A Review on Analytical methods for the Simultaneous Estimation of Aceclofenac, Paracetamol and Serratiopeptidase in bulk and combined dosage forms

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***Abstract*—The simultaneous estimation of Aceclofenac, Paracetamol, and Serratiopeptidase in bulk and combined dosage forms has gained significant importance due to their widespread use in the management of pain and inflammation. Numerous analytical methods have been developed to ensure accurate, precise, and reproducible quantification of these drugs in multicomponent formulations. This review summarizes various analytical techniques including UV-visible spectrophotometry, Reversed-phase High-Performance Liquid Chromatography (RP-HPLC), High-Performance Thin-Layer Chromatography (HPTLC) and stability-indicating methods reported for the combined analysis of these three drugs. The article highlights method development strategies, selection of wavelengths, mobile phases, chromatographic conditions, validation parameters and the advantages and limitations of each method. Special emphasis is placed on sensitivity, specificity, linearity, range and robustness as per ICH guidelines. This review aims to provide comprehensive insights into existing analytical approaches that can support routine quality control and research applications for Aceclofenac, Paracetamol, and Serratiopeptidase in Pharmaceutical formulations.**

***Keywords*—Aceclofenac, Paracetamol, Serratiopeptidase, UV Spectrophotometry, RP-HPLC, HPTLC.**

I. INTRODUCTION:[1-5]

Aceclofenac, Paracetamol, and Serratiopeptidase are widely prescribed in combination for their complementary therapeutic actions in pain, inflammation, and musculoskeletal disorders. Aceclofenac is a non-steroidal anti-inflammatory agent that is a preferential COX-2 inhibitor primarily employed for its potent anti-inflammatory and analgesic properties. It acts by selectively inhibiting cyclooxygenase-2 (COX-2), thereby reducing the synthesis of prostaglandins responsible for pain and inflammation. Paracetamol (Acetaminophen) is a para-amino phenol derivative used as analgesic and antipyretic agent for the symptomatic relief of mild to moderate pain and fever. Serratiopeptidase (Serrapeptase) is a proteolytic enzyme derived from Serratia marcescens, widely used to combat various kinds of inflammation and inflammatory disorders. recognized for its anti-inflammatory disorders. The combination of these three agents offers a synergistic effect, providing faster and more effective relief in conditions such as osteoarthritis, rheumatoid arthritis, postoperative pain, dental pain, and soft-tissue injuries. Due to their co-administration in

multicomponent formulations, the development of reliable analytical methods for their simultaneous estimation is crucial. Accurate quantification ensures quality control, therapeutic efficacy, and patient safety. Over the years, several analytical techniques such as UV-spectrophotometry, HPLC, RP-HPLC, and HPTLC have been reported for the determination of Aceclofenac, Paracetamol, and Serratiopeptidase in combined dosage forms. This review focuses on summarizing these analytical approaches, highlighting their optimization parameters, validation criteria, and practical applicability in pharmaceutical analysis.

Mechanisam of Action:[6-11]

Aceclofenac is a nonsteroidal anti-inflammatory drug (NSAID) that works mainly by inhibiting cyclooxygenase (COX-1 and COX-2) enzymes, which are responsible for converting arachidonic acid into prostaglandins—the key mediators of pain, inflammation.By reducing prostaglandin synthesis,

Aceclofenac decreases pain, swelling, and redness. It shows preferential inhibition of COX-2, providing effective anti-inflammatory and analgesic activity

Additionally, Aceclofenac suppresses inflammatory cytokines (like TNF-α and IL-1β), reduces oxidative stress, and exhibits a chondroprotective effect, helping to slow cartilage degradation in arthritis.
Paracetamol (acetaminophen) is a centrally acting analgesic and antipyretic agent that exerts its effect mainly within the central nervous system. Its mechanism of action involves the inhibition of prostaglandin synthesis by blocking a variant of the cyclooxygenase enzyme (COX-3) in the brain and spinal cord. Unlike non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol shows minimal peripheral inhibition of COX enzymes, which explains its weak anti-inflammatory activity and low risk of gastric irritation. It also acts on the hypothalamic heat-regulating center, promoting vasodilation and sweating to enhance heat loss, thereby reducing fever. Furthermore, paracetamol is believed to activate descending serotonergic pathways, which enhance the modulation of pain at the spinal level. Through these combined central actions, paracetamol effectively reduces pain and fever without significant effects on platelet function or gastric mucosa.

Serratiopeptidase is a proteolytic enzyme that works by breaking down inflammatory proteins such as bradykinin, histamine, and serotonin at the site of inflammation. This action helps to reduce swelling, pain, and edema. It also promotes the drainage of inflammatory fluids by thinning mucus and exudates, thus speeding up tissue repair. In addition, serratiopeptidase decreases neutrophil migration and cytokine release, which helps control inflammation without affecting prostaglandin synthesis. Overall, it provides anti-inflammatory, analgesic, and fibrinolytic effects, making it useful in post-surgical swelling and joint pain.

UV–Visible Spectroscopy[12-13]
UV–Visible spectroscopy is a simple and widely used analytical technique that measures the absorption of ultraviolet and visible light by chemical substances. Molecules that contain chromophores absorb specific wavelengths of light, leading to electronic transitions such as $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$. The amount of light absorbed is directly proportional to the concentration of the analyte, as described by Beer–Lambert's law, making the technique highly suitable for quantitative analysis.

It is commonly used in pharmaceutical analysis, biochemistry, environmental monitoring, and quality control due to its high sensitivity, rapid analysis, minimal sample preparation, and cost-effectiveness. UV–Visible spectroscopy is also frequently used to determine λmax, purity of compounds, and estimation of drugs in single or combination dosage forms.

UV–Visible spectroscopy works on the principle that molecules absorb UV or visible light when electrons in their chromophores transition from lower to higher electronic energy levels. When a beam of light passes through the sample, part of it is absorbed and part transmitted. The instrument measures this decrease in intensity as absorbance. According to Beer–Lambert's law ($A = \varepsilon bc$), absorbance is directly proportional to the concentration, path length, and molar absorptivity of the substance. Because of its accuracy and reproducibility, this technique is routinely used for both qualitative and quantitative estimations of pharmaceutical compounds.

Reverse-Phase HPLC (RP-HPLC)[14-15]
Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) is the most widely used chromatographic technique for the separation, identification, and quantification of pharmaceutical compounds. In RP-HPLC, the stationary phase is non-polar (commonly C18 or C8 bonded silica), while the mobile phase is relatively polar, typically a mixture of water, buffer, and organic solvents such as methanol or acetonitrile.

Separation occurs based on hydrophobic interactions: less polar (more hydrophobic) compounds retain longer on the column, while more polar compounds elute earlier. RP-HPLC provides excellent resolution, high sensitivity, reproducibility, and suitability for thermally unstable and non-volatile compounds. It is widely used for routine quality control, impurity profiling, and simultaneous estimation of multicomponent drug formulations.

Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) operates on the principle of hydrophobic interactions between the

analytes and a non-polar stationary phase (commonly C18) while using a polar mobile phase such as water, methanol, or acetonitrile. When a sample mixture is injected into the system, components with higher hydrophobicity interact more strongly with the stationary phase and are retained longer, while less hydrophobic or polar compounds elute earlier. Separation occurs due to these differences in retention time.

As the mobile phase passes through the column under high pressure, analytes are differentially partitioned between the stationary and mobile phases. Gradient or isocratic elution can be used to optimize separation. Detection is commonly done using UV or PDA detectors. RP-HPLC provides high resolution, sensitivity, reproducibility, and is widely used for the analysis of pharmaceuticals, especially compounds with moderate to high polarity.

High-Performance Thin-Layer Chromatography (HPTLC) [16]

HPTLC is an advanced form of thin-layer chromatography that offers improved resolution, reproducibility, and sensitivity. It uses pre-coated plates with uniform particle size and automated sample application, development, an densitometric scanning. HPTLC is simple, cost-effective, and suitable for analyzing multicomponent formulations such as Aceclofenac, Paracetamol, and Serratiopeptidase.

Table: 1 Reorted Spectroscopic and Chromatographic methods of Aceclofenac and Its Combination

Sr. No.	Method	Title and Abstract		
1.	UV	UV- Spectrophotometric Determination of Aceclofenac in Tablets[18]		
		Linearity	2-10 µg/ml	
		Detection wavelength	203 nm	
		r^2	0.9981	
		Solvent	Methanol	
2.	UV	Validated Spectroscopic Method for Estimation of Aceclofenac from Tablet Formulation[19]		
		Linearity	2-20 µg/mL	
		Detection wavelength	273 nm	
		r^2	0.9998	
		Solvent	Methanol	
3.	UV	Spectrophotometric simultaneous Determination of paracetamol and Aceclofenac in Tablet Dosage Form[20]		
		Drugs	Paracetamol	Aceclofenac
		Linearity	12.5 µg/Ml	2.5 µg/mL
		Detection wavelength	247 nm	276 nm
		r^2	0.9990	0.9990
		Solvent	Methanol	Methanol
4.	UV	Simultaneous Spectrophotometric Estimation of Diacerein and Aceclofenac in tablet dosage form[21]		
		Drugs	Diacerein	Aceclofenac
		Linearity	2-14 µg/Ml	4-28 µg/mL
		Detection wavelength	258.5nm	274 nm
		r^2	0.9998	0.9995
		Solvent	Methanol	Methanol
5.	RP-HPLC	Simple RP-HPLC method for Aceclofenac quantitative analysis in pharmaceutical tablets[22]		
		Column	C8 column (250 × 4.6 mm , 5 µm particle size), LaChrom, Hitachi, Japan with C8 guard column (23 mm X	

			4 mm; 3 µm), LaChrom, Hitachi, Japan
		Mobile Phase	Phosphate Buffer: methanol (30:70% v/v)
		Detection wavelength	272 nm
		Flow rate	1.0 ml/min
6.	RP-HPLC	Reversed-Phase HPLC Determination of Aceclofenac in Bulk Powder and its Pharmaceutical Tablets[23]	
		Column	Eurosphere-100 C18, column (250 mm × 4.5 mm ID, 5 µm) (Knauer, Germany)
		Mobile Phase	Acetonitrile: methanol :phosphate buffer (30:17:53)
		Detection wavelength	280 nm
		Flow rate	1.0ml/ min
7.	RP-HPLC	Stability indicating HPLC method for simultaneous determination of Drotaverine and Aceclofenac[24]	
		Column	RP-18 column(250×4.6 mm,5 µm particle size)
		Mobile Phase	0.1% trifloro acetic acid : acetonitrile (45:55)
		Detection wavelength	275 nm
		Flow rate	1 ml/min
8.	RP-HPLC	RP-HPLC Method development and validation for the simultaneous estimation of Aceclofenac and Rabeprazole Sodium in the bulk and marketed formulation[25]	
		Column	C18 (250 x 4.6mm dimensions 5µm particle size)
		Mobile Phase	Methanol : Water : Acetonitrile (60:30:10 v/v)
		Detection wavelength	283 nm
		Flow rate	1 min/ml
9.	HPTLC	HPTLC Method Development And Validation Of For Aceclofenac in Bulk And Marketed Dosage Forms[26]	
		Stationary Phase	Precoated silica gel 60 F254 TLC plates
		Mobile Phase	N-butanol: 1, 4-Dioxane in the ratio (6.5:3.5 v/v)
		Detection wavelength	275 nm
10.	HPTLC	Simultaneous HPLC Determination of Paracetamol and Aceclofenac in Tablet Dosage Form[27]	
		Stationary Phase	Precoated silica gel $60F_{254}$
		Mobile Phase	Toluene : isopropyl alcohol :ammonia (20:20:3v/v)
		Detection wavelength	254 nm

Table: 2 Reported Spectroscopic and Chromatographic methods of Paracetamol and Its Combination

Sr. No.	Method	Title and Abstract	
1.	UV	UV Spectrophotometric Method Development and Validation for Quantitative Estimation of Paracetamol[28]	
		Linearity	2-12 µg/ml
		Detection wavelength	244 nm
		r^2	0.9999
		Solvent	Methanol
2.	UV	UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation[29]	
		Linearity	5-30 µg/Ml
		Detection wavelength (nm)	257 nm

		r^2	0.998	
		Solvent	Methanol	
3.	UV	Simultaneous Estimation of Paracetamol and Aceclofenac in Tablet Dosage form using UV Spectroscopy[30]		
		Drugs	Paracetamol	Aceclofenac
		Linearity	3-30 µg/mL	2-20 µg/mL
		Detection wavelength	248 nm	276 nm
		r^2	0.999	0.998
		Solvent	Methanol	Methanol
4.	RP-HPLC	Analytical Method Development and Validation of Simultaneous Estimation of Paracetamol, Aceclofenac and Serratiopeptidase by RP-HPLC[31]		
		Column	Phenomenex C18 (250mm× 4.6mm, 5µm)	
		Mobile Phase	Heptane-1-Sulphonic acid:acetronitrile (90:10v/v)	
		Detection wavelength	226 nm	
		Flow rate	1 ml/min	
5.	RP-HPLC	Method development and validation of Paracetamol drug by RP-HPLC[32]		
		Column	C18 column [4.6×250mm, particle size 5µm]	
		Mobile Phase	ACN :Water (25:75)	
		Detection wavelength	207 nm	
		Flow rate	1 ml/min	
6.	RP-HPLC	A validated RP- HPLC method for the simultaneous estimation of Paracetamol and Naproxen in tablet formulation[33]		
		Column	Eclipse XDB C18 column (150 ×4.6 mm i.d., 5 µm)	
		Mobile Phase	Water (pH - 2.5 adjusted with orthophosphoric acid): acetonitrile (87:13)	
		Detection wavelength	263 nm	
		Flow rate	1.0 ml/min	
7.	HPTLC	Simultaneous determination of Paracetamol and Ibuprofen from combined dosage formulation by HPTLC method[34]		
		Stationary Phase	Silica gel 60F, 20 x10 cm,Merck no. 5642	
		Mobile Phase	Ethyl acetate: acetone: butanol : ammonia in (30: 40: 30: 10 v/v)	
		Detection wavelength	254 nm	
8.	HPTLC	Validated Stability Indicating HPTLC of Paracetamol[35]		
		Stationary Phase	Silica gel 60F-254 as the stationary phase.	
		Mobile Phase	Toluene: methanol: triethylamine (6.5: 4.0: 0.1 v/v/v)	
		Detection wavelength	243 nm	
9.	HPTLC	High-performance thin-layer chromatographic determination of Paracetamol, Diclofenac, and Caffeinein combined tablet dosage form[36]		
		Stationary Phase	Silica gel aluminum plate 60 F254 (10×10) with 250 µm	
		Mobile Phase	Ethyl acetate: Methanol: ammonia (8: 2:0.1 v/v/v)	
		Detection wavelength	263 nm	

Table 3: Reported Spectroscopic and chromatographic methods for Serratiopeptidase and Its Combination

Sr. No.	Method	Title and Abstract		
1.	UV	New Visible Spectrophotometric Method for Estimation of Serratiopeptidase from Tablet Formulations[37]		
		Linearity	0.5-5 µg/mL	
		Detection wavelength	531 nm	
		r^2	0.9993	
		Solvent	Phosphate buffer	
2.	UV	Formulation and development of Serratiopeptidase enteric coated tablets and analytical method validation by UV Spectroscopy[38]		
		Linearity	25–150 µg/mL	
		Detection wavelength	265 nm	
		r^2	0.999	
		Solvent	Distilled water	
3.	UV	Development and Validation of UV Spectroscopic First Derivative Method for simultaneous Estimation of Aceclofnac and Serratiopeptidase in Synthetic Mixture[39]		
		Drugs	Aceclofenac	Serratiopeptidase
		Linearity	10-50 µg/mL	1.5-7.5 µg/Ml
		Detection wavelength	274 nm	279 nm
		r^2	0.986	0.989
		Solvent	Methanol :water	Methanol: water
4.	UV	Development and Validation of Analytical Method for Simultaneous Estimation of Diclofenac Sodium and Serratiopeptidase in Bulk and Tablet Dosage Form[40]		
		Drugs	Serratiopeptidase	Diclofenac Sodium
		Linearity	25-150 µg/Ml	5-30 µg/Ml
		Detection wavelength	295.20 nm	264.20 nm
		r^2	0.9989	0.9994
		Solvent	Water	Water
5.	HPLC	HPLC Spectroscopy Method Development and Validation For Simultaneous of Ibuprofen, Paracetamol, Serratiopeptidase[41]		
		Column	C8, 150× 4.6mm, 5um	
		Mobile Phase	Acetonitrile: water (70:30v/v) 0.1% glacial acetic acid	
		Detection wavelength	227 nm	
		Flow rate	1.0 ml/min	
6.	RP-HPLC	Analytical Method Development and Validation of Simultaneous Estimation of Paracetamol, Aceclofenac and Serratiopeptidase by RP-HPLC[42]		
		Column	Phenomenex C18 (250mm× 4.6mm, 5µm)	
		Mobile Phase	Heptane-1-Sulphonic acid: acetronitrile (90:10v/v)	
		Detection wavelength	226 nm	
		Flow rate	1 ml/min	
7.	RP-HPLC	Development and Validation of new Chromatographic Method for the simultaneous estimation of Serratiopeptidase, Aceclofeanc and Paracetamol by RP-HPLC[43]		
		Column	C18 Column (4.6×150mm× 5µm)	
		Mobile Phase	Water: methanol (50:50v/v)	
		Detection wavelength	327 nm	

		Flow rate	0.4 ml/min
8.	RP-HPLC	Development and Validation of RP-HPLC method for simultaneous estimation of Paracetamol, Aceclofenac and Serratiopeptidase in combined tablet dosage form[44]	
		Column	C18 column (250mm× 4.6mm, 5µm)
		Mobile Phase	Acetonitrile :water (70:30 v/v)
		Detection wavelength	227 nm
		Flow rate	1.0 ml/min
9.	HPTLC	Simultaneous HPLC Determination of Paracetamol and Aceclofenac in Tablet Dosage Form[45]	
		Stationary Phase	Silica gel 60F_{254} on aluminium plate
		Mobile Phase	Acetonitrile :toluene :acetic acid (6:4:0.1 v/v)
		Detection wavelength	270 nm
10.	RP-HPLC	A new Reversed-Phase High-Performance Liquid Chromatography Method for the Simultaneous estimation of Serratiopeptidase and Diclofenac Sodium in bulk and Tablet Dosage Form[46]	
		Column	Kromasil C18 column (250mm× 4.6 mm, 5µm particle size)
		Mobile Phase	O-phosphoric acid: methanol: acetonitrile (5:4:1 %v/v)
		Detection wavelength	270 nm
		Flow rate	1.0 ml/min

II. CONCLUSION

The review presents the reported spectroscopic and chromatographic methods developed and validated for the estimation of Aceclofenac, Paracetamol and Serratiopeptidase. As per the literature review, various analytical methods have been reported for the estimation of these drugs in bulk and combined dosage form also it was found that mobile phase containing methanol, water, acetonitrile, toluene, orthophosphoric acid phosphate buffer were common for the most of the chromatographic methods and methanol and water were used for most of the spectroscopic methods.

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