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Three spectrophotometric approaches for measuring ratio spectra of Ivabradine and Carvedilol in a binary mixture using green analytical principles

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A B S T R A C T

The development of three simple, precise, efficient, and accurate spectrophotometric techniques that manipulate ratio spectra is undertaken to measure Ivabradine and Carvedilol simultaneously in bulk and tablet formulation. Applying mathematical calculations like the ratio derivative, first-order ratio derivative and ratio derivative subtraction method has been used for determination of CVD and IBD. For Carvedilol, the calibration curve is linear between concentrations of 40–65 µg/mL and 50–81.25 µg/mL for Ivabradine. These strategies have been put into practice and used for analyzing marketed pharmaceutical formulations. Further, the outliers were statistically assessed by using Grubb's test and found, as null hypothesis cannot be rejected. The suggested approach was evaluated using four green evaluation approaches with which these tools found an excellent green outcome. Altogether, the proposed method was found to be a simple, sensitive, accurate and eco-friendly method for the analysis of drugs that have overlapping properties in the UV spectrum.

1. Introduction

Chemically Ivabradine (IBD) 2H-3-Benzazepin-2-one, 3-[3-[[[(7S)-3,4-dimethoxybicyclo [4.2.0] octa-1,3,5-trien-7-yl] methyl] methylamino] propyl]-1,3,4,5-tetrahydro-7,8-dimethoxy hydrochloride **(Fig. 1a)**. As a function, its activity is based on a direct influence on the sinus node, resulting in a lower inclination of the endogenous diastolic depolarization. The sinus node is selectively affected if an inhibitor is utilized in the therapy of angina pectoris in people who are unable to take beta-blockers.

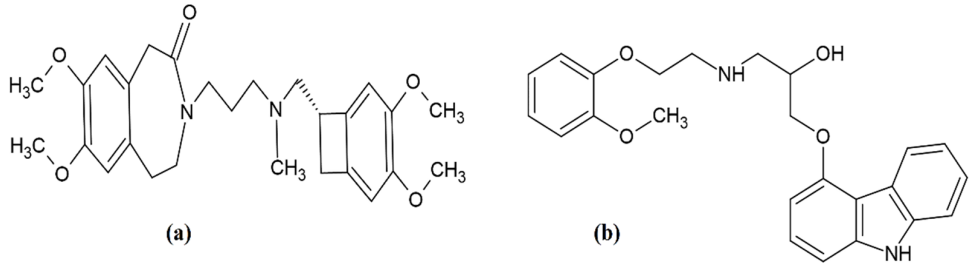

Fig. 1. Structure of (a) Ivabradine (IBD) and (b) Carvedilol (CVD)

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Carvedilol (CVD) is Chemically 2-Propanol, 1-(9H-carbazol-4-yloxy)-3- [[2-(2-methoxy phenoxy) ethyl] amino], **(Fig. 1b)** is an efficient α1 non-selective, β1 and β2-adrenoreceptor antagonist in the therapy of systolic heart failure. It has been shown to exhibit simultaneous reactive oxygen species (ROS) scavenger and ROS suppressing properties. The study showed that daunorubicin- (DNR-) caused cardiac toxicity was prevented by the presence of lower levels of oxidative damage and apoptosis. [1,2]

The use of Carvedilol and Ivabradine mostly in medication of angina and for treatment of heart failure should be for patients with coronary artery disease and patients with heart failure. The literature review shows that no analytical techniques have been employed for the detection of CVD and IBD, which have been determined each independently or in mixture among other drugs, in tablet dosage forms or biofluids via analytical methods so as spectroscopy,[3] capillary electrophoresis,[4] HPLC,[5–9] UPLC,[10] HPTLC,[11] and LCMS.[12,13]

The proposed research work's main objective is to develop an eco-friendly UV method using two different techniques for concurrent and speedy evaluation of CVD and IBD in bulk (API), tablet dosage forms, and further validated the proposed method according to ICH Q2 (R1) guideline. Developing a technique that utilizes eco-friendly solvents [14–18] in UV spectroscopy for drugs with overlapping properties will significantly strengthen method adaptation for rapid screening in the pharmaceutical industry and commercial labs for these two drugs.

1.1. Ratio derivative and subtraction method

The ratio subtraction method [19] begins by analyzing the zero-order absorption spectrum for prepared standards. The linearity within the absorbance at the specific wavelength is then calculated, and the associated drug concentration is then determined. The method is based on fact when a mixture of A and B has a more extended-spectrum. A is determined by scanning mixture of A and B as zero-order absorption spectra, followed by dividing them precisely with chosen concentration of prepared and scanned B standard solution (B′ = divisor), and creating a unique ratio spectrum which represents (A/B) + constant (without any absorbance). So, finding an absorbance of these constants (B/B′) in the plateau is subtracted, and the acquired spectra were multiplied by (B′) using the divisor. Ultimately, the original spectra of (A) were collected, which were used to determine directly and calculate the concentration using the related regression equation. A linear regression equation is achieved within the absorbance, followed by a corresponding concentration at particular wavelength.

1.2. First derivation ratio spectra

The approach developed by Salinas et al.[20] considers the ratio-spectra derived from the binary mixture of spectrum in the derivation of the spectrophotometric solution. The significant advantage of the first derivative ratio absorption spectrophotometric method is making simple computations with the associated peak wavelengths. To as follows Often, the incidence of a large number of maximum and minimum creates a benefit for quantifying active substances, which could interfere with other compounds and excipients . The ratio difference spectra of the sample mix are divided by a standard solution of one of the components, and the absorbance spectrum of the mixture is then used to find the first derivative of the ratio absorption spectra. From a calibration graph, the concentration of the other factor is then calculated.

1.3. Application of recommended techniques for determining CVD and IBD in the marketed formulation

The amounts of CVD and IBD in the formulated mixtures are determined using the three techniques like ratio spectrum, Ratio subtraction method and first derivative ratio subtraction method has applied.

1.4. Proposed Method validation

The intended approaches were validated in accordance with the ICH guidelines Q2R1 and validated by using the parameters like linearity, accuracy, precession, the limit of detection and quantification.

2. Results and Discussion

(Cardivas® IN) tablets are formulated with IBD in addition to CVD. The general use in the management of hypertension makes it particularly effective in this role. This research seeks to discover a simple method to quantify IBD and CVD simultaneously in tablets. In pharmaceutical formulations, UV absorption spectroscopy has been widely utilized for the improvement of analytical methods. This method has a limitation due to the majority of active pharmaceuticals absorbing in the UV and exhibiting overlapping spectra, making it difficult to measure them simultaneously. Both IBD and CVD have zero-order absorption spectra that overlap **(Fig. 2)**. To nullify this first and second derivative spectrophotometric methods were applied and found that the drug spectrums were still overlapping, making those drugs difficult to analyze. Since the application of the techniques like ratio derivative methods made the analysis simple and easy to separate the prescribed set of drug formulation.

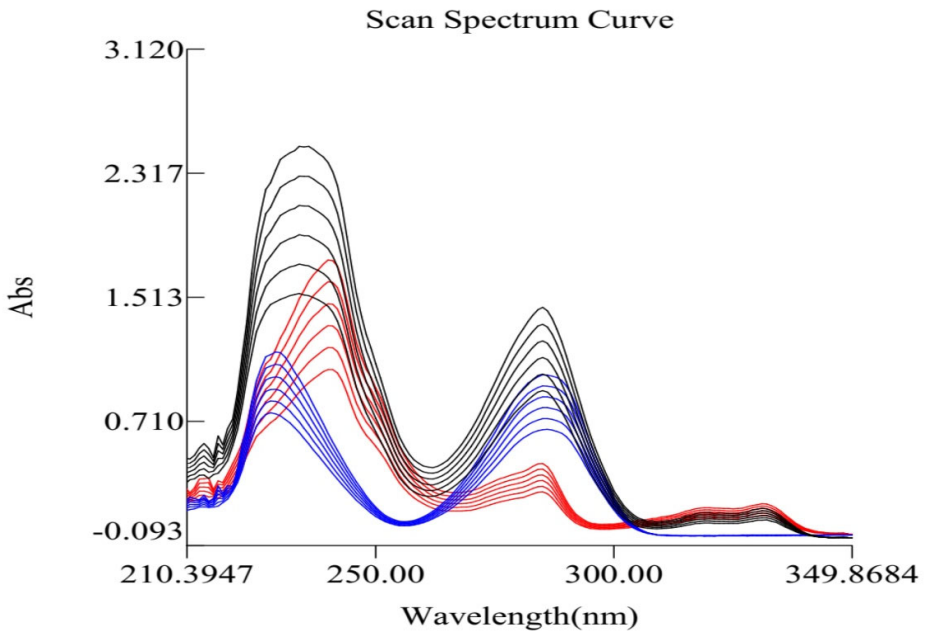

Fig. 2. Zero-order UV-spectrum of 40–65 µg/mL CVD (Blue) and 50–81.25 µg/mL IBD (Red). Mixture (Black)

2.1. Ratio derivative and subtraction technique for CVD and IBD

The ratio subtraction method[19] begins by analyzing the zero-order absorption spectrum for prepared standards of CVD. Different aliquots corresponding to 40–65 µg/mL and 50–81.25 µg/mL of CVD and IBD is prepared from the stock solutions in separate 10-mL volumetric flasks and diluted to volume using diluent. For Determining the concentration of CVD in the combination of IBD, the saved CVD spectrum are separated by using 50 µg/mL IBD spectrum, and the absorbance discrepancy between the ratio difference spectra (CVD/IBD) at 296 ($\Delta M_{287–302.15}$) is shown against the relevant (CVD) quantities and depicted in **Fig. 3a**. For determining the presence of IBD in CVD, the saved IBD spectra are divided by the 40 µg/mL CVD spectrum, the subsequent ratio absorption spectra will be smoothed with $\Delta\lambda$ = 4 nm, and the absorbance discrepancy between the ratio spectra (IBD/CVD) at 255 nm ($\Delta M_{247-266}$) is mapped toward corresponding IBD concentrations and depicted in **Fig. 4a**. This generates the ratio derivative spectra of CVD and IBD at different concentrations. For obtaining ratio subtraction spectrums the constant has been subtracted from the ratio spectrum (to obtain pure spectrum of CVD or IBD) which was obtained as CVD/CVD' or IBD/IBD' as shown in **Fig. 3b and 4b** for CVD and IBD. The linearity within the absorbance at the specific wavelength of 296 nm is then calculated, and the associated CVD concentration is then determined. A linear regression equation is achieved within the absorbance, followed by a corresponding concentration of CVD (296 nm). The approach was repeated for the IBD and the concentration of IBD is determined by using linear regression equation.

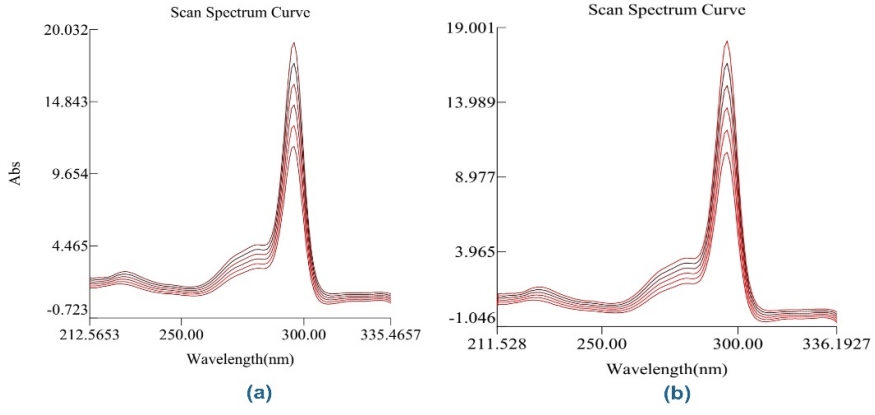

Fig. 3. (a) Smoothed ratio spectrum of CVD (40–65 µg/mL) with 50 µg/mL of IBD as a divisor and (b) Ratio subtraction spectrum of CVD (40–65 µg/mL) with 50 µg/mL of IBD as a divisor

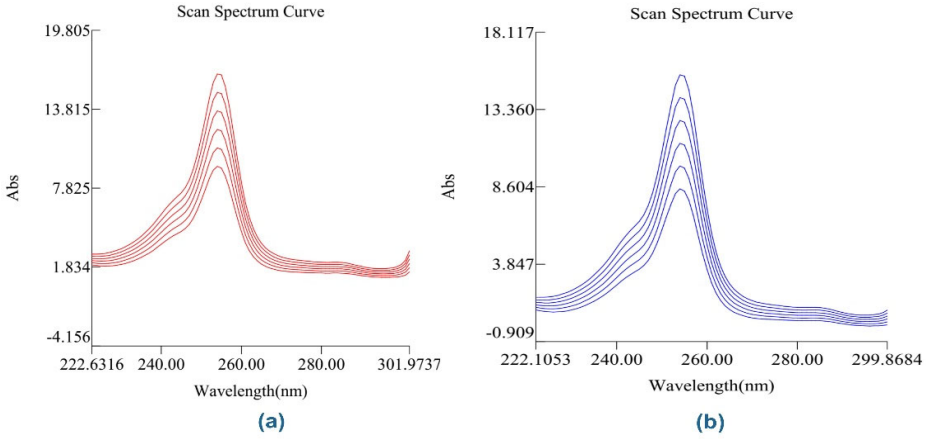

Fig. 4. (a) Smoothed ratio spectrum of IBD (50–81.25 µg/mL) with 40 µg/mL of CVD as a divisor and (b) Ratio subtraction spectrum of IBD (50–81.25 µg/mL) with 40 µg/mL of CVD as a divisor

2.2. First-order derivative approach

For the first derivative technique, multiple dilutions equivalent to 40–65 µg/mL and 50–81.25 µg/mL of CVD and IBD is prepared by transferring stock solutions into individual groups of 10-mL flasks and then volumetrically filled using diluent. The optimized standard solutions' Zero order absorption spectrum was measured around 210 to 350 nm and processed by the help of software using diluent as a blank. For determining the presence of CVD in the mixture, the stored IBD at 50 µg/mL were utilized and generated ratio derivative spectra. Further, these have been smoothed and derivatives using $\Delta\lambda = 4$ nm, generating the first derivative ratio spectrum. The first derivative peak of (CVD/IBD) has an absorbance of 292.78 nm. For determining the presence of IBD in the mixture, the saved spectra of CVD at 40 µg/mL concentration have been utilized repeatedly like the above process. At 250.15 nm, the magnitude of the first derivative peak (IBD/CVD) is estimated. A linear curve is built by comparing specific peak absorption at 250.15 nm to the identical IBD concentrations in µg/mL. The divisors concentration, scanning speed, wavelength, the wavelength enhancement on which the derivative is generated, and the smoothing role are all closely measured to determine their effect on the shape of the ratio spectra. Ratio spectra are revealed in **Fig. 3** and Fig. **4**, as well as the first derivative spectrum of the obtained ratio absorption spectra seen in **Fig. 5**. The explanations given for **Fig. 5** may very well back up this interpretation. The influence of scanning speed on wavelength is examined. It is discovered that fast scanning produces noisy spectra; therefore, when scanning at a low speed, noise is decreased; however, measuring stint is increased; thus, quantifications are performed at a medium scanning speed. The divisor concentrations of 40 and 50 µg/mL of IBD and CVD, respectively, were the finest when it comes to the prediction of CVD and IBD concentrations in bulk and prepared laboratory mixtures from the marked formulation.

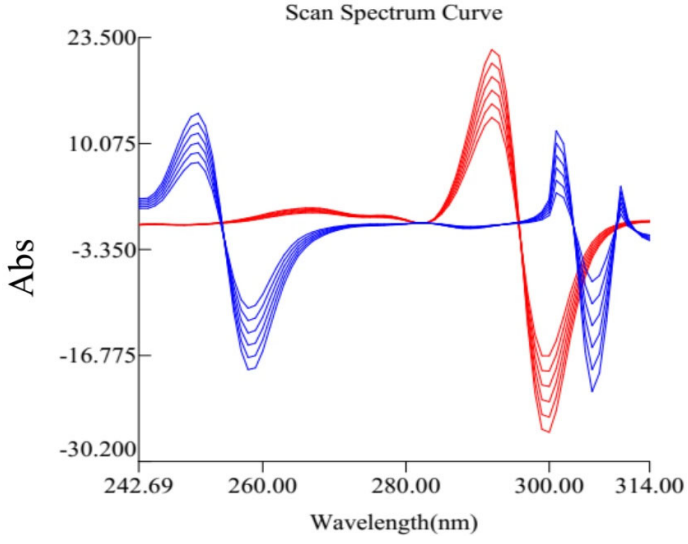

Fig. 5. Smoothed 1st ordered ratio derivative spectrum of CVD (40–65 µg/mL) with 50 µg/mL of IBD as a divisor (Red), IBD (50–81.25 µg/mL) with 40 µg/mL of CVD as a divisor (Blue)

2.3. Method validation

Method validation has performed by using the ICH guidelines Q2R1 and the results of their specific parameters were as follows

2.3.1. Accuracy and precision

The results were validated for accuracy by applying the proposed methods to multiple blind samples of CVD with IBD. Different concentrations were determined using appropriate regression models to determine the % recovery, with the mean % recovery displayed in **Table 1**. The accuracy of both procedures was additionally enhanced through the practice of standard addition procedure, which included adding known amounts of standard CVD and IBD to marketed preparation, an assay of the resultant mixes, and comparing the obtained results to the predicted findings **(Table 4)**. The high recovery rates for the usual addition procedure revealed that the proposed approaches were accurate.

Repeatability: Three CVD (25, 50, 75 µg/ml) and three IBD (31.25, 62.5, 95.75 µg/ml) concentrations were evaluated intra-daily using the suggested techniques. We estimated the % recoveries and the relative standard deviation.

Intermediate precision: The above processes were performed three times daily on three separate days to analyze the three concentrations selected. We estimated the % recoveries and the RSD.

Table 1. Accuracy and precision outcomes for CVD and IBD

Parameters	Ratio absorption method		Ratio subtraction method		First derivative method	
	CVD	IBD	CVD	IBD	CVD	IBD
Accuracy (mean ± SD)	99.52 ± 1.12	98.46 ± 1.65	99.42 ± 0.98	98.53 ± 1.12	97.34 ± 1.24	98.87 ± 1.35
Precision						
Repeatability	98.96 ± 0.91	99.92 ± 1.02	98.85 ± 1.70	99.95 ± 1.2	99.74 ± 1.54	98.53 ± 1.64
Intermediate precision	98.47 ± 0.98	98.53 ± 1.12	98.48 ± 0.98	98.73 ± 1.6	99.68 ± 1.63	98.85 ± 1.74
Robustness	98.46 ± 1.65	98.95 ± 1.27	99.42 ± 1.02	98.82 ± 0.58	99.65 ± 1.24	98.24 ± 1.53

2.3.2. Linearity

The methods' linearity was determined by assessing seven CVD and IBD concentrations ranging from 40 –65 µg/mL and 50–81.25 µg/mL, respectively **(Fig. 6)**. Three repetitions were repeated for each concentration. The assay was conducted under the previously specified experimental conditions **(Table 2)**. The proposed methods were further authenticated by using Grubb's test with an H_0 (no outlier in the data) and Ha (where the minimum or maximum value is an outlier) showing that as the computed p-value is greater than the significance level alpha = 0.05, one cannot reject the null hypothesis H0. And the results are depicted in **Table 3**. The Z score also indicated that the values were inside the outliers.

Table 2. Linearity data for the proposed techniques.

Parameters	Ratio absorption method		Ratio subtraction method		First derivative method	
	CVD	IBD	CVD	IBD	CVD	IBD
Linearity range (µg/mL)	40 - 65	50 - 81.25	40 - 65	50 - 81.25	40 - 65	50 - 81.25
Slope	0.236	0.2796	0.238	0.2791	0.293	0.2449
Correlation coefficient	0.9994	0.9994	0.9996	0.9997	0.9994	0.9995
STERYX	0.076	0.071006	0.5877	0.0531	0.0913	0.0581
LOD	1.074	0.838	0.815	0.628	1.028	0.784
LOQ	3.254	2.540	2.469	1.904	3.117	2.376
%RSD	0.50	0.55	0.41	0.44	0.52	0.55

Table 3. Outlier determination by Grubb's test for the proposed methods

	Ratio Absorbance method		Ratio Subtraction Method		First order ratio subtraction method	
	CVD	IBD	CVD	IBD	CVD	IBD
Data Point with Largest G	11.48	16.49	10.606	8.443	12.98	7.532
Data Index with Largest G	1	6	1	1	1	1
G Statistic	1.3706	1.3543	1.3312	1.3409	1.3352	1.3558
Critical Value	1.8871	1.8871	1.8871	1.8871	1.8871	1.8871
Approximate P Value	0.8649	0.9049	0.9625	0.9380	0.9524	0.9010
Significance	0	0	0	0	0	0

2.3.3. Selectivity

The methods' selectivity has shown by analyzing the various laboratory-prepared mixes of CVD and IBD within the linearity range.

2.3.4. Stability

It has shown that CVD and IBD sample solutions were free of any spectrophotometric alterations for almost three weeks when kept at 4 °C and seven days when kept at room settings compared with the freshly prepared standard solutions.

2.4. Assay of marketed formulations

The assay for a commercialized tablet has developed using proposed spectrophotometric methods that have confirmed to be accurate and reliable. The mean drug content of both drugs in two different formulations was 98.1 to 100.8 % in two formulations, namely Cardivas varied concentrations of IBD I and II. No interference peaks have seen in the spectra, showing that the drug has estimated without concern for excipients and the results were depicted in **Table 4**.

Table 4: The suggested spectrophotometric techniques application of standard addition approach has used to confirm CVD and IBD in Cardivas® IN tablets

Brand	Contents	Present (µg/mL)	Added (µg/mL)	Standard addition			
				First derivative method		Ratio subtraction method	
				Found (µg/mL)	Recovery percentage	Found (µg/mL)	Recovery percentage
Cardivas® IN	CVD	50	25	25.04	100.16	25.14	100.56
			50	49.92	99.84	50.32	100.64
			75	74.95	99.93	74.35	99.13
	IBD	62.5	31.25	31.22	99.904	31.42	100.54
			62.5	62.45	99.92	62.35	99.76
			93.75	93.79	100.04	93.89	100.14
Cardivas® IN	CVD	50	25	25.19	100.77	25.11	100.44
			50	50.92	101.84	50.12	100.24
			75	74.45	99.26	74.45	99.26
	IBD	31.25	15.62	15.55	99.52	15.925	101.92
			31.25	31.15	99.68	31.45	100.64
			46.87	46.67	99.57	46.67	99.57

2.5. Green assessment of proposed method

Greenness assessment of the proposed method was done using four greenness assessment tools called (National Environmental Method Index) NEMI, Green Analytical Procedure Index (GAPI), Analytical eco-scale, and AGREE metrics.

2.5.1. NEMI [21,22]

NEMI assessment is represented by a circular NEMI pictogram, where it consists of four quadrants coded with green if it fulfils the criteria. First quadrant deals with persistent, bioaccumulative, and toxic (PBT)-list- if a chemical or solvent used in the method was not listed in the PBT list, then the quadrant is coded with green colour. Second quadrant with Environmental protection act list (EPA) list. The third quadrant with pH and chemicals should be within the range of 2 – 12 to make this quadrant green. Finally, the fourth quadrant deals with the waste generated from the method should be less than 50 mL, represented by green colour.

In the proposed method the chemicals used were only 1 % acetic acid which may produce negligible effect and waste so all the quadrant was represented with green colour and the NEMI pictogram was depicted in **Fig. 6**.

2.5.2. GAPI [22,23]

GAPI is a colour coding representation like NEMI but extended with procedural application makes this a more reliable qualitative method. By applying the required data in the 11 quadrants of GAPI, the output representation will give an idea regarding the method greenness and the chemicals list that need to be checked in this GAPI was according to National Fire Protection Act (NFPA). The final GAPI output was represented in **Fig. 6**.

2.5.3. Analytical Eco-scale [24,25]

Analytical eco-scale is a semi-quantitative method deals with the penalty points (PP) scored by the method and minus 100. Basics of AES will follow four steps as follows

- The reagent and amount used was less than 10 mL per analysis - 0 PP due to usage of acetic acid, which is less than 1 mL for complete analysis.
- **Hazard PP-** pictograms of reagents - the acetic acid contains a danger symbol with two pictograms. So, two penalty points and multiplied by the amount PP (0) gives 2 PP.

- **Energy consumption** for UV is less than 0.1 kWh - 0 PP.
- **Occupation hazard** - 0 PP for acetic acid and water.
- **Wastage** < 1 mL - 0 PP – as less than 1 ml of acetic acid was used for the whole study.
- Finally, the total lost PP for proposed method is 2+0+0+0 =2
- AES =100 – PP = 98.

The method that received a score of 75 was determined to be greener; however, the suggested approach received a score of 98, which shows the influence the technique will have on future use concerning environmental friendliness.

2.5.4. AGREE metrics [26, 27]

AGREE metrics is a quantitative approach based on the software. The required details were applied to the software. The results were depicted in **Fig. 6**.

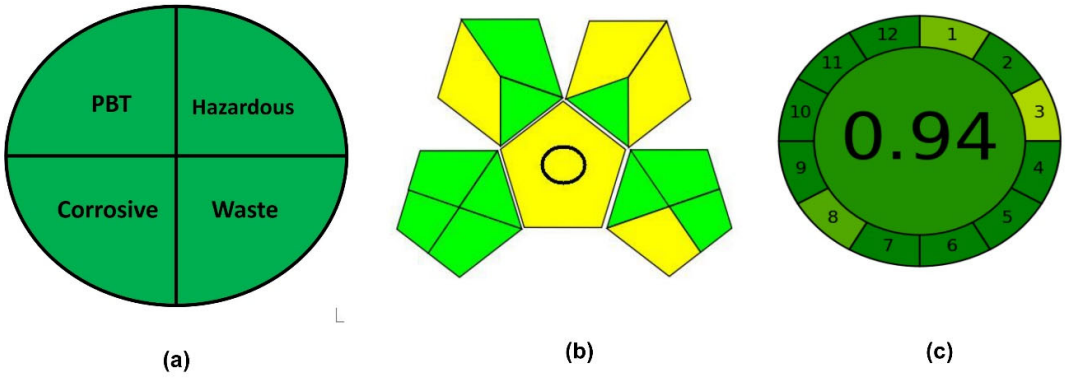

Fig. 6. Green assessment for the proposed method by using (a) NEMI (b) GAPI (c) AGREE

3. Conclusions

As a result of the preceding discussion, it is clear that the proposed approaches are simple, easy, and do not require any specialized methods or tools. Furthermore, these methods are sensitive and specific, and their available dose form allows for regular detection of CVD and IBD. Additionally, the approaches are applicable and valid in labs without liquid chromatographic equipment. It turns out that the selection of three different methods is based on a principle known as Multi analytical technique, and three methods were performed using a single solution in the current study, indicating that the methods can be easily transferred from one method to another and confirming that these are the best approaches to green analytical chemistry principles available. The proposed approach was then evaluated for its environmental friendliness using green assessment tools and determined to be an environmentally friendly technique. As a result, the pharmaceutical industry and the quality control department can readily use this strategy for routine research and development that is long-term.

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4. Experimental

4.1. Apparatus

The LAB INDIA UV 3092 double beam UV-VIS spectrophotometer sealed and quartz coated with Czerny-Turner monochromator optics with Wavelength range: 190 to 900 nm, Spectral bandwidth: Continuous slit 0.1 - 5.0 nm with 0.1 nm interval. Wavelength accuracy: ± 0.3 nm. Automatic eight-cell changer. Tungsten and deuterium lamp as detector and UV Win Lab Version 5.1.1 Software for data output has used.

4.2. Materials

Pure standards: Sun pharmaceuticals, Goregaon, Mumbai, kindly supplies IBD and CVD medications. The purity of IBD and CVD is labelled as 99.66% and 99.64 %, respectively, with a certificate of analysis.

4.3. Pharmaceutical formulation

IBD and CVD Cardivas® IN (Batch No. EMX2581) are made by Sun pharma labs ltd contains 6.25 mg IBD and 6 mg CVD. India's East Sikkim.

4.4. Reagent

Acetic acid was supplied by Union Drug & Chemical Company- China.

4.5. Preparation of diluent

Diluent composed of 1 % acetic acid

4.6. Procedures

4.6.1. Standard solutions

Accurately weighed 50 and 62.5 mg of pure CVD and IVD and transferred individually in a 100 mL calibrated flask, a few mL of diluent was added, sonicated for 15 minutes to achieve complete dissolution and made up the final volume with the diluent. Additionally, 50 and 62.5 µg/mL dilutions were prepared using the same diluent and labelled as working standards.

4.6.2. Preparation sample solutions from the marketed formulations

Weighed accurately 20 tablets of marketed formulation, gridded finely followed by weighing the powder equivalent to 5 and 6.25 mg of CVD and IBD and transferred to 100 mL volumetric flask and added 25 mL of diluent, sonicated, and make upon the volume with the diluent. Further dilutions were made from the above solutions to prepare a final solution of 50 and 62.5 µg/mL of CVD and IBD and marked as sample solutions.

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