu g/ml$, $30\mu g/ml$, $50\mu g/ml$ of 2-Hydroxybenzoic Acid analyzed and %RSD was calculated at different time intervals on same day and %RSD was found to be less than 2%

Table 6- Intraday precision data of 2-Hydroxybenzoic Acid

Concentration	absorbance		Standard deviation	%relative standard deviation
10 μg	Abs 1hr	0.912	0.002	0.104
	Abs 4hr	1.90	0.006	0.356
	Abs 8hr	1.90	0.01	0.652
30 μg	Abs 1hr	2.304	0.01	0.06
	Abs 4hr	2.302	0.002	0.086
	Abs 8hr	2.30	0.01	0.66
50 μg	Abs 1hr	2.913	0.001	0.05
	Abs 4hr	2.30	0.002	0.0685
	Abs 8hr	2.90	0.007	0.240

^{*=}Average absorbance of three replicates

Interday precision: For Interday precision three replicates of solution containing concentration $10\mu g/ml$, $30\mu g/ml$, $50\mu g/ml$ of 2-Hydroxybenzoic Acid analyzed and %RSD was calculated on three consecutive

days. And the calculated %RSD was found to be less than 2%

Table 7 - Interday precision data of 2-Hydroxybenzoic Acid

Concentration	Absorbance*		Standard deviation	%relative standard deviation
10 μg	Day 1	0.912	0.002	0.104
	Day 2	1.90	0.007	0.39
	Day 3	1.92	0.015	0.792
30 μg	Day 1	2.304	0.001	0.06
	Day 2	2.29	0.01	0.66
	Day 3	2.31	0.02	0.865

ISSN: **2229-7359** Vol. 11 No. 5S, 2025

https://www.theaspd.com/ijes.php

50 μg	Day 1	2.916	0.001	0.104
	Day 2	2.913	0.002	0.06
	Day3	2.916	0.015	0.52

^{* =} Average absorbance of three replicates

Ruggedness: Ruggedness was determined by performing the same proposed method on different instrument i.e. UV-1800 and UV-1900and it is carried out by different analyst to check the reproducibility which showed %RSD less than 2% and indicates that the method developed is rugged

Table 8- Ruggedness data of 2-Hydroxybenzoic Acid

Concentration	Absorba	nce*	Standard deviation	% relative standard deviation
10 μg	Analyst 1	1.92	0.015	0.792
	UV-1800			
	Analyst 2	1.90	0.01	0.652
	UV-1900			
30 μg	Analyst 1	2.31	0.02	0.865
	UV-1800			
	Analyst 2	2.30	0.01	0.66
	UV-1900			
50 μg	Analyst 1	2.916	0.015	0.52
	UV-1800			
	Analyst 2	2.90	0.007	0.240
	UV-1900			

^{* =} Average absorbance of three replicates

Robustness: Methanol is used as solvent because 2-Hydroxybenzoic Acid is soluble in methanol. Maximum Absorbance of 2-Hydroxybenzoic Acid is found at 212nm,232nm,300nm. Robustness is done by doing the sonication for 30min

Table 9- Robustness data of 2-Hydroxybenzoic Acid

Concentration	Absorba	ance*		Standard deviation	% relative standard deviation
10 μg	Change in	211nm	0.911	0.002	0.219
	Wavelength	212nm	0.912	0.002	0.104
	sonication for 30min	213nm	1.926	0.015	0.792
30 μg	Change in	211nm	2.31	0.02	0.865
	Wavelength	212nm	2.304	0.001	0.06
	sonication for	1			
	30min	213nm	2.31	0.002	0.086
50 μg	Change in	211nm	2.910	0.0020	0.071
	Wavelength	212nm	2.916	0.001	0.05
	sonication for				
	30min	213nm	2.916	0.003	0.102

ISSN: **2229-7359** Vol. 11 No. 5S, 2025

https://www.theaspd.com/ijes.php

Table 10 -System precision data of 2-Hydroxybenzoic Acid (232nm)

Concentration	Absorbance*	Standard deviation	% relative standard deviation
10 μg	0.912	0.002	0.104
30 μg	2.304	0.001	0.06
50 μg	2.916	0.001	0.05

^{* =} Average absorbance of three replicates

Intraday precision: For intraday precision three replicates of solution containing concentration 10µg/ml, 30µg/ml, 50µg/ml of 2-Hydroxybenzoic Acid analyzed and %RSD was calculated at different time intervals on same day and %RSDwas found to be less than 2%

Table 11- Intraday precision data of 2-Hydroxybenzoic Acid

Concentration	Absorbance		Standard deviation	%relative standard deviation
10 μg	Abs 1hr	0.459	0.02	0.43
	Abs 4hr	0.460	0.002	0.5
	Abs 8hr	0.460	0.002	0.45
30 μg	Abs 1hr	1.37	0.002	0.15
	Abs 4hr	1.37	0.001	0.11
	Abs 8hr	1.375	0.001	0.11
50 μg	Abs 1hr	2.32	0.02	1.08
	Abs 4hr	2.34	0.02	1.07
	Abs 8hr	2.33	0.1	0.6

^{*=}Average absorbance of three replicates

Interday precision: For Interday precision three replicates of solution containing concentration $10\mu g/ml$, $30\mu g/ml$, $50\mu g/ml$ of 2-Hydroxybenzoic Acid analyzed and %RSD was calculated on three consecutive days. And the calculated %RSD was found to be less than 2%

Table 12 Interday precision data of 2-Hydroxybenzoic Acid

Concentration	Abso	orbance*	Standard deviation	%relative standard deviation	
10 μg	Day 1	0.459	0.02	0.43	
	Day 2	0.46	0.003	0.65	
	Day 3	0.46	0.004	0.90	
30 μg	Day 1	1.37	0.002	0.15	
	Day2	1.35	0.001	0.11	
	Day 3	1.37	0.002	0.15	
50 μg	Day 1	2.32	0.02	1.08	
	Day 2	2.34	0.02	1.07	
	Day 3	2.33	0.01	0.65	

^{* =} Average absorbance of three replicates

^{* =} Average absorbance of three replicates

ISSN: **2229-7359** Vol. 11 No. 5S, 2025

https://www.theaspd.com/ijes.php

Ruggedness: Ruggedness was determined by performing the same proposed method on different instrument i.e. UV-1800 and UV-1900and it is carried out by different analyst to check the reproducibility which showed %RSD less than 2% and indicates that the method developed is rugged

Table 13 - Ruggedness data of 2-Hydroxybenzoic Acid

Concentration	Absorba	nce*	Standard deviation	% relative standard deviation
10 μg	Analyst 1	0.46	0.003	0.65
	UV-1800			
	Analyst 2	0.44	0.004	0.90
	UV-1900			
30 μg	Analyst 1	1.37	0.001	0.11
	UV-1800			
	Analyst 2	1.37	0.002	0.15
	UV-1900			
50 μg	Analyst 1	2.34	0.02	1.07
	UV-1800			
	Analyst 2	2.33	0.015	0.65
	UV-1900			

^{* =} Average absorbance of three replicates

Robustness: Methanol is used as solvent because 2-Hydroxybenzoic Acid is soluble in methanol. Maximum absorbance of 2-Hydroxybenzoic Acid is found at 212nm, 232nm, 300nm.Robustness is done by doing the sonication for 30min

Table 14 -robustness data of 2-Hydroxybenzoic Acid

Concentration	Absorban	ce*		Standard	% relative standard
				deviation	deviation
10 μg	Change in	231	0.452	0.002	0.55
	Wavelength	232	0.454	0.002	0.43
	sonication for	233	0.456	0.002	0.45
	30min				
30 μg	Change in	231	1.36	0.004	0.29
	Wavelength	232	1.37	0.002	0.15
	sonication for				
	30min	233	1.37	0.001	0.110
50 μg	Change in	231	2.22	0.02	1.13
	Wavelength	232	2.28	0.01	0.43
	sonication for				
	30min	233	2.32	0.02	1.08

^{* =} Average absorbance of three replicates

Table 15- System precision data of 2-Hydroxybenzoic Acid (300nm)

Concentration	Absorbance*	Standard deviation	% relative standard deviation
10 μg	0.912	0.002	0.104
30 μg	2.304	0.001	0.06
50 μg	2.916	0.001	0.05

ISSN: **2229-7359** Vol. 11 No. 5S, 2025

https://www.theaspd.com/ijes.php

Intraday precision: For intraday precision three replicates of solution containing concentration $10\mu g/ml$, $30\mu g/ml$, $50\mu g/ml$ of 2-Hydroxybenzoic Acid analyzed and %RSD was calculated at different time intervals on same day and %RSD was found to be less than 2%

Table 16- Intraday precision data of 2-Hydroxybenzoic Acid

Concentration	Absorb	ance	Standard deviation	%relative standard deviation
10 μg	Abs 1hr	0.266	0.002	0.94
	Abs 4hr	0.263	0.002	0.76
	Abs 8hr	0.260	0.002	0.96
30 μg	Abs 1hr	0.749	0.002	0.26
	Abs 4hr	0.744	0.002	0.27
	Abs 8hr	.0737	0.002	0.34
50 μg	Abs 1hr	1.28	0.02	1.95
	Abs 4hr	0.737	0.02	0.34
	Abs 8hr	1.21	0.015	1.25

^{*=}Average absorbance of three replicates

Interday precision: For Interday precision three replicates of solution containing concentration 10µg/ml, 30µg/ml, 50µg/ml of 2-Hydroxybenzoic Acid analyzed and %RSD was calculated on three consecutive days. And the calculated %RSD was found to be less than 2%

Table 17- Interday data of 2-Hydroxybenzoic Acid

Concentration	Absorbance*		Standard deviation	%relative standard deviation
10 μg	Day1	0.266	0.002	0.94
	Day 2	0.265	0.002	0.783
	Day3	0.265	0.002	0.754
30 μg	30 μg Day 1 0.749		0.002	0.26
	Day 2	0.747	0.002	0.267
	Day 3	0.745	0.001	0.204
50 μg	50 μg Day 1 1.28		0.002	1.95
	Day 2	1.27	0.02	1.97
	Day 3	1.27	0.01	1.19

^{* =} Average absorbance of three replicates

Ruggedness: Ruggedness was determined by performing the same proposed method on different instrument i.e. UV-1800 and UV-1900and it is carried out by different analyst to check the reproducibility which showed %RSD less than 2% and indicates that the method developed is rugged

Table 17 - ruggedness data of 2-Hydroxybenzoic Acid

Concentration	Absorbance*		Standard deviation	% relative standard deviation	
10 μg	Analyst 1	0.265	0.002	0.754	
	UV-1800				
	Analyst 2	0.266	0.002	0.752	

^{* =} Average absorbance of three replicates

ISSN: **2229-7359** Vol. 11 No. 5S, 2025

https://www.theaspd.com/ijes.php

	UV-1900			
30 µg	Analyst 1 UV-1800	0.745	0.001	0.20
	Analyst 2 UV-1900	0.742	0.001	0.20
50 μg	Analyst 1 UV-1800	1.27	0.01	1.19
	Analyst 2 UV-1900	1.24	0.01	1.1

^{* =} Average absorbance of three replicates

Robustness: Methanol is used as solvent because 2-Hydroxybenzoic Acid is soluble in methanol. Maximum absorbance of 2-Hydroxybenzoic Acid is found at 212nm, 232nm, 300nm.Robustness is done by doing the sonication for 30min

Table 18 - Robustness data of 2-Hydroxybenzoic Acid

Concentration	Absorbar	nce*		Standard deviation	% relative standard deviation
10 μg	Change in	299	0.260	0.001	0.58
	Wavelength	300	0.266	0.002	0.94
	sonication for 30min	301	0.273	0.002	0.73
30 μg	Change in	299	0.742	0.002	0.33
	Wavelength	300	0.749	0.002	0.26
	sonication for				
	30min	301	0.753	0.002	0.265
50 μg	Change in	299	1.22	0.02	1.64
	Wavelength	300	1.28	0.22	1.95
	sonication for				
	30min	301	1.34	0.02	1.49

^{* =} Average absorbance of three replicates

Accuracy: Accuracy was determined by performing recovery experiments in which determination of % mean recovery of sample by standardization method at three different levels50%, 100% and 150% of the sample solutions were prepared. And the percent recovery is found in the range of 100-112%

 Table 19 - Recovery data of 2-Hydroxybenzoic Acid(212nm)

Total conc	Standard conc	Sample conc	Absorbance (212nm)		Conc (µg/ml)	Sample concentration	%recovery
(μg/ml)	(µg/ml)	(μg/ml)	standard	sample		n difference (μg/ml)	
10 μg	7	3	0.910	0.912	10.02	3.02	100.7
				0.918	10.08	3.08	102.9
				0.915	10.05	3.05	101.8
30 μg	27	3	2.303	2.309	30.07	3.07	102.6
				2.305	30.02	3.02	100.8

ISSN: **2229-7359** Vol. 11 No. 5S, 2025

https://www.theaspd.com/ijes.php

				2.310	30.09	3.09	103.03
50 μg	47	3	2.913	2.915	50.03	30.3	101.14
				2.910	49.9	2.94	98.28
				2.917	50.06	3.06	102.28

Table 20 -Recovery data of 2-Hydroxybenzoic Acid(232nm)

Total conc	Standard conc	Sample conc	Absorband (232nm)	Absorbance (232nm)		Sample Conc	%recovery
(μg/ml)	(µg/ml)	(μg/ml)	standard	sample	(μg/ml)	difference (µg/ml)	
10 μg	5	5	0.459	0.455	9.912	4.912	98.24
				0.460	10.02	5.02	100.4
				0.457	9.95	4.95	99
30 μg	25	5	1.372	1.370	29.95	4.95	99.12
				1.375	30.05	5.06	101.3
				1.379	30.15	5.15	103.06
50 μg	45	5	2.3	2.32	50.43	5.43	108.6
				2.31	50.2	5.21	104.3
				2.33	50.6	5.65	113.04

Table 21 - Recovery data of 2-Hydroxybenzoic Acid(300nm)

Total	Standard	Sample	Absorbance		Conc	Sample	%recovery
conc	conc	conc	(300nm)	(300nm)		conc	
(µg/ml)	(µg/ml)	(µg/ml)	standard	sample		difference	
						(µg/ml)	
10 μg	7	3	0.264	0.261	9.88	2.88	96
				0.2	10.41	3.41	113.6
				0.269	10.18	3.18	106.31
30 μg	27	3	0.747	0.742	29.79	2.79	93
				0.751	30.6	3.16	105.33
				0.749	30.08	3.08	102.66
50 μg	47	3	1.26	1.262	50.07	3.079	102. 64
				1.265	50.19	3.198	106.61
				1.27	50.39	3.39	113.2

CONCLUSION

The developed UV spectrophotometric method for estimating 2-Hydroxybenzoic Acid (SA) in marketed formulations is **simple**, **sensitive**, **accurate**, **precise**, **and reproducible**, validated per ICH Q2(R1) guidelines. Its simplicity lies in minimal sample preparation and cost-effective instrumentation. Accuracy, confirmed through recovery studies (90–120%), and precision, with intra- and inter-day relative standard deviations (RSD < 2%), ensure reliability across analyses. The method's specificity was proven by the absence of spectral interference from excipients in commercial formulations, validated via placebo comparisons and forced degradation studies. Reproducibility was affirmed through consistent results under varied conditions. Additionally, the method aligns with green chemistry principles, using eco-friendly solvents and reducing

ISSN: **2229-7359** Vol. 11 No. 5S, 2025

https://www.theaspd.com/ijes.php

waste, making it sustainable for routine use. By overcoming challenges like matrix interference and offering compliance with pharmacopeial standards, it serves as a robust, accessible tool for quality control in pharmaceutical and cosmetic industries. This approach bridges the gap between analytical rigor and industrial practicality, ensuring batch consistency, regulatory compliance, and patient safety, while providing a viable alternative to complex chromatographic techniques. Future applications could extend to novel SA formulations or multi-component analyses, further enhancing its utility.

REFERENCES:

- 1. Klebeko J, Ossowicz-Rupniewska P, Świątek E, Szachnowska J, Janus E, Taneva SG, et al. 2-Hydroxybenzoic Acid as Ionic Liquid Formulation May Have Enhanced Potency to Treat Some Chronic Skin Diseases. Mol 2022, Vol 27, Page 216 [Internet]. 2021 Dec 30 [cited 2025 May 10];27(1):216. Available from: https://www.mdpi.com/1420-3049/27/1/216/htm
- 2. Arif T. Clinical, Cosmetic and Investigational Dermatology 2-Hydroxybenzoic Acid as a peeling agent: a comprehensive review Tasleem Arif Clinical, Cosmetic and Investigational Dermatology Dovepress 2-Hydroxybenzoic Acid as a peeling agent: a comprehensive review. Clin Cosmet Investig Dermatol [Internet]. 2015 [cited 2025 May 10];8:455-61. Available from: https://www.tandfonline.com/action/journalInformation?journalCode=dcci20
- 3. Wiśniewska J, Klasik-Ciszewska S, Duda-Grychtoł K. 2-Hydroxybenzoic Acid and its use in cosmetology. Aesthetic Cosmetol Med. 2023;12(3):91–5.
- 4. Herrick G, Fritts H, Forsyth A, Johnson A, Saunooke J. Advancements in Topical Treatments for Acne Vulgaris: A Comprehensive Review of Efficacy, Safety, and Management. Ameri J Clin Med Re. 2024;163.
- 5. Trigo G, Coelho M, Ferreira CB, Melosini M, Lehmann IS, Reis CP, et al. Exploring the Biological Activity of Phytocannabinoid Formulations for Skin Health Care: A Special Focus on Molecular Pathways. Int J Mol Sci [Internet]. 2024 Dec 1 [cited 2025 May 10];25(23):13142. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11641943/
- 6. Kowalska M, Woźniak M, Kijek M, Mitrosz P, Szakiel J, Turek P. Management of validation of HPLC method for determination of acetyl2-Hydroxybenzoic Acid impurities in a new pharmaceutical product. Sci Reports |. 123AD;12:1.
- 7. Bulduk I, Akbel E. A comparative study of HPLC and UV spectrophotometric methods for remdesivir quantification in pharmaceutical formulations. 2021 [cited 2025 May 10];15(1):507–13. Available from: https://www.tandfonline.com/action/journalInformation?journalCode=tusc20
- 8. Kumar Kosuru S, Srinivasa Rao V, Suvarna T, Jhansi C, Santhosh Kumar G, Kumar Kosuru MPharm S, et al. A Review on Analytical Challenges in Complex Formulations. J Clin Pharm Res [Internet]. 2023 Oct 24 [cited 2025 May 10];3(4):32–3. Available from: https://jcpr.in/index.php/journal/article/view/111
- 9. P. Shinde K, D. Rajmane A. A Review UV Method Development and Validation. Asian J Pharm Anal. 2023 Jun 3;122–30.
- 10. Verch T, Campa C, Chéry CC, Frenkel R, Graul T, Jaya N, et al. White Paper Analytical Quality by Design, Life Cycle Management, and Method Control.
- 11. Hebballi AP, Pujar B, Honnalli SS, Hiremath SI, Menasinakai A, Bakale A, et al. Establishment and Validation of a Robust Reversed-Phase HPLC Method for the Determination of Calotropis gigantea in Bulk Material and Marketed Product. Curr Pharm Anal [Internet]. 2024;20(8):920–31. Available from:
 - https://www.benthamdirect.com/content/journals/cpa/10.2174/01157341293438582410070734 50