DEVELOPMENT AND VALIDATION OF Q-ABSORBANCE RATIO UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF IBUPROFEN AND CAFFEINE

[1]BATTU RAMA MADHURI,[2] M.SWAPNA, [3]R.INDU, [4]T.KAVYA, [5]B.MONIKA PRIYA

[1] Assistant Professor, Assistant Professor, [3] Assistant Professor
[1] Department of Pharmaceutical Analysis,
[1] Viswanadha Institute of Pharmaceutical Sciences , Visakhapatnam, India

Abstract : A simple, accurate, and precise UV spectrophotometric method based on the Q-absorbance ratio technique has been developed and validated for the simultaneous estimation of two drugs, Ibuprofen and Caffeine, in combined dosage form. The method is based on the measurement of absorbance at two selected wavelengths: the isoabsorptive point ([λ_1] nm) and the λ max of one of the components ([λ_2] nm).The concentrations of the drugs were calculated using the Q-absorbance ratio equation.

The method was validated according to ICH guidelines for parameters such as linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ). Both drugs showed good linearity in the concentration ranges of 4-20 µg/mL and 4-20µg/mL, respectively, with correlation coefficients (R^2) greater than 0.999. The recovery studies indicated accuracy within 98–102%, and the %RSD for precision was found to be less than 2%, indicating good reproducibility of the method.

IndexTerms **- Ibuprofen, Caffeine, Q-Absorbance Ratio, UV Spectrophotometry, Method Validation.**

1.INTRODUCTION

Q-Absorption ratio method in UV-spectrophotometry. Absorption ratio method is used for the ratio of the absorption at two selected wavelengths one of which is iso-absorptive point and other being the λ max of one of the two components. The aim of this article is to give brief idea about various method developed using Q-Absorption ratio method on combined dosage forms. It shows selection of wavelength, linearity study and choosing solvent for give combine dosage form. This study also gives brief idea about practical and industrial application of Q-Absorption ratio method. The absorbance ratio method is modification of the simultaneous equations procedure. It depends on the property that, for a substance which obeys Beer's Law at all wavelength, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length. For example, two different dilutions of the same substance give the same absorbance ratio A1/A2, 2.0. In the USP, this ratio is referred to as a Q value. The British Pharmacopoeia also uses ratio of absorbance at specified wavelength in certain confirmatory tests of identity.

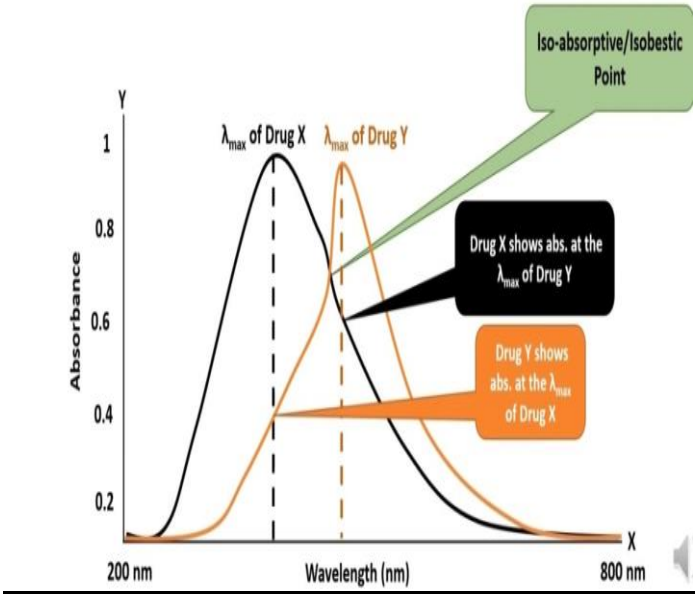
Q –absorbance ratio method formula

C x = [(QM-QY)/QX-QY)] × (A1/Ax1)
C y = [(QM-QX)/QY-QX)] × (A1 /Ay1)
Q –Values

- **QM=** A2/**A1**
- **QX=**ax2/ax1
- **QY=**ay2/ay1

TERMS

- **Cx=**Concentration of drug x
- **Cy=**Concentration of drug y
- **Qm=** Absorbance ratio of mixture
- **QX=** Absorptivty ratio of drug x
- **QY=** Absorptivity ratio of drug y
- **A1=**Absorbance of mixture at Isoabsorptive point (λ1)
- **A2** Absorbance of mixture at λ–max one of the two componenets (λ2)
- **ax1=**Absorptivity of drug x at (λ1)
- **ay1=**Absorptivity of drug y at (λ1)
- **ax2=** Absorptivity of drug x at (λ2)
- **ay2=** Absorptivity of drug y at (λ2)



Q-Absorption ratio method

2. LITERATURE REVIEW

Nyola N. et al. [8] developed a simple, accurate, precise, and reproducible RP-HPLC method for the simultaneous estimation of ibuprofen and famotidine in tablet dosage forms. Chromatographic separation was achieved using a C18 column with an isocratic mobile phase consisting of methanol, water, and phosphate buffer (70:20:10, v/v/v). Detection was carried out at 284 nm with a flow rate of 1.0 mL/min. Ibuprofen and famotidine exhibited retention times of 3.6 min and 7.8 min, respectively. The method showed linearity in the concentration range of 2–10 µg/mL for both drugs and demonstrated excellent accuracy with recoveries ranging from 99–101%. The method was validated according to ICH guidelines and successfully applied to the analysis of combined tablet formulations.

Supe K.S. and Patil J.J. [9] developed and validated a UV spectroscopic method for the quantitative estimation of ibuprofen in bulk and tablet dosage forms using the absorption maxima technique. Ibuprofen was characterized through solubility studies, melting point determination, and FTIR analysis. Methanol was employed as the solvent, and the drug exhibited a maximum absorbance (λmax) at 226 nm. The method showed good linearity over a concentration range of 6–36 µg/mL. Validation studies performed according to ICH guidelines demonstrated satisfactory accuracy, precision, and linearity, with percentage relative standard deviation (%RSD) values below 2%. The study concluded that the method is simple, rapid, economical, and suitable for routine quality control analysis of ibuprofen.

Ahmad Bhawani S. et al. [10] reviewed various spectrophotometric methods developed for the determination of caffeine in environmental and consumer products, including beverages and pharmaceutical formulations. Caffeine, a naturally occurring alkaloid, is widely consumed through coffee, tea, energy drinks, and medications. Excessive intake may lead to adverse physiological effects, necessitating accurate analytical methods for its quantification. The review highlighted the application of UV-Visible spectrophotometry, derivative spectrophotometry, chemometric techniques, and spectral derivatization methods, which improve analytical selectivity and minimize spectral interferences. These methods have been successfully applied across diverse sample matrices for caffeine determination.

Mohammed O.J. et al. [11] developed a simple, accurate, and validated RP-HPLC method for the simultaneous estimation of paracetamol and caffeine in standard mixtures and tablet formulations. Separation was achieved using a C18 column and a mobile phase comprising methanol and water (40:60, v/v) adjusted to pH 4.0. The method exhibited excellent linearity with correlation coefficients (R^2) greater than 0.999 for both analytes. Recovery studies yielded values ranging from 99.57% to 100.36%, confirming the accuracy of the method. The short run time of 10 minutes and reliable analytical performance make the method suitable for routine pharmaceutical quality control.

Reid I.O., Osman S.M., and Bakheet S.M. [12] presented a comprehensive review of analytical methods reported for the quantification of ibuprofen and paracetamol in bulk drugs and fixed-dose combination (FDC) formulations. The combination of ibuprofen and paracetamol is widely used due to its synergistic analgesic effect and established safety profile. The review systematically compiled analytical techniques from major scientific databases, including HPLC, gas chromatography (GC), high-performance thin-layer chromatography (HPTLC), UV-Visible spectrophotometry, Fourier-transform infrared spectroscopy (FTIR), and micellar electrokinetic chromatography (MEKC). Among these, HPLC and UV-Visible spectrophotometry were the most frequently reported techniques, each accounting for 37.9% of the studies reviewed.

Wani Y.B. and Patil D.D. [13] developed two simple, accurate, precise, reproducible, and economical spectrophotometric methods for the simultaneous estimation of ibuprofen and famotidine in bulk drugs and synthetic mixtures. The first method employed the Q-absorbance ratio technique using wavelengths of 263 nm and 273.8 nm, while the second method utilized derivative spectrophotometry with measurements at 252.8 nm and 304 nm in 0.1 N NaOH. Both methods obeyed Beer–Lambert's law over concentration ranges of 150–750 µg/mL for ibuprofen and 5–25 µg/mL for famotidine. Validation according to ICH Q2(R1) guidelines confirmed the accuracy, precision, and suitability of the methods for routine quality control analysis.

Patel A., Firke S.D. et al. [14] developed a simple Q-absorbance ratio UV spectrophotometric method for the simultaneous estimation of naproxen and paracetamol in pharmaceutical dosage forms. The analysis was carried out using 0.1 N NaOH as the solvent system. Two wavelengths, 257.0 nm (λmax of paracetamol) and 234.0 nm (isoabsorptive point), were selected for quantitative estimation. The isoabsorptive point was identified from the overlay spectra of both drugs. The method exhibited linearity in the concentration ranges of 2.5–5.0 µg/mL for paracetamol and 1.5–3.0 µg/mL for naproxen. Recovery studies showed values of 97.91% and 98.64% for paracetamol and naproxen, respectively, indicating that the method was accurate, precise, and suitable for routine analysis of the combined formulation.

3. MATERIALS AND METHODS

3.1 DRUG PROFILE

3.1.1 IBUPROFEN

Drug name: Ibuprofen

IUPAC NAME: 2-(4-(2-methylpropyl) phenyl) propanoic acid

Category: Non-steroidal anti-inflammatory drug (NSAID)

Molecular weight: 206.28 g/mol

Molecular formula: C13H18O2

Melting point: 75–78°C

Dosage form: Tablets, capsules, suspension

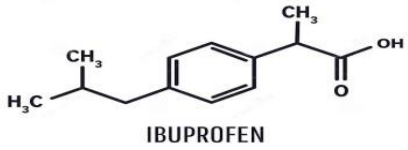
Description: White or almost white crystalline powder

Solubility: Slightly soluble in water, freely soluble in ethanol and methanol

Route of administration: Oral route

Storage: Stored in a closed container at room temperature, protected from light and moisture

Structure:



IBUPROFEN

Fig.3.1: chemical structure of ibuprofen

Mechanism of action: Ibuprofen acts by inhibiting cyclooxygenase (COX-1 and COX-2) enzymes, leading to decreased synthesis of prostaglandins. This results in reduced inflammation, pain, and fever.

Pharmacokinetic properties: Elimination half-life: 2–4 hours

Absorption: After oral administration, ibuprofen is rapidly absorbed from the gastrointestinal tract. Peak plasma concentration (Cmax) is reached within 1–2 hours. The bioavailability of ibuprofen is approximately 80–100%.

Distribution: Ibuprofen is extensively bound to plasma proteins (about 99%) and is widely distributed throughout body tissues and fluids.

Metabolism: Ibuprofen is primarily metabolized in the liver by oxidation to inactive metabolites, mainly through the cytochrome P450 (CYP2C9) enzyme system.

Elimination: Ibuprofen and its metabolites are mainly excreted through the urine, with a small amount eliminated in feces. Approximately 90% of the administered dose is excreted within 24 hours.

Side effects: Nausea, vomiting, abdominal pain, heartburn, dizziness, headache, skin rash

Uses: Treatment of pain, inflammation, and fever in conditions such as arthritis, musculoskeletal disorders, headache, dental pain, and dysmenorrheal.

3.1.2 CAFFEINE

Drug name: Caffeine

IUPAC NAME: 1,3, 7-Trimethylxanthine

Category: Central nervous system stimulant

Molecular weight: 194.19 g/mol

Molecular formula: C8H10N4O2

Melting point: 235–238°C

Dosage form: Tablets, capsules, beverages

Description: White crystalline powder with a bitter taste

Solubility: Sparingly soluble in water, freely soluble in ethanol and chloroform

Route of administration: Oral route

Storage: Stored in a well-closed container at room temperature, protected from moisture

Structure:

Fig.3.2 chemical structure of caffeine

Mechanism of action: Caffeine acts primarily by antagonizing adenosine receptors in the central nervous system, leading to increased neuronal activity and release of neurotransmitters such as dopamine and norepiephrine. This results in stimulation, increased alertness, and reduced fatigue.

Pharmacokinetic properties: Elimination half-life: 3–7 hours

Absorption: After oral administration, caffeine is rapidly and completely absorbed from the gastrointestinal tract. Peak plasma concentration (C max) is achieved within 30–60 minutes, with an oral bioavailability close to 100%.

Distribution: Caffeine is widely distributed throughout body tissues, including the brain. It readily crosses the blood–brain barrier and the placenta, and is moderately bound to plasma proteins.
Metabolism: Caffeine is extensively metabolized in the liver, primarily by the cytochrome P450 enzyme CYP1A2, into metabolites such as paraxanthine, theobromine, and theophylline.

Elimination: Caffeine and its metabolites are mainly excreted through the urine. Less than 5% of caffeine is excreted unchanged.

Side effects: Insomnia, nervousness, restlessness, palpitations, headache, gastrointestinal irritation

Uses: Used as a central nervous system stimulant to reduce fatigue and drowsiness; also used as an adjuvant in headache and migraine preparations.

Table 3.1.Marketed formulations of Ibuprofen and Caffeine

Marketed drugs	Manufactured by	Types of doses
Brufen -200mg	Abbott India Ltd.	Tablet
Ibugesic-200mg	Cipla Ltd.	Tablet
NoDoz-100mg	Glaxo SmithKline	Tablet
Vivarin-200mg	SmithKline Beecham	Tablet

Table 3.2 List of Chemicals used

S. no.	Materials	Grade	Company
1.	Methanol	Rankem	Qualigens
2.	Phosphate buffer	Rankem	LR(Laboratory reagent)
3.	HCl	Rankem	LR(Laboratory reagent)

Table3.3 Working standards /API 'S

S.no	Working standard	Company
1.	Ibuprofen	A gift sample from the Hetero labs
2.	Caffeine	A gift sample from the Hetero labs

Table3.4 List of equipments

S.no.	Name of the equipment	Company
1.	UV-VIS Spectrophotometer	Systronics 2202
2.	Weighing balance	DIGI

3.2 EXPERIMENTAL WORK:

3.2.1 DETERMINATION OF λ MAX OF IBUPROFEN

Preparation of stock solution: 10mg of Caffeine was accurately weighed and transferred to a 10 ml volumetric flask, and dissolved in 10 ml of methanol to obtain a concentration (1000µg/ml).

Preparation of standard solution: From the above stock solution 1ml was pipetted and transferred into a 10 ml volumetric flask, and made up to 10ml of methanol to obtain a concentration (100µg/ml).

Further, 3 ml of the 100 µg/ml solution was diluted to 10 ml with methanol to obtain a concentration of 30µg/ml.

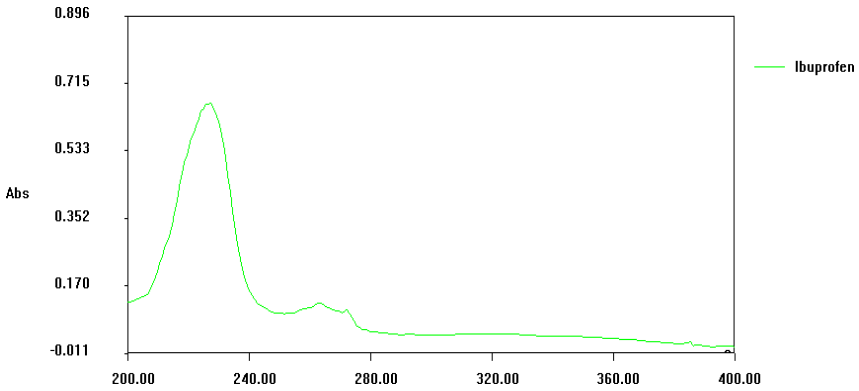


Fig.3.2.1 Lambda max of ibuprofen 226nm

3.2.2 DETERMINATION OF λ MAX OF CAFFEINE

Preparation of stock solution: 10mg of Caffeine was accurately weighed and transferred to a 10 ml volumetric flask, and dissolved in 10 ml of methanol to obtain a concentration (1000µg/ml).

Preparation of standard solution: From the above stock solution 1ml was pipetted and transferred into a 10 ml volumetric flask, and made upto 10ml of methanol to obtain a concentration (100µg/ml).

Further, 3 mL of the 100 µg/mL solution was diluted to 10 mL with methanol to obtain a concentration of 30µg/ml.

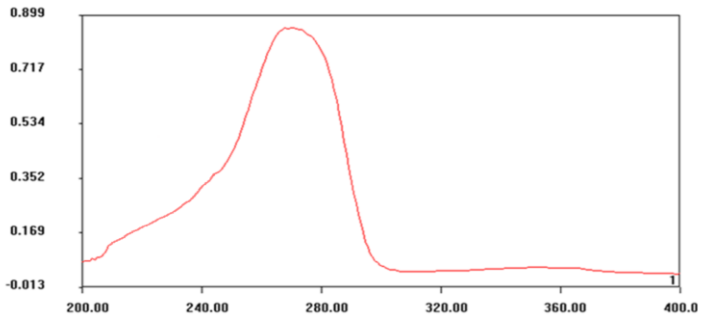


Fig.3.2.2 Lambda max of caffeine 272nm

3.2.3 ISO ABSORPTIVE POINT

An iso-absorptive point **is** the wavelength in the UV–Visible spectrum at which the absorptivity of two components is equal, resulting in identical absorbance values for both substances at that wavelength**,** which is useful for the simultaneous estimation of drugs in a mixture using spectrophotometric methods**.**

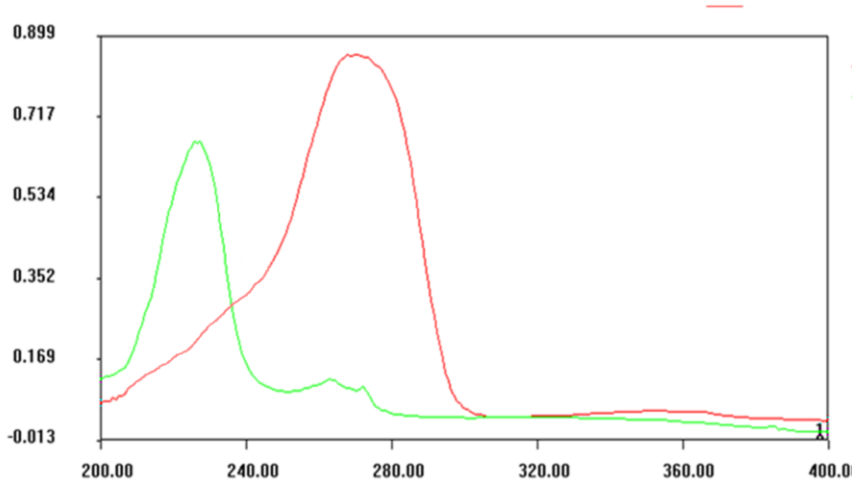


Fig.3.2.3 Iso-absorptive Point was Observed at 232nm wavelength.

3.2.4 Q ABSORBANCE METHOD DEVELOPMENT

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ - max of one of the two components. From the overlay spectra of two drugs, it is that Caffeine and Ibuprofen show an isoabsorptive point 232nm. The second wavelength used is 272nm, which is the λ -max of caffeine. Working standard solutions having concentrations 4, 8, 12, 16 and 20µg/ml for Ibuprofen and 4, 8, 12, 16 and 20µg/ml for Caffeine were prepared in methanol and the absorbances at 232nm (Isoabsorptive point) and 272nm(λ max of Caffeine) were measured and absorptivity coefficients were calculated using calibration curve.

Q –absorbance ratio method formula

$$Cx = [(QM-QY)/QX-QY)\ ]\times(A1/Ax1)$$

$$Cy = [(QM-QX)/QY-QX)]\times(A1/Ay1)$$

By using this formula to find out the Cx and Cy values

3.3 METHOD VALIDATION

3.3.1 LINEARITY

PREPARATION OF SOLUTIONS OF IBUPROFEN

Preparation of stock solution: 10mg of Ibuprofen was accurately weighed and transferred to a 10 ml volumetric flask, and dissolved in 10 ml of methanol to obtain a concentration (1000µg/ml).

Preparation of standard solution: From the above stock solution 1ml was pipetted and transferred into a 10 ml volumetric flask, and made upto 10ml of methanol to obtain a concentration (100µg/ml).

Preparation of blank solution : Methanol is used as a blank solution.

PREPARATION OF LINEARITY SOLUTIONS OF IBUPROFEN:

4µg/ml: 0.4ml was pipetted from standard solution and transferred into a 10ml volumetric flask and made upto 10ml of methanol to obtain a concentration (4µg/ml).

8µg/ml: 0.8ml was pipetted from standard solution and transferred into a 10ml volumetric flask and made upto 10ml of methanol to obtain a concentration (8µg/ml).

12µg/ml: 0.12ml was pipetted from standard solution and transferred into a 10ml Volumetric flask and made upto 10ml of methanol to obtain a concentration (12µg/ml).

16µg/ml: 0.16ml was pipetted from standard solution and transferred into a 10ml Volumetric flask and made upto 10ml of methanol to obtain a concentration (16µg/ml).

20µg/ml: 0.20ml was pipetted from standard solution and transferred into a 10 ml Volumetric flask and made upto 10ml of methanol to obtain a concentration (20µg/ml).

PREPARATION OF SOLUTIONS CAFFEINE

Preparation of stock solution:10mg of Caffeine was accurately weighed and transferred to a 10 ml volumetric flask, and dissolved in 10 ml of methanol to obtain a concentration (1000µg/ml).

Preparation of standard solution: From the above stock solution 1ml was pipetted and transferred into a 10 ml volumetric flask, and made upto 10ml of methanol to obtain a concentration (100µg/ml).

Preparation of blank solution : Methanol is used as a blank solution.

PREPARATION OF LINEARITY SOLUTIONS OF IBUPROFEN:

4µg/ml: 0.4ml was pipetted from standard solution and transferred into a 10ml volumetric flask and made upto 10ml of methanol to obtain a concentration (4µg/ml).

8µg/ml: 0.8ml was pipetted from standard solution and transferred into a 10ml volumetric flask and made upto 10ml of methanol to obtain a concentration (8µg/ml).

12µg/ml: 0.12ml was pipetted from standard solution and transferred into a 10ml volumetric flask and made upto 10ml of methanol to obtain a concentration (12µg/ml).

16µg/ml: 0.16ml was pipetted from standard solution and transferred into a 10ml volumetric flask and made upto 10ml of methanol to obtain a concentration (16µg/ml).

20µg/ml: 0.20ml was pipetted from standard solution and transferred into a 10 ml volumetric flask and made upto 10ml of methanol to obtain a concentration (20µg/ml)

3.3.2 PRECISION

Precision is defined as “The degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample”. The precision of the assay was determined by repeatedly (intra-day) and intermediate precision (inter-day). Two replicates of the sample solutions were prepared and analyzed

PRECISION OF IBUPROFEN

Preparation of 12µg/ml solution: 12ml was pipetted from standard solution and transferred into a 10ml volumetric flask and made upto 10 ml of methanol to obtain a concentration (12µg/ml).

Preparation of blank: Methanol is used as a solvent.

Intraday precision: 6 determinations of 12µg/ml solution were done 3 times in a day and observed for absorbance.

Interday precision: 6 determinations of 12µg/ml solution were done in consecutive days and observed for absorbance.

PRECISION OF CAFFEINE

Preparation of 12µg/ml solution: 12ml was pipetted from standard solution and transferred into a 10ml volumetric flask and made upto 10 ml of methanol to obtain a concentration (12µg/ml).

Preparation of blank: Methanol is used as a solvent.

Intraday precision: 6 determinations of 12µg/ml solution were done 3 times in a day and observed for absorbance.

Interday precision: 6 determinations of 12µg/ml solution were done in consecutive days and observed for absorbance.

3.3.3 ACCURACY

To check the accuracy of the proposal method, recovery studies were carried out by applying standard addition method. A known amount of standard Ibuprofen and Caffeine corresponding to 80, 100 and 120 **%** of the label claim was added to pre-analyzed sample of the tablet. The recovery studies were carried out

Accuracy Of Ibuprofen: Accuracy was performed at 3 different levels i.e 80 % , 100%,120 %.

%Recovery = Amount found X 100
Amount taken

The % recovery should be between 99.67-100.10%

Accuracy Of Caffeine: Accuracy was performed at 3 different levels i.e 80 % , 100%,120%.

$$\%\text{Recovery} = \frac{\text{Amount found}}{\text{Amount taken}} \times 100$$

The % recovery should be between 98.89-100.21%

Criteria: %RSD should be less than 2.

3.3.4.RUGGEDNESS

RUGGEDNESS OF IBUPROFEN : 6 determinations of 12µg/ml solution was done by two different analysts and observed for absorbances.

CRITERIA: %RSD should be less than 2.

RUGGEDNESS OF CAFFEINE: 6 determinations of 12µg/ml solution was done by two different analysts and observed for absorbances.

CRITERIA: %RSD should be less than 2.

3.3.5 LOD AND LOQ
LOD AND LOQ OF IBUPROFEN:

Limit of detection: 3.3x σ/S
Limit of quantification: 10x σ/S

Where,
σ is standard deviation
S is slope.

LOD AND LOQ OF CAFFEINE:

Limit of detection: 3.3x σ/S
Limit of quantification: 10x σ/S

Where,
σ is standard deviation
S is slope.

IV. RESULTS AND DISCUSSION

4.1. LINEARITY :

4.1.1. Linearity of Ibuprofen

Concentration(µg/ml)	Absorbance
4	0.082
8	0.164
12	0.247
16	0.329
20	0.412

Table 4.1.1:Linearity data of Ibuprofen

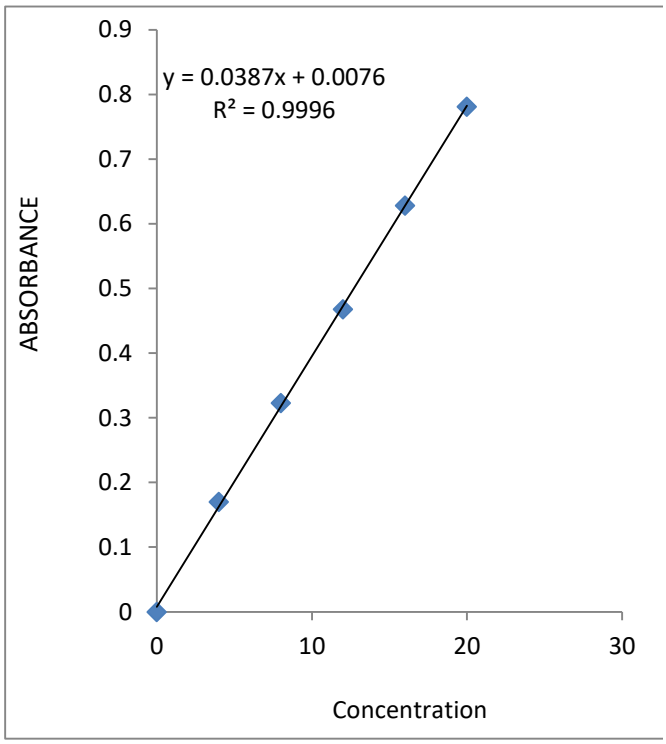


Fig.4.1.1.CalibrationcurveofIbuprofen

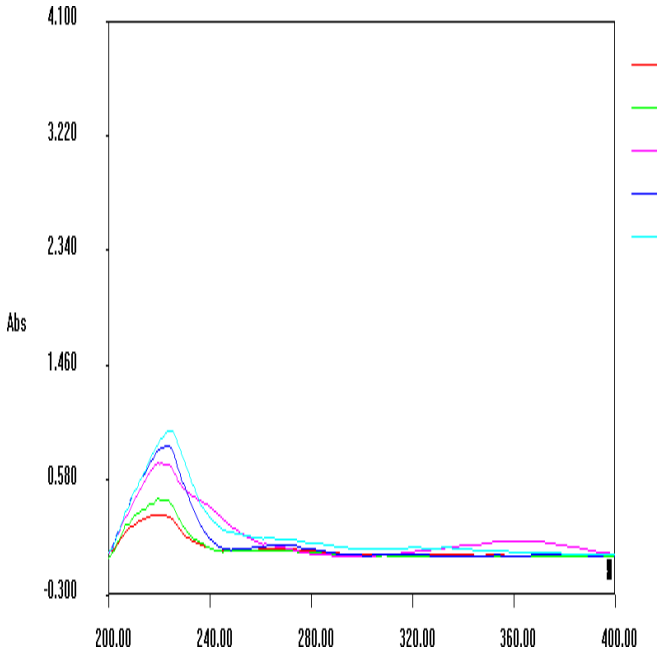
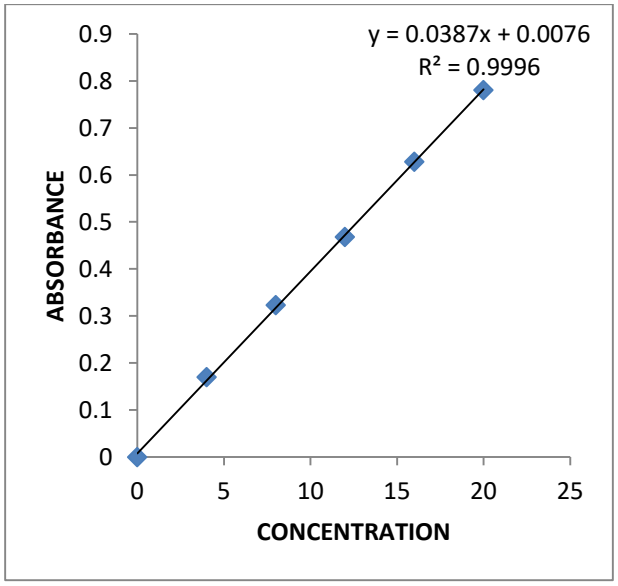


Fig.4.1.2 Overlay spectra of ibuprofen drug

4.1.2. Linearity of Caffeine

Concentration(µg/ml)	Absorbance
4	0.170
8	0.323
12	0.468
16	0.628
20	0.781

Table 4.1.2 Linearity data of Caffeine



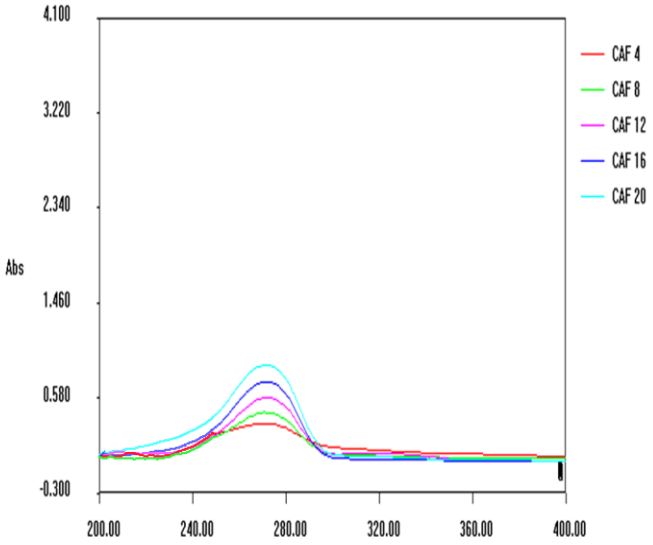


Fig. 4.1.3 calibration curve of caffeine

Fig.4.1.4 Overlay spectra of Caffeine drug

4.2 Accuracy:

To check the accuracy of the proposal method, recovery studies were carried out by applying standard addition method. A known amount of standard **Ibuprofen** and **Caffeine** corresponding to **80, 100** and **120 %** of the label claim was added to pre-analyzed sample of the tablet. The recovery studies were carried out at each level and the results are shown in table below

Drug	**Amount of sample taken(mg)**	**Spiked level**	**Amount of pure drug added(mg)**	**Average amount recovered (mg) (n=3)**	**% Recovery**
Ibuprofen	12µg/ml	80%	9.6µg/ml	9.59µg/ml	99.89%
		100%	12µg/ml	12.01µg/ml	100.08%
		120%	14.4µg/ml	14.43µg/ml	100.21%
Caffeine	12µg/ml	80%	9.6µg/ml	9.61µg/ml	100.10%
		100%	12µg/ml	11.34µg/ml	98.67%
		120%	14.4µg/ml	14.33µg/ml	99.51%

Table No 4.2.Accuracy 0f Ibuprofen and Caffeine

4.3. Precision:

Precision is defined as the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. The precision of the assay was determined by repeatedly (intra-day) and intermediate precision (inter-day).Two replicates of the sample solutions were prepared and analyzed. The values of intra-day and inter-day precision were shown in the table.

Precision	**Caffeine (12µg/ml)**			**Ibuprofen (12µg/ml)**		
	Intraday					
n=5	**Morning**	**Afternoon**	**Evening**	**Morning**	**Afternoon**	**Evening**
1	0.320	0.318	0.315	0.164	0.161	0.158
2	0.318	0.315	0.313	0.166	0.164	0.161
3	0.321	0.319	0.316	0.163	0.160	0.158
4	0.319	0.317	0.315	0.165	0.162	0.159
5	0.322	0.319	0.317	0.164	0.162	0.160
6	0.320	0.317	0.314	0.166	0.163	0.162
Average	0.3200	0.3175	0.3150	0.1647	0.1620	0.2595
RSD	0.00141	0.00152	0.00141	0.00121	0.00141	0.00138
%RSD	0.44%	0.48%	0.45%	0.74%	0.87%	0.86%
Accepted Criteria				**NMT2%**		

TableNo4.3.1. Intraday Precision

Precision n=5	Caffeine (12µg/ml)			Ibuprofen (12µg/ml)		
	Inter day					
	Day-1	Day-2	Day-3	Day-1	Day-2	Day-3
1	0.319	0.316	0.314	0.165	0.162	0.160
2	0.323	0.320	0.317	0.167	0.165	0.162
3	0.318	0.315	0.313	0.163	0.160	0.158
4	0.321	0.318	0.315	0.166	0.163	0.161
5	0.324	0.321	0.318	0.164	0.162	0.159
6	0.320	0.317	0.315	0.168	0.165	0.162
Average	0.3208	0.3178	0.3153	0.1655	0.1628	0.16603
RSD	0.00232	0.00232	0.00186	0.00187	0.00194	0.00163
%RSD	0.72%	0.73%	0.59%	1.13%	1.19%	1.02%
Accepted Criteria			NMT2%			

Table No. 4.3.2 Inter day Precision

4.4.Limit of Detection and Limit of Quantification

LOD and LOQ were calculated based on the standard deviation of the analytical response and the slope of the calibration curve using the equations

LOD=3.3/S and

LOQ = 10 /S,

Where is the SD of the response and S is the slope of calibration curve.

Statistical parameter	Ibuprofen(µg/ml)	Caffeine(µg/ml)
LOD	0.176	0.118
LOQ	0.534	0.359

Table 4.4 Sensitivity of proposed method

4.5. RUGGEDNESS:

6 determinations of 12µg/ml of solutions of Ibuprofen and Caffeine was done by two different analysts and observed for absorbances .

Caffeine		Ibuprofen	
Analyst-1	Analyst-2	Analyst-1	Analyst-2
0.246	0.245	0.467	0.466
0.247	0.247	0.468	0.466
0.248	0.246	0.469	0.467
0.248	0.247	0.469	0.468
0.246	0.246	0.468	0.467
0.247	0.245	0.467	0.468

Table 4.5 Robustness data of Caffeine and Ibuprofen

- %RSD of Ibuprofen was found to be 0.41%

- %RSD of Caffeine was found to be 0.21%

5. SUMMARYAND CONCLUSION

Table5.1: Summary table

PARAMETERS	IBUPROFEN	CAFFEINE
Wavelength(nm)	226.04nm	272nm
Linearity(µg/ml)	4-20µg/ml	4-20µg/ml
Regression equation Slope (m) Intercept(c)	y=(mx+c) 0.020625 -0.007	y=(mx+c) 0.038175 0.0159
Correlation coefficient	0.999	0.999
Precision(%RSD) 1. Intraday 2. Interday	 0.82% 1.1%	 0.45% 0.68%
Accuracy(%Recovery) 1 80% 2 100% 3 120%	 99.89% 100.08% 100.21%	 100.10% 98.67% 99.51%
Ruggedness(%RSD) 1. Analyst-1 2. Analyst-2	 0.40% 0.42%	 0.20.5% 0.23%
Limit of detection(µg/ml)	0.176	0.118
Limit of quantification(µg/ml)	0.534	0.359

Conclusion:

The proposed **Q-Absorption Ratio Method** was found to be simple, accurate, precise, and linear for the simultaneous estimation of **Ibuprofen and Caffeine**. The method showed good recovery, reliable results, and uses simple, easily available reagents and instruments. Hence, it is suitable for routine quality control (QC) analysis, API analysis, and accelerated stability studies in pharmaceutical formulations.

Acknowledgment

The authors express their sincere gratitude to the management and faculty of the institution for providing the necessary facilities, infrastructure, and continuous support to carry out this research work. The authors are also thankful to the laboratory staff for their technical assistance during the experimental studies. The authors gratefully acknowledge the dedicated efforts and valuable contributions of the project students, whose enthusiasm, commitment, and assistance in conducting the experimental work, data collection, and analysis significantly contributed to the successful completion of this research. The authors also extend their appreciation to their colleagues and well-wishers for their encouragement and support throughout the study.

REFERENCES

1. A Textbook of Pharmaceutical Analysis. Dr. S. Ravi Shankar. Edition – 4. Pg.No: 1-1, 1-4 & 1-5.
2. Instrumental Analysis. Skoog, Holler, Crouch. Pg.No: 13-14.
3. Instrumental Method of Analysis. Gurdeep R. Chatwal, Sham K. Anand. Himalaya Publishing House. Revised 5th edition. Pg. No: 2.107 - 2.112.
4. Instrumental Method of Analysis. Willard, Merritt, Dean, Settle. 7th Edition. Pg. No: 118– 154.

5. Pharmaceutical Drug Analysis. Ashutosh Khar. New Age International Publishers Revised 2nd Edition. Pg. No: 303-304.

6. N. Karnakar, H. Ramana, P. Amani, D. Sri Tharun, M. Nagaraju, Saurabh Bodhgaya Sharma. International Journal of Multidisciplinary Research and Development. 2020, 7,23-24.

7. Panchumarthy Ravisankar, MD. Shaheem Sulthana, P. S. Babu, SK. Afzal Basha, R.Aswini, V. Swathi, SK. Mahamuda Sultana, M. Sri Lakshmi Prasanna, N. Navyasri, I. M.Thanuja. Comprehensive Review of Important Analytical Reagents used inSpectrophotometry. Indo American Journal of Pharmaceutical Research. 2017, 7(5),8716-8744.

8. Nyola N. Simultaneous estimation of ibuprofen and famotidine in pure and combination dosage form by RP-HPLC. Journal of Applied Pharmaceutical Science. 2025 Jul 3.

9. Supe KS, Patil JJ. Analytical method development and validation of ibuprofen by UV spectroscopy. World J. Pharmaceut. Res.. 2022 Aug 1;11:1420-30.

10. Ahmad Bhawani S, Fong SS, Mohamad Ibrahim MN. Spectrophotometric analysis of caffeine. International journal of analytical chemistry. 2015;2015(1):170239.

11. Mohammed OJ, Hamzah MJ, Saeed AM. RP–HPLC method validation for simultaneous estimation of paracetamol and caffeine in formulating pharmaceutical form. Research Journal of Pharmacy and Technology. 2021 Sep 1;14(9):4743-8.

12. .Analytical Techniques for the Determination of Paracetamol and Ibuprofen Combination Imad O. Abu Reid, Sayda M. Osman, Somia M. Bakheet

13. Wani YB, Patil DD. Development and validation of spectrophotometric methods for the estimation of ibuprofen and famotidine. Int. J. Pharmacy Pharmaceutical Sci.. 2013;5(3):358-63.

14. Patel A, Firke SD, Bari SB, Ranoliya JR. Development and Validation of Uv-spectrophotometric method for simultaneous estimation of naproxen and paracetamol by Q-absorbance ratio method. International Journal. 2014;3(1):57-63.

15. Khamar JC, Patel SA. Q-absorbance ratio spectrophotometric method for the simultaneous estimation of rifampicin and piperine in their combined capsule dosage. Journal of applied pharmaceutical science. 2012 Apr 30(Issue):137-41.

16. Kumar PR, Prakash PB, Krishna MM, Yadav MS, Deepthi CA. Simultaneous estimation of domperidone and pantoprazole in solid dosage form by UV spectrophotometry. Journal of Chemistry. 2006;3(3):142-5.

17. Patel NV, Prajapati AM. Q-absorbance ratio spectrophotometric method for the simultaneous estimation of ciprofloxacin and metronidazole in their combined dosage form. JPSBR. 2012 May;2(3):118-22.

18. .Singh G, Kumar D, Sharma D, Singh M, Kaur S. Q-Absorbance ratio spectrophotometric method for the simultaneous estimation of prednisolone and 5-Amino salicylic acid in tablet dosage form. Journal of applied pharmaceutical science. 2012 Jul 30;2(7):222-6.

19. .Pandey G, Mishra B. A New Analytical Q-Absorbance Ratio Method Development and Validation for Simultaneous Estimation of Lamivudine and Isoniazid. International Scholarly Research Notices. 2013;2013(1):912376.

20. Palabiyik IM, Dinç E, Onur F. Simultaneous spectrophotometric determination of pseudoephedrine hydrochloride and ibuprofen in a pharmaceutical preparation using ratio spectra derivative spectrophotometry and multivariate calibration techniques. Journal of pharmaceutical and biomedical analysis. 2004 Feb 18;34(3):473-83.