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Research Article

Machine Learning-Assisted Simultaneous Estimation of Aspirin and Dipyridamole Using UV–Visible Spectrophotometer

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Abstract

Simultaneous estimation of Aspirin (ASP) and Dipyridamole (DIP) was developed using UV–visible spectrophotometer. Multicomponent analysis mode was used for the estimation of both drugs at their respective wavelength maxima. Both drugs were found to be linear within the concentration range of 5-30 µg/mL. The correlation coefficient (R^2 value) of ASP and DIP were found to be 0.995 and 0.996 respectively. Method validation parameters such as accuracy, precision, and recovery studies were found to be within acceptable ICH limits. H point standard addition method (HPSAM) was developed for the estimation of both the drugs at the selected isosbestic points namely 218 and 226nm. Python code was developed to calculate the validation parameters of the developed methods. The percentage purity for both methods was found to be within the range of 94.4 to 106.84%. The relative standard deviation (RSD) of ASP and DIP was found to be 2.26 and 2.21 respectively and the percentage recovery was in the range from 93.41 to 94.59%. Green metrics was performed using AGREE software and the developed methods show greenness with a score of 0.79. Both the methods were found to be accurate and precise. The developed Python code offers additional support in plotting the graph and in the calculation of different parameters. An attempt was made to develop an APP using the optimized Python code for calculating HPSAM and Multi-component method validation parameters.

Keywords: Simultaneous estimation, HPSAM, isosbestic points, UV Vis spectroscopy, Python, Multicomponent Analysis

1. INTRODUCTION

Aspirin (ASP), chemically known as 2-Acetoxybenzoic acid (Figure 1), is a nonselective cyclooxygenase (COX) inhibitor, thereby preventing the synthesis of prostaglandins and thromboxanes. This inhibition results in anti-inflammatory, analgesic and antipyretic effects [1]. Dipyridamole (DIP), 2,2',2'',2'''-(4,8-di(piperidin-1-yl) pyrimido [5,4-*d*] pyrimidine 2,6diyl) bis(azanetriyl) tetra ethanol (Figure 2) is a coronary vasodilator and platelet aggregation inhibitor. The combination of dipyridamole and aspirin exerts additive or synergistic effects in preventing blood clot formation [2]. The combination of ASP and DIP provides better protection against the formation of blood clots than either of the medicine is used. Aspirin inhibits platelet aggregation by blocking thromboxane A2 synthesis, while dipyridamole enhances the antiplatelet effect by increasing cAMP levels. This dual mechanism provides more comprehensive protection against thrombotic events.

Few methods were reported were reported in the literature for the estimation of ASP based on spectrophotometric, HPLC and HPTLC individually or in combination with other drugs . There has been few reports on the determination of DIP invidually and in combination with drugs using spectroscopy and chromatography techniques. In this paper, we report a simple and precise methods for simultaneous estimation of these two drugs. These methods include Multicomponent Analysis and H-Point Standard Addition Method (HPSAM) [4]. Both the methods were developed and validated by conventional method. In order to improve the sensitivity of the methods, Python programming language was used to develop codes for validation parameters [5]. To ascertain the eco-friendliness of the developed method, green metrics was implemented using AGREE software [6].

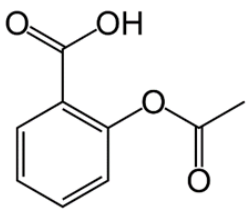


Figure 1: Structure of Aspirin

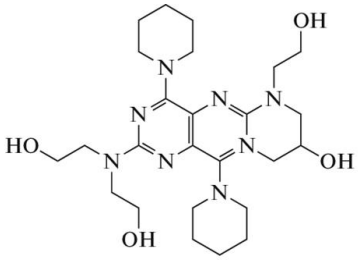


Figure 2: Structure of Dipyridamole

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Pure standard drugs of ASP and DIP were procured as gift samples from Micro Labs, Bangalore. The tablets containing both the drugs were procured from the local market.

2.2 Instrument

Shimadzu UV-1800, Double beam UV-VIS spectrophotometer was employed in the study.

2.3 Software tools

Python (Spyder), MS Excel

2.4 Standard Solutions

The stock solutions (1000 µg/ml) were prepared separately by dissolving accurately about 50 mg of each drug in 20 ml of methanol and the volume was made up to 50ml with methanol. Mixed standard solutions were prepared in both the methods by placing 50mg of DIP and 6.75mg of ASP in same 50ml volumetric flask and the volume was made up to the mark with methanol to get 1000 µg/ml of DIP and 135 µg/ml of ASP respectively.

2.5 Sample Preparations

For the preparation of sample solutions, ten tablets were weighed and powdered. An amount of powder equivalent to 100 mg was weighed and transferred to the volumetric flask. The volume was made up to 100 ml with water after appropriate continuous shaking for 15 mins with 20 ml of water. The solution was filtered and labelled for further dilutions. The dilutions were appropriately made for different validation parameters.

2.6 Individual Calibrations

To verify the linearity of both the drugs, six different dilutions were made in the concentration range from 5 to 30 µg/ml for ASP and 2-12 µg/ml for DIP in separate volumetric flasks. Absorbance values of all the solutions were recorded at their respective wavelength maxima namely 227 and 237 for DIP and ASP respectively. Calibration curves were constructed and the correlation coefficient values were calculated.

2.6.1 Method 1 – Multicomponent Analysis

The overlay spectrum of both the drugs at different concentration levels is shown in Figure 3. For the estimation of drugs by Multicomponent mode, a mixed standard solution was prepared by placing 50 mg of pure standard DIP and 6.75 mg of ASP in a 50 mL volumetric flask. The volume was made up to the mark, filtered and labelled as stock solution. The working standard solution was prepared by diluting 5 ml of the stock solution to 50 ml with methanol. Six different mixed dilutions were made with distilled water to get the concentrations ranging from 5, 6, 7, 8 and 9 µg/ml of DIP and 0.675, 0.75, 0.875, 1 and 1.125 µg/ml of ASP respectively. The absorbance of all the solutions were recorded at their respective wavelength maxima namely 227 and 237 for DIP and ASP respectively. Estimation of both the drugs were made using the developed Python code. The output comprising the concentrations of both the drugs is shown in the Figure 4.

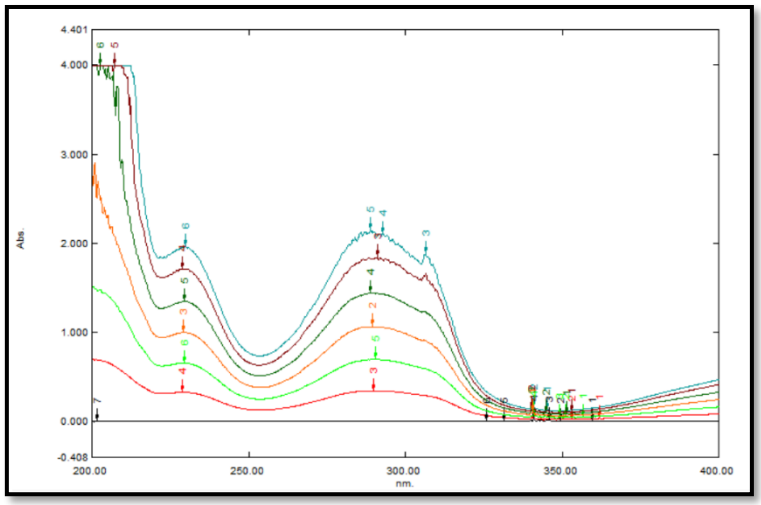
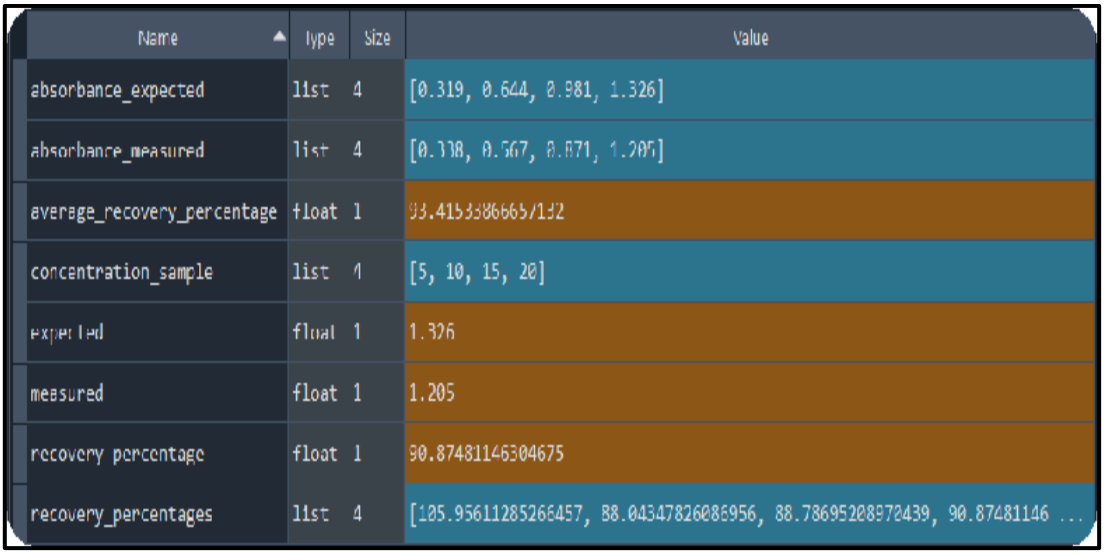


Figure 3: Overlay Spectrum of ASP and DIP at different mixed concentration levels



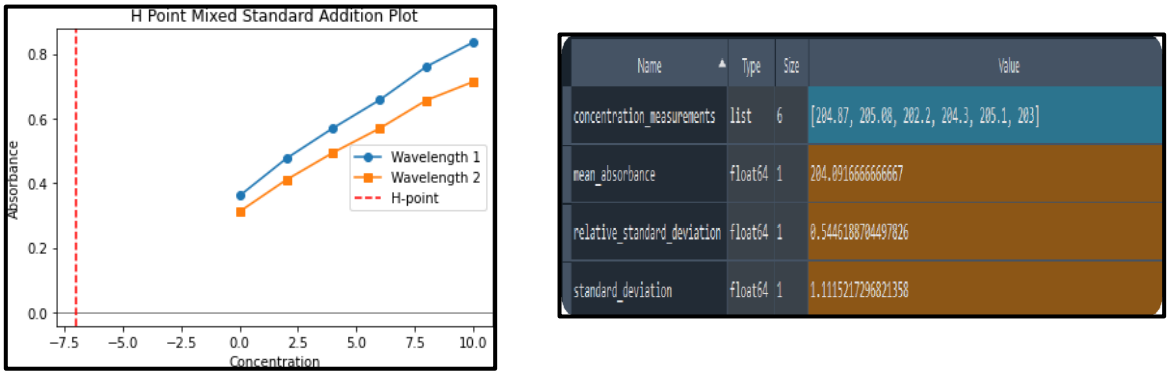
Name	Type	Size	Value
absorbance_expected	list	4	[0.319, 0.644, 0.981, 1.326]
absorbance_measured	list	4	[0.338, 0.667, 0.871, 1.205]
average_recovery_percentage	float	1	93.41533866657132
concentration_sample	list	4	[5, 10, 15, 20]
expected	float	1	1.326
measured	float	1	1.205
recovery_percentage	float	1	90.87481146304675
recovery_percentages	list	4	[105.95611285266457, 88.04347826086956, 88.78695208970439, 90.87481146 ...

Figure 4: Python output in Multicomponent Analysis

2.6.2 Method 2 – H-Point Standard Addition Method (HPSAM)

Three pairs of isosbestic points (208 & 226nm, 209 & 218nm, 218 & 226nm) were obtained from the overlapping spectra of ASP and DIP of different concentrations. From the three pairs, 218 and 226nm were selected for the method optimization. Different aliquots of the working standard solutions of DIP and ASP were taken and several dilutions were made with distilled water to get the concentrations ranging from 2-12 µg/ml for DIP and 5-30 µg/ml for ASP. All the solutions were subjected to measurement of absorbance at the selected wavelengths, 218 and 226nm. Linearity was verified for both the drugs at the two selected wavelengths as shown in Table I.

For the estimation using HPSAM, known amount of DIP was added to the mixed samples of various ASP and DIP concentration ratios [7]. A series of 10 dilutions were prepared to get different concentration ratios of mixed working standards for ASP and DIP as given in Table 2. The absorbance values were measured at the selected isosbestic wavelengths 218 nm and 226 nm. The H- point graphs were determined using the developed Python code and the concentrations of ASP were obtained as C_H from the graph and the absorbance of DIP was found from the graph as A_H. (Figure 5)



Name	Type	Size	Value
concentration_measurements	list	6	[204.87, 205.08, 202.2, 204.3, 205.1, 203]
mean_absorbance	float64	1	204.09166666666667
relative_standard_deviation	float64	1	0.5446188704497826
standard_deviation	float64	1	1.1115217296821358

Figure 5: Output of HPSAM graph and Data in Python

3. RESULTS AND DISCUSSION

3.1 Requirements for applying HPSAM

For the simultaneous estimation of two drugs, one drug is considered as analyte X and the other as interferent Y. Determination of the concentration of X by HPSAM requires the selection of two wavelengths λ_1 and λ_2 at which the interfering species, Y should have the same absorbance. Then known amounts of X are successively added to the mixture and the resulting absorbances are measured at the two wavelengths and expressed by equations (1) and (2), where $A_{(\lambda 1)}$ and $A_{(\lambda 2)}$ are the analytical signals measured at λ_1 and λ_2 respectively. The b0 and A0 (b0 ≠ A0) are the original analytical signals of X at λ_1 and λ_2 respectively and b and A' are the analytical signals of Y at λ_1 and λ_2 respectively. $M_{\lambda 1}$ and $M_{\lambda 2}$ are the sloped of the standard addition calibration lines at λ_1 and λ_2 respectively, C_i is the added concentration of analyte X. The two straight lines obtained intersect at the so called H Poso-called A_H]. (eqs (1) and (2)) [8].

$A_{(\lambda 1)} = b_0 + b + M_{(\lambda 1)}\, C_i$ ---------- (1)

$A_{(\lambda 2)} = A_0 + A' + M_{(\lambda 2)}\, C_i$ ---------- (2)

At the H-Point ($C_i = -C_H$), Eqs. (3) and (4) follow from Eqs. (1) and (2), since $A_{(\lambda 1)} = A_{(\lambda 2)}$.

$b_0 + b + M_{\lambda 1}(-C_H) = A0 + A' + M_{\lambda 2}(-C_H)$ ---------- (3)

$-C_H = [(A_0 - b_0) + (A' - b) / M_{\lambda 1} - M_{\lambda 2}$ ---------- (4)

From Eqn (4), the following conclusions can be drawn:

If component Y is the known interferent and the analytical signal corresponding to Y, b (at λ_1) and A' (at λ_2) do not change with the addition of analyte X, that is b = A' = constant, and then see Eqns (5) – (8).

$\mathbf{-C}_x = (A_0 - b_0) / M_{\lambda 1} - M_{\lambda 2} = b_0 / M_{\lambda 1} = A_0 / M_{\lambda 2}$ ---------- (5)

If $C_H = \mathbf{-C}_x$ then,

$\mathbf{-C}_H = (A_0 - b_0) / M_{\lambda 1} - M_{\lambda 2} = b_0 / M_{\lambda 1} = A_0 / M_{\lambda 2}$ ---------- (6)

if the value of –*C*H is included in *Eqn*. (1), then

$A_H = b_0 + b + M_{\lambda 1}(-C_H)$ ---------- (7)

b0 = $-M_{\lambda 1}$ CH [Eqn. (4)], then

$A_H = b$ ---------- (8)

and similarly $A_H = A'$

Hence, the *A*H value is only related to the signal of the interfering species Y at the two selected wavelengths and *C*H is independent of the concentration of interfering species. Fig. shows the effect of change in Aspirin concentration at H-point. According to the above discussion, at H-point *C*H is independent of the concentration of interferent and so AH is also independent of the analyte concentration.

For the selection of appropriate wavelengths for applying HPSAM, the following principles were followed. At the two selected wavelengths, the analyte signals must be linear with the concentration, and the analyte signal obtained from a mixture containing the analyte and the interferent signal should be equal to the sum of individual signals of the two species. In addition, the difference in the slopes of the two straight lines measured at two selected wavelengths, λ_1 and λ_2 respectively must be as large as possible in order to get good accuracy and sensitivity 9.

In the current system, the analyte is Dipyridamole and Aspirin is the interferent. Several wavelength pairs were examined and the wavelength pair of 218 and 226 nm was selected. Under optimal conditions, determination of DIP and ASP was carried out using HPSAM. The concentration of interferent was calculated in each test solution by the calibration method with a single standard and the ordinate value of the H-point (*A*H). Several synthetic mixtures with different concentration ratios of valsartan and hydrochlorothiazide were analyzed by the proposed method. The results are given in Table 1.

Table 1: Linearity results of both the drugs at selected wavelength pair

Drug	Lambda max (nm)	R^2	Slope	Intercept	Linear range (µg/ml)
ASP	218	0.9974	0.0525	0.0293	5-30
DIP	218	0.9970	0.1470	0.0039	2-12
ASP	226	0.9978	0.0536	0.0131	5-30
DIP	226	0.9992	0.1307	0.0026	2-12

3.2 Repeatability of the HPSAM

To check the repeatability of the method, six replicate experiments of ASP and DIP were carried out (Table 2). The analyte concentration was obtained as C_H and the concentration of the interferent was calculated in each test solution using the ordinate value of the H-Point (A_H). the relative standard deviations for six replicate measurements of the mixture were found to be 1.8554 and 0.5446 % for ASP and DIP respectively [9, 10].

Table 2: Various concentrations of ASP and DIP in HPSAM

S.no.	Mixed standard (µg/ml)		Standard addition of DIP (µg/ml)	S.no.	Mixed standard (µg/ml)		Standard addition of DIP (µg/ml)
	DIP	ASP			DIP	ASP	
1	5	0.675	0	6	10	1.125	0
			2				2
			4				4
			6				6
			8				8
2	6	0.750	0	7	11	1.375	0
			2				2
			4				4
			6				6
			8				8
3	7	0.875	0	8	12	1.5	0
			2				2
			4				4
			6				6
			8				8
4	8	1	0	9	13	1.625	0
			2				2
			4				4
			6				6
			8				8
5	9	1.125	0	10	14	1.75	0
			2				2
			4				4
			6				6
			8				8

3.3 Recovery and Precision Studies for both the methods

The accuracy and precision for the analysis of ASP and DIP in the proposed synthetic mixtures at three different concentrations (5.0, 10.0, 15.0 µg/ml for ASP and 0.625, 1.25, and 1.875 µg/ml for DIP) were tested in intra-day and inter-day experiments. Good accuracy and precision were observed for both drugs. The standard addition method was used to observe the selectivity of the proposed HPSAM and Multicomponent analysis methods [9]. Appropriate volumes of the standard stock solutions of ASP and DIP at three different concentrations were added to the analyzed tablet solutions and re-analyzed by the proposed methods. This procedure was repeated six times for each concentration level.

Table 3: Results of accuracy and precision in formulation

S.No	mount found (mg)		% Purity(w/w)		RSD (HPSAM)		RSD (Multicomponent)	
	DIP	ASP	DIP	ASP	DIP	ASP	DIP	ASP
A1	201.96	25.272	100.98	101.08	1.051	0.582	1.082	0.423
A2	212.08	26.505	106.04	106.08	0.982	0.587	1.024	0.542
A3	201.10	26.015	100.55	104.06	0.998	0.547	1.035	0.624

Label Claim – DIP- 200 mg and ASP – 25 mg RSD – Relative Standard Deviation

3.4 Analysis of Commercial Tablets

Results obtained by the application of Multicomponent and HPSAM to the analysis of ASP and DIP in tablet formulation are summarized in Table 4.

Table 4: Simultaneous Determination of ASP and DIP in commercial tablets by HPSAM and Multicomponent Analysis

Sample	Label Claim (mg)		HPSAM (mg)*		Multicomponent (mg)*	
	DIP	ASP	DIP	ASP	DIP	ASP
Arreno	200	25	201.25 ± 0.81	25.21 ± 0.75	201.32 ± 0.23	25.23 ± 0.45

*mean ± SD, n=3

3.5 Greenness of the Methods

Measurement of the Greenness of the developed analytical methods is essential in assessing the environmental impact of various analytical procedures. This is determined according to the occupational hazards, amounts of reagents and solvents, waste generation, and energy consumption [11]. These factors are involved in the calculation of penalty points, which was established by the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). The Green and Analytical Calculator (AGREE metric) was created by the Gdańsk University of Technology. The twelve parameters that determine the result of this software's calculation match the twelve GAC principles [12]. Each parameter or principle has a score ranging from 0 to 1, which is determined by the degree of risk associated with a certain principle's greenness. The principles of SIGNIFICANCE are used to compute the final score. The ultimate score with each criterion for analytical process performance and the user-assigned weights are all displayed in a pictogram as the outcome (Figure 6).

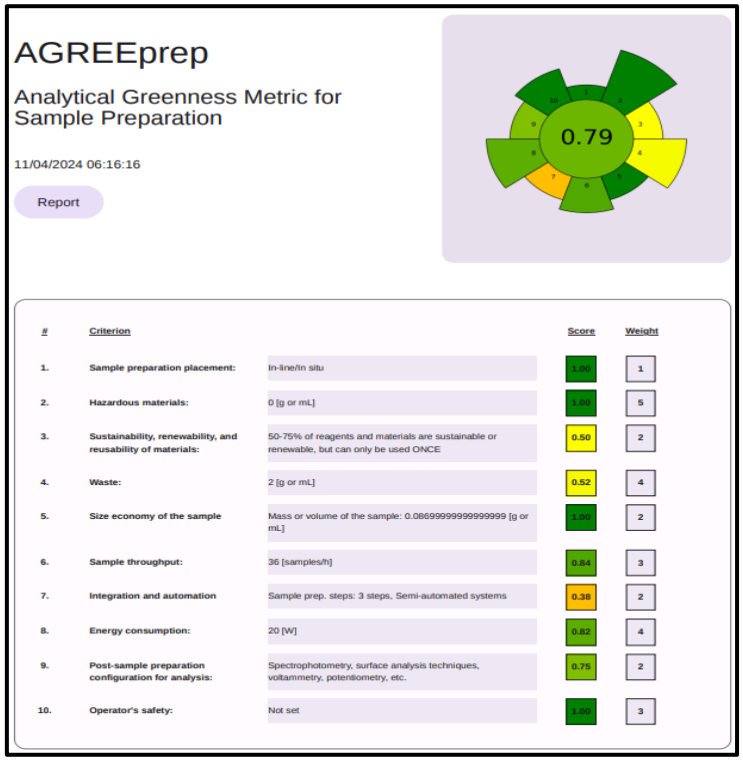


Figure 6: Analytical greenness report

4. CONCLUSION

The above results show that HPSAM and Multicomponent Analysis allow rapid, accurate, and simple resolution of Aspirin and Dipyridamole mixtures. HPSAM can be used in complex samples with matrix effects because the standard addition method has the capability of removing these effects. The application of Python, a versatile machine learning tool overcomes the need for additional software for the estimation of these drugs using UV Visible Spectrophotometer.

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