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Stability indicating HPLC method development and validation for quantification of salicylic acid impurity in acetaminophen aspirin caffeine oral solid dosage forms

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ABSTRACT: The present study discusses the development of simple isocratic reversed-phase HPLC method was developed and validated for the quantification of Salicylic acid, itwas amajor degradant impurity of Aspirin, in the Acetaminophen Aspirin Caffeine Tablet dosage formulations. The analytes were separated on a C18 Zodiac column (100mm x 4.60 mm i.d., 5 µm particle size). A mobile phase, water, MeOH, Glacial acetic acid in the ratio of (690:280:30) (v/v/v) were used. The column temperature 45℃ and flow rate 2.0ml/mint.The UV detection was made at 302 nm for Salicylic Acid. The HPLC method was obtained highly specific with linearity concentration ranging between 0.373-112.079µg/mL of salicylic Acid, and the correlation coefficient was found to be >0.999 The method showed high accuracy more than 97%. The limit of detection(LOD) for Salicylic acid was0.41µg/ml, respectively. The limit of quantification (LOQ) for salicylic acid was 1.25µg/ml,respectively. Validation acceptance criteria were met in all cases. The developed method was validated as per international ICH guidelines with respect to specificity, linearity, precision, accuracy and robustness. In stress studies the Acetaminophen Aspirin and caffeine tablets were found to be sensitive inAcid, Base stress conditions and Oxidation stress condition. Theproposed method was established to be precise and stability indicating as no interfering peaks of degrades and excipients were observed. The proposed method is suitable for purpose in quality control laboratories forquantitative analysis of salicylic acid in Acetaminophen Aspirin and caffeine tablet, as it is simple and rapid with tremendous precision and accuracy.

KEYWORDS: Acetaminophen Aspirin Caffeine,Salicylic Acid, HPLC, method development, Validation

I. INTRODUCTION

The FDA recognized the combination of Acetaminophen (250 mg), Aspirin(250mg) and Caffeine (65 mg) as safe and effective in treating acute headaches, especially migraine, and was also considered effective by the American Headache Society. This combination is well-tolerated in episodic tension-type headaches and considered superior to acetaminophen; all components in this combination medication are considered safe during breastfeeding and can be taken orally for acute migraine attacks. The mechanism of action of this combination is from the accumulation of the components' effects; each component has a different mechanism of action. This activity reviews the indications, contraindications, activity, adverse events, and other key elements of acetaminophenaspirincaffeine therapy in the

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clinical setting as it relates to the essential points needed by members of an interprofessional team managing the care of patients with migraine headaches and its related conditions and sequelae.
The three-drug combination AAC was the first OTC medicationregistered for the treatment of migraine in the United States in 1998and is recommended as first-linemigraine treatment by the US,German, Swiss, and Austrian headache societies [1].

Acetaminophen

Acetaminophen, or paracetamol(Fig. 1), chemically designated as n-(4-hydroxyphenyl) ethanamideis a white crystalline powder which is soluble in boiling water, freely soluble in alcohol. Its chemical formula is $C_8H_9NO_2$.The most used analgesic and antipyretic drug worldwide. The drug may be used without a prescription and is the drug of choice in patients who cannot have treatment with non-steroidal anti-inflammatory drugs (NSAIDs) as well as those patients with bronchial asthma, peptic ulcer disease, hemophilia, salicylate-sensitized people, children under 12 years of age, and pregnant or breastfeeding women. Acetaminophen is used alone or combined with other medications to treat acute primary headaches; it is combined with aspirin and caffeine for migraine, tension-type headaches, and tramadol for cluster headaches.

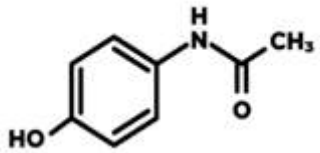

Fig. 1 Acetaminophen

Aspirin

Aspirin, or Acetylsalicylic Acid(Fig. 2) chemically designated as 2-acetyloxybenzoic acid,is a white crystalline powder which is soluble in boiling water, freely soluble in alcohol. Its chemical formula is $C_9H_8O_4$. Aspirinis metabolized into salicylic acid (SA) and used at doses of less than or equal to 325 mg per day to reduce the risk of cardiovascular events, whereas it is used at higher doses (500 to 1000 mg as a single dose and 3000 to 4000 mg per day) to reduce pain, fever, and inflammation.

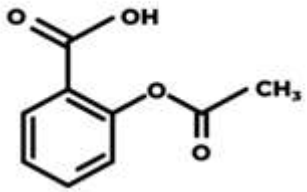

Fig. 2 Aspirin

Caffeine

Caffeine (Fig. 3) chemically designated as 1,3,7-trimethylpurine-2,6-dione is a white crystalline powder which is soluble in water and freely soluble in alcohol.Its chemical formula is $C_8H_{10}N_4O_2$. Caffeineis legal, cheap, and not regulated in almost all parts of the world; it can be found as over the counter (OTC) medication or in other sources such as coffee, tea, sodas, gum, and candy.

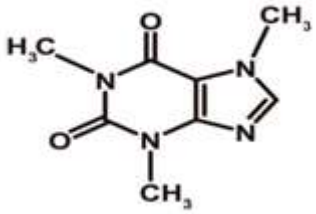

Fig.3 Caffeine

Salicylic acid

Salicylic acid (Fig. 4) is chemically 2-hydroxy benzoic acid that has antiseptic, antifungal and keratolytic properties. It is used to treat warts, psoriasis, corns and other skin conditions. It works by softening and loosening dry, scaly, or thickened skin so that it falls off or can be removed easily [2].Salicylic acid is white or almost white, crystalline powder or white or colorless, acicular crystals, slightly soluble in water, freely soluble in ethanol, sparingly soluble in methylene chloride, Its molecular formula is $C_7H_8O_3$.

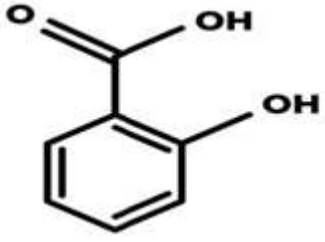

Fig.4 Salicylic acid

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The estimation of assay of the drug product is critical to understand the drug product's behavior.The lower assay or purity results could affect bioavailability and safety. Therefore, theestimation of drug products is essential in regulated pharmaceutical industry.

Till date severalanalytical methods, majorly for the estimation ofsalicylic acid using different analyticaltechniques.(Vincenzo puccialet al., 2004; Dalibor satin sky et al., 2004;Rendon Mi et al.,2010;Sanjay A. Patila et al.,2015;Vichare V. S et al., 2017;Ahmed Mahdi Saeedet al.,2018; Ling liang et al., 2020;). In these methods there are a few HPLC methods available for the quantification of individual salicylic acid.There were no reported analytical methods for simultaneous quantification of Salicylic acid impurity and qualification of Acetaminophen aspirin caffeine in combination dosage forms in presence of their degradation products. Hence an author made an attempt to develop stability indicating specific, sensitive, accurate and precise RP-HPLC method for quantification of salicylic acid impurity with isocratic elution mode.

II. MATERIALS AND METHODS

Chemicals and reagents

The AR grade Glacial Aceticacid procuredfrom Ankit Raj organochemicals LTD, India. The HPLC grade of Methanol (J.T. beaker) with certified purity of99.9% was purchased from Avantor performance materials, Hyderabad, Telangana. High quality In-House purity water was used for the experiments (TOC <500ppb, pH about 7.0, Conductivity < 1.0µs/cm, finally exposed to UV radiation and followed filtered through 0.2µm filter). Salicylic acid was procured from Sigma-Aldrichpharma chemprivate limited,Ahmadabad, India.

Instruments and software

Waters HPLC system Alliance e2695 separation module with auto injector, temperature controller for sample storage and column was used for current analysis. The signal output was observed through Empower 3. The LC column is Zodiac packC18,100 mm_4.6mm,5.0 µm, is manufactured by waters. Analytical balance model AX205 (make: Mettler Toledo), sonicator (make: ENERTECH), Rotary shaker (make: REMI; model: RS – 24BL) were employed in this work.

Preparation of diluent

Mixed Methanol and Formic acid in the ratio of (990:10) (v/v).

Preparation of standard solution

Accurately weigh and transfer about 50 mg of Salicylic Acid standard into a 50-mLvolumetric flask. Add diluent about 60% volume of the flask, sonicate to dissolve, dilute to volume with diluent. The concentration is about 1000 µgmL^{-1}, dilute to volume with diluent, Pipet 2.0 mL of Salicylic Acid stock solution into a 100-mL volumetric flask, dilute to the volume with diluent and mix well. The finalconcentration ofSalicylic Acid is about 20 µgmL^{-1}.**Preparation of Sample**

Accurately weigh and transfer 20tablets offine powder of Acetaminophen Aspirin and Caffeineequivalent to 500mg of Aspirin into a 200-mL volumetric flask. Add 150mlof diluent flask, keep on rotary shaker for30minutes with intermittent shaking. Dilute to volume with diluent, mix well and label as SampleSolution. The concentration is about 2500µgmL^{-1} of Aspirin.

Method Development and optimization

The main aim of the current method was to separation of Acetaminophen having impurities i.e. Acetaminophen related compound- B, Acetaminophen related compound C, Acetaminophen related compound- D, Acetaminophen related compound J, p-Aminophenol and Aspirin having impurity salicylic acid as main degradant impurity and Caffeine having impurity theobromine, and placebo in Acetaminophen Aspirin and Caffeine tablets. A systematic study of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant for development of the analytical method. Both Salicylic acid and Aspirin were soluble in polar solvents; therefore, RP-HPLC was chosen. The selection of stationary phase has been done based on back pressure, resolution, peak shape, theoretical plates and day to day reproducibility in retention time resolution between Aspirin and Salicylic acid peaks. After evaluating all these factors, Enable Zodiac pack C18column (100 × 4.6 mm 5µ) was chosen for the analysis. The selection of bufferwas based on the chemical structure of selected drug molecules

Using mixed water,methanol and glacial acetic acid in the ratio of (690:280:30) (v/v/v) as mobile phase.HenceKnown impurities wereseparated and resolution between aspirin and

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salicylic acid were well satisfied. See**Table 1** for Method development experiments.

A series of aqueous and organic modifiers used as a diluent, finally decided to methanol and glacial acetic acid in the ratio of 990:10(v/v) as a diluent, in this diluent aspirin was stable. All impurities of Acetaminophen, Aspirin, & Caffeine are separated from Aspirin & Salicylic acid.Blank and placebo interference was not observed at retention time of any impurity of AcetaminophenAspirinCaffeine. So,a methodwas found specific for estimation of salicylic acid.

Table :1 Optimized Method development experiments

Mobile phase	Column	Flow rate(ml/min), elution mode, column temp and injection volume	Observation	Inference
0.02M Ammonium dihydrogen phosphate pH 3.5 as buffer and methanol in the ratio of (60:40) (v/v) as mobile phase	Zodiac C18, 100mm_ 4.6 mm, 5µm	1.0ml/mint isocratic 30℃,10µL	All peaks were not separated and coeluted with same retention time	Rejected
0.02M potassium dihydrogen phosphate and add adjust pH 2.5 and methanol in the ratio of 70:30 as mobile phase.	Zodiac C18, 100mm_ 4.6 mm, 5µm	1.0ml/mint isocratic 30℃,10µL	All peaks were not showing any separation and coeluted with same retention time	Rejected
Transferred 800ml of water and 200ml methanol in the ratio of (80:20) (v/v) as mobile phase	Zodiac C18, 100mm_ 4.6 mm, 5µm	1.5ml/mint Isocratic 45℃,10µL	all peaks were not separated from salicylic acid peak, and resolution between the aspirin and salicylic acid were not satisfied	Rejected
Transferred 700ml of water and 300ml methanol in the ratio of (70:30) (v/v) as mobile phase	Zodiac C18, 100mm_ 4.6 mm, 5µm	2ml/mint Isocratic 45℃,10µL	All peaks were separated from salicylic acid and aspirin, but the resolution was not satisfied between the aspirin and salicylic acid.	Rejected
Transferred 690ml of water add 280ml of methanol and 30ml of glacial acetic acid and	Zodiac C18, 100mm_ 4.6 mm, 5µm.	2ml/mint Isocratic, sample cooler 5℃, 45℃,10µL	Salic acid and aspirin and placebo peaks were well separated and all impurities detected	Approved

Chromatographic conditions

The chromatographic separation was achieved using mixed water, methanol and glacial acetic acid in the ratio of (690:280:30) (v/v/v) as the mobile phase. The flow rate was 2.0mL min^{-1}. Column temperature 45℃ and sampler cooler 5℃. The LC column was used Zodiac packC18,100 mm _4.6mm,5.0 µm. The isocratic elution, the injection volume was 10µL and detection of components were made at 302 nm.

III. METHOD VALIDATION

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The method was validated based on International Conference on Harmonization (ICH) Q2(R1)

Guidelines (ICH 2005). (Sreenivas, P. et al., 2022, Pippala et al., 2023,). Validation parameters included linearity, precision, accuracy, specificity and forced degradation (Jyothsna et al., 2024a,2024b, Teja Kami Reddy et al., 2024).

System suitability

System suitability parameters (tailing factor,number of theoretical plates)were assessed by injecting a blank diluent followedby salicylic acid solution (20 µgmL^{-1}).

Specificity

Prepared blank, known impurities spec level and placebo as per the optimized testprocedure and verified the interference of peaks at retention time of activepeak (Vaishnavi 2024).

Linearity

To prove the linearity of the optimized method, A sequence of concentrations was made for salicylicacid (0.373µg mL^{-1} to 112.019µg mL^{-1}) from the concentration range (0.5% to 150%) by using suitable amounts of the mixture of stock solutions. A curve was created by mapping the peak response and concentration.

Sensitivity

The limit of detection (LOD) and Limit of quantification (LOQ) values for salicylic acid was established by calibration curve method. The LOD and LOQ results were shown in Table 3 and calculated using the formulas

$$LOQ = \frac{10 \times \text{Standard Deviation}}{\text{Slope of Calibration Curve}}$$

$$LOD = \frac{3.3 \times \text{Standard Deviation}}{\text{Slope of Calibration Curve}}$$

Precision

Precision provides a degree of agreement between the individual test results by applying the procedure or method to the homogeneous sample (Sreenivas et al. 2024a and Sreenivas et al. 2024b,PrasannakumarLankalapalli et al.2024). Typically, it is expressed as variance, SD. In normal conditions, it is a measure of the degree of repeatability or reproducibility.

Six individual samples were taken from a homogenous mixture of samples and were spiked with them at the 100% level to check the reproducibility. The precision of the analysis was calculated on the RSD values of the six impurity- spiked samples results. To check the ruggedness (intermediate precision) of the method, the analysis was repeated on different days. The precisions for the analysis of each individual impurity at 100% concentrationlevel.The sample and standard solutions stable up to 24 hours on Benchtop.

Accuracy

They prove the accuracy of optimized method prepared three levels (LOQ,50, 100 & 150%). Preparedeach sample in triplicatepreparation rangefrom 50% level and 150% level and concentrations 37.438µgmL^{-1},112.314µgmL^{-1}, and six sample preparation ranges from LOQ and 100% level and concentration3.530µg mL^{-1} and 74.876µg mL^{-1} of Salicylic acid. The recovery was calculated in terms of the amount added againstthe amount estimated.

Robustness

The robustness of the proposed RP-HPLC method was carried out by altering the experimental conditions such as analytical column, column temperature, flow rate,and mobile phase composition. The method was established by introducing small changes in experimental conditions like wavelength ± 1 nm.

Degradation studies

Degradation studies were conducted to determine specificity and stability-indicating properties of the suggested method. That these stress conditions were performed under the following acidic, alkaline, oxidative, and Water conditions. Weighed 20 tablets of AcetaminophenAspirinCaffeineand calculated the average weight. Transferred into mortar vessel and crushed into fine powder.

weighed and transferred equivalent to 500 mg of Aspirininto 200 ml volumetric flask, added 10 mL of 0.5N HCl or 0.5N NaOH , mixed, kept on water bath at 60°C for about 30 min, neutralized with 10 mL of 0.5N NaOH or 0.5 N Hcl , then added about 120 ml of diluent, kept on rotary shaker for 30 minutes, diluted to volume with diluent and mixed well.

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For peroxide sample Weighed and transferred equivalent to 500 mg of Aspirininto 200 ml volumetric flask, added 10 mL of 10% peroxide solution, mixed, kept on water bath at 60°C for about 30 min, then added about 120 ml of diluent, kept on rotary shaker for 30 minutes, diluted to volume with diluent and mixed well. For water sample Weighed and transferred equivalent to 500 mg of Aspirininto 200 ml volumetric flask, added 10 mL of water solution, mixed, kept on water bath at 60°C for about 30 min, then added about 120 ml of diluent, kept on rotary shaker for 30 minutes, diluted to volume with diluent and mixed well. Centrifuged above all degradation solutions at 3500 rpm for 10 minutes. Supernatant solution was injected into HPLC system.

IV. RESULTS AND DISCUSSIONS

System suitability

In system suitability study the peak showed good tailing factor (1.05) and sufficient plate count (5352). The retention time was 4.522minutes, thearea of % RSD below 5%. The results were shown in table 2.

Table 2systemsuitability study

Parameter	Result	Acceptance limit
Retention time of Salicylic acid peak	4.522	NA
Average Area of six standard Injections	152703	NA
USP tailing factor of Salicylic acid	1.05	NMT 2.0
USP Plate count of Salicylic acid	5352	NLT 2500
% RSD of six replicate injections of Salicylic acid peak is	0.17	NMT 5.0

Specificity

The method proved to be specific with no interference of mobile phase or impurities or excipients. (Fig.5-11). The peak purity for the standard and sample solution was 1.666 and 1.723(Table 3)

Figure :5 Typical Chromatogram of Blank

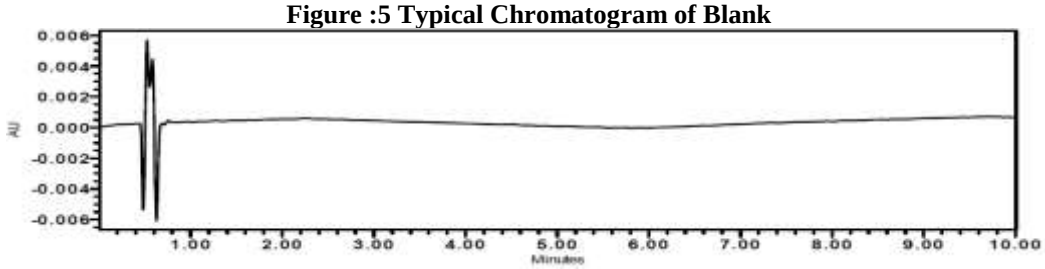

Figure :6 Typical Chromatogram of Aspirin Placebo

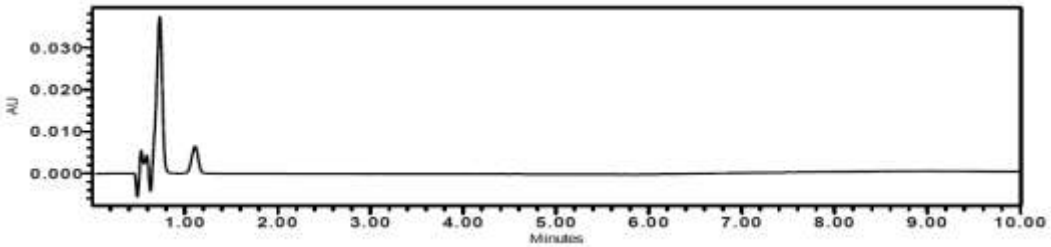

Figure :7 Typical Chromatogram of Salicylic acid Standard

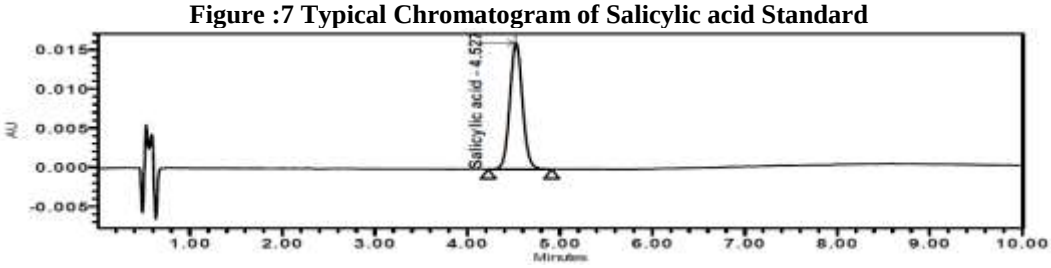

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Figure:8 Typical Chromatogram of Sample

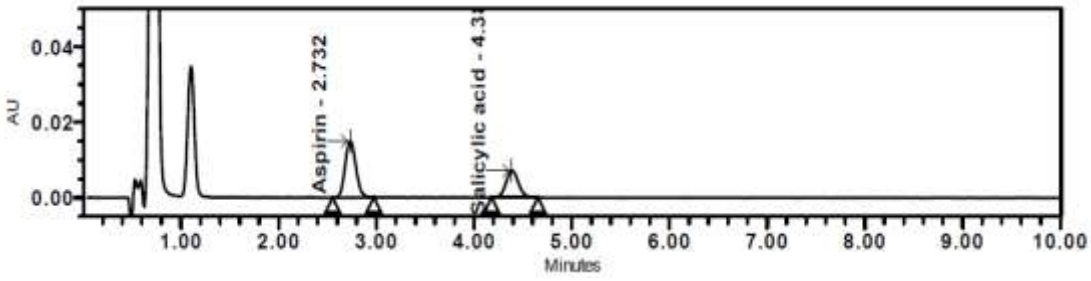

Figure:9Typical Chromatogram of spiked sample

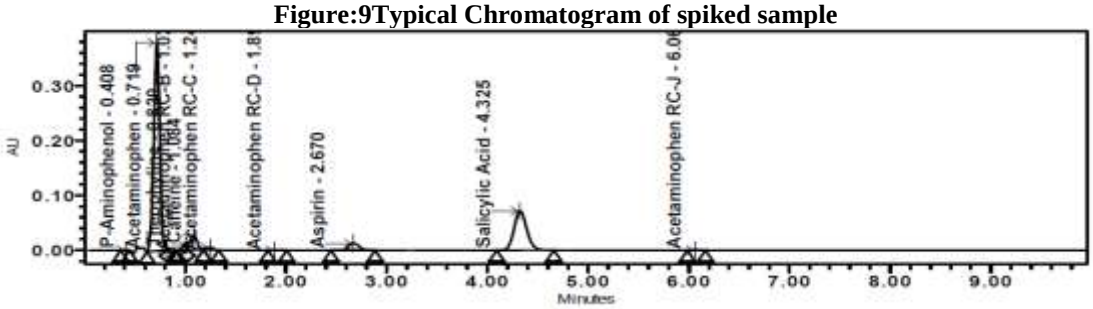

Figure:10Typical Chromatogram of Acetaminophen

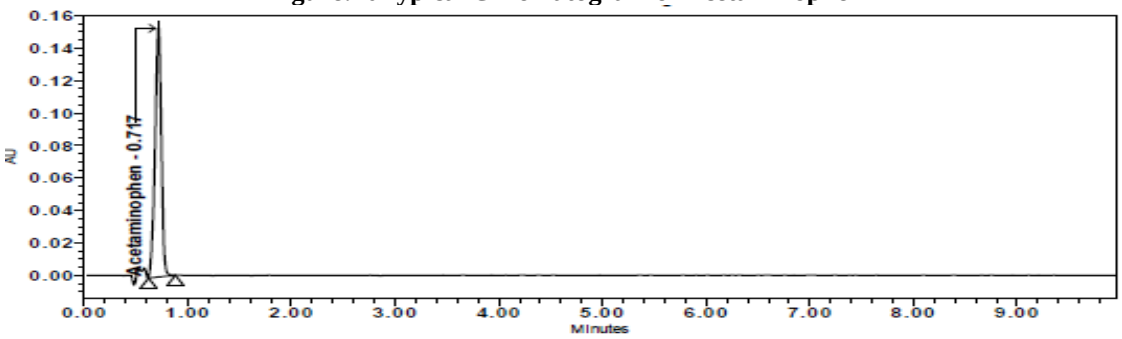

Figure:11Typical Chromatogram of Caffeine

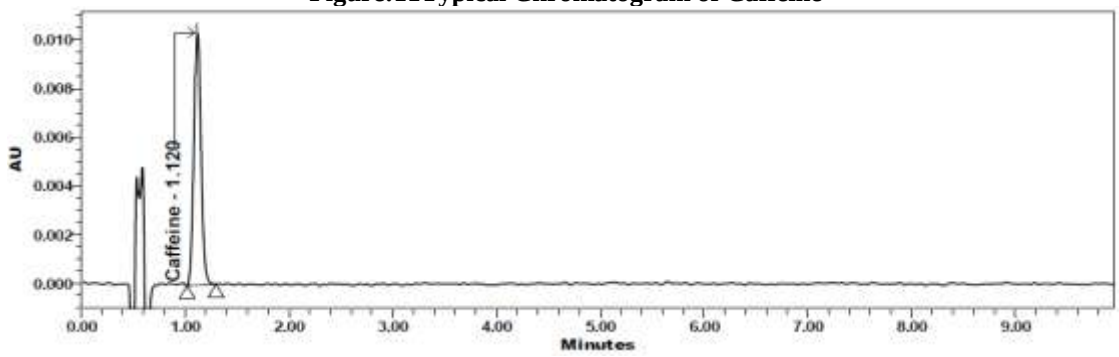

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Table :3 specificity study

Blank	No interference at RT of Salicylic acid in blank
Standard solution	Peak purity was 1.666
Placebo	No interference at RT of Salicylic acid in placebo
Impurities	No interference at RT of Salicylic acid in impurities
Sample solution	Peak purity was 1.723

Linearity

For linearity range, correlation coefficient was 0.99999.(Table 4).

Table: 4 Linearity results

S.No	**Level (%)**	**Salicylic acid**	
		Conc. (ppm)	**Area**
1	0.5	0.373	2227
2	2	1.494	10818
3	5	3.734	27706
4	10	7.468	56590
5	50	37.340	279425
6	100	74.679	570197
7	125	93.349	704206
8	150	112.019	845935
Correlation coefficient (r)			**0.9999**
Slope			**7567.71728**
Intercept			**461.6978**
Bias			**0.08**
STEYX (Standard Deviation)			**2671.46872**

Sensitivity

The limit of detection (LOD) and Limit of quantification (LOQ) values for salicylic acid was established by calibration curve method (Table 5).

Table: 5 sensitivity results

STEYX from Linearity solution	**Slope from Linearity solution**	**LOQ Conc.(ppm)**	**LOD Conc.(ppm)**
2671.46872	7567.71728	3.53	1.16

Precision

Repeatability and intermediate precision studied showed %RSD 1.6 and 1.4.(Table6)

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Table: 6 precision study repeatability intermediate precision

Samplename	%(w/w) salicylic acid repeatability	%(w/w) salicylic acid intermediate precision
Sample 1	3.307	3.256
Sample 2	3.285	3.201
Sample 3	3.398	3.301
Sample 4	3.349	3.289
Sample 5	3.418	3.334
Sample 6	3.327	3.268
Average	**3.347**	**3.275**
%RSD	**1.6**	**1.4**

Accuracy

The mean recovery should be within the range of 85.0 % -115.0 % and found to be within the range.

Table: 7 Accuracy study

Recovery level	Recovery	Average Recovery	% RSD
LOQ-1	102.1	102.0	0.8
LOQ-2	101.6		
LOQ-3	100.9		
LOQ-4	102.9		
LOQ-5	101.7		
LOQ-6	103.1		
50%-1	101.0	101.9	0.9
50%-2	101.8		
50%- 3	102.9		
100%-1	101.4	101.5	0.8
100%-2	100.7		
100%-3	102.3		
100%-4	100.8		
100%-5	101.2		
100%-6	102.8		
150%- 1	101.7	101.3	0.4
150%- 2	100.9		
150%- 3	101.4		

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Robustness

Making a deliberate changein flow rate, column temperature and wavelength was taken place and RSD found to be less than 5, specify that the method was robust.

Table :8 Robustness study

Parameter	Deviation n=3	Salicylic Acid				
		USP tailing	USP plate count	RRT salicylic acid w.r.to Aspirin	Average area of standard	%RSD
Flow rate(ml/mint)	2.2ml	1.09	5511	1.623	144960	1.15
	1.8ml	1.11	5834	1.627	176514	1.43
Column temperature (℃)	50℃	1.12	5416	1.579	155384	0.78
	40℃	1.09	5896	1.671	155977	1.50
Wavelength(nm)	304nm	1.09	5568	1.62	155245	0.71
	300nm	1.10	5489	1.63	154729	1.44

Degradation studies

No purity flag was observed in all stressed conditions. This indicates that there is no interference from degradants in quantifying Aspirin/ Salicylic acidin Acetaminophen/Aspirin/Caffeine tablets and there is no interference at the retention time of Aspirin/Salicylic acid from stressed placebo. Thus, this method is considered as "Stability Indicating".

2500 ppm of Aspirin and salicylic acid were processed at 302 nm due to maximum absorbance of Salicylic acid, and Aspirin was processed at 275 nm due to maximum absorbance of Aspirin for mass balance(100ppm).The results were shown in table 9.

Table: 9 Forced degradation conditions studies

Nature of Stress	% of Salicylic acid	% Assay of Aspirin stressed Test	Mass Balance	Salicylic acid		Aspirin		
				PA	PT	PA	PT	PF
Unstressed	0.04	100.8	NA	5.099	7.053	0.244	0.472	No
Acid	10.75	87.7	97.7	0.069	0.262	0.260	0.487	No
Base	4.02	94.5	97.7	0.098	0.309	0.173	0.363	No
Peroxide	3.37	98.4	100.9	0.180	0.368	0.261	0.421	No
Water	0.79	101.0	100.9	0.413	0.664	0.188	0.429	No

Discussion

The current method was to separation of Acetaminophen having impurities Acetaminophen related compound- B, Acetaminophen related compound C, Acetaminophen related compound-D, Acetaminophen related compound J, p-Aminophenol and Aspirin having impurity salicylic acid as main degradant impurity and Caffeine having impurity theobromine, and placebo(Fig.5-10 andTable 1). The method showed good system suitability with a tailing factor as 1.05, %RSD for peak area less than 5 and theoretical plates more than 5352 (Table 2).Salicylic acid is insoluble in water and freely soluble in organic solvents like alcohol, but aspirin was not stable in only methanol so added 1% of Formic acid. Hence, mixed methanol and formic acid in the ratio of(990:10)(v/v) was chosen as a diluent for sample preparation. Salicylic acid was adequately extracted in the diluent, and excipients did not cause interference in the analysis. mixed water, methanol and glacial acetic acid in the ratio of (690:280:30) (v/v/v) as the mobile phase. Presence of methanol in the mobile phase ensured no plug formation after injection of sample solution. The analysis was carried out using a UV detector at a wavelength of 302 nm where salicylic acid showed maximum absorbance. High peak purity with no interference from solvent system or excipient or impurities indicated results specific to the salicylic acid(Fig. 5(a-g) and Table 3). The specifications for the column used in this study (zodiac C18 column, 100 mm × 4.6 mm, 5 µm) are frequently found in columns used in laboratories and are commonly

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available in the market. Use mobile phase and carefully selected composition of organic solvents ensured early elution of Salicylic acid. The low retention time of 4.52 min ensured quick analysis. With short run time, more samples could be analyzed in the given time, making the process cost-effective. With correlation coefficient of 0.9999, linearity between peak area and analyte concentration. (Table 4). The method was sensitivity the limit of detection (LOD) and Limit of quantification (LOQ) values for salicylic acid was established by calibration curve method. (Table 5),the method was precise with respect to intra-day and inter-day precision (Table 6), the method was found to be accurate with test results close to the true values (Table 7). The method was robust. Change in wavelength, flow rate and column temperature did not affect the analysis significantly (Table 8) this method considered as a stability indicating, In stress studies the Acetaminophen Aspirin and caffeine tablets in aspirin was found to be sensitive in Acid, Base stress conditions and Oxidation stress condition(Table 9).

V. CONCLUSION

A simple, economical, rapid and stability indicating RP—HPLC method has been effectivelyoptimized for estimation of Salicylic Acidin Acetaminophen Aspirin Caffeine Tablets. The optimized method was further validated for specificity, linearity, selectivity, precision,accuracy, robustness and ruggedness parameters. The method was developed and validated in the quality control lab for stability analysis.

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Author contributions
Prasanna Kumar lankalapalli, Pranitha Sambu, Srinivasulu Kasa and Rama Krishna Myneni conceptualized and collected the necessary data from literature, designed, developed the analytical methodology, performed the validation, wrote the main manuscript draft, Hareesh Divadari, Teja kami Reddy and Vijaykumar chollety, Ashok Morsuperformed the validation studies and wrote the main manuscript draft. All three authors have proofread the whole manuscript and are responsible for the data integrity. The authors declare that they have read and agreed to the published version of the manuscript.

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REFERENCES

[1]. Ailani J, Burch RC, Robbins MS, Board of Directors of the American Headache Society. The American HeadacheSociety Consensus Statement: update on integrating new migraine treatments into clinical practice. Headache. 2021 Jul;61(7):1021-1039. doi: https://doi.org 10.1111/head.14153

[2]. Vincenzo Pucci, Roberto Mandrioli, Maria Augusta Raggi, Salvatore Fanali(2004) Reversed-phase capillary electrochromatography for the simultaneous determination of acetylsalicylic acid, paracetamol, and caffeine in analgesic tablets, 2004 Feb;25(4-5):615-21. doi: https://doi.org/10.1002/elps.200305631.

[3]. Dalibor satin sky, Isabel Neto, Petr Solich, Hana Sklenarová, M. Conceição, B. S. M. Montenegro, Alberto N. Araujo(2004) Sequential injection chromatographic determination of paracetamol, caffeine, and acetylsalicylic acid in pharmaceutical tablets ;27(7-8):529-36. Doi: https://doi.org/10.1002/jssc.200301644

[4]. Rendon Mi, Berson DS, Cohen JI, Roberts WE, Starker I, Wang B. Evidence and considerations in the application of chemical peels in skin disorders and aesthetic resurfacing. J Clin Aesthet Dermatol. 2010 Jul;3(7):32-43. https://pmc.ncbi.nlm.nih.gov/articles/PMC2921757/

[5]. Sanjay A. Patila, Bhikan J. Khairnara, Dhananjay V. Maneb, Bhata R. Chaudharia A (2015) validated stability-indicating HPLC related substances method for salicylic acid in bulk drug and dosage form published World J Pharm Sci 2015; 3(6): 1025-1282

[6]. Vichare V. S., Choudhari V.P., Reddy MV (2017)" Simultaneous Estimation of Mometasone Furoate and Salicylic Acid in Topical Formulation by UV-Visible Spectrophotometry", Int J Chem Sci 2017; 15(2):129doi:10.2174/1573412914666180418162143

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 6 Nov - Dec 2024, pp: 747-758 www.ijprajournal.com ISSN: 2456-4494

[7]. Ahmed Mahdi Saeed, Mohammed Jassim Hamzah, Noor Qasim Ahmed (2018) Quantitative assay of Aspirin and (Salicylic acid and heavy metals as impuraties) in IRAQI's market aspirin tablets using different analytical methods September 2018International Journal of Applied Pharmaceutics 10(5):167-172doi: http://dx.doi.org/10.22159/ijap.2018v10i5.26820

[8]. Ling Liang, Yaojing Huang, Wenren Liu, Weiyuan Zuo, Fanggui Ye and Shulin Zhao State Colorimetric Detection of Salicylic Acid in Aspirin Using MIL-53(Fe)Nanozyme Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, College of Chemistry and Pharmaceutical Science of Guangxi Normal University, Guilin, China September 2020 Volume 8 - 2020 | https://doi.org/10.3389/fchem.2020.00671.

[9]. Jyothsna, M., Vaishnavi, Ch., Leela Prasad, K., Surya Prakash Rao, P. et al., (2024a) Quality by design tool evaluated green stability-indicating UPLC content determination method for the Olanzapine and Samidorphan dosage form. Microche J., 197, 109835. https://doi.org/10.1016/j.microc.2023.109835.

[10]. Jyothsna, M., Vaishnavi, Ch., Phani Raja, K., Naresh Kumar, K., et al. (2024b). Quality by Design Tool Assessed Ultraperformance Liquid Chromatography Method for the Analysis of Remogliflozin and Teneligliptin in Oral Dosage Form. ACS Omega,2024 Mar 7;9(11):12553–12563.https://pubs.acs.org/doi/10.1021/acsomega.3c04589.

[11]. Pippalla, S., Nekkalapudi, A.R., Jillellamudi, S.B., Reddy, M.P., Kumar, C.V. (2023). A stability-indicating, reversed-phase HPLC method for quantification of assay and organic impurities in doxycycline hyclate bulk and parenteral dosage forms. Biomed Chromatogr., 2023 e5626. https://doi.org/10.1002/bmc.5626.

[12]. Sreenivas, P., Arjuna Rao, N., Suresh Babu, J. (2022) Stability Indicating RP-UPLC Method for Quantification of Glycopyrrolate, Methylparaben and Propylparaben Assay in Liquid Oral Formulation. American Journal of Analytical Chemistry, 13, 538-552. https://doi.org/10.4236/ajac.2022.1312036.

[13]. Sreenivas, P., Arjuna Rao, N., Venugopal Reddy, K. (2024) A validated stability-indicating reversed-phase-UPLC method for simultaneous estimation of promethazine hydrochloride, methylparaben, propylparaben and sodium benzoate assay of cough suppressant and antihistamine liquid oral dosage forms. 14 July 2024 https://doi.org/10.1002/bmc.5944.

[14]. Teja Kamireddy, Pranitha Sambu, Prasanna Kumar Lankalapalli, Rama Krishna Myneni, Hareesh Divadari (2024) Stability-indicating RP-HPLC method development and validation for the quantification of amlodipine besylate and valsartan tablets in solid oral dosage form Biomed Chromatogr. 2024 Sep 26: e601714https://doi.org/10.1002/bmc.6017 Publication Date: Sep 26, 2024.

[15]. Vaishnavi Ch. (2024). Stability indicating reversed-phase-high-performance liquid chromatography method development and validation for pyridostigmine bromide and sodium benzoate in oral solution. Biomed Chromatogr.,2024 Mar ;38(3):e5800. https://doi.org/10.1002/bmc.5800.

[16]. Prasanna kumarLankalapalli,Teja Kamireddy, Pranitha Sambu(2024) A Simple and Rapid HPLC Method Development for Quantification of Sodium Benzoate Content in Chlorzoxazone Oral Solid Dosage Forms.Biomed chromatogr.,2024 November https://doi.org/10.1002/bmc.6048.