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Development and validation of UV spectrophotometric method for simultaneous estimation of caffeine and paracetamol in tablate formulation

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Abstract
A simple new, rapid and precise UV spectrophotometric method was developed and validated for simultaneous estimation of caffeine and paracetamol. Two influences, precise, accurate and simple UV spectrophotometric methods have been developed for simultaneous estimation of Paracetamol (PARA) and Caffeine (CAF) in pharmaceutical dosage forms. Method A tangled simultaneous equation method. The two wavelengths 257nm (λmax of Paracetamol) and 272 nm (λmax of Caffein) in their solution in sodium hydroxide 0.1 N NaoH. were selected for the formation of Simultaneous equations. involved formation of Q-absorbance equation at isosbestic point (259.5 nm). Linearity was observed in the concentration range of 2-10mcg/ml for paracetamol and 2-10 mcg/ml for caffeine by these methods. The proposed methods have been applied successfully to the analysis of cited drugs in pharmaceutical formulations. Recovery study was performed to confirm the accuracy of the methods. The methods were validated as per ICH guidelines.

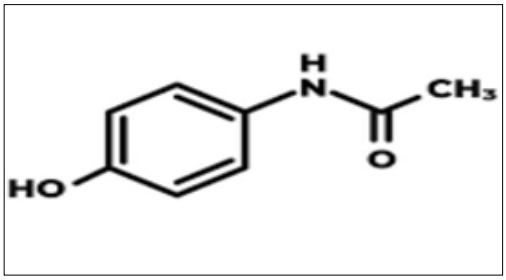
Keywords: paracetamol caffeine, simultaneous estimation method devolvement, UV spectroscopy, validation

Introduction
caffeine is a well known central nervous system stimulant and most widely used consumed psychoactive substance in the world [1]. caffeine is a methyl xanthine derivative which has various pharmacological activities, such as blockade of adenosine receptor, inhibition of phosphodiesterase, inhibition of 5-nucleotidase, and modulation of intracellular calcium movement, which may effect neuronal functions [2]. caffeine seems to exert is effects directly on the central nervous system by blocking A1 and A2A Adenosine receptors.it is a relatively nonspecific adenosine antagonist but seems to have the greatest affinity for A1 receptors [3]. paracetamol (4-acetamidophenol) is one of the most common drugs used in the world. It causes reduction in the amount of prostaglandin, therefore, helps to prevent headache and other pain like migraine headache, muscular aches, neuralgia, backache, joint pain, rheumatic pain, general pain, toothache, teething pain, period pain, and also used for the reduction off ever of bacterial or viral origin. It is suitable for most people, including elderly and young children, because it has very few side effects [4]

Litreture review
Litreture review revealed by that there are different ways available for caffeine, individually or another drugs using uv spectrometry [5] reverse phase HPLC [6]. FTIR [7] high performance liquid chromatography [8], and LC-MS, liquid chromatography-mass spectrophotometry
Similarly, Paracetamol was estimated by uv – spectroscopy [9], RP- HPLC [10], HPLC [11] and LC-MS [12], electrochemical [13-15], spectrochemical [16-18] hence, from Litreture review it is clear that there is nit a single uv method is reported so far for Simultaneous analysis of caffeine and Paracetamol in tablet formulation. precise, linear, simple, rapid, validated and cost effective UV-spectrometric method for the estimation of caffeine and Paracetamol in a single formulation.

Rational / Objectives
1. When taken at recommended doses, both paracetamol and the combination of paracetamol and caffeine are safe and effective treatments for primary periods. Consistent with results from other acute pain states, caffeine acts as an analgesic adjuvant and enhances the efficacy of paracetamol.
2. Caffeine has been added to common analgesics such as paracetamol, ibuprofen, and aspirin, in the belief that it enhances analgesic efficacy
3. Litreture review reveals that there is few analytical method reported for the analysis of paracetamol and caffeine by simultaneous estimation by RP-HPLC, spectrophotometry, HPTLC are the reported analytical methods for compounds either individually or in combination with other drugs form. hence, it is a analytical method development for the simultaneous estimation of paracetamol and caffeine by uv spectrophotometry in pharmaceutical dosage form

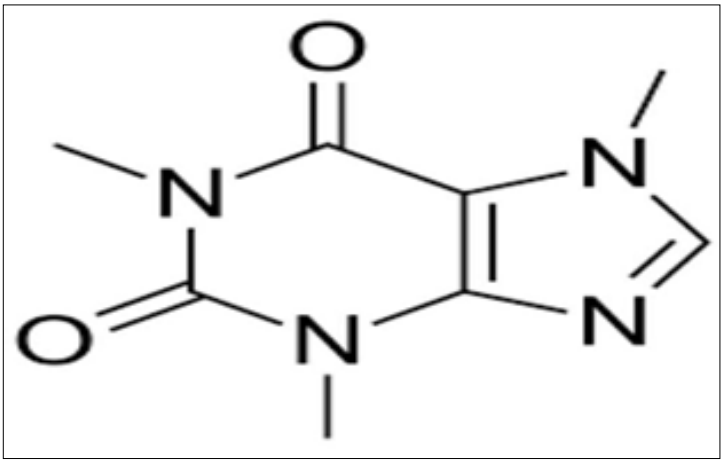
1 Drug Profile
1.1 Paracetamol
Structure



IUPAC Name: N-(4-hydroxyphenyl) acetamide
Molecular formula: C8H9NO2
Molecular weight: 151.163 g/mol
PKa: 9.5
Melting point: 165.6 °c
Boiling point: 420 ° c
Category: Analgesic and antipyreitic drug

Pharmacokinetic and Pharmacodynamics:
Paracetamol is a p-aminophenol derivative that exhibits analgesic and antipyretic activity. It does not possess anti-inflammatory activity.

2. Caffeine
Structure



IUPAC Name: 1, 3, 7-Trimethylpurine-2,6-dione
Molecular formula: (C8H10N4O2)
Molecular weight: 194.19 g/mol
PKa: 10.4.
Melting point: 235 °c
Boilingpoint: 173 °c
Category: Methylxanthine
Pharmacokinetics and Pharmacodynamics: it has been classified both as a food and as a drug based on where it travels in the body (pharmacokinetics) and the resulting effects it has (pharmacodynamics) once ingested.

Materials and methods
Apparatus
A double beam UV-spectrophotometer, attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wave length accuracy of 0.5nm and pair of 1cm matched quartz cells, digital weighing balance, ultrasonicator, volumetric flask and pipettes of borosilicate glass were used for the development and validation of proposed analytical method.

Material and methods
Caffeine, paracetamol pure API, pacimol tablet, water

Selection of solvent
The solubility of drugs was determined in a various of solvents as per Indian pharmacopoeia (Ip) standards for selection of common solvent solubility was carried out in polar to non- polar solvents. The common solvent was formed to be methanol, ethanol, H2O, NaoH. The NaoH which was used for the analysis of both caffeine and Paracetamol for the proposed method.

Determination of λ_{max}
Preparation of stock solution:
The solution was prepared by dissolving 10 mg caffeine and Paracetamol in 100 ml NaoH. which gives 100mcg/ml respectively. The UV spectrum was recorded using uv visible Double beam spectrophotometer in value range of 400-200 nm using NaoH as a blank.

Preparation of standard solutions
1. Preparation of standard solutions of paracetamol
   --Dissolve 10 mg (0.1gm) of Paracetamol in 100 ml volumetric flask in 0.1N NaoH (100 ppm)
   -from above solution take 1 ml and dilute up to 10 ml with 0.1 N NaoH (10 ppm)
   1ml /10 ml ×100 = 10mcg/ml = 10ppm

- **Preparation of standard caffeine**
  --Dissolve 10 mg (0.1gm) of caffeine in 100 ml volumetric flask in 0.1N NaoH (100 ppm)
  -from above solution take 1 ml and dilute up to 10 ml with 0.1 N NaoH (10 ppm)
  1ml /10 ml ×100 = 10mcg/ml = 10ppm

Then the sample was scanned with Uv- visible spectrophotometer in the range 200- 400 nm against NaoH as blank and the wavelength corresponding to maximum absorbance was noted which is its $\lambda_{max.}$ i.e at 272 nm for caffeine and 257 nm for paracetamol

- **Preparation of calibration curves**

The standard stock solution of caffeine and Paracetamol were prepared by dissolving 10 mg of each drug in 0.1N NaoH, the final volume was adjusted with the same solvent in 100 ml of volumetric flask to get a solution containing 100mcg/ml of each drug. Working standard solution (2,4,6,8 & 10/mcg for each drug) were prepared and scanned in the entire UV range of 400 -200 nm (figure 1 and figure 2).calibration curve as concentration v/s Absorbance were constructed taking concentration on x- axis and absorbance on y-axis which showed a straight line obeyed Linearity in the concentration range of 2-10/mcg/ml of both drugs. The correlation coefficient was found to be 0.9789 for caffeine and 0.9871 for paracetamol.

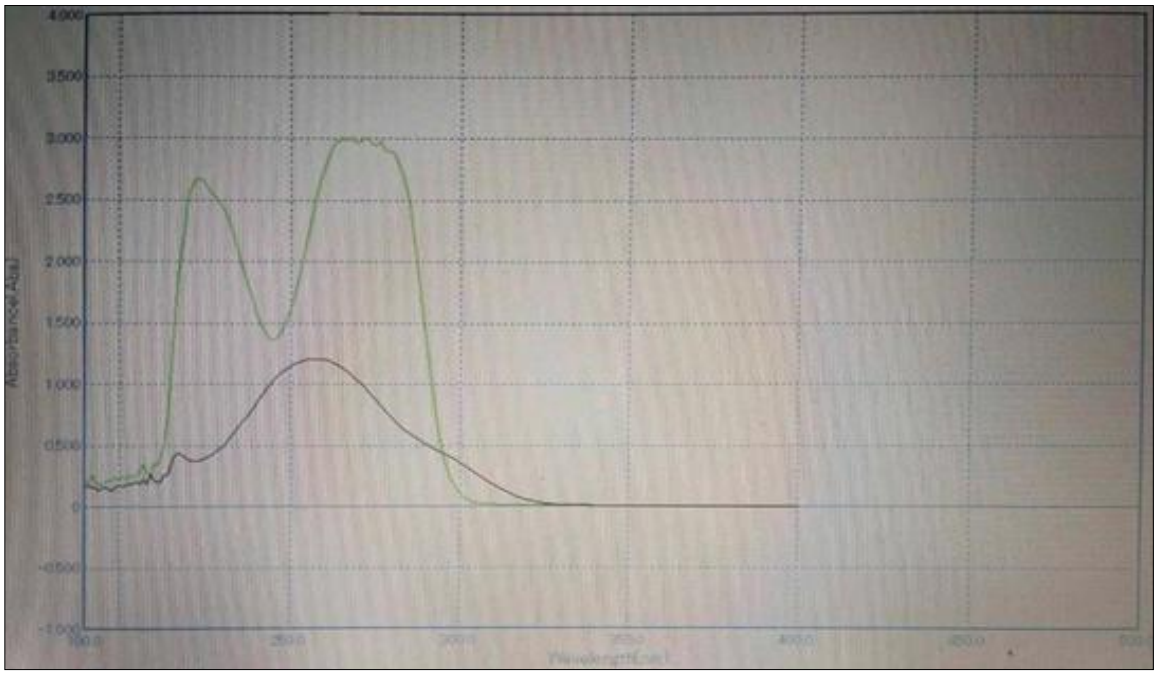


Fig 1: overlay spectra of Paracetamol and Caffeine

Simultaneous equation method

Simultaneous estimation of drug is very important method as the new combined formulation approved in market. The main aim behind the quantitative estimation is to ensure that whether a particular drug contains the same amount of drug as mentioned because if the dose given will be high then it will cause over dosage side effects and if it is less, then the patient will not get the required dose [19]. When combination of drug contains two drug or more than two drugs in combined dosage form then the simultaneous equation or Vierordt's method were applied to that formulation [20].

From the overlay spectra (Figure 1) of Caffeine (10µg/ml) and Paracetamol (10µg/ml), two wavelengths i.e. 272 nm as λmax of Caffeine and 257nm as λmax of paracetamol were selected as the working wavelength, at which both drugs showed absorbance for each other. The absorptivity of these two drugs was determined at 257nm and 272nm. A set of two simultaneous equation were formed using absorptivity values as given in equation (1) and (2), at selected wavelengths. The concentrations of two drugs in tablet formulation were calculated using following two simultaneous equations.

Cx= (A2ay1-A1ay2)/ (ax2ay1-ax1ay2) ------(1) [20]
Cy= (A1ax2-A2ax1)/ (ax2ay1-ax1ay2) ------(2) [20]

equation (1) and (2). Validity of the equation was checked by using mixed standard of pure drug sample of two drugs, measuring their absorbance at respective wavelength and calculating concentration of two components.

Methodology

Analysis of tablet formulations 20 tablets were weighed and ground to fine powder. An accurately weighed powder equivalent to 650 mg of Paracetamol and 50mg of caffeine was transferred to a 100 ml of volumetric flask containing 10 ml 0.1 N NaoH. and 50 ml of distilled water and ultrasonicated for about 15 min. The volume was made up to the mark with distilled water. The solution was filtered through Whatman filter paper no. 41. appropriate aliquots were subjected to Method A The amounts of PARA and CAF were determined.

Simultaneous determination

The standard solutions of PARA and CAF (10mcg/ml) were scanned seperately in the range of 200to 400 nm against distilled water as blank and wavelengths of maximum absorbance were determined. The absorbances of all dilutions were recorded at selected wavelengths (257 for PARA) and (272 for CAF) and calibration curves were plotted. The overlay spectrum of these drugs is shown drugs were determined at both wavelengths and. Simultaneous equations were formed. 257nm (ℨ max for PARA), 272 nm (ℨ max of caffeine) and 259.5 nm (isobestic point)

The concentrations of drugs were determined using following equations.

Cx = (A2. ay1 – A1.ay2) / (ax2.ay1 – ax1.ay2)
Cy (A1. ax2 – A2.ax1) / (ax2.ay1 – ax1.ay2)

Where
Cx = Concentration of Paracetamol in gms/lit
Cy = Concentration of caffeine in gms/lit
A2 =Absorbance at 272nm
A1 =Absorbance at 257nm
ax1= absorptivity of PARA at 257 nm
ay1= absorptivity of CAF at 272 nm
ax2= absorptivity of PARA at 257nm
ay2= absorptivity of CAF at 272 nm

Analysis of the tablet formulation

Tablet solution (1ml) containing equivalent to 10 mg of both drugs was weighed and transferred to volumetric flask and Then drug dissolved in 0.1N NaoH. The sample solution was then filtered through what man filter paper. This solution was appropriately diluted to get approximate concentration of 10 µg/ml of Caffeine and Paracetamol each, the absorbance of sample solution was measured at 272 nm and 257 nm against blank solution [21].

Validation of the developed method

1. Linearity

For each drug, appropriate dilution of standard stock solution were assayed as per the developed methods. The Beer- lamberts concentration range for both the, was found to be 2-10 mcg/ml. The Linearity data for method is presented in table 3.

2. Accuracy

Accuracy means test output match with true value. To study the accuracy of proposed method, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Here to a pre-analyzed sample solution, standard drug solution was added and then percentage drug content were calculated. The % recovery of the added pure drug was calculated as % recovery= [(Ct-Cs)/Ca] x 100, where Ct is the total drug concentration measured after standard addition; Cs, drug concentration in the formulation sample; Ca, drug concentration added to formulation [21] The result of recovery studies are reported in Table 5.

3. Precision

Inter-day and Intra-day precision

The repeatability of the method was confirmed by the formulation analysis, repeated for six times with the same concentration. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intra-day and inter-day analysis i.e. the analysis of the formulation was repeated three times in the same day at an interval of one hour and on three successive days, respectively. The amount of drug was determined and % RSD was also calculated [21]. The results of both inter and intraday precision studies are reported in Table 6.

4. Ruggedness Study

It expresses the precision within laboratories variations like different analyst. Ruggedness of the method was assessed by for the standard 3 times with diff. analyst by using same equipment [21]. The result was indicated as %RSD & given in Table 7.

5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were separately determined based on calibration curve. The residual standard deviation of a regression line or the standard deviation of y- intercepts of regression lines were used to calculate the LOD and LOQ. The LOD and LOQ were calculated by using the average of slope and standard deviation of response (Intercept). The LOD and LOQ of Caffeine and Quercetin by proposed methods were determined using calibration standards. LOD= 3.3σ/S and LOQ= 10σ/S Where, S is the slope of the calibration curve and σ is the standard deviation of response (intercept) [16]. The results of LOD and LOQ are shown in Table 3.

Results and discussion

The proposed methods for simultaneous estimation of PARA and CAF in combined dosage form were found to be accurate, simple and rapid which can be well understood from validation data. The % R.S.D. was found to be less than 2, which indicates the validity of method. Linearity was observed by linear regression equation method for PARA and CAF in different concentration range. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. The assay results obtained by proposed methods are in fair agreement, hence it can be used for routine analysis of two drugs in combined dosage forms. There was no interference from tablet excipients was observed in these methods. It can be easily and conveniently adopted for routine quality control analysis. Both methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic and are validated as per ICH guidelines.

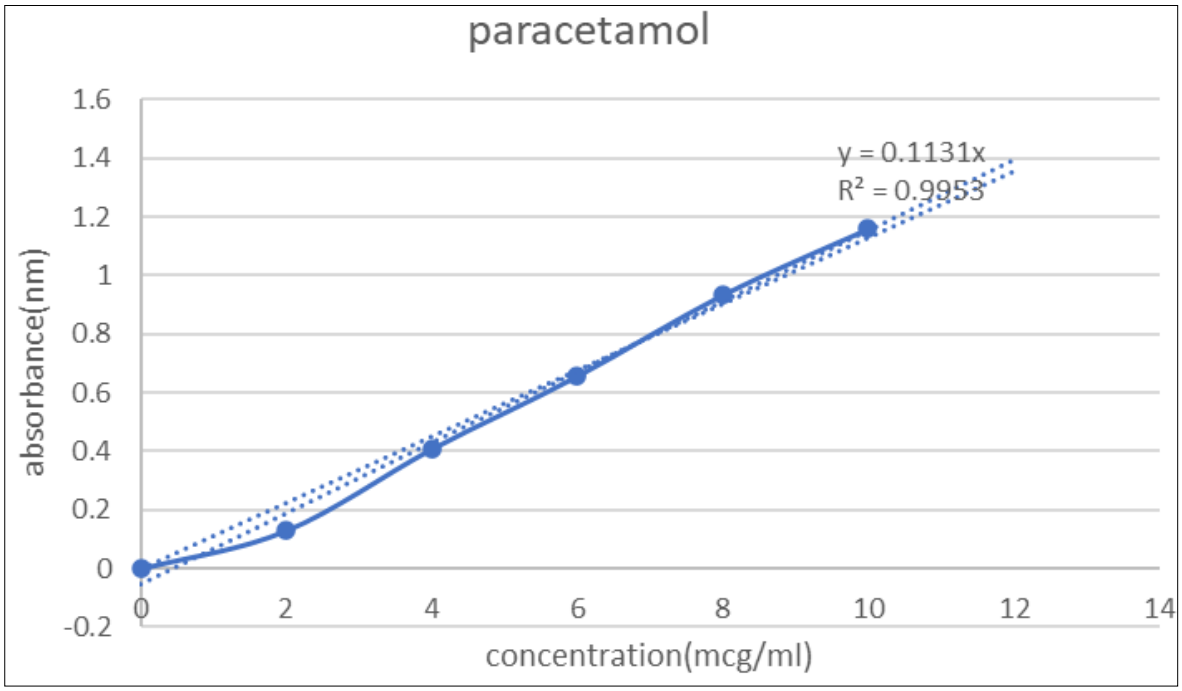


Fig 2: Calibration curve of paracetamol

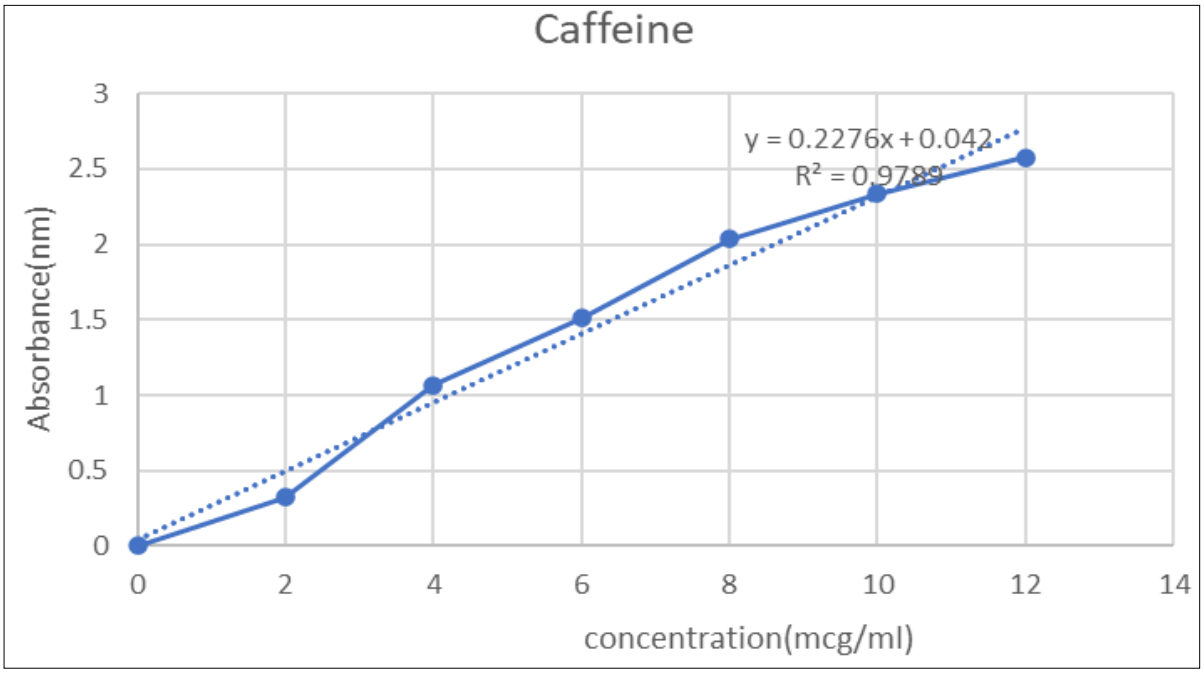


Fig 3: Calibration curve of caffeine

Table 1: Result of validation parameter

Parameter	Caffeine	Paracetamol
Λmax (nm)	272nm	257nm
Linearity range(µg/ml)	2-10µg/ml	2-10µg/ml
Linearity equation	Y=0.2276x+0.042	Y=0.1131x+0.0559
Correlation coefficient	0.9789	0.9871
Slope (b)	0.2276	0.1131
Intercept (a)	0.042	0.0559
LOD	14.40	14.23
LOQ	43.6379	43.1445

Table 2: Drug recovery

Drug concentration(µg/ml)	%recovery	Amount added(µg/ml)	Total amount recovered(µg/ml)	%Recovered
Caffeine (10µg/ml)	80	8	1.95	97.5 ±0.64
	100	10	4.90	98.2 ±0.87
	120	12	7.89	98.5 ±0.37
Paracetamol (10µg/ml)	80	8	1.90	98.01 ±0.51
	100	10	4.96	99.00 ±0.44
	120	12	7.92	99.00 ±0.39

Table 3: Analysis of tablet formulation

Drug	Labeled amount(mg/ml)	Amount found (mg/ml) ± SD	% Label claim
Caffeine	50	49.70	99.02
Paracetamol	650	638.3	97.46

Table 4: precision study

Precision	%Estimation of caffeine ± SD	% RSD	%Estimation of Paracetamol ± SD	% RSD
Interday precision	0.45	0.48	0.75	0.78
Intraday precision	0.94	0.96	0.40	0.41

Table 5: Ruggedness study

Ruggedness	Caffeine		Paracetamol	
	Mean SD	%RSD	Mean SD	%RSD
Analyst 1	0.452 ± 0.0015	0.33	0.598 ± 0.049	0.82
Analyst 2	0.453 ± 0.0005	0.12	0.601 ± 0.0005	0.09

Conclusion

A simple UV spectrophotometric method was developed for the simultaneous determination of Paracetamol and caffeine in bulk and tablet formulation without any interference from the excipients. To the best of our knowledge, the present study is the first report for the purpose. The present methods succeeded in adopting a simple sample preparation that achieved satisfactory extraction recovery and facilitated its application in coformulated formulation. The results of our study indicate that the proposed UV spectroscopic methods are simple, rapid, precise and accurate. Statistical analysis proves that, these methods are repeatable and selective for the analysis of PARA and CAF. It can therefore be concluded that use of these methods can save much time and money and it can be used in small laboratories with accuracy.

Motivation

1. Curiosity about new things.
2. Desire to get intellectual joy of doing some creative work.
3. Desire to be service to socity.
4. Desire to get respectability.

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