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DEVELOPMENT AND VALIDATION OF ABSORPTION RATIO UV SPECTROPHOTOMETRIC TECHNIQUE FOR DICYCLOMINE HYDROCHLORIDE AND MEFENAMIC ACID EVALUATED USING GREEN TOOLS

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ABSTRACT
A new green spectroscopic technique was designed and validated for the measurement of mefenamic acid and dicyclomine hydrochloride in a synthetic mixture. Dicyclomine hydrochloride and mefenamic acid both displayed an isosbestic point at 250 nm in methanol. The developed method was determined using the absorption maxima of dicyclomine HCL at 214 nm, the point of equal absorbance at 250 nm, and the absorption maxima of mefenamic acid at 210 nm. Both drugs met the requirement that absorbance be directly proportional to concentration within the range of 2 to 10 µg/mL. They demonstrated linearity with R^2=0.9992 and R^2=0.9991 at λmax 214 nm and λmax 250 nm, respectively, and R^2=0.999 and R^2=0.9996 at λmax 214 nm and λmax 250 nm, respectively. The method was precise, and the % RSD was less than 2. It was shown that the percentage recovery for DCY was between 99.9 and 101.2%, and the percentage recovery for MEF was between 99.94 and 102.3%. This spectroscopic method was highlighted using green tools such as AGREE, GAPI, and BAGI. It was further confirmed to conform with ICH requirements, and the validation parameter's %RSD was less than 2.
Keywords: Q Absorption Ratio Method, Green Tool, Spectroscopic Method, BAGI
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INTRODUCTION

Dicyclomine is a tertiary amine anticholinergic medication used to treat irritable bowel syndrome, morning sickness, and dysmenorrhea.[1] Mefenamic acid, a nonsteroidal anti-inflammatory drug medication, mainly serves as an analgesic. By treating both inflammation and muscular spasms, dicyclomine hydrochloride and mefenamic acid are primarily used to treat menstrual discomfort (dysmenorrhea) and cramping in the abdomen. While dicyclomine, an antispasmodic, relaxes gut muscles, mefenamic acid, an NSAID, suppresses molecules that produce pain for disorders like IBS, colic, and general period discomfort. Mefenamic acid is known chemically as 2-(2,3-dimethylanilino) benzoic acid. Dicyclomine hydrochloride is known chemically as 2-diethylaminoethyl [1'-bi(cyclohexane)]-1-carboxylate hydrochloride. This combination effectively treats biliary colic, ureteric colic, intestinal colic, and spasmodic dysmenorrhea. Mefenamic acid is one medication that helps reduce pain in the body. An antipyretic is a drug that reduces fever by restoring the body's normal temperature. The main purpose of anti-inflammatory medications is to control pain and lower inflammation. We can estimate two drugs simultaneously using the q-absorption ratio method without first separating them. This combination of drugs has a synergistic effect. For this combination, the absorption ratio method's green spectroscopic approach is inaccessible. The literature review indicates that no analytical technique for the Q-absorption ratio measurement of mefenamic acid and dicyclomine hydrochloride in a combined formulation has been documented. The current research project intends to develop an absorbance ratio technique, a novel, simple, accurate, sensitive, repeatable, and economical analytical method for estimating mefenamic acid and dicyclomine hydrochloride in their combination dosage form in routine analysis as per ICH guidelines. Spectrophotometric analysis is based on the absorption of light by molecules in the UV region of the electromagnetic spectrum. A portion of UV light is absorbed at specific wavelengths that correlate to electronic changes inside molecules when it is administered to a sample solution that contains molecules with conjugated double bonds or chromophores. UV spectrophotometers create a spectrum by

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measuring the amount of light absorbed by the sample at various wavelengths. According to Beer-Lambert's law, the concentration of the absorbing species in the sample determines the absorption strength at a particular wavelength. Chemistry, biochemistry, pharmacology, and environmental science are among the disciplines that frequently employ this method for both qualitative and quantitative material examination. Furthermore, a new spectroscopic technique needs to be developed to address wavelength overlap problems with Green Tools. The proposed approach was validated using ICH Guideline Q2R1, and it is mapped with SDG 12, as it is an eco-friendly analytical technique with minimal waste production. Absorbance spectroscopy is a widely available and moderately cost-effective analytical instrument when compared to more sophisticated methods like chromatography. In many commercial formulations, mefenamic acid and dicyclomine HCl are often prescribed combined, especially for the treatment of conditions like stomach pain, menstrual cramps, and irritable bowel syndrome.[2] Simultaneous estimation of these two drugs is necessary for these formulations to guarantee proper dosage and therapeutic efficacy. Traditional methods for measuring mefenamic acid and dicyclomine HCl separately can be time-consuming, labour-intensive, or require specialised equipment. By developing a single method that can measure both drugs simultaneously, pharmaceutical analysis can be made more efficient and beneficial. These AGREE and GAPI tools are intended to improve a green analytical approach. The AGREE value of the greenness factor ranges from 0 to 1. AGREE offers a way of evaluation that emphasises the environmental impact of the strategy. The GAPI primarily examines solvent use, energy consumption, and waste formation during the analysis process in order to evaluate how ecologically sustainable analytical processes are. BAGI determines the method's practical efficacy, which consists of ten variables with an image and a score. Furthermore, BAGI assigns a score ranging from 0 to 100; a high score denotes a highly useful analytical procedure.[3,4] This work's primary goal was to create an absorption ratio spectroscopic green technique for this combination, as it is not available.

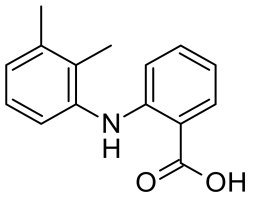

Fig.1: Structure of Mefenamic Acid

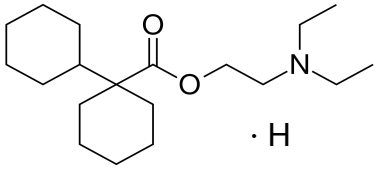

Fig.2: Structure of Dicyclomine Hydrochloride

EXPERIMENTAL

Material and Methods

A Shimadzu UV-1900 series device came with two matching 1 cm quartz cells. A computerized analytical balance and an ultrasonic Sonicator (LMUC-6, LABMAN) were used in the study. We employed 100, 250, and 500 mL borosilicate glass beakers; 10 and 100 mL calibrated flasks; and 1, 2, and 5 mL calibrated pipettes. Venus Pharma Chem, India, supplied a gift sample of dicyclomine hydrochloride, while Dhamtec Pharma and Consultants supplied mefenamic acid powder API. The local pharmacy sold us 500 mg of Metformin Hydrochloride Mefiway tablets. We bought methanol and other solvents from Merck. Every chemical reagent was of analytical purity.

Preparation of Standard Solution

Both standard drugs, mainly 50 mg were weighed and diluted with methanol, mainly 25 mL. Standard stock solutions were prepared mainly at 1000 PPM separately for both drugs. Several dilutions were done to prepare 100 PPM of each standard drug.

Methodology

The absorbance ratio method is a multi-component analytical approach that uses a UV spectrophotometer. It is also known as the Q-value method and is a modified version of the simultaneous equation approach. This is determined by the properties of the material that adhere to the Beer-Lambert law. Q-value, or Hufner's Quotient, for every wavelength. It involves measuring absorbance at two different wavelengths: the drug's maximum wavelength ($\lambda_{2\ max}$) and the point of equal absorbance ($\lambda_{1\ max}$). It is quicker and less expensive than chromatographic techniques. The absorbance ratio method is a derivative spectrophotometric technique for concurrently identifying two or more analytes in a mixture. It is a multicomponent analysis method. works by measuring absorbance at two wavelengths: a wavelength unique to one of the components and the equal absorption point, which is the point where the components' separate spectra intersect. For instance, a mixture of mefenamic acid has an equal absorption point of 250 nm, while dicyclomine HCl has a second wavelength of 214 nm. Calculating the ratio of absorbances at different wavelengths increases quantification accuracy and precision by reducing interference from formulation excipients and variations in sample preparation.[5,6,7]

$$Cx = \frac{(Qm-Qy)}{(Qx-Qy)} \times \frac{(A_1)}{(ax_1)}$$

$$Cy = \frac{(Qm-Qx)}{(Qy-xy)} \times \frac{(A_1)}{(ay_1)}$$

Where,

Qm = sample absorbance at 214 nm divided by sample absorbance at 250 nm.

Qx = Mefenamic acid's absorbance at 214 nm divided by its absorptivity at 250 nm.

Qy = Dicyclomine Hydrochloride's absorbance at 214 nm divided by its absorptivity at 250 nm.

Preparation of Test Solution for Assay

Twenty Mefiway pills (250 mg of mefenamic acid and 10 mg of dicyclomine hydrochloride) were carefully weighed and averaged. Absorbance was measured at 250 nm and 214 nm at the same time after several dilutions and a full scan for 200 - 400 nm. This mixture was diluted with methanol for analysis.[8]

Linearity

To acquire concentrations of 2 - 10 ppm for each medication separately, aliquots ranging from 0.2 - 1.0 mL were pipetted from the 100 ppm primary standard solution and diluted to 10 mL with methanol for both drugs.[9]

Application of Green Tools

Green tools make it much easier to choose safer solvents by replacing them with more ecologically friendly ones, which highlights how beneficial the approach is for the environment. Choosing the right green tool for the method is quite important. Since every green tool is distinct in its own way. In this method, AGREE, GAPI, and BAGI were used.

AGREE

It provides a colour-coded circular image, a score between 0 and 1, and evaluates analytical procedures with respect to all 12 green chemistry principles.[10]

GAPI

It is a visual analytical source that assesses the environmental significance of every stage of a process, from sample collection to final analysis, using unique symbols. GAPI employs a colour-coded system and the green character for analytical procedures. The use of pictograms in GAPI allows for quick recognition of the intensive environmental steps involved in a procedure or analytical pathway, for example, solvents, sample preparation, and energy. GAPI is very beneficial for comparing alternative procedures, as environmental similarities provide much more information than absolute environmental values. It mostly aids in reducing energy use and garbage output. As it is very simple in nature, it is mainly used in laboratory and academic research purposes.[10]

BAGI

In analytical chemistry, it is an important indicator, especially in white chemistry. It focuses mostly on the WAC's "blue" values. The method is more practical if the score is more than 60. It assesses the suitability and effectiveness of analytical techniques by examining a number of performance criteria. A high BAGI score indicates that the procedure is very dependable and appropriate for the intended analytical objective. This approach assesses procedures based on ten different factors, producing a pictogram and numerical score that together show the methods' overall usefulness.[10]

Method Validation

According to ICH standards Q2 (R1), the method was validated.[11,12]

Linearity and Range

Standard stock solutions were diluted, and linearity was assessed. For linearity, separate concentrations of 2 - 10 PPM were created for each medication. The absorbance of both drugs was measured at 214 and 250 nm for each solution. The standard curves for both drugs were plotted.

Precision

Repeatability, within-day, and interval-of-days precision were used to evaluate the precision. (n = 6). To verify this, quantities of mefenamic acid (5 µg/mL) and dicyclomine hydrochloride (0.2 µg/mL) were used (n = 6). The results were reported using the percentage RSD.

Accuracy

The procedure was carried out three times at each percentage level, or n = 9. Pre-spiking, post-spiking, and measured concentrations were recorded and reported as a recovery percentage. Following the addition of known quantities of both APIs in accordance with 80, 100, and 120% levels independently, a recovery study was carried out. For every concentration, three sets were made and measured twice.

LOD and LOQ

Both values were computed using the regression line and slope.

RESULTS AND DISCUSSION

Linearity

The R^2 value is closer to 0.999 for DCL and MEF at λ1 and λ2. Fig. 3 displayed the determination of the isosbestic point. The DCY calibration curves at 214 nm and 250 nm, respectively, were displayed in Fig.4 and 5. The calibration curves for MEF at 214 nm and 250 nm were displayed in Fig. 6 and 7, respectively. Table 1 includes a summary of all the validation parameters.

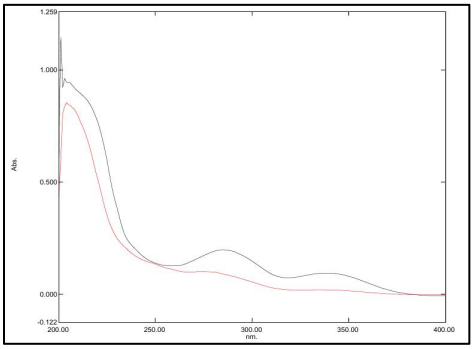

Fig. 3: The Determination of the Isosbestic Point (λmax = 250 nm)

Precision

The % RSD was found to be less than 2 for both drugs.[13]

Accuracy

Recovery studies were carried out at three stages of the conventional addition process. It was shown that the percentage recovery for DCY was between 99.9 and 101.2%, and the percentage recovery for MEF was between 99.94 and 102.3%.

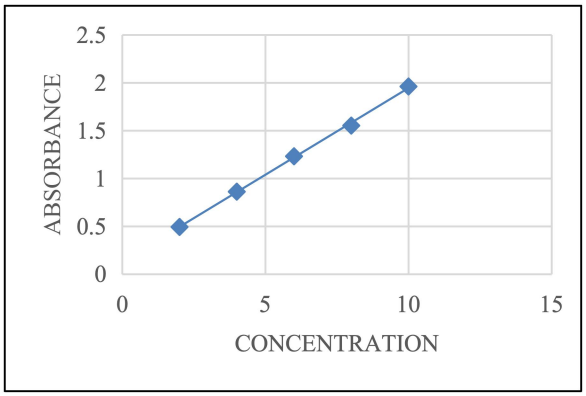

Fig. 4: Calibration Curve DCY at 214 nm

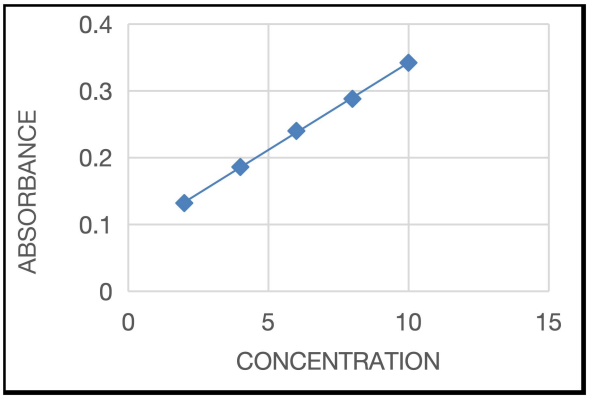

Fig. 5: Calibration Curve DCY at 250 nm

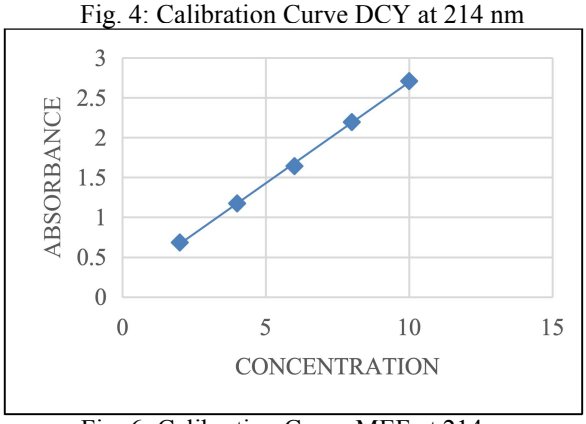

Fig. 6: Calibration Curve MEF at 214 nm

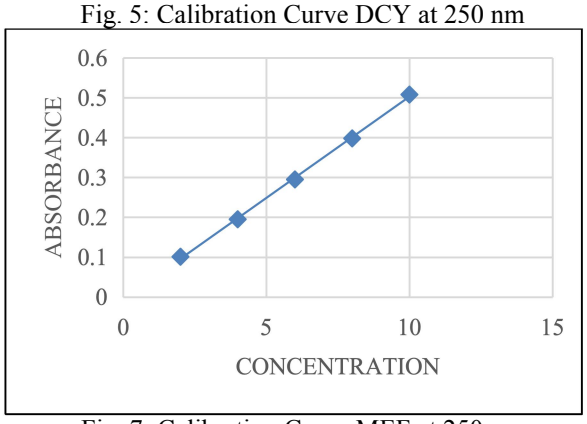

Fig. 7: Calibration Curve MEF at 250 nm

LOD and LOQ

Both values (µg/mL) were shown in Table 1.

Table 1: Validation Data Summary

Parameter	Dicyclomine HCl		Mefenamic acid	
	λ_{max} 214 nm	λ_{max} 250 nm	λ_{max} 214 nm	λ_{max} 250 nm
Equation for linear regression	y = 0.1815x + 0.1307	y = 0.0261x + 0.081	y = 0.2536x + 0.1595	y = 0.0509x - 0.0057
Determination coefficient	R^2 = 0.999	R^2 = 0.9996	R^2 = 0.9992	R^2 = 0.9991
Precision				
Repeatability	1.3 %	1.1 %	1.4 %	1.8 %
Intraday	1.66 %	1.63 %	1.2 %	1.3 %
Interday	1.58 %	1.50 %	1.4 %	1.6 %
Accuracy (%Recovery)	99.9 - 101.2			99.94 - 102.3
LOD	0.401	0.563	0.799	0.825
LOQ	1.215	1.701	2.421	2.499

Application of Green Tools

To support the method's greenness, green parameters were introduced.

AGREE

The method's greenness was evaluated by optimizing the data in the 12 AGREE principles.[14] Sample preparation, batch analysis procedures, direct analytical methods, and reducing external sample pre-treatment. Sample preparation involved three or fewer crucial steps, and analytical processes required less time to utilize the least amount of reagent and energy. 3 mL of analytical waste was produced using single-analyte techniques. Because it takes less time, the suggested method raises the greenness score. The computed score was 0.71 after taking into consideration each of these factors. The method is an acceptable green method, according to this score. In Fig.8, the AGREE tool was shown.

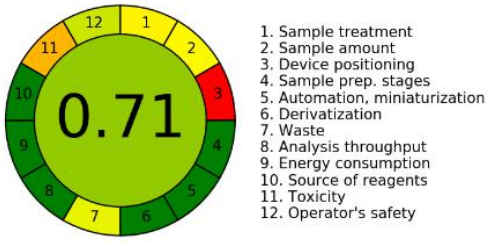

Fig. 8: AGREE Tool

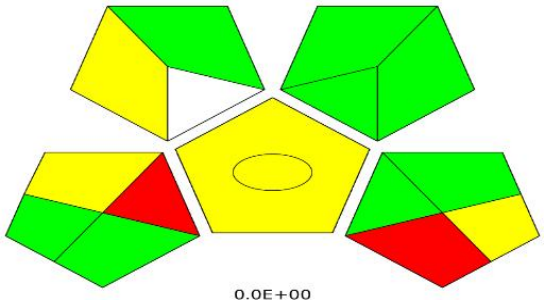

Fig. 9: GAPI Tool

GAPI

It makes use of instruments, technique types, reagents, solvents, and sample preparation.[15] The GAPI tool was updated using the suggested method's parameters. Using colour coding such as red, yellow, and green to indicate risk, moderate, and eco-friendly, this approach demonstrated eco-friendliness. The extraction process, which is not adhered to in the suggested method, is indicated by the empty spot. The GAPI tool was displayed in Fig.-9.

BAGI

White analytical chemistry is the source of the BAGI concept. BAGI describes the applicability of tests that have been evaluated using a score, colour scale, and pictogram.[16] This statistic has a score range of 25 to 100. The calculated BAGI score of 80 indicates how useful the process is. Here, a score of 80 indicates the method has a very high level of greenness and sustainability. The BAGI score was shown in Fig.-10. A BAGI score of 80 denotes an extremely dependable, effective, and practical analytical approach. The application of green concepts and spectroscopic techniques together helped to achieve SDG-12.

Fig.10: BAGI Score

CONCLUSION

When using the Q absorption ratio spectroscopic approach to detect DCY and MEF, the spectrophotometric green method is linear, accurate, and specific. Because it combines spectrophotometric and green methods, this approach is novel and distinct. UV spectroscopy helps to achieve SDG 12 by lowering chemical usage and hazardous waste formation and enabling efficient quality control in pharmaceutical analysis. This Q-absorption ratio method is better suited for determining mefenamic acid and dicyclomine HCl because it uses inexpensive and readily available solvents. It has been confirmed that the suggested method satisfies ICH Q2 R1 requirements. Eco-friendliness has been validated by the developed approach utilizing novel green instruments. The use of AGREE, GAPI, and BAGI increases environmental friendliness.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-12: Responsible Consumption and Production*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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