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Review Article

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A Review on Methods Developed for Estimation of Paracetamol in Combination with Other Drugs

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ABSTRACT

In this ever-growing world, it is crucial to improve upon the formulations in terms of potency, patient acceptability, fewer side effects, and quicker relief. Due to these requirements, the market is flooded with various combination dosage forms, with a constant increase in number. Paracetamol is a commonly used non-steroidal anti-inflammatory drug (NSAID) that has antipyretic and analgesic action. This drug is available in a wide range of combinations. It acts by inhibiting the production of prostaglandins, which combat pain and inflammation. A simultaneous multicomponent analysis is used to determine the estimation of medicines that are available in combination. Different analytical techniques are available for their determination, one of which includes the use of UV spectrophotometric methods. This review focuses on a variety of paracetamol combinations with drugs like Domperidone, Aceclofenac, Diclofenac Sodium, Etodolac, Ibuprofen, Piroxicam, Caffeine, Aspirin, and their simultaneous estimation by different UV methods viz. Simultaneous equation method, Absorbance ratio (Q-Analysis), Difference spectrophotometry, Derivative spectroscopy method, and a few other chemometric methods. This manuscript would provide the platform to have exhaustive literature on methods used for the estimation of paracetamol with different drugs using a spectrophotometer. It would help the researchers and scholars who are working in the area.

Key words: *Combination drugs, Multicomponent analysis, Paracetamol combinations, UV spectroscopy, Simultaneous estimation.*

INTRODUCTION

Various drugs are prepared in various combinations and dosage forms because a large number of diseases that have harmful effects on humanity are universal. These multi-component formulations are frequently favored because they have higher patient acceptance, enhanced efficacy, various actions, minimal side effects, and provide faster relief when handled appropriately [1]. Pharmaceutical formulations with a combination of drugs have shown promising benefits by counteracting other symptoms specific to a drug and formulation, and therefore the quantitative evaluation of such multi-component formulations is critical.

One of the much more desired and extensively used equipment accessible for quantitative analysis is Absorption spectroscopy. The extent of light absorption is a result of an increase in the number and effectiveness of light-absorbing molecules at a given wavelength [2]. The relation between the Concentration of the analyte and the quantity of light absorbed is the basis of the majority of analytical uses of molecular spectroscopy [3, 4]. Beer-Lambert Law states the same via the following expression –

$A = \log(I_0/I_T) = \varepsilon Cl$ [2, 3, 5] (1)

A is the absorbance of the compound at a given wavelength.
I_0 is the Intensity of incident light on the cuvette.
I_t refers to the amount of light that is passed through the cuvette.
The molar concentration of the solute is represented by **c**.
l is the path length i.e., the distance traveled by the light inside the sample cell in cm.
Ɛ is the molar absorptivity. It is specific for every molecule undergoing electronic transition.

As a result of changes in the electronic energy of molecules or atoms brought on by energy absorption in the UV band (200–400 nm), electrons are excited from lower to higher energy levels **(Figure 1)**. The amount of energy required for the transition of valence electrons in the molecule to happen is very precise and definite for the matter to be analysed [6].

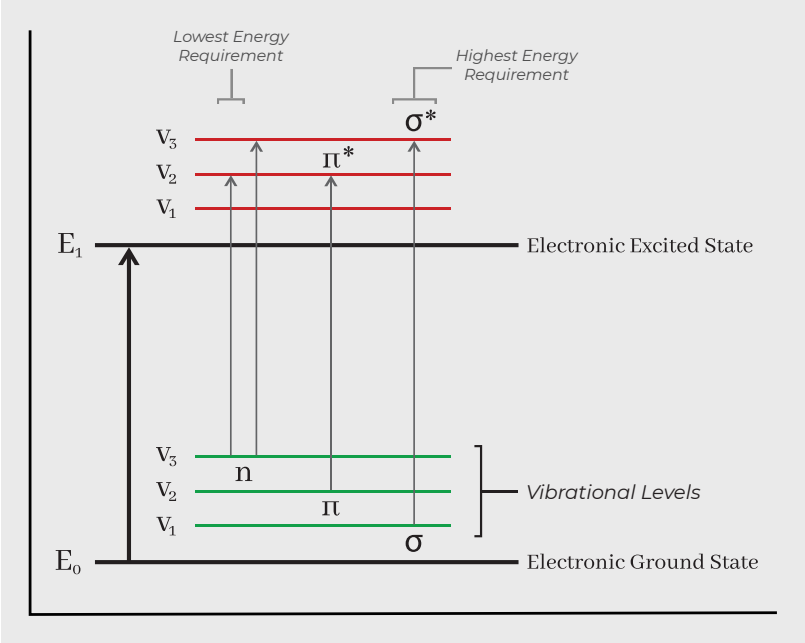


Figure 1. Electronic and Vibrational Transitions

These transitions are divided into two categories:

I. *Allowed transitions:* Have an equal to or higher molar extinction coefficient ($Ɛ_{MAX}$) than 10^4. These are -
- $\sigma \rightarrow \sigma^*$
- $n \rightarrow \sigma^*$
- $\pi \rightarrow \pi^*$

II. *Forbidden Transitions:* These are the transitions for which the $Ɛ_{MAX}$ value is lesser than 10^4.
- $n \rightarrow \pi^*$

$\sigma \rightarrow \sigma^*$ transitions have the highest energy requirement, while $n \rightarrow \pi^*$ transitions have the least energy requirement [7].

Multicomponent analysis

One of the most sensitive and commonly used measurement techniques for quantitative and qualitative analysis is the simultaneous analysis of multiple components through absorbance measurements based on ultraviolet. This process avoids previous separation methods involving extraction, the concentration of components, and purification steps that make the process time-consuming, and is fast, accurate, and simple; wide applicability to both organic and inorganic systems.

Simultaneous equation method

The concentration of different components with the additive nature of the absorbance present in the given mixture can be determined by solving a set of simultaneous equations even if their spectra overlap **(Figure 2)**.

If a multi-component system consists of two components M and N, each of which absorbs at λmax of the other, where λ_1 is the wavelength of maximum absorbance of M (λ_{max} M) and λ_2 is the Wavelength of maximum absorbance of N (λ_{max} N)

The information required is:

1. a_{m1} and a_{m2} are the drug M's absorptivity at λ_1 and λ_2 respectively.
2. A_{n1} and a_{n2} are the drug N's absorptivity at λ_1 and λ_2 respectively.
3. A_1 and A_2 represent the diluted sample's absorbance at wavelengths λ_1 and λ_2 respectively.

C_M and C_N represent the concentrations of M and N in the sample, respectively.

At λ_1,

$$\mathbf{A_1 = a_{M1}\, b\, C_M + a_{N1}\, b\, C_N} \quad (2)$$

At λ_2,

$$\mathbf{A_2 = a_{M2}\, b\, C_M + a_{N2}\, b\, C_N} \quad (3)$$

If the cell is 1 cm, then b=1

$$\mathbf{C_N = (A_1\, a_{M2} - A_2\, a_{M1})/ (a_{N1}\, a_{M2} - a_{N2}\, a_{M1})} \quad (4)$$

Similarly,

$$\mathbf{C_X = (A_2\, a_{N2} - A_1\, a_{N1})/ (a_{N1}\, a_{M2} - a_{N2}\, a_{M1})} \quad (5)$$

Using the above-mentioned simultaneous equations, the drug concentrations of M and N in the combination may be simply computed.

Absorbance ratio method/Q-analysis

This approach is a variation of the Simultaneous equation technique. Its premise is based on the fact that given a chemical obeying Beer's Law, the absorbance ratios at any two wavelengths produce a constant value regardless of analyte concentration or path length [5]. A component at two distinct dilutions produces the same absorbance ratio of A_1/A_2. This is known as the k/a Q-Value ratio. In a two-component analysis, absorbance is measured at two wavelengths; one being the isosbestic point of the two substances (λ_1), the other being the wavelength of maximum absorption of any of the two components (λ_2) **(Figure 2)**.

Two equations are constructed as in the previous method with $a_{M1} = a_{N2}$ at λ_1 and b = 1 cm;

$$\mathbf{A_1 = a_{M1}C_M + a_{M1}C_M} \quad (6)$$

$$\frac{\mathbf{A_2}}{\mathbf{A_1}} = \frac{\mathbf{a_{M2}C_M + a_{M2}C_N}}{\mathbf{a_{M1}C_M + a_{M1}C_N}} \quad (7)$$

The concentration of each component (C_X & C_Y) in the sample can be calculated

$$\mathbf{C_M} = \frac{(\mathbf{Q_A - Q_N})\mathbf{A_Q}}{(\mathbf{Q_M - Q_N})\mathbf{a_{M1}}} \quad (8)$$

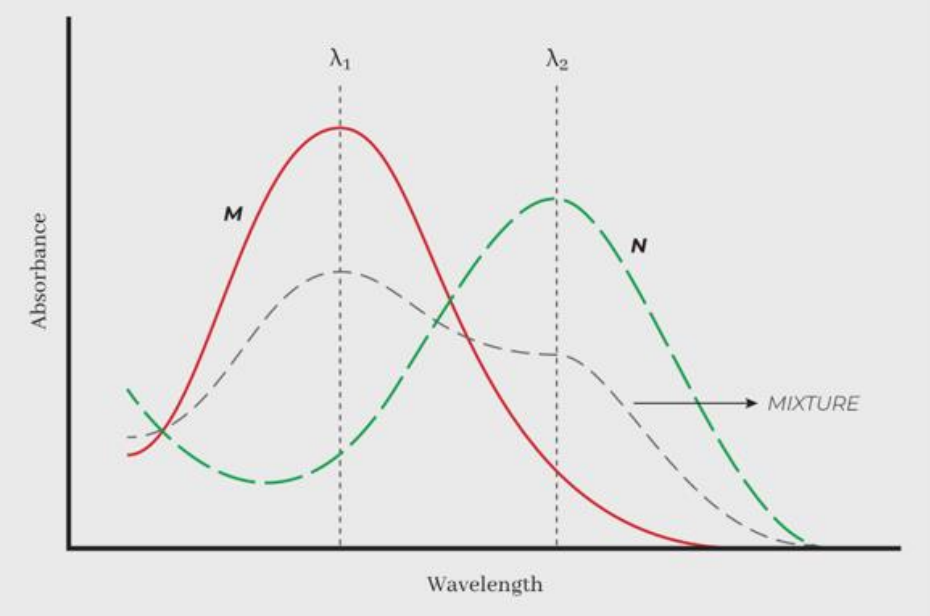
$$\mathbf{C_N} = \frac{(\mathbf{Q_A - Q_M})\mathbf{A_Q}}{(\mathbf{Q_M - Q_N})\mathbf{a_{N1}}} \quad (9)$$

$$Q_N = \frac{\text{Absorbance of sample solution at } \lambda 2}{\text{Absorbance of sample solution at } \lambda 1} \quad (10)$$

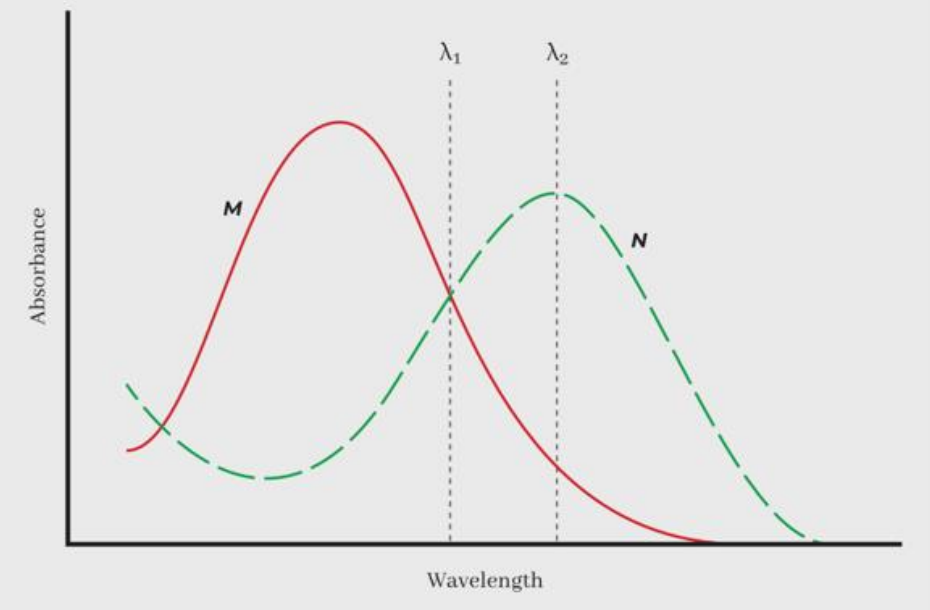
$$Q_M = \frac{\text{Absorptivity of pure component M at } \lambda 2}{\text{Absorptivity of pure component M at } \lambda 1} \quad (11)$$

$$Q_N = \frac{\text{Absorptivity of pure component N at } \lambda 2}{\text{Absorptivity of pure component N at } \lambda 1} \quad (12)$$

A_Q = Absorbance of the sample at isosbestic (λ_1) wavelength
a_{M1} = Absorptivity of components M at isosbestic (λ_1) point
a_{N1} = Absorptivity of components N at isosbestic (λ_1) point
The precision of the dilutions of the sample solution and standard solution of M and N determines the accurate absorption and absorptivity measurements, respectively.



a) Vierordt's Method



b) Absorption Ratio Method

Figure 2. Absorption spectra of substances M, N, and mixture

Derivative spectrophotometry

Derivative spectroscopy is based on the principle of transition of simpler absorption spectrum into the first, second, or higher spectrum depending on their wavelength. This spectroscopic approach employs Gaussian bands to depict the modifying spectral data. It is also used for spectrum analysis to characterize any chemical configuration. The zeroth order spectrum, or fundamental absorption spectrum, is represented by the symbol $\mathbf{D^0}$ [5].

Zero-order spectra are simpler to understand than derivative spectra. The rate at which absorbance varies with wavelength is graphically depicted in a first-order derivative spectrum. A first-order derivative begins and ends at the zero point, passing through it at the absorbance band's maximum. Across the same wavelength, the upper side of this point exhibits a positive band, while the lower exhibits a negative band including both maxima as well as minima values; hence, this location is known as the inflection point.

The absorbance of a sample is discriminated against concerning wavelength to create the first, second, or higher-order derivatives **(Figure 3)**.

$$A = f(\lambda)\text{: Zero order} \quad (13)$$

$$dA/d\lambda = f(\lambda)\text{: First order} \quad (14)$$

$$d^2A/d\lambda^2 = f(\lambda)\text{: Second order} \quad (15)$$

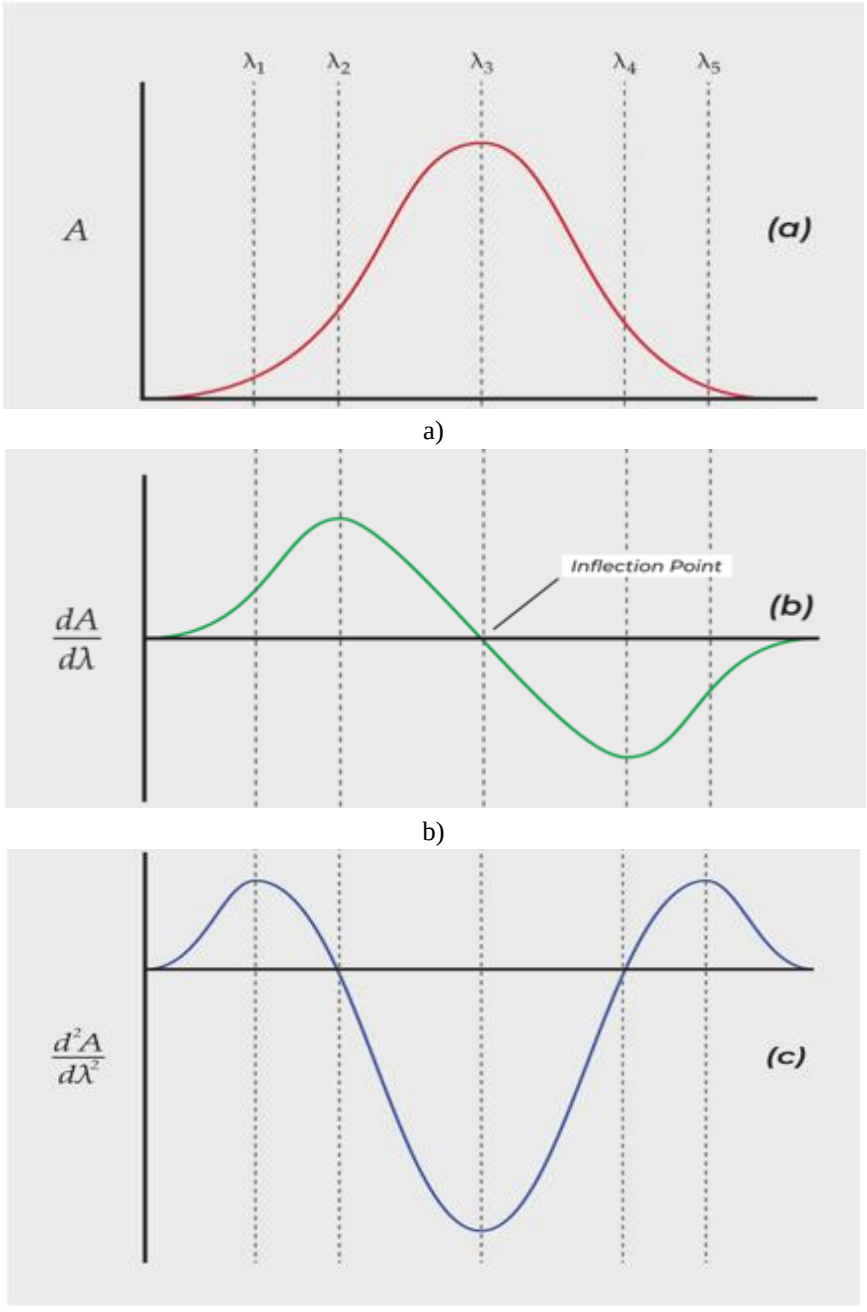


Figure 3. Zeroth (a), first (b), and second (c) derivate spectra

An absorption band's first derivative spectrum has a maximum, and a minimum, as well as, a cross-over point at its λ_{max}. Finding the zero crossover point or wavelengths for each component is easily achieved with the use of the derived spectra. Absorbances of varying concentrations derived from stock solutions of separate components are measured at their corresponding zero crossover values acquired from their derivative spectra [7]. Regression analysis is carried out in conjunction with the plotting of calibration curves. The components are estimated by solving regression equations.

The derivative technique's key characteristics comprise increased information richness, differentiation against background noise, and more specificity in quantitative analysis [6].

Difference spectrophotometry

This method is based on the concept that between any two wavelengths, The concentration of the interest component on a mixed spectrum determines the absorbance difference (ΔA), which is independent of the concentration of an interfering component given that the absorbance difference at the preferred wavelengths is zero [5]. Two wavelengths (λ_1 & λ_2) are chosen for component X in a manner to ensure that the absorbance is the same at both wavelengths of interfering component Y. The calibration curves are obtained by plotting the absorbance difference (ΔA) of each standard and sample mixture at λ1 and λ2 against the corresponding concentration. In the case of binary mixtures, the wavelength is chosen to ensure that the value of each component stands zero at the wavelength where the other components display maximum absorbance **(Figure 4)**.

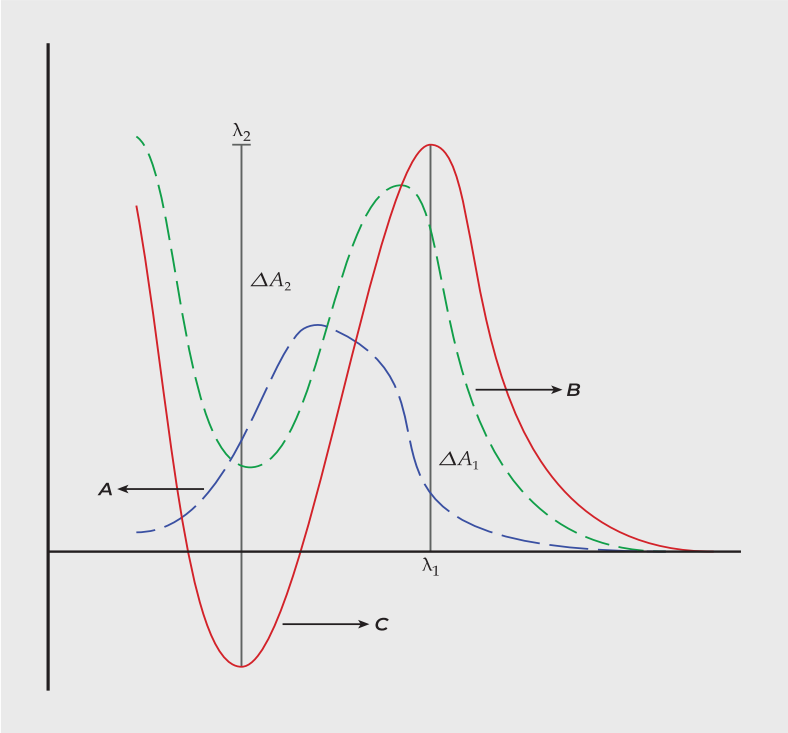


Figure 4. Individual absorption spectra of substances A and B; Difference absorption spectra C

(Table 1) outlines several instances of different UV spectroscopic analytical methods in pharmaceutical applications.

Table 1. Applications of different UV analytical methods

Applications	Method Used	Ref.
Acetaminophen and Chlorzoxazone	Difference Spectrophotometry, Q-Absorbance Method	[8]
Allopurinol and Lesinurad	Simultaneous Equation Method	[9]
Ambroxol, Salbutamol, and Theophylline	Simultaneous Equation Method	[10]
Bromfenac and Ofloxacin	Derivative Spectrophotometry	[11]
Esomeprazole and Naproxen	Derivative Spectrophotometry	[12]
Fluorescein and Benoxinate	Simultaneous Equation Method	[13]

Fluticasone and Formoterol	Simultaneous Equation Method, Q-Absorbance Method	[14]
Furazolidone and Metronidazole	Q-Absorbance Method	[15]
Hydrochlorothiazide and Carvedilol	Q-Absorbance Method	[16]
Ledipasvir and Sofosbuvir	Derivative Spectrophotometry	[17]
Levosulpiride and Rabeprazole sodium	Derivative Spectrophotometry	[18]
Metformin HCl and Anagliptin	Q-Absorbance Method	[19]
Nalidixic acid and Metronidazole	Difference Spectrophotometry	[20]
Pamabrom, Mefenamic Acid, and Dicyclomine Hydrochloride	Simultaneous Equation Method	[21]
Quinfamide and Mebendazole	Q-Absorbance Method	[22]
Sofosbuvir and Velpatasvir	Simultaneous Equation Method	[23]
Sumatriptan and Naproxen	Simultaneous Equation Method	[24]
Telmisartan and Hydrochlorothiazide	Q-Absorbance Method	[25]
Tinidazole and Norfloxacin	Difference Spectrophotometry	[26]
B-Carotene and Lycopene	Simultaneous Equation Method	[27]

Paracetamol

Paracetamol (PCM), widely known as *Acetaminophen* is an OTC medicine having analgesic and antipyretic properties used in mild to moderate pain and fever. It is chemically N-(4-hydroxyphenyl) acetamide **(Figure 5)**. PCM comes under the category of *non-steroidal anti-inflammatory drugs (NSAIDs).*

It is considered to be a weak inhibitor of Prostaglandins (PGs). It works primarily by specifically inhibiting COX-1 and COX-2 through peroxidase's metabolizing activity (in-vivo). This results in inhibition in the formation of phenoxyl radical which is critical for prostaglandin production and cyclooxygenase activity of COX-1, COX-2.

The world's most commonly used pain reliever, recommended by the *World Health Organization (WHO)* as a first-line treatment drug in anti-inflammatory therapy is Acetaminophen (paracetamol), commonly known as *Tylenol*. It is also used for its antipyretic properties, which help bring down a fever. Paracetamol is often found in combination with other medications in cold medicines, more than 600 *over-the-counter (OTC)* allergy medicines, pain relievers, sleep aids, and other products.

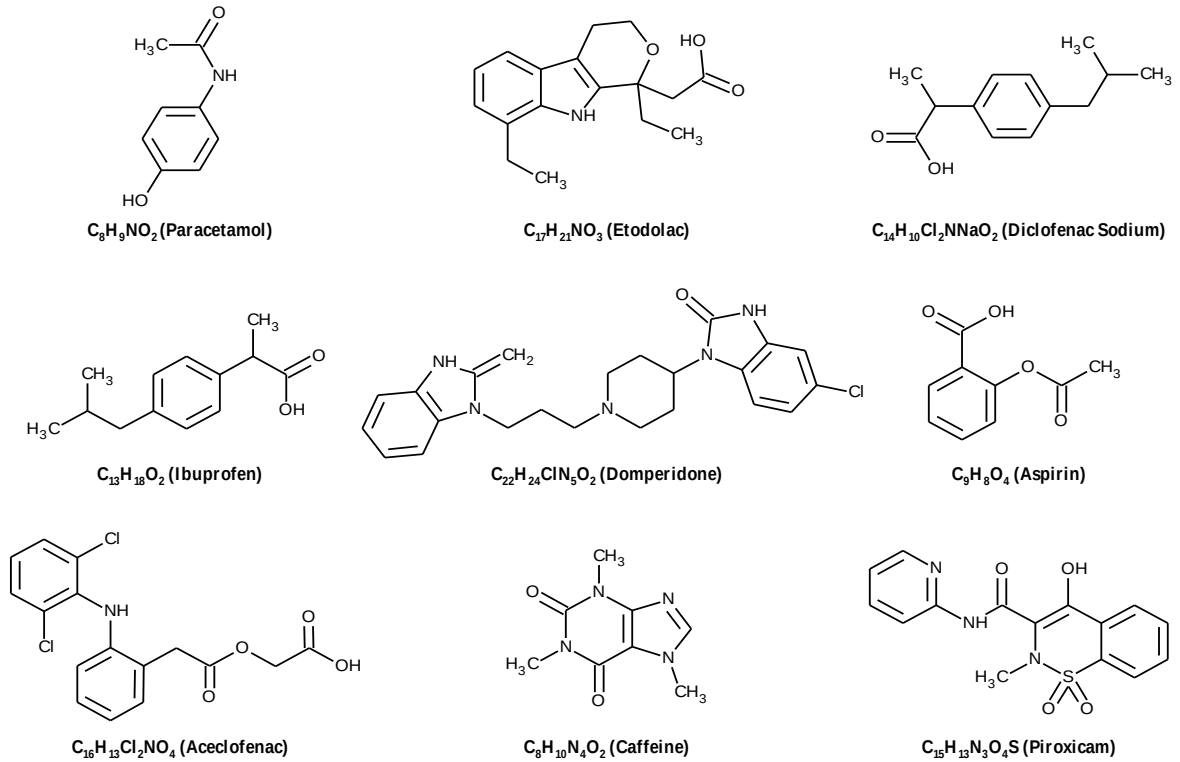


Figure 5. Structures of drugs used with PCM in combination

Estimation methods of paracetamol combinations

Paracetamol + Etodolac

Etodolac (ETO) is an NSAID with antipyretic and analgesic activity being used for chronic arthritis and acute pain. Its chemical name is 1,8-Diethyl-1,3,4,9-tetrahydropyran (3,4-b) indole-1-acetic acid. Similar to other NSAIDs, etodolac provides its anti-inflammatory effect by inhibition of the enzyme cyclooxygenase (COX) preferably COX-2 (about 5-50 times more selective than COX-1). This results in the decrease of peripheral prostaglandins involved in mediating inflammation. Etodolac binds to the active site of the COX enzyme and prevents arachidonic acid from entering the active site.

A combination of 400mg Etodolac and 500mg Paracetamol is available in the tablet dosage form commercially. It has been found, from an extensive literature survey, that only a few UV spectroscopic and some RP-HPLC methods are available for simultaneous estimation of this combination.

By taking Triethylammonium phosphate buffer as a solvent with the pH adjusted to 10 using 30% v/v orthophosphoric acid, Ashok Kumar, *et al.* (2015) utilized the simultaneous equation method of estimation [28]. The wavelength selected for ETO and PCM were 227nm and 252nm respectively. The developed method was validated for linearity which lay in the range of 5-15µg/ml for Etodolac and 6.25-18.75µg/ml for Paracetamol.

In the ratio of 60:40 v/v as the common solvent for both drugs in the formulations, Alpa *et al.* (2013) and Shaikh *et al.* (2017) used methanol and water [29, 30]. The λ_{max} observed for the drugs were 247nm and 280nm for PCM and ETO respectively by Alpa *et al.* (2013) and 256nm and 286nm by Shaikh *et al.* (2017). The derivative spectroscopic method was used by both researchers with achieving zero cross points at 224.28nm and 219.27nm for Etodolac and Paracetamol respectively at First-order spectra out of the four derivatized. The method was validated for linearity, precision, and accuracy with concentration ranges of 5-25µg/ml (PCM) and 2-18µg/ml (ETO).

Balan *et al.* (2011) also used the simultaneous equation method for the estimation of the combination [31]. Phosphate buffer with pH 7.4 was used as the solvent instead of methanol. The maximum absorptive wavelength for PCM and ETO was found to be 242.5nm and 223.5nm respectively. The method was validated for linearity in the range of 2-10µg/ml for ETO and 2-14µg/ml for PCM.

Paracetamol + Diclofenac Sodium

Diclofenac Sodium (DIC) is an NSAID used in the condition of inflammation and acute and chronic pain with cases including osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis. Diclofenac belongs to the family of phenylacetic acids having an analgesic, antipyretic and anti-inflammatory activity [32]. DIC is a competitive, reversible, and non-selective inhibitor of cyclooxygenase (COX-1 and COX-2), which subsequently blocks the conversion of arachidonic acid to prostaglandin precursors. This inhibits the formation of prostanoids such as (PGE2) prostacyclin, and thromboxane, which are essential for response involved in pain, inflammation, and fever.

Paracetamol is a poorly water-soluble drug. From the literature study, it has been found that in the past years, a few Hydrotropic solubilization methods are used for simultaneous estimation with Diclofenac sodium.

Sharma *et al.* (2010) used 1.0 M Urea solution as a hydrotropic solubilizing agent to solubilize PCM for its spectrophotometric analysis [33]. Six methods in total in different studies were used. For the simultaneous equation method, the λ_{max} values of PCM and DIC were found to be 247nm and 276nm respectively. For Q-analysis, the isosbestic point was found to be at 268nm and λ_{max} of Diclofenac (276nm) was used as the second wavelength. Another method used was the Dual wavelength (Difference Spectroscopy) method. In this method, the *Zero-difference* wavelengths of PCM (245 and 249nm) and DIC (257 and 294nm) were selected for their estimation. The linearity range was within the range of 2-40µg/ml for both drugs.

In another study by Sharma *et al*, the Derivative spectroscopic method was used and calibration curves were plotted for PCM (2-40µg/ml) at 247nm and Diclofenac (2-40µg/ml) at 276nm [34]. For Area Under Curve Method (AUC), the regions selected (245-249nm) for PCM and (276-280nm) for DIC were used for the calculation of their concentrations. The aliquots were scanned at 247nm and 276nm and overlain spectra of mixed standards were obtained. The methods were validated for accuracy, precision, repeatability, and recovery study with standard deviation being <1.0% and RSD values being <2.0%. The linearity was within the concentrations selected.

Sharma *et al.* (2011) and Vandana Gupta *et al.* (2019) also used Urea as the Hydrotropic solubilizing agent in the concentrations 5M and 8M respectively [35, 36]. Sharma *et al.* (2011) used the simultaneous equation method with λ_{max} values being 247.8nm and 261.1nm for PCM and DIC respectively. The method was validated for

accuracy, precision, repeatability, and recovery. The Beer's law limit was found to be in the concentration range of 5-35µg/ml for both PCM and DIC.

Gupta *et al*. (2019) used the simultaneous equation method with λ_{max} at 243 and 276nm. In the Q-analysis method, the wavelengths selected were 264.4nm (λ_1-isosbestic point) and 276nm. Which was further estimated by the Derivative spectrophotometric method in the First order derivative. The zero crossing points for PCM and DIC were 319.4nm and 276.8nm respectively. The methods were validated with %RSD value <1.0% in all three methods and a linearity limit between 5-25µg/ml.

Phaneemdra and Nagamalleswari (2012) used the first-order derivative method with zero crossing points at 275.6nm (Diclofenac) used for Paracetamol and 242.69nm (Paracetamol) for Diclofenac [37]. Phosphate Buffer pH 6.8 was used as a common solvent. For the simultaneous equation method, the λ_{max} of observed at 243nm and 281nm. The linearity range was 2-10µg/ml and 5-25µg/ml for PCM and DIC respectively.

Ganesh *et al*. (2015) and Patel *et al*. (2020) used Distilled Water as a common solvent in determining the drug concentrations by the simultaneous equation method [38, 39]. The wavelengths selected were 247nm (PCM) and 276nm (DIC). Ganesh also used the Q-Absorbance ratio method using the same solvent with selected wavelengths of 247nm and 265nm (isosbestic point). The proposed methods were validated for accuracy, linearity (6-30µg/ml), and precision with %RSD <2.0%.

Sebaiy *et al*. (2020) used the absorption subtraction method, ratio difference method, and derivative method. The solvent used is 90% Methanol [40]. For the advanced absorption subtraction method, the wavelengths were selected at 225nm (Isosbestic point) and 267nm (zero difference in absorbance of PCM). In the ratio difference method, selected wavelengths were 283nm and 270nm for Diclofenac and 251nm and 240nm for PCM. The first-order derivative of the ratio difference curve was calculated and resulting spectra were measured at 273nm for DIC and 254nm for PCM. The absorption difference method is also incorporated by Chakravarthy *et al*. (2004) using methanol as solvent and the selected wavelengths at 230 and 254nm with zero absorbance difference for PCM and 260 and 292nm having zero difference for DIC [41].

In another study by Sebaiy *et al*., the H-Point assay method is used [42]. The wavelengths 225nm and 265nm were selected as zero difference points for PCM and shows a significant difference in absorption for DIC. The linearity was within the range of 7.5-4.5µg/ml for DIC and 4-22µg/ml for PCM in both studies. The correlation coefficient was found to be >0.9990 for both drugs and specificity values were 100.32% ± 0.51 for PCM and 100.25% ± 1.29 for Diclofenac.

Paracetamol + Ibuprofen

Ibuprofen (IBU) is a commonly used NSAID that is considered to be one of the safest in the category. At low doses (800-1,200 mg/day) it is approved for over-the-counter sales and is generally safer to use. Ibuprofen is a derivative of propionic acid that has anti-inflammatory, analgesic, and antipyretic properties because it inhibits cyclo-oxygenase I and II non-selectively, which reduces prostaglandin production, by prostaglandin synthase, the main physiologic effect of ibuprofen. Ibuprofen can also inhibit platelet aggregation by decreasing the formation of thromboxane A2.

From an extensive literature survey, it has been found that various methods and approaches have been used for the simultaneous determination of PCM and IBU in the combined dosage form. The simultaneous equation method is used by Gondalia *et al*. (2010) for combination drugs present in soft gelatine capsule dosage form [43]. Methanol was used as a common solvent and the wavelengths selected were 224nm and 248nm. The method was validated for linearity which was found to be in the range of 4-14µg/ml (IBU) and 2-12µg/ml (PCM), and accuracy with a %recovery of 99.70 ± 1.08 and 100.16 ± 1.02 for IBU and PCM, respectively. %RSD values were 1.44 and 0.95 for the same.

Harshini *et al*. (2014) and Gaikwad *et al*. (2017) also used the simultaneous equation method with different solvents i.e., Ethanol and 0.1N NaOH respectively [44, 45]. In both studies, the λ_{max} of PCM and IBU were found to be at 240nm and 220nm. The developed methods were validated with linearity in the range of 2-20µg/ml for IBU and 1-15µg/ml for PCM.

Tejashree *et al*. (2020) used Methanol as a common solvent for both drugs [46]. For the simultaneous equation method, the wavelengths selected were 256nm and 222.4nm as λ_{max} of PCM and IBU respectively. 226.4nm was observed as the isoabsorptive point for the Q-analysis method. 5-30µg/ml was the linearity concentration range for both drugs. The recovery study resulted in the values 102.65% for PCM and 100.83% for IBU. %RSD values were 0.58 and 0.47.

Ostwal *et al*. (2012) and T. Mamatha et, al. (2013) used the dissolution method using Phosphate buffer (pH 5.8 and 7.2 respectively) as the dissolution medium [47, 48]. The wavelengths selected were 222.4nm (λ_{max} IBU) and 226.4nm (Isoabsorptive point) by Ostwal and 221.8nm and 213.8nm by Mamatha for estimation by absorbance ratio method using the concentration range within the linearity limit of 2-21µg/ml for IBU and 2-14µg/ml for PCM.

Hassan, (2008) used chemometric methods including ratio derivative and multivariate methods (Classical Least Square and Principal components regression analysis) for simultaneous determination of the drug combination [49]. Methanol was used to prepare the aliquots, 290nm and 230nm were observed as zero-crossing points for IBU and PCM, respectively. For the first derivative, the amplitudes measured at 280nm and 270nm were found linear to the concentrations of IBU and PCM, respectively. For multivariate analysis, ten solutions were prepared with a linearity concentration range of 5-60 and 10-100µg/ml for ibuprofen and paracetamol, respectively. The Calibration K matrix was obtained from the absorption data in the range of 100-40nm. The methods were validated for accuracy, precision, and repeatability with %RSD values being within the range.

The ratio spectra method is also used in another development by Zayed *et al*. (2011) with getting Mean recovery % of 96.83(IBU) and 97.59(PCM) in the first derivative; and 97.16(IBU) and 96.62(PCM) in the second derivative spectra [50]. The linearity was found between the range of 2-32 (IBU) and 2-24µg/ml (PCM). The same solvent was used as in the previous study.

Another study by Hoang *et al.* (2014) also used derivative spectroscopy along with wavelet transforms [51]. Phosphate buffer pH 7.2 was used as the solvent. 249.3 and 242.0 nm were observed as zero-crossing points for IBU and PCM, respectively. The beer's law limit was within the concentration range of 12-32µg/ml (IBU) and 20-40µg/ml (PCM). The spectrophotometric results were found to be 95% accurate when statistically compared with the HPLC method taken as standard.

Omray *et al*. (2007) used the absorbance difference method for the simultaneous determination of the combination [52]. Ethanol was used as a common solvent. Absorbance was scanned over a range of 200 – 600 nm. Two wavelengths 220 and 231nm were selected with absorbance difference for IBU being zero. Similarly, 241 and 255nm were selected for having zero absorbance difference for PCM. The method was validated in terms of linearity (6-12µg/ml), accuracy, precision, specificity, and reproducibility of the sample applications.

El-Maraghy and Lamie (2019) also used the ratio difference method for the resolution of overlapped zero-order spectra [53]. Methanol was used as a common solvent to achieve a concentration of 2-20µg/mL for PCM and 2-50µg/mL for IBU which was proven for linearity. The zero-order spectra were measured over the range of 200-400nm. Two wavelengths each with a maximum difference in peak amplitudes for PCM (236 and 248 nm) and IBU (210.6 and 216.4 nm) were selected and a calibration curve was plotted. %RSD was found to be 0.650 and 0.778; and the Mean recovery% values were 99.91 and 100.18 for PCM and IBU, respectively.

Paracetamol + Domperidone

Domperidone (DOM) is a dopamine antagonist with antiemetic, gastrokinetic, and galactagogue activities. It binds to the D2 receptor in the chemoreceptor trigger zones which inhibits dopamine binding and D2R-mediated signaling affecting the motor functions of the GIT and relieving various gastrointestinal (GI) symptoms, such as nausea and vomiting.

A literature survey has revealed that only a few validated methods have been developed for simultaneous estimation of Paracetamol and Domperidone as a combination drug therapy from the year 2009 till 2016 and no recent development has taken place since.

Kapil *et al*. (2009) used the simultaneous equation method for the determination [54]. Methanol is used as a common solvent and the λ_{max} was measured at 250nm and 285nm for PCM and DOM, respectively. The method was validated for accuracy, precision, specificity, and ruggedness with recovery study values found to be 99.45±0.47% for PCM and 100.67±0.18 for DOM. The linearity range was observed to be 5-30µg/ml (PCM) and 0.8-5µg/ml (DOM).

Babar *et al*. (2012) used two simple methods, the simultaneous equation method with wavelengths selected were 243.4 nm and 284.12 nm as the corresponding λ_{max} of PCM and DOM [55]. For the absorption ratio method, 270nm was recorded as the absorptive point of the two drugs. The method was validated and recovery studies were performed with linearity concentrations to be found in the range between 15-30µg/ml (DOM) and 11-16µg/ml (PCM).

Appasaheb *et al*. (2013) also used the simultaneous equation method taking 258nm and 292nm as maximum absorption wavelengths for PCM and DOM, respectively. 0.1N NaOH was taken as a common solvent [56]. The

dual wavelengths with zero absorbance difference for DOM (247nm and 269nm) and PCM (288nm and 296nm) were selected for the absorbance difference method. Another method developed was Area under curve method with sampling wavelength ranges selected 242nm-275nm for PCM and 284nm-302nm for DOM from the calibration curve. The methods were validated for accuracy and precision with obtaining linearity concentration between the range 5-30µg/ml for both PCM and DOM.

Mali *et al*. (2016) also used the AUC method for the simultaneous determination of the combination drugs with a wavelength range of 220-274nm for Paracetamol and 262-304nm for Domperidone from the calibration curve [57]. The maximum wavelengths 248nm (PCM) and 286nm (DOM) were used to plot the calibration curve by simultaneous equation method. In a separate study [58], *Mali A.* used the First order derivative overlain spectra for further resolution of the zero-order spectrum overlapping. The zero crossing points 262nm (PCM) and 297nm (DOM) were used to measure the first-order derivative values of paracetamol and domperidone, respectively. Both studies revealed that the linearity for both drugs was observed in the range of 5-25µg/ml by all three methods.

Paracetamol + Aceclofenac

Aceclofenac (ACF) is a Phenylacetic acid derivative that is the carboxymethyl ester of Diclofenac. It is NSAID with anti-inflammatory and analgesic properties and is used in the management of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, low back pain (LBP), scapulohumeral periarthritis, extraarticular rheumatism, odontalgia. It is reported to have higher Anti-inflammatory action and is well tolerated with a more favorable GI profile than other NSAIDs.

From a comprehensive literature survey, it has been found that several methods have been developed for the estimation of the combination of Aceclofenac and Paracetamol in the last two decades including Viedort's method, Q-analysis, and Ratio derivative method.

Mishra and Garg (2006) used the simultaneous equation method and Q-analysis method by taking Ethanol as a common solvent [59]. The absorption maxima of PCM and ACF were observed at wavelengths 256nm and 275nm, respectively. 230nm was observed as the isosbestic point for the two drugs. The method showed linearity within the concentration range of 1-10µg/ml. The recovery study was well within the range of 99-100%.

Pawar *et al*. (2010) also used the simultaneous equation method utilizing 274nm and 248nm as the estimation wavelengths for PCM and ACF respectively [60]. Methanol: Glass distilled water was used as the common solvent. The linearity concentrations were within the range of 1-5µg/ml (ACF) and 5-25 (PCM).

Jain *et al*. (2007) and Gharge *et al*. (2010) also used the same above-mentioned vierodt's and Q-analysis methods but with Methanol (pure and 80%, respectively) as a common solvent [61, 62]. The wavelengths selected were 249nm, 276nm, and 270nm [61]; 245nm, 276nm, and 267.5nm [62]. The Linearity concentrations observed for PCM and ACF were 2-25µg/ml and 1-30µg/ml respectively by *Jain A.*; and 2-20µg/ml and 5-40µg/ml by *Gharge D.*

Mahapare *et al*. (2007) used the Difference spectroscopy method and AUC method for determination using AR-grade Methanol as the solvent [63]. 274.5nm and 244nm were the selected wavelengths (λ_{max} of ACF and PCM). For AUC overlain spectrum was obtained and the concentrations were measured using the selected wavelength ranges, 224 to 260 nm (ACF) and 254 to 294 nm (PCM). For the absorption difference method, the wavelengths selected were 221.5nm and 257nm for ACF and, 261nm and 278nm for PCM. The methods were validated in terms of linearity of absorbance in the concentration range of 2-20 µg/ml (ACF) and 5-40 µg/ml (PCM) at their respective maxima.

The absorbance difference method was also implied by Pradhan et, al. (2019) using the same solvent as above. 245nm and 214nm were observed as the absorbance maxima of PCM and ACF respectively [64]. The wavelengths selected from the spectrum were 245 and 270nm for the estimation of PCM and for the estimation of ACF wavelengths 214 and 242nm were chosen as λ_1 and λ_2. The range for linearity was found to be 3-40µg/ml for PCM and 3-10µg/ml for ACF.

Gandhi *et al*. (2008) used the Ratio Derivative method with selected wavelengths, 256nm (PCM) and 268nm (ACF) from the first-order derivative spectra [65]. Linearity was found in the range of 10-50µg/ml with high correlation coefficients for both the drugs and %RSD <1.5.

A similar method, The First-order derivative method was used by Nimbekar *et al*. (2014) with zero-crossing points observed at 276nm for ACF and 248nm for PCM [66]. vierodt's method was also implied using the respected absorbance maxima. The linearity was found to be in the range, of 3-30µg/ml (PCM) and 2-20µg/ml (ACF).

Kumar *et al*. (2011) and Mishra *et al*. (2014) used the Q-analysis absorbance ratio method using wavelengths 275.4nm (λ_{max} ACF) and 266.1nm (isosbestic point); and 268nm (isosbestic) and 238nm (λ_{max} PCM), respectively

[67, 68]. In the former study, the linearity range was achieved between 1-35µg/ml for ACF and 1-15µg/ml for PCM. *Ganesh Mishra* also used the derivative method with zero-crossing points at 238nm (ACF) and 268nm (PCM) in the first-order derivative spectra. The linearity found between the concentration range is 5-50µg/ml. Both studies showed good recovery within the range of 99-102% and %RSD <2%.

Paracetamol + Caffeine

Caffeine (CAF) is a CNS stimulant methylxanthine alkaloid, structurally related to adenosine, and primarily acts as an adenosine receptor antagonist. It has psychotropic and anti-inflammatory activities with increased energy metabolism throughout the brain but induced brain hypoperfusion. It reduces myocardial blood flow and limits adenosine-mediated vasodilation by inhibiting A1, A2A, and A2B adenosine receptors in blood vessels. The anti-inflammatory effect is caused due to competitive inhibition of PDE (Phosphodiesterase) which leads to an increase in the amount of cAMP, protein kinase activation, and inhibited leukotrienes synthesis which ultimately assists in reducing inflammation.

Paracetamol and caffeine as a combination act as a good analgesic and antipyretic drug therapy. During the last two decades, several methods have been developed for the estimation of the combination simultaneously by UV spectrophotometer. Due to huge variables and a large number of absorbance values, chemometric-assisted methods have been preferred for rapid and precise estimation.

Multivariate methods like principal component regression (PCR), partial least-squares regression (PLS), and artificial neural networks (ANN) were used by Dinç & Baleanu (2002); and Aktaş and Kitiş, (2014) [69, 70]. *Dinc and Baleanu* measured the absorbances at an interval of 15λ in the region of 215 – 285 nm. 0.1 M HCl was used as the common solvent. 'MAPLE V' software was used for solving complex regression equations. *Aktaş and Kitiş* used 'Minitab 16' software using the same 0.1 N HCl as a common solvent. The absorption spectra were measured in the spectral region 205-305nm with a much smaller Δλ value, set to 0.1nm.

In a more recent study by Karim *et al*, (2019) Partial least square regression and artificial neural network methods are used for the simultaneous essay of PCM and CAF [71]. The spectra region 205-300nm was used for recording the absorbance with an interval of 1nm and preferred common solvent Methanol. The software 'MATLAB 2014' and 'Unscrambler® X' has been used for ANN and PLS respectively. Both drugs showed an R^2 value of 99.28% for prediction and 99.13% for the validation set.

Tavallali and Sheikhaei, (2009) used the H-point standard addition method for the simultaneous estimation of the drug combination [72]. The wavelength used is of the visible region; 453nm. Acetic acid buffer pH 5.0 is used as the reagent for the essay. The linearity was within the range of 0.1-3µg/ml for CAF and 1.5-7µg/ml for PCM.

Vichare *et al*., (2010) used the simpler simultaneous equation method and absorption ratio method for the estimation of the combination [73]. 243nm and 273nm were observed as λ_{max} of PCM and CAF, respectively and wavelength 259.5nm was the isosbestic point. The stocks were prepared by dissolving the drugs in distilled water. 2-32 and 2-16µg/ml were the linearity range for CAF and PCM respectively.

Sharma *et al*. (2015) used the Dual wavelength method with selected wavelengths 260nm and 281nm for PCM and 234nm and 249nm for CAF [74]. Methanol was taken as solvent. The linearity concentration ranges were 10-60 and 3-18 µg/mL for paracetamol and caffeine, respectively.

Paracetamol + Aspirin

Aspirin (ASP) also known as acetylsalicylic acid is an orally administered NSAID most widely used in the condition of pain, fever, myocardial infarction, osteoarthritis, and ischemia [75].

It has anti-inflammatory and antipyretic activity caused by non-selective inhibition of COX leading to lowered prostaglandin levels. Unlike other NSAIDs, it binds irreversibly to COX II and also blocks thromboxane A2 on platelets, preventing platelet aggregation [76].

From an exhaustive literature survey, it has been found that only a couple of studies have been performed for simultaneously estimating aspirin and paracetamol in the combined dosage form.

Samnani *et al*. (2007) used Vierordt's method for the determination of aspirin and paracetamol in treated sewage water [77]. Double Distilled Water (DDW), Methanol, and 0.1N HCl were used to prepare separate stock solutions for both drugs. The wavelength used for recording the absorbance was 225nm for ASP and 244nm for PCM. The results were compared to that of HPLC. The method was validated for linearity, precision, and accuracy with %RSD less than 0.008 for both drugs and correlation coefficient being 0.9626 (ASP) and 0.9989 (PCM).

Murtaza *et al*. (2010) also used the simultaneous equation method with selected wavelengths 265nm and 257nm as λ_{max} of ASP and PCM respectively [78]. The solvent was prepared by mixing 0.1N HCl and Methanol in equal parts. The linearity was between the concentration range of 2 to 64µg/ml.

Paracetamol + Piroxicam

Piroxicam (PIR) is an NSAID of the oxicam class used for its anti-inflammatory, antipyretic, and analgesic activity. Piroxicam non-selectively bind to cyclooxygenase enzymes inhibiting prostaglandin synthesis. It reversibly stops the conversion of arachidonic acid into prostaglandin precursors which leads to inflammation. It is used to treat chronic ankylosing spondylitis, osteoarthritis, rheumatoid arthritis, soft-tissue disorders, acute gout, and also in postoperative pain [79].

Not a lot of methods have been developed for this combination of drugs. It's been revealed that only two studies have been performed so far regarding the same.

Shirkhedkar *et al*. (2008) used the Q-Absorbance method with selected wavelengths 257nm (λmax of PCM) and 320nm (the absorptive point) [80]. 0.01N NaOH was used as the common solvent for dissolving both drugs [81]. The linearity range was 4-12µg/ml and 4-40µg/ml.

In a more recent study, the chemometric Partial least square method has been implied by *Pretty Falena Atmanda* Kambira *et al*. (2020). 0.1N NaOH was used as a common solvent. A wavelength range of 200-500nm (UV-Visible combined) was used for recording the absorbance with an interval of 1nm [82]. Software 'UV Probe v2.52' was used for interpreting the data. The Root mean square of error cross-validation (RMSEC) values are 0.125 and 0.087.

CONCLUSION

At present, various analytical methods are available for the simultaneous estimation of combination drugs, yet further studies regarding the same should be performed to develop newer, simpler, economic, and robust methods with good linearity and recovery. UV-visible spectroscopy offers a straightforward, less time-consuming, accurate, and very sensitive approach for estimating various medication combinations for which no method of estimation has yet been published.

This compilation study will provide the researchers working in the field with extensive knowledge and data about the already developed UV spectroscopic methods and will assist them further in their research **(Table 2).**

Table 2. Estimation examples of different combinations of paracetamol

S. NO.	STUDIES	METHOD USED	WAVELENGTH (nm)		LINEARITY LIMIT (µg/ml)		SOLVENT USED
			λ1	λ2	DRUG	PCM	
PARACETAMOL + ETODOLAC							
1	Shailaja *et al*. (2015) [28]	Simultaneous Equation Method	252	227	5.00-15.00	6.25-18.75	Triethylammonium phosphate buffer pH 10
2	Alpa *et al*. (2013) [29]	Derivative Spectroscopic Method	280	247	2.00-18.00	5.00-25.00	Methanol and water (60:40)
3	Saikh *et al*. (2017)	Derivative Spectroscopic Method	280	247	2.00-18.00	5.00-25.00	Methanol and water (60:40)
4	Balan *et al*. (2011) [31]	Simultaneous Equation Method	223.5	242.5	2.00-10.00	2.00-14.00	Phosphate buffer pH 7.4
PARACETAMOL + DICLOFENAC SODIUM							
1	Sharma *et al*. (2010) [33]	Simultaneous Equation Method	247	276	2-40	2-40	1.0 M Urea
		Q-Analysis	268	276			
		Difference Spectroscopy	259, 294	245, 249			
2	Jain & Sharma, (2010) [34]	Derivative Spectroscopy	247	276	2-40	2-40	1.0 M Urea

		Area Under Curve	245-249	276-280			
		Multicomponent Method	247	276			
3	Sharma *et al*. (2011) [35]	Simultaneous Equation Method	247.8	261.1	5-35	5-35	5 M Urea
4	Gupta *et al*. (2019) [36]	Simultaneous Equation Method	243	276	5-25	5-25	8 M Urea
		Q-Analysis	264.4	276			
		Derivative Spectroscopy	243	276			
5	Phaneemdra & Nagamalleswari (2012) [37]	Derivative Spectroscopy	275.6	242.69	2-10	5-25	Phosphate Buffer pH 6.8
		Simultaneous Equation Method	243	281			
6	Ganesh *et al*. (2015) [38]	Simultaneous Equation Method	247	276	6-30	6-30	Distilled Water
		Q-Analysis	247	265			
7	Patel *et al*. (2020)[39]	Simultaneous Equation Method	247	276	6-30	6-30	Distilled Water
8	Sebaiy *et al*. (2020) [40]	Absorption Subtraction	227	267	7.5-45	4-22	Methanol 90%
		Difference Spectroscopy	283, 270	251, 240			
		Derivative Spectroscopy	273	254			
9	Sebaiy *et al*. (2020) [42]	H-Point Essay	225	265	7.5-45	4-22	Methanol 90%
10	Saheb *et al*. (2004) [41]	Difference Spectroscopy	230, 254	260, 292			Methanol
		PARACETAMOL + IBUPROFEN					
1	Gondalia *et al*. (2010) [43]	Simultaneous Equation Method	224	248	4-14	2-12	Methanol
2	Harshini *et al*. (2014) [44]	Simultaneous Equation Method	240	220	2-20	1-15	Ethanol
3	Gaikwad *et al*. (2017) [45]	Simultaneous Equation Method	240	220	2-50	2-80	0.1 N NaOH
4	Tejashree *et al*. (2020) [46]	Simultaneous Equation Method	256	222.4	5-30	5-30	Methanol
		Q-Analysis	256	226.4			
5	Ostwal *et al*. (2012) [47]	Q-Analysis	222.4	226.4			Phosphate Buffer pH 5.8
6	Tirunagari et, al. (2013) [48]	Q-Analysis	221.8	213.8	2-21	2-14	Phosphate Buffer pH 7.2
7	Hassan (2008) [49]	Derivative Spectroscopy	230	290	5-100	10-100	Methanol
8	Hoang *et al*. (2014) [50]	Derivative Spectroscopy	249.3	242	12-32	20-40	Phosphate Buffer pH 7.2
9	Omray *et al*. (2007) [52]	Difference Spectroscopy	220, 231	241, 255			Ethanol

10	El-Maraghy & Lamie (2019) [53]	Difference Spectroscopy	210.6, 216.4	236, 248	2-50	2-20	Methanol
			PARACETAMOL + DOMPERIDONE				
1	Kapil *et al*. (2009) [54]	Simultaneous Equation Method	250	285	0.8-5	5-30	Methanol
2	Babar *et al*. (2012) [55]	Simultaneous Equation Method	243.4	284.12	15-30	11-16	Methanol
		Q-Analysis	243.4	270			
3	Appasaheb *et al*. (2013) [56]	Simultaneous Equation Method	258	292	5-30	5-30	0..1 N NaOH
		Difference Spectroscopy	247, 269	288, 296			
		Area Under Curve	284-302	242-275			
4	Mali *et al*. (2016) [57]	Area Under Curve	262-304	220-274	5-25	5-25	Methanol
		Simultaneous Equation Method	286	248			
		Derivative Spectroscopy	262	297			
			PARACETAMOL + ACECLOFENAC				
1	Mishra & Garg (2006) [59]	Simultaneous Equation Method	275	256	1-10	1-10	Ethanol
		Q-Analysis	275	230			
2	Pawar *et al*. (2010) [60]	Simultaneous Equation Method	274	248	1-5	5-25	Methanol
3	Jain *et al*. (2007) [61]	Simultaneous Equation Method	276	249	1-30	2-25	Methanol
		Q-Analysis	276	270			
4	Gharge *et al*. (2010) [62]	Simultaneous Equation Method	276	245	5-40	2-20	Methanol 80%
		Q-Analysis	276	267.5			
5	Mahaparale *et al*. (2007) [63]	Difference Spectroscopy	221.5, 257	261, 278	2-20	5-40	Methanol
		Area Under Curve	224-260	254-294			
6	Basnett *et al*. (2019) [64]	Difference Spectroscopy	214, 242	245, 270	3-10	3-40	Methanol
7	Nikam *et al*. (2008) [65]	Derivative Spectroscopy	268	256	10-50	10-50	Methanol
8	Chaudhari *et al*. (2014) [66]	Simultaneous Equation Method	276	248	2-20	3-30	Methanol: Distilled Water
		Derivative Spectroscopy	276	248			
9	Kumar *et al*. (2011) [67]	Q-Analysis	275.4	266.1	1-35	1-15	Methanol
10	Mishra *et al*. (2014) [68]	Q-Analysis	268	238	5-50	5-50	2 M Urea & 5 M Sodium Acetate (20:30)

		Derivative Spectroscopy	238	268			
PARACETAMOL + CAFFEINE							
			λ Range	**Δλ**	**DRUG**	**PCM**	
1	Aktaş and Kitiş, (2014) [70]	Principal component regression (PCR), Partial least-squares regression (PLS), Artificial neural networks (ANN)	205-305	0.1nm	-	-	0.1 N HCl
2	Dinç & Baleanu (2002) [69]	PCR, PLS	215-285	15nm	-	-	0.1 M HCl
3	Uddin *et al*, (2019) [71]	PLS, ANN	205-300	1nm	-	-	Methanol
			λ1	**λ2**	**DRUG**	**PCM**	
4	Tavallali & Sheikhaei, (2009) [72]	H-Point Essay	453	453	0.1-3.0	1.5-7.0	Acetic Acid Buffer pH 5
5	Vichare *et al*., (2010) [73]	Simultaneous Equation Method	243	273	2-32	2-16	Distilled Water
		Q-Analysis	243	259.5			
6	Sharma *et al.* (2015) [74]	Difference Spectroscopy	234, 249	26, 281	3-18	10-60	Methanol
PARACETAMOL + ASPIRIN							
1	Samnani *et al.* (2007) [77]	Simultaneous Equation Method	225	244			Double Distilled Water (DDW) / Methanol / 0.1N HCl
2	Ghulam *et al.* (2010) [78]	Simultaneous Equation Method	257	265	2-64	2-64	0.1N HCl + Methanol (1:1)
PARACETAMOL + PIROXICAM							
1	Shirkhedkar *et al*. (2008) [80]	Q-Analysis	320	257	4-40	4-12	0.01N NaOH
2	Kambira *et al*. (2020) [82]	PLS	200 to	500	-	-	0.1N NaOH

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