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Analytical Method Development and Validation of Stability Indicating RP-HPLC Method For Assay and Related Substances of Paracetamol and Caffeine Effervescent Tablets: Review

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ABSTRACT:
The ongoing, interdependent tasks related to the departments of research and development, quality control, and quality assurance are the creation and validation of analytical methods. Analytical methods are essential for risk management and equivalency assessments. It aids in the development of acceptability standards unique to a product and the consistency of outcomes. Validations establish whether the analytical process is appropriate for the goal for which it was designed. A review of the literature indicates that paracetamol and caffeine can be determined individually or in combination with other medications using analytical techniques based on UV spectroscopy, RP-HPLC, and HPTLC. The parameters were checked for correctness, precision, robustness, and other aspects of analytical validation in accordance with ICH guidelines. The discovered techniques can be applied to the bulk and tablet dosage form analysis of paracetamol and caffeine in effervescent form since they are straight forward, sensitive, and repeatable. The review also outlines the applicability and constraints of numerous published analytical techniques for paracetamol and caffeine analysis. Stability studies have to be conducted on time and in compliance with the standards issued by the World Health Organization, International Conference of Harmonization, and other bodies. The capacity of a pharmaceutical product to meet the physical, chemical, microbiological, toxicological, protective, and informational requirements of a specific formulation in a particular container-closure system is known as its stability. It also ensures that a pharmaceutical product's effectiveness, safety, and performance will be maintained for the duration of its shelf life, which is seen as a need for approval and acceptability. The requirement for ongoing quality and purity monitoring of medications and products gave rise to various stability test techniques. This review covers the various drug substance stability categories as well as the applicability of various techniques for drug substances that are stable, the applicability of various methods used to test the pharmaceutical product's stability, guidelines released to test the pharmaceutical product's stability, protocols for stability testing that outline the essential elements of a well-managed and regulated stability test, and other aspects of stability are all covered in this review. The researcher working on paracetamol and caffeine in effervescent form will greatly benefit from this thorough review study.

KEYWORDS: Validation, Stability, HPLC.

INTRODUCTION:

Caffeine and paracetamol (acetaminophen) are two commonly used pharmacological ingredients with different medical uses. Caffeine is recognized for its stimulating effects and capacity to augment the analgesic effects of other medications, whereas paracetamol is a powerful analgesic and antipyretic substance. Pharmaceutical companies frequently combine coffee with paracetamol to increase the effectiveness of pain treatment[1]. But for quality assurance and therapeutic efficacy, it is crucial to make sure that these chemicals are precisely and accurately quantified in pharmaceutical formulations. Because of its adaptability and effectiveness in separating complicated combinations, High-Performance Liquid Chromatography (HPLC) stands out as a main analytical technique for the validation of paracetamol and caffeine. High sensitivity and specificity simultaneous quantification is made possible by HPLC, which uses a stationary phase and a mobile phase to separate analytes according to how they interact with the stationary phase[2].Furthermore, UV detection and HPLC are frequently combined, utilizing the UV absorption characteristics of caffeine and paracetamol for measurement[3]. Liquid chromatography-mass spectrometry (LC-MS), an alternative to HPLC, shows promise as a potent method for the validation of caffeine and paracetamol[4]. By combining the mass analysis and separation powers of mass spectrometry with liquid chromatography, LC-MS offers improved sensitivity and selectivity for the identification and quantification of compounds[5].

Mass spectrometry's great specificity makes it possible to identify paracetamol and caffeine in complicated matrices with little to no interference from other substances[6]. Even if chromatographic methods are more precise than ultraviolet (UV) spectroscopy, the latter is nevertheless a useful approach for verifying the presence of caffeine and paracetamol in pharmaceutical formulations. The basis of UV spectroscopy is the analytes' absorption of UV light, which enables quantitative analysis using the Beer-Lambert law. UV spectroscopy offers a quick and affordable way to quantify by detecting the absorbance of caffeine and paracetamol at particular wavelengths[7].Moreover, Fourier-transform infrared (FTIR) spectroscopy provides additional data to support the validity of coffee and paracetamol. Based on the molecules' functional groups' absorption of infrared light, FTIR spectroscopy helps to clarify structures. This method helps determine the identity of caffeine and paracetamol as well as evaluate if pharmaceutical formulations contain any contaminants or degradation products[8].To summarize, the verification of the presence of caffeine and paracetamol in pharmaceutical formulations requires the application of exacting analytical methods such UV, FTIR, LC-MS, and HPLC. Together, these techniques guarantee drug quantification's accuracy, precision, and dependability, protecting pharmaceutical industry product quality and therapeutic efficacy[9].

MATERIAL AND METHOD

RP-HPLC, caffeine, and paracetamol were the keywords used in a literature search that was conducted across many database sources, including PubMed and ScienceDirect. To locate the most pertinent articles for the purpose of this review, the search was customised by applying the appropriate filter.

REVIEW OF LITERATURE:

Ahmed Mahdi Saeed et al., Aspirin (ASP), caffeine (CAF), and paracetamol (PCM) have all been determined using an accurate, effective, and repeatable isocratic reversed-phase high-performance liquid

chromatographic (RP-HPLC) approach that has been developed and validated in this study. Using a Shimadzu RP-HPLC, model LC-20-A, Japan, a phenomenex C18 column (L, 15 cm, I.D., 0.46 cm, and Size of particle, 5µm) was used to separate a mixture of medicines as reference material and in formulation tablets. The experiment and design helped to optimize the eluent phase. Elution was carried out using an eluent phase that consisted of a mixture of water (H2O), methanol (MOH), and acetonitrile (ACN) at a ratio of (60: 20: 20, V/V), with acetic acid to adjust the pH to 4.0 and a pumping flow rate of 0.5 mL/min. At 254 nm, the medicines were separated using a UV-VIS detector for 8 min. The recorded elution times for PCM, CAF, and ASP were 3.980, 4.366, and 6.894 minutes, respectively. PCM, CAF, and ASP were tested at concentrations ranging from 1 to 100µg/mL in order to validate the procedure. It was discovered that the procedure was precise, strong, and resistant to even the smallest experimental variations in the wavelength, pH, and flow rate values. The LOD values for PCM, CAF, and ASP were determined to be 0.01µg/mL, 0.03µg/mL, and 0.05µg/mL, respectively. For PCM, CAF, and ASP, the LOQ values were 0.033µg/mL, 0.099µg/mL, and 0.165µg/mL, respectively. [10]

Boyka G. Tsvetkova et al., For the simultaneous measurement of caffeine and paracetamol in a model tablet formulation, a high-performance liquid chromatography analytical method has been devised. Using UV detection at 230 nm, the separation was accomplished on a C8 column with a flow rate of 1.0 ml/min. Methanol (65:35 v/v) and 1 mM phosphate buffer pH 7.0 made up the mobile phase. Through examination of analytical criteria such specificity, linearity, accuracy, precision, LOD, and LOQ, the method was validated. For paracetamol and caffeine, the linearity of the technique was examined in the concentration ranges of 31.25-250 µg/ml (r = 0.9999) and 4.06-32.50 µg/ml (r = 0.9998), respectively. It was discovered that the approach was accurate, with recoveries falling between 99.57% and 99.87%. A 500 mg paracetamol and 65 mg caffeine model tablet formulation were the subject of stability tests for one year at 25 ± 2°C and RH = 60 ± 5% (long-term storage) and 40 ± 2°C and RH = 75 ± 5% (accelerated storage). Key physical and chemical characteristics of the tablets, including their mechanical strength, disintegration speed, drug release kinetics, and drug content, were assessed. None of the assessed tablet's attributes showed any appreciable alterations. It was determined that the manufactured pills had good stability qualities, enabling the product to be scaled up and put through clinical investigation. [11]

Hamad M. Adress Hasan, et al., For the simultaneous measurement of ascorbic acid (ASC), methionine (MET), paracetamol (PAR), and caffeine (CAF), a straightforward reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been devised and validated. Using a Brownlee Bio C18 column (250 x 4.6 mm, 5µm) and isocratic elution of water-acetonitrile (85:15) (v/v) mobile phase flowing at 1.0 mL min-1 at room temperature, the stated components are entirely separated. Sequentially, the spectrophotometric detection is done for ASC at 260 nm (2 min), MET at 200 nm (1 min), PAR at 240 nm (1.5 min), and CAF at 270 nm (1.5 min). For each sample, the total chromatographic analysis duration was about six minutes. 40-160µg mL-1, 40-200µg mL-1, 20-400µg mL-1, and 40-160µg mL-1 are the linear range of determination for ASC, MET, PAR, and CAF, respectively. Consequently, the suggested approach can be effectively used to analyse pharmaceutical preparations including the aforementioned medications without any excipient interference. 96.46 to 102.70%, 2.65% for ASC, 96.33 to 103.43 %, 2.93 % for MET, 98.31 to 102.73 %, 2.09 % for PAR, and 95.82 to 102.13 %, 2.68% for CAF are the recovery ranges. [12]

J.T. Franeta et al., This work presents an HPLC method that uses a chromatographic system that includes a Bio Rad 18 01 solvent pump, Rheodine 71 25 injector, and Bio Rad 18 01 UV/Vis Detector to determine the levels of acetylsalicylic acid, paracetamol, caffeine, and phenobarbital in tablets simultaneously. Using

a Bio SiL HL C18, 5 mm, 250/4.6 mm column, separation was accomplished. As a mobile phase, a 25:75 v/v acetonitrile/water mixture that had been pH-adjusted using phosphoric acid was utilized at a flow rate of 2.0ml/min. The UV was detected at a range of 207 nm, 0.01 AUFS. The amount of salicylic acid might be found under the same circumstances. It was decided what the chromatographic characteristics were, including selectivity, resolution, peak asymmetry, capacity factor, and retention times. The validation parameters were found to be satisfactory: linearity (r ~/0.998), intra-day precision (RSD: 0.36}/1.89%) and inter-day precision (RSD: 0.58~/2.18%), sensitivity (LOD: 9/10}/1.7}/10}4 mg ml}1 and LOQ: 2.5/10/4}/5.6}/10}4 mg/ml); accuracy (recoveries: 98.35}/99.14%) and reproducibility (recovery values: 98.74}/102.08% for acetylsalicylic acid, 99.93~/102.11% for paracetamol, 98.25}/102.12% for caffeine, and 98.15}/102.3% for phenobarbital) (RSD: 1.21}/1.85%). The contents of Mellophonium tablets were determined using the suggested HPLC method to include acetylsalicylic acid, caffeine, paracetamol, and phenobarbital. Within 0.99~/1.21%, the obtained RSD values were achieved. [13]

Jinendra Sardiya et al., There are now affordable and straightforward spectrophotometric techniques available for estimating dexibuprofen (DXB) and paracetamol (PCM) from tablet dose forms simultaneously. The solvent used was 95% ethanol. The first method, Method-I, entails creating a Q-absorbance equation at the isoabsorptive point, which is 235.5 nm, and the maximum absorbance point, which is 249.5 nm. The second method, Method-II simultaneous equation method, measures the absorbances at two wavelengths, 223 nm (max of dexibuprofen) and 249.5 nm (max of paracetamol), and the third method, Method-III multicomponent mode of analysis, involves measuring the absorbances at the two wavelengths, 223 nm (max of dexibuprofen) and 249.5 nm (max of paracetamol). For all three methods, the linearity falls between 2–7 g/ml for dexibuprofen and 4–14 g/ml for paracetamol. The methodologies' precision and accuracy were established and verified statistically. Good recovery and reproducibility were demonstrated by all the methods, with % RSD less than 1. All of the methods were discovered to be quick, precise, accurate, and specific, and they may all be effectively used for the regular analysis of paracetamol and dexibuprofen in bulk and mixed dosage forms. [14]

Mohamed Arief S et al., In order to gain some additional advantages over other methods previously developed for this combination, a quick and stability-indicating RP-HPLC approach was created for the simultaneous quantification of paracetamol and caffeine in soft gelatin capsule form. In terms of accuracy, precision, specificity, linearity, robustness, stability of the solution, sensitivity, forced degradation, and system applicability, the method was verified in accordance with ICH guidelines. This was accomplished by maintaining an isocratic condition of mobile phase consisting of phosphate buffer (pH 3.5) and acetonitrile in a ratio of 15:85, v/v over a YMC C18 column (250 × 4.6 mm, 5µm) at a flow rate of 1 mL/minute at room temperature. With correlation coefficient (R2) values for paracetamol and caffeine, respectively, of 0.999 and 1.0, which were within the range of correlation coefficient (R2 > 0.995), the approach demonstrated an outstanding linear response. The acceptability limit of 98.0–102.0% was determined to be within the percent recoveries for two medicines. The percentage RSD values for intermediate precisions, reproducibility, and repeatability were all less than 2.0. In order to determine the stability-indicating characteristic of this method and to provide important information regarding the degradation pathways, degradation products, and how the quality of a drug substance and drug product changes over time under the influence of different stressing conditions, forced degradation of the drug product was carried out. [15]

Nafiu Aminu et al., To determine paracetamol (PCM) and caffeine (CF) simultaneously in solid dose form, a high-performance liquid chromatography (HPLC) approach that is easy to use, affordable, fast,

dependable, and stable-indicating has been designed and validated. A Waters Symmetry W C18 column (5µm, 4.6 × 150 mm) was used for the chromatographic separations. A mobile phase consisting of methanol and water (40:60, v/v) was used, and the elution mode was isocratic, with a flow rate of 0.8 mL/min and ultraviolet (UV) detection set at 264 nm. The column's oven temperature was established and kept at 35 °C. The approach showed good linearity over the concentration ranges of 15–300 µg/mL (PCM) and 2.5–50 µg/mL (CF), with correlation coefficients of 1 and 0.9999 for PCM and CF, respectively, after validation in accordance with International Conference on Harmonization (ICH) guidelines. For PCM and CF, the retention time (tR) was determined to be 2.6 ± 0.001 and 3.5 ± 0.002 minutes, respectively. Analytes were exposed to a range of stress conditions, including acidic and alkaline hydrolysis, as well as oxidative, photolytic, and thermal degradations, in order to undertake extensive stress degradation experiments. It was discovered that the procedure effectively separated the peaks of the analytes from those of the degradation products, with no change in the retention durations. For PCM and CF, the relative standard deviation (RSD) values of all recoveries were less than 1.3%. [16]

Wasim Ahmad et al., The most popular over-the-counter treatment for fever and headache is a fixed-dose combination of paracetamol (PCM) and caffeine (CAF) pills or capsules. This study presented a straightforward, dependable, fast, sensitive, and stability-indicating ultra-performance liquid chromatography (UPLC) analytical technique for evaluating PCM and CAF in pharmaceutical formulations at the same time. The Acquity UPLC® CSHTM C18 column was used to create the UPLC method. The column oven temperature was kept at 35 ± 5 ◦C utilizing isocratic elution using a methanol and water (30:70, v/v) solution. At 272.5 nm, the highest absorbance of PCM and CAF was noted. The PCM and CAF separation process took two minutes to complete at a flow rate of 0.2 mL/min and an injection volume of 1 µL. The suggested UPLC method showed excellent linearity with correlation coefficients of 0.9995 and 0.9999 over the concentration ranges of 40–400 and 7–70 ng/mL for PCM and CAF, respectively, after it was validated in accordance with the ICH criteria. For PCM and CAF, the mean retention periods were found to be 0.82 ± 0.0 and 1.16 ± 0.02 respectively. For PCM and CAF, the corresponding limits of detection and quantification were 16.62 and 3.86, and 50.37 and 11.70, respectively. Acidic, alkaline, oxidative, phytochemical, dry-heat, and wet-heat degradation processes were applied to PCM and CAF. With no changes to retention durations, the technique was found to effectively distinguish the peaks of the analytes from the degradation peaks. The suggested approach is robust, linear, exact, accurate, and particular. It can be used quickly to the quantitative evaluation of pharmaceutical formulations containing PCM and CAF and show stability. [17]

Binh Thuc Tran et al., This study simultaneously measured the amounts of paracetamol (PAR), ibuprofen (IBU), and caffeine (CAF) in tablets using the classical least-squares (CLS) approach in conjunction with molecular absorption spectrophotometric measurement. With a 0.5 nm step, the absorbance spectra of the standard solutions and samples were measured over this wavelength range. Tested on mixed standard laboratory samples with varying ratios of PAR, IBU, and CAF concentrations, the procedure and the CLS-Excel software demonstrated minimal mistakes and satisfactory repeatability. For tablets containing PAR, IBU, and CAF, an analytical method was created. By comparing the mean active ingredient contents in the tablets acquired from the analytical process with the HPLC method, as well as by demonstrating the recovery and repeatability of the analysis results using an actual tablet sample, the procedure's dependability was demonstrated. Comparing the procedure to the HPLC standard method, it is less expensive and simpler. [18]

M. N. Uddin et al., This study used spectrophotometric data to create and compare two chemometric methods for the simultaneous assay of paracetamol (PCT) and caffeine (CAF) in pharmaceutical formulations: partial least-square regression (PLSR) and artificial neural network (ANN). Using an orthogonal experimental design (OED), mixed solutions of standard samples were created with six different doses of caffeine and paracetamol. These mixtures' UV spectra were taken between 205 and 300 nm in relation to a solvent blank, and samples of the digital absorbance were taken at intervals of 1 nm. Six commercially available tablets were used to test the created models after drug concentrations and experimental spectra of 36 mixed solutions were used for model building and validation. ANN outperforms PLSR in terms of prediction efficiency, with R2 values of 99.28% for prediction and 99.13% for validation set. After these two models were successfully applied to commercial pharmaceutical formulations, ANN discovered that the medications met their label claims for caffeine content (77–92%) and paracetamol content (75–86%). Both of the suggested approaches are quick and easy to employ as a substitute analytical method for evaluating the quality of medications. [19]

A. Hakan Aktaş et al., The spectrometric multicomponent analysis of the medication containing paracetamol (PCT) and caffeine (CAF) was conducted without any separation step. Three multivariate calibration-prediction techniques, principal component regression (PCR), partial least squares (PLS), and artificial neural networks (ANN), were applied. The variables' selection was examined. PCR, PLS, and ANN prediction abilities were tested using a series of synthetic solutions with varying concentrations of PCT and CAF. We are highly encouraged to use these procedures for routine medication analysis and quality control based on the investigation's outcomes. The effectiveness of the PCR, PLS, and ANN techniques in producing dependable analysis results has been used to validate them. As a result, 15 artificial mixes with various proportions of PCT and CAF were created as a separate validation set. Three chemometric techniques were proposed for the simultaneous determination of PCT and CAF in their binary mixtures in spectrometric analysis: PCR, PLS, and ANN. Commercial pharmaceutical tablets were successfully treated with these procedures. The application of PCR, PLS, and ANN approaches resulted in the resolution of highly overlapping drug combinations. a choice of working wavelength with strong concentration correlation values caused by interference from extra analytes or the matrix sample outside the working range. The suggested chemometric methods don't require time-consuming chemical separation ahead of time and can be used for routine medication analysis in tablet formulations. [20]

Hemaraj Sharma et al., A dual wavelength spectrophotometric approach that is easy to use, precise, and accurate has been devised for the simultaneous measurement of caffeine and paracetamol in a combination pharmaceutical dosage form. The concentration of the component of interest is exactly proportional to the absorbance difference between two spots on the mixture spectrum. The interfering component has the same absorbance during the selection of two wavelengths, whereas the component of interest exhibits a notable variation in absorbance with concentration. This method was created because, as the literature study indicates, no dual wavelength method had been devised for this combination of medications. The wavelengths that were chosen were 234 nm and 249 nm for the determination of caffeine, and 260 nm and 281 nm for the determination of paracetamol. Since there is relatively little caffeine in the formulation, conventional caffeine API is added to make the caffeine observable in terms of absorbance. We used methanol as a solvent. A regression analysis using Beer's plots revealed a strong association between the concentration ranges of 3–18 µg/mL for caffeine and 10–60 µg/mL for paracetamol. A method's accuracy was found to be between 98% and 102%. The method's precision (within the day, between days, and

between analysts) was found to be within acceptable bounds (% CV < 2). The suggested technique was successfully used to identify these medications in store-bought tablets. [21]

Vijaya Vichare et al., Two straightforward, sensitive, accurate, precise, and accurate UV spectrophotometric techniques have been devised for the simultaneous measurement of caffeine (CAF) and paracetamol (PARA) in pharmaceutical dosage forms. The simultaneous equation method was used in Method A. For the creation of simultaneous equations, the two wavelengths 243 nm (the λmax of paracetamol) and 273 nm (the λmax of caffeine) were chosen. Method B, on the other hand, involved creating the Q-absorbance equation at the isobestic point, or 259. nm. Using these techniques, linearity was seen in the concentration range of 2–16 mcg/ml for paracetamol and 2-32 mcg/ml for caffeine. The suggested techniques have been effectively used to analyse pharmaceutical formulations that contain cited medicines. A recovery study was carried out to verify the approaches' accuracy. ICH guidelines were followed in the validation of the procedures. [22]

Asim Najmi et al., The purpose of this study was to determine the caffeine content of a selection of coffee samples that were available in the local Jazan market in Saudi Arabia using high performance liquid chromatography (HPLC) and an analytical quality by design (AQbD) technique. A Raptor C-18 column-equipped Waters HPLC system was used for the experiment. A mobile phase consisting of acetonitrile and ammonium acetate buffer (10 mM; pH 4.0) in a 90:10 v/v ratio was utilized, with a flow rate of 1 mL/min for 5 minutes at 274 nm, to accomplish chromatographic separation. A central composite design (CCD) was employed in conjunction with AQbD to optimize chromatographic conditions. Stat-Ease Inc.'s Design Expert 13.0.3.0 program, Minneapolis, was utilized using a factorial design of 23 (two factors and three replies). Using ICH and USP guidelines, the HPLC method was validated and found to meet all acceptance criteria. The process was demonstrated to be linear in the dilution range of 2–28 µg/mL. All five samples' caffeine contents (5 g/100 mL) ranged from 20.59 to 25.38 mg, which is below the USFDA's suggested threshold of toxicological concern. [23]

Fernando J. Pereira et al., Three significant medications that are frequently used for a variety of clinical objectives are tramadol hydrochloride (TRA), caffeine (CAF), and paracetamol (acetaminophen) (PAR). The most important thing is to figure out what's within. In this regard, an isocratic RP-HPLC method with photodiode array detection that is rapid, easy to use, and sensitive was created for the detection of paracetamol, caffeine, and tramadol in pharmaceutical formulations. A fluorescence detector device was also used to develop a more sensitive tramadol process. The apparatus utilized was a C18 column with a methanol/phosphate mobile phase. Using photodiode-array detection, the LODs for tramadol hydrochloride, caffeine, and paracetamol were determined to be 0.2 µg/mL, 0.1 µg/mL, and 0.3 µg/mL, respectively. As an alternative, using the fluorescence detector reduced the LOD for tramadol to 0.1 µg/mL. The linear concentration ranges for the same ordered analytes (including the fluorescence detector) are 0.8–270 µg/mL, 0.4–250 µg/mL, and 1.0–300 (0.2–40) µg/mL. These are additional noteworthy analytical figures of merit. The suggested technique was effectively used to quantify the three medications in tablet dose forms. [24]

Kuldeep Delvadiya et al., In the presence of rasagine as an internal standard, this study suggests using liquid chromatography (RPHPLC) for the quantitative measurement of the most commonly prescribed combination of caffeine, propyphenazone, and paracetamol in tablet dose form. A Gracesmart C18 column (5µm, 250mm x 4.6mm i.d.) was used for the chromatography, and the mobile phase consisted of a combination of water and 2-propanol in a ratio of 80:20 v/v. 1% o-phosphoric acid was used to bring the aqueous phase's pH down to 3.0, and a 1.5 ml/min flow rate was kept constant. The absorbance at 210 nm

was used to identify the analytes. For every sample, the chromatographic analysis period was maintained at roughly 20 minutes, during which the compounds propyphenazone, paracetamol, caffeine, and rasagide were eluted at 1.63, 2.36, 3.17, and 11.22 minutes, respectively. The plots of calibration were acquired between. The range of calibration plots for paracetamol, propyphenazone, and caffeine was 3–90µg/ml, 1.5–45µg/ml, and 0.5–15µg/ml, respectively. The approach was determined to be specific, accurate, exact, and simple to use for routine analysis after it was validated in compliance with ICH standards. [25]

Hany Ibrahim et al., In the presence of two PC impurities, namely 4-aminophenol and 4-nitrophenol, two chromatographic methods were validated for the determination of the commonly prescribed analgesic and antipyretic drug combination of propyphenazone (PZ), caffeine (CF), and paracetamol (PC), which was recently integrated into the supportive treatment of COVID-19. The development of a high-performance liquid chromatography method using a "dual-mode" gradient allowed for the achievement of separation by the modification of both the flow rate and the composition of the ternary mobile phase, which comprised acetonitrile, methanol, and water. This allows for a satisfactory resolution in a comparatively shorter analytical time. Zorbax Eclipse XDB column C18, 5µm (250 × 4.6 mm), was used for the analysis, and the UV detector's wavelength was adjusted to 220 nm. The other technique is a thin-layer chromatography densitometry approach, in which the following components of the mobile phase were used to produce the separation: methanol, acetic acid, toluene, ethyl acetate, and chloroform (6: 6: 1: 2: 0.1, by volume). On silica gel 60 F254 plates, densitometric detection was carried out at a wavelength of 220 nm. The developed methods were completely validated in accordance with the ICH criteria and shown their accuracy, robustness, specificity, and suitability for use as methods for routinely showing purity in pharmaceuticals containing PZ and CF in quality control laboratories, or in the analysis of PC in pure form. [26]

Almaz Arage et al., The simultaneous detection of paracetamol, caffeine, phenylephrine, and chlorpheniramine in tablets was accomplished through the development and validation of a high-performance thin layer chromatographic technique that is fast, precise, and selective. Glass plates that had been previously coated with silica gel 60 F254 as a stationary phase were used for the chromatographic analysis. Methanol: n-butanol: toluene: acetic acid (8:6:4:0.2 v/v) was the ideal mobile phase. A 15-minute saturation period was employed with a 10-by-20-cm TLC chamber. The retardation factor (RF) values for paracetamol, caffeine, phenylephrine, and chlorpheniramine were determined to be 0.15 ±0.02, 0.29 ±0.02, 0.50 ± 0.02, and 0.68 ± 0.02, in that order. The wavelength of detection was 212 nm. ICH Q2 (R1) guidelines were followed in the validation research. The calibration plots regression data demonstrated a strong linear relationship, with R^2 5 0.997 for caffeine over the 300–1,500 ng band^{-1} concentration range, R^2 5 0.996 for phenylephrine over the 100–500 ng band^{-1} concentration range, R^2 5 0.996 for chlorpheniramine over the 200–600 ng band^{-1} concentration range, and R^2 5 0.998 for paracetamol over the 400–2,400 ng band^{-1} concentration range. The technique's recovery, precision, and accuracy were confirmed. For caffeine, phenylephrine, chlorpheniramine, and paracetamol, the minimum detectable amounts were determined to be 304.9 ng band^{-1}, 87.88 ng band^{-1}, 117.18 ng band^{-1}, and 143.06 ng band^{-1}, respectively. The limit of quantification was determined to be 923.95 ng band^{-1}, 266.32 ng band^{-1}, 355.11 ng band^{-1}, and 433.53 ng band^{-1}, in the same order. Two marketed tablets were successfully analyzed using the technology in a reproducible and selective way. [27]

Marzieh Rahimi et al., Today, because of the pharmaceutical industry's expansion and the widespread use of pharmaceuticals, it is crucial to monitor drug residues in environmental samples due to their detrimental effects on both persons and the environment. The current study used a quick and easy

technique called ultrasound-assisted emulsification-microextraction in conjunction with high-performance liquid chromatography to simultaneously determine the levels of caffeine and paracetamol in aqueous samples. Effective factors influencing these analytes' extraction efficiencies were examined and optimized, including the kind and volume of the extraction solvent, the pH of the sample solution, the concentration of salt, sonication, and centrifuging durations. The calibration graphs were linear under ideal circumstances, ranging from 30.0 to 1100.0 ng/mL for paracetamol and from 50.0 to 400.0 ng/mL for caffeine. For paracetamol and caffeine, the limits of detection were 9.7 and 15.2 ng/mL, respectively. The suggested technique was effectively used to identify these medications in various aqueous samples. In the current investigation, a USAEME method combined with HPLC was created for the first time to determine caffeine and paracetamol in aqueous samples simultaneously. The suggested technique for detecting traces of these medications in various water sources is sensitive and quick. In contrast to the dispersive liquid-liquid microextraction (DLLME) method, the USAEME method uses less organic solvent by substituting ultrasonic waves for the dispersive solvent. The suggested method's high per-concentration factor makes it possible to monitor extremely low concentrations of target analytes in ambient waters. The usefulness of the method for actual sample analysis is demonstrated by comparing the figures of merit of the current method with those from earlier papers. [28]

E. Dinc et al., Without the need for a chemical separation process, the simultaneous analysis of tablets containing acetaminophen and caffeine was suggested utilizing principal component regression (PCR) and classical least-squares (CLS) approaches. Using a training set of the mixtures of both medications in 0.1 M HCl, the absorbance values at the 15 wavelengths in the spectral area of 215–285 nm were measured to prepare the chemometric calibrations. Acetaminophen and caffeine concentrations in samples were estimated using the chemometric calibrations that were obtained. The program "MAPLE V" was used to carry out the numerical computations. The mean recoveries and relative standard deviations in the CLS and PCR procedures were determined to be 99.5 and 1.29, 99.7 and 1.00% for acetaminophen and 99.9 and 1.92, 100.0 and 1.178% for caffeine, respectively, after applying two techniques to synthetic combinations. We compared our results to those that one of us had previously gotten using the HPLC method as a reference method. Despite the fact that the zero-order spectra of the two medications overlap in the spectral region of 210–310 nm, the chemometric identification of both pharmaceuticals in the synthetic mixture and tablets was successfully accomplished using the CLS and PCR procedures. Compared to the conventional procedures outlined in literature, it was discovered that CLS and PCR approaches, which do not require a preprocessing step such separation, spectra derivation, and division, are more straightforward, accurate, and cost-effective. These methods are quite simple to use; all that is needed is the use of robust software for data processing, abstract vector space manipulation, and regression analysis. Our application of these methodologies for a routine study of two medications in pharmaceutical manufacture is strongly encouraged by the results achieved in this paper. [29]

CONCLUSION:

A crucial component of pharmaceutical quality assurance is the validation of High-Performance Liquid Chromatography (HPLC) procedures for the measurement of caffeine and paracetamol in combination medication compositions. In order to evaluate the efficacy and safety of pharmaceutical goods, researchers must guarantee the accuracy and dependability of analytical results through meticulous technique development and validation. Because HPLC can separate complicated combinations well, it is a very useful method for quantifying caffeine and paracetamol at the same time. HPLC precisely separates and

quantifies these active components by taking use of differences in molecular features like size and polarity. This allows for an accurate evaluation of medication quantities in formulations. In order to assess method performance criteria including accuracy, precision, specificity, and robustness, extensive testing is required as part of the validation process. Through a methodical evaluation of these criteria, researchers verify that the method is appropriate for the intended use and build trust in the dependability of the analytical output produced. In the end, proven HPLC techniques are essential to the research, production, and regulatory compliance of pharmaceuticals. In the end, they improve patient outcomes and public health by facilitating batch-to-batch repeatability, ensuring consistency in medication quality, and upholding safety requirements.

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