RESEARCH PAPER

Analytical Method Development and Validation of HPLC and UPLC Methods for Simultaneous Estimation of Aceclofenac, Paracetamol, and Chlorzoxazone (Kapnac MR Tablets)

Janorious Winka J[1], S.P. Dhanabal*[1], Nalin D[2], Archana Anebarassane[2], Veera Venkata Satyanarayana Reddy Karri[2] and Ramesh Jagadeesan[3]

[1,1]Department of Pharmacognosy & Phytopharmacy, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, Nilgiris, Tamil Nadu, India*
[2]Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, Nilgiris, Tamil Nadu, India
[3]Vice President & Business Head, Aragen Life Sciences, Hyderabad, Telangana, India
***Corresponding Author Mail:** spdhanabal@jssuni.edu.in*

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ABSTRACT

Fixed-dose combinations (FDCs) containing analgesic, anti-inflammatory, and muscle-relaxant agents are widely used in the management of pain and musculoskeletal disorders. Despite the presence of different formulation preparations, including KAPNAC MR tablets with aceclofenac, paracetamol, and chlorzoxazone, these preparations have no official pharmacopeia monographs, which creates challenges to standard and reproducible quality control analysis. The present study was intended to design and establish isocratic high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) methods to determine aceclofenac, paracetamol, and chlorzoxazone in KAPNAC MR tablets simultaneously, as required by ICH Q2(R2). In addition, the transferability of the methods was tested in a comparative evaluation of the chromatographic performance, the efficiency of the analytics, and the consumption of solvents using both platforms. The chromatographic separation was ultimately achieved by using a C18 stationary phase and an isocratic mobile phase consisting of acetonitrile and 0.1 per cent orthophosphoric acid in water (55:45, v/v) and detected at 275 nm. The optimized HPLC technique was then migrated into a UPLC system, which utilised an ACQUITY Premier HSS C18 column (2.1×100 mm 1.8 µm), in place of the scale of the column. System suitability, specificity, linearity, accuracy, precision, robustness, and solution stability were all proved to both procedures. With an HPLC environment, a retention time of about 2.8, 4.5, and 10.8 minutes was obtained with Paracetamol, chlorzoxazone, and aceclofenac, respectively, whereas the analysis time decreased significantly when the UPLC system was applied. Both techniques demonstrated good linearity ($R2 > 0.999$) and good accuracy (recoveries of 98 to 102 %) and precision (%RSD ≤ 2 %). Comparison of assay results taken statistically using one-way analysis of variance did not display the presence of any significant difference between the two procedures ($p > 0.05$). The UPLC methodology offered better chromatographic separation, reduced run time, and reduced solvent usage. Validated HPLC and UPLC methods were successfully established for the simultaneous estimation of aceclofenac, paracetamol, and chlorzoxazone in KAPNAC MR tablets. While both methods were suitable for routine quality control, the UPLC method offered improved analytical efficiency and reduced solvent usage, making it a practical alternative for high-throughput pharmaceutical analysis of similar fixed-dose combinations.

Keywords: Aceclofenac, Paracetamol, Chlorzoxazone, Fixed-dose combination, High-performance liquid chromatography, Ultra-performance liquid chromatography, Analytical method validation.

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INTRODUCTION

Fixed-dose combinations (FDCs) are widely prescribed in the management of pain and musculoskeletal disorders because they offer complementary therapeutic effects, improve patient compliance, and reduce overall treatment costs[1]. KAPNAC MR tablets contain aceclofenac, paracetamol, and chlorzoxazone, combining anti-inflammatory, analgesic, and muscle-relaxant activities in a single oral dosage form. Although this formulation is approved for marketing in India, it does not have an official pharmacopeial monograph. The absence of compendial standards complicates routine quality control, as analytical laboratories are required to rely on in-house methods whose performance, robustness, and reproducibility may vary [2].

**Author for Correspondence: S.P. Dhanabal*

From an analytical perspective, the simultaneous determination of aceclofenac, paracetamol, and chlorzoxazone presents inherent challenges due to differences in their physicochemical properties, including polarity, solubility, and ultraviolet absorption characteristics[3-5]. Aceclofenac, a nonsteroidal anti-inflammatory drug with preferential cyclooxygenase-2 inhibition, is commonly analysed using reversed-phase high-performance liquid chromatography with ultraviolet detection in the range of 275–280 nm[6]. Paracetamol, a centrally acting analgesic and antipyretic agent, has been extensively quantified using ultraviolet spectrophotometry and liquid chromatographic techniques, particularly in combination products [7]. Chlorzoxazone, a centrally acting skeletal muscle relaxant, exhibits ultraviolet absorption at higher wavelengths and is susceptible to interference from formulation excipients and co-formulated drugs [8].

Several analytical methods have been reported for the estimation of these drugs either individually or in dual combinations. Most published procedures for aceclofenac–paracetamol or paracetamol–chlorzoxazone combinations employ gradient elution, extended run times, or solvent-intensive mobile phases, and some exhibit limited chromatographic resolution between closely eluting components [9,10]. Although a limited number of studies have reported simultaneous estimation of all three analytes, such methods are not harmonized across laboratories and are not incorporated into major pharmacopeia's, including the Indian Pharmacopoeia, British Pharmacopoeia, or United States Pharmacopeia [11]. As a result, a validated, efficient, and reproducible analytical method applicable to a standard quality control of this fixed-dose combination is still required.

The modern pharmaceutical analysis is shifting towards approaching efficiency of methods, transferability, and low consumption of solvents in accordance with the principles of green analytical chemistry [12]. Unlike the conventional high-performance liquid chromatography, ultra-performance liquid chromatography uses columns packed with particles that are less than 2um, and so provides enhanced chromatographic efficiency, sharper peaks and fewer analysis times [13]. The transferability of the method between high-performance liquid chromatography and ultra-performance liquid chromatography is thus of practical applicability in laboratories aiming to enhance the performance of the method and still achieve better throughput.

In this context, the present study aimed to develop and validate reliable high-performance liquid chromatography and ultra-performance liquid chromatography methods for the simultaneous determination of aceclofenac, paracetamol, and chlorzoxazone in KAPNAC MR tablets. Although simultaneous RP-HPLC methods for this combination have been previously reported, most available studies focus primarily on method validation using a single chromatographic platform and provide limited systematic evaluation of method transfer or comparative analytical performance. Furthermore, the quantitative assessment of chromatographic efficiency and solvent consumption during transfer from conventional high-performance liquid chromatography to ultra-performance liquid chromatography has received limited systematic evaluation for this fixed-dose combination. Accordingly, the present work was designed not only to develop and validate isocratic HPLC and UPLC methods in accordance with ICH Q2(R2) [14], but also to critically evaluate method transferability by comparing chromatographic performance parameters, analytical efficiency, and solvent usage across both platforms. This approach provides a practical framework for laboratories seeking to transition validated HPLC methods to UPLC systems for routine quality-control testing of non-compendial fixed-dose combinations.

MATERIALS AND METHODS

Chemicals and reagents:

Aceclofenac, paracetamol, and chlorzoxazone working standards were obtained from certified pharmaceutical suppliers and were of stated purity suitable for analytical use. Acetonitrile of high-performance liquid chromatography grade and orthophosphoric acid of analytical reagent grade were used throughout the study. Purified water was obtained using a Milli-Q water purification system. KAPNAC MR tablet samples were procured from the local pharmaceutical market and used for assay analysis.

Instrumentation:

High-performance liquid chromatography analysis was performed using a Waters Alliance system equipped with a quaternary pump and an ultraviolet/photodiode array detector. Ultra-performance liquid chromatography analysis was carried out using a Waters ACQUITY UPLC system controlled by Empower 3 chromatography software. An ultrasonic bath was used for solution preparation, and an analytical balance with appropriate sensitivity was used for weighing. A pH meter was employed for monitoring solution acidity where required. All solutions were filtered through 0.2 µm nylon membrane filters prior to injection.

Chromatographic conditions:

Chromatographic conditions were optimized to achieve adequate resolution, symmetrical peak shape, and reproducible retention for all three analytes. Separation was performed on a C18 reversed-phase column (250 × 4.6 mm, 5 µm) for high-performance liquid chromatography and on an ACQUITY Premier HSS C18 column (2.1 × 100 mm, 1.8 µm) for ultra-performance liquid chromatography.

The mobile phase consisted of acetonitrile and 0.1% orthophosphoric acid in water in the ratio of 55:45 (v/v), delivered under isocratic conditions. The flow rate was maintained at 1.0 mL/min for high-performance liquid chromatography and 0.2 mL/min for ultra-performance liquid chromatography. Detection was carried out at 275

nm. The injection volume was set at 20 µL for high-performance liquid chromatography and 5 µL for ultra-performance liquid chromatography. The column temperature was maintained at 25 °C. Total run times were 15 minutes and 10 minutes for high-performance liquid chromatography and ultra-performance liquid chromatography, respectively. The optimized chromatographic conditions selected for both methods are summarized in Table 1.

Table.1 Optimized chromatographic conditions for high-performance liquid chromatography and ultra-performance liquid chromatography methods used for the simultaneous estimation of aceclofenac, paracetamol, and chlorzoxazone

Parameter	HPLC	UPLC
Column	C18 (250×4.6 mm, 5 µm)	ACQUITY Premier HSS C18 (2.1×100 mm, 1.8 µm)
Mobile phase	Acetonitrile: 0.1 % orthophosphoric acid in water (55:45, v/v)	Acetonitrile: 0.1% orthophosphoric acid in water (55:45, v/v)
Flow Rate	1.0 mL/min	0.2 mL/min
Detection	275 nm	275 nm
Injection Volume	20 µL	5 µL
Column Temperature	25°C	25°C
Run Time	15 min	10 min

Under these conditions, paracetamol, chlorzoxazone, and aceclofenac eluted at approximately 2.8, 4.5, and 10.8 minutes, respectively, in the high-performance liquid chromatography system. Upon method transfer to ultra-performance liquid chromatography, retention times were reduced to approximately 1.2 minutes for paracetamol, 2.0 minutes for chlorzoxazone, and 6.4 minutes for aceclofenac, while maintaining adequate chromatographic resolution.

Preparation of solutions:

The mobile phase was used as the diluent for all standard and sample preparations. Aceclofenac, paracetamol, and chlorzoxazone (100mg each) were weighed very carefully and put in a 100-mL volumetric flask to create a standard stock solution. About 50ml of mobile phase was included and the mixture was sonicated after 10minutes so that the mixture could be fully dissolved. The solution was then left to cool to room temperature and mixed up to volume with mobile phase.

A standard working solution was prepared by taking 5.0 mL of the stock solution as a standard and adding the stock solution through a 50 ml volumetric flask up to volume with the mobile phase.

To prepare a sample, a specific weight of one sample tablet corresponding to one tablet was weighed and placed in a volumetric flask with a volume of about 75 mL of mobile phase. The mixture was sonicated within 15 minutes to aid extraction of the analytes, and cooled to room temperature then diluted to volume with mobile phase. A 0.2 µm nylon membrane filter would filter the resulting solution. A 5.0 mL aliquot of the filtrate was diluted again to 50mL of mobile phase before being added to the chromatography column [15,16].

Method optimization:

Optimization of the method was achieved through a systematic assessment of the effects of mobile phase composition, detection wavelength and flow rate on chromatography. Various concentrations of aqueous and acetonitrile orthophosphoric acid solution were tested to obtain sufficient resolution and acceptable symmetry of peaks of all the three analytes. The detection wavelength was selected according to the conjugated ultraviolet absorption properties of aceclofenac, paracetamol, and chlorzoxazone. The wavelength of detection used was 275nm because it gave reasonable response to all the analytes without much interference to the baseline. The flow rates used gave unchanged retention times and equalized shapes of peaks and theoretically more than 2000 plates per analyte, which met the requirements of system suitability.

Statistical analysis:

Each experiment was conducted 3 times, and the results are stated as an average with the standard deviation. Assays results obtained by using high-performance liquid chromatography and ultra-performance liquid chromatography were compared by the one-way analysis of variance. A p-value below 0.05 was taken as statistically significant. Microsoft Excel 365 and GraphPad Prism software (version 10.0) were used to perform statistical calculations.

Forced degradation studies:

Forced degradation experiments were done to evaluate possible interference with degradation products at stressed conditions. Aceclofenac, paracetamol and chlorzoxazone were exposed to acidic (0.1 N HCl), alkaline (0.1 N NaOH) hydrolysis, oxidative (3% hydrogen peroxide) degradation, thermal (60 o C) and photolytic (UV light) degradation. Before analysis, the samples were neutralized to acidic and alkaline. All the stressed samples were diluted properly using mobile phase and ran under the optimized chromatographic conditions. Chromatograms were assessed based on the separation of the peak, change in retention time and the occurrence of other degradation peaks proving that degradation products did not interfere with the measurement of the analyte.

To determine any interference caused by degradation products, forced degradation experiments were conducted; quantitative degradation kinetics, mass balance analysis and impurity profiling were not part of this analysis.

Validation parameters (ICH Q2(R2)):

The high-performance liquid chromatography and ultra-performance liquid chromatography methods were justified through the international council for harmonisation (ICH) guideline; Q2(R2) on the validation of analytical methods. The validation was conducted to prove that the methods were suitable in the quantitative determination of aceclofenac, paracetamol, and chlorzoxazone in tablet dosage form. The main validation properties under consideration were system suitability, specificity, linearity, accuracy, precision, robustness, stability of the solution, limits to detection, limits to quantification, and ruggedness.

System Suitability:

Before analysis of samples, system suitability testing was conducted to confirm whether the chromatographic system was adequately performing. The working standard solution was repeatedly injected, and the system suitability parameters including retention time consistency, peak area repeatability, tailing factor and theoretical plate count were analyzed individually per analyte.

Linearity:

Linearity was assessed by determining standard solutions of various levels of concentration covering a specified range of percent (50 to 150%) of target assay concentration. Calibration curves were built by plotting the area of the peak with the concentration of each analyte and the linear correlation between the detector response and the concentration was studied.

Accuracy (recovery):

Accuracy has been established through recovery studies in which a recovery of known amounts of each of the analytes were added to the placebo matrix at different concentration levels compared to the nominal assay concentration. The processed samples were treated and examined through the proposed methods to determine the proximity of the sampled values on the nominal values.

Precision:

Precision was tested using two levels, which are repeatability and intermediate precision. The ability to repeat it was determined by using numerous replicate preparations of the sample under the same conditions of the experiment. To determine more about intermediate precision, the analysis was done on various days and at times, by various analysts and instruments.

Specificity:

Specificity was evaluated to establish the capacity of the methods to measure aceclofenac, paracetamol, and chlorzoxazone unambiguously in the presence of formulation excipients and possible interferences. Blank solutions, placebo solutions, standard solutions and sample solutions were tested to test any form of interference at the retention time of the analytes.

Robustness:

Strong robustness was attained by deliberately applying small alterations to the chromatographic parameters like flow rate, column temperature. The effect of these variations on the performance of chromatography was determined to determine the reliability of the methods in the normal operation conditions.

Solution stability:

To investigate the stability of the standard and sample solutions in the normal analysis, solution stability was tested. Ready-made solutions were kept in room temperature and studied at specific time intervals to establish any change in analysis response.

Limit of detection and limit of quantification:

The detection and quantification limits were estimated using the slope of the calibration curve and the standard deviation of the analytical response and in line with ICH Q2(R2) requirements. These parameters were decided to be able to test the sensitivity of the methods.

Ruggedness:
An assessment of ruggedness was made by evaluating the reproducibility of the assay results during normal but varying laboratory conditions, such as analyst, day of analysis or instrument change.

RESULT

Chromatographic performance and comparison of HPLC and UPLC methods:

The chromatographic performance of the developed methods was evaluated using both HPLC and UPLC for the simultaneous determination of aceclofenac, paracetamol, and chlorzoxazone. In both chromatographic systems, a consistent elution order was observed, with paracetamol eluting first, followed by chlorzoxazone and aceclofenac. Under optimized HPLC conditions, paracetamol, chlorzoxazone, and aceclofenac eluted at approximately 2.8, 4.5, and 10.8 minutes, respectively. Upon transfer of the method to the UPLC platform, retention times were reduced to approximately 1.2 minutes for paracetamol, 2.0 minutes for chlorzoxazone, and 6.4 minutes for aceclofenac, while maintaining baseline separation of all analytes.

System suitability testing demonstrated acceptable chromatographic performance for both HPLC and UPLC methods. Parameters including retention time repeatability, peak area precision, tailing factor, and theoretical plate count met predefined acceptance criteria, with percentage relative standard deviation values not exceeding 2.0% for any analyte. Statistical comparison of assay results obtained using HPLC and UPLC was performed using one-way analysis of variance (ANOVA). No statistically

significant difference was observed between the mean assay values generated by the two methods ($p > 0.05$), indicating comparable quantitative performance within the evaluated conditions.

Key analytical performance characteristics obtained using the UPLC method are summarized in Table 2. Table 3 is a comparison of the chromatographic and operational characteristics of the UPLC and the HPLC methods to indicate the differences between the two methods in regards to solvent use and the ability to generate an analysis.

Table 2. Ultra-performance liquid chromatography performance parameters for the simultaneous estimation of aceclofenac, paracetamol, and chlorzoxazone

Parameter	Aceclofenac	Paracetamol	Chlorzoxazone
Retention time (UPLC)	6.4 min	1.2 min	2.0 min
Linearity (R^2)	0.9997	0.9998	0.9996
Recovery (%)	99.2–101.5	98.5–101.8	98.0–100.9
System precision (%RSD)	0.8	0.7	0.9
% Assay	99.3	101.1	100.5

Table 3. Comparative chromatographic and operational characteristics of HPLC and UPLC methods.

Parameter	HPLC	UPLC
Run time (min)	15	10
Flow rate (mL/min)	1.0	0.2
Solvent consumption per run (mL)*	15	2
Theoretical plates (Aceclofenac)	>2000	Increased relative to HPLC
Resolution (critical pair)	≥ 2.0	≥ 2.0

*Solvent consumption per run calculated based on flow rate and total run time.

The comparative data presented in Table 3 demonstrate that the UPLC method achieved a marked reduction in run time and mobile phase consumption as a consequence of lower flow rate and reduced column dimensions, without compromising chromatographic resolution or system suitability requirements. The preservation of adequate resolution for the critical pair confirms that comparable chromatographic performance was maintained following method transfer.

Validation study results:

Linearity was analyzed over a predetermined concentration range that is 50-150 per cent of the target assay concentration of each analyte. Calibration curves showed a linear correlation, and coefficient of determination above 0.999, which indicates strong linearity over the studied range. Recovery studies were performed to assess the accuracy with regards to different concentrations. The aceclofenac, paracetamol and chlorzoxazone recoveries were within the acceptable range of values, meaning the analysis procedures are effective in quantifying all the three analytes despite the presence of excipients in the formulations.

The level of precision was determined through repeatability and intermediate precision. Repeatability experiments had low variability between and among replicate sample preparations that were run under the same conditions. Intermediate precision was measured over days and between different analysts also exhibited tolerable variability and thus confirmed the reproducibility of the procedures in normal laboratory procedures. The detection and quantification limits also showed that it had enough sensitivity to the planned usage. Calibrated limits showed that low concentrations of each of the three analytes can be detected and measured with acceptable accuracy using the methods. The robustness testing revealed that a small purposeful change in chromatography conditions, such as flow rate and column temperature, did not result in significant changes of chromatographic response. Similarly, stability of solutions investigations showed that standard and sample solutions were stable during the period of study and no radical changes of the analytical response were detected. The summary of the validation results to both the procedures is presented in Table 4.

Table 4. Summary of validation results for the developed HPLC and UPLC methods

Validation parameter	Acceptance criteria	HPLC	UPLC
System suitability	%RSD ≤ 2.0, tailing ≤ 2.0, plates ≥ 2000	Met	Met
Specificity	No interference at analyte RT	Met	Met
Linearity range	50–150% of assay concentration	Met ($R^2 > 0.999$)	Met ($R^2 > 0.999$)
Accuracy (recovery)	98–102%	Met	Met
Repeatability	%RSD ≤ 2.0	Met	Met

Intermediate precision	%RSD ≤ 2.0	Met	Met
LOD	Method-dependent	Determined	Determined
LOQ	Method-dependent	Determined	Determined
Robustness	No significant effect	Met	Met
Solution stability	Stable up to 24 h	Met	Met
Ruggedness	%RSD ≤ 2.0	Met	Met

Forced degradation and specificity evaluation:

Forced degradation studies were also conducted to determine the abilities of the developed methods to provide information on stability. Under the applied stress conditions, aceclofenac, paracetamol, and chlorzoxazone exhibited varying degrees of degradation, with more pronounced degradation observed under acidic, alkaline, and oxidative conditions. Thermal and photolytic stress resulted in comparatively lower levels of degradation.

In all stressed samples, degradation products were well resolved from the principal analyte peaks, and no co-elution was observed at the retention times corresponding to aceclofenac, paracetamol, or chlorzoxazone. Peak purity analysis using photodiode array detection confirmed the spectral homogeneity of all analyte peaks under stressed conditions, indicating the absence of interference from degradation products or formulation excipients.

Table 5. Summary of forced degradation behaviour of aceclofenac, paracetamol, and chlorzoxazone

Stress condition	Degradation observed	Peak separation
Acidic	Yes	No interference
Alkaline	Yes	No interference
Oxidative	Yes	No interference
Thermal	Minor	No interference
Photolytic	Minor	No interference

The results summarized in Table 5 confirm that degradation products generated under all applied stress conditions were adequately separated from the analyte peaks, demonstrating that degradation products were adequately resolved from the analyte peaks under the applied stress conditions. Forced degradation studies were performed to assess potential interference from degradation products; quantitative degradation kinetics, mass balance evaluation, and impurity profiling were beyond the scope of this study.

Method transfer evaluation between HPLC and UPLC:

Method transfer from HPLC to UPLC was evaluated to demonstrate analytical equivalence between the two chromatographic platforms. Transfer was performed by maintaining identical stationary phase chemistry and mobile phase composition, while adjusting column dimensions, flow rate, and injection volume in accordance with chromatographic scaling principles.

Following transfer, the elution order of aceclofenac, paracetamol, and chlorzoxazone was preserved, and adequate resolution was maintained for all analyte peaks. Resolution values for the critical peak pair remained above the acceptance criterion (Rs ≥ 2.0) for both chromatographic systems, and no meaningful change in chromatographic selectivity was observed. These findings indicate that chromatographic separation was not compromised by differences in column geometry or flow conditions.

Table 6. Method transfer evaluation parameters between HPLC and UPLC

Parameter	HPLC	UPLC
Stationary phase chemistry	C18	C18 (HSS)
Mobile phase composition	Acetonitrile: 0.1% OPA (55:45, v/v)	Same
Elution mode	Isocratic	Isocratic
Column internal diameter scaling	Reference	Scaled down
Flow rate scaling approach	Reference	Adjusted proportionally
Elution order	Preserved	Preserved
Resolution acceptance criterion	≥ 2.0	≥ 2.0 (met)
Selectivity changes after transfer	—	No significant change

The data presented in Table 6 confirm that the applied transfer strategy achieved analytical performance between HPLC and UPLC while enabling improved operational efficiency with reduced analysis time and solvent consumption.

DISCUSSION

The present research evaluated the viability of the procedure transfer between high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) and determine and optimized chromatographic procedures of simultaneous determination of aceclofenac, paracetamol and chlorzoxazone in KAPNAC MR tablets. The development of an appropriate analytical technique which can be utilized repeatedly as an element of quality-control analysis is tremendously imperative in a practical sense, since there exists no official monograph of a pharmacopeia to this fixed-combination of doses.

The performance of the two chromatographic techniques was found to be satisfactory according to the acceptance criteria of standard system suitability, linearity, accuracy, precision, specificity, robustness, and stability of the solution as required in the International Council for Harmonisation (ICH) guideline Q2(R2). System-suitability parameters such as maximum Symmetry, maximum count of the theoretical plate counts and maximum-area repeatability were used to confirm that the chosen chromatographic parameters ensured sufficient resolution and reproducible characteristics in all three analytes. These findings justify the applicability of the developed techniques to the quantitative analysis in the normal laboratory environment. Compared to the previous reports that center mainly the validation issue on a single analytical platform, the current study goes further to conduct a systematic assessment of the transfer of the method in HPLC to UPLC. Following method transfer, retention behaviour, order of elution, linearity, precision, and assay values were maintained and significant savings were made in terms of the analysis time and mobile-phase consumption. These observations have shown that similar analytical behaviours were observed regardless of the change in column geometry and flow rate, thus confirming the relevance of the proposed method to labs that want to achieve better throughput without losing data quality. The method transfer was achieved through keeping the same chemistry under stationary phases and the same composition under mobile phases on both platforms. The implementation of the column filled with smaller particle size in UPLC system had a reduced retention time and greater chromatographic efficiency shown by higher theoretical plate counts. Notably, there was no change in the order of elution of the three drugs (aceclofenac, paracetamol and chlorzoxazone) following transfer thereby showing that the chromatography was selective. The lack of interference by formulation excipients on the retention times of the analytes of interest was confirmed by specificity studies, which is most pertinent when the ultraviolet absorption properties of the analytes of interest vary in polarity, a phenomenon that can increase the risk of co-elution in fixed-dose combinations. Robustness and ruggedness tests also showed that intentional changes in chromatographic conditions, analyst and day of analysis did not produce negative effects on method so that the reliability of the proposed methods under normal laboratory practice was showcased to be dependable. The ability of the method to remove analytes of degradation products at various stress conditions also justifies its high adaptation as a technique in quality-control situations associated with stability. Compared to the analytical protocols that have been reported in the past, which are mostly concerned with individual drugs or binary mixtures, the current methods have presented the viable solution in estimating all three components at the same time with isocratic elution and moderate run times. Compared to conventional HPLC, the UPLC methodology consumes less time and less solvent, but does not compromise the accuracy and precision of the analysis as compared to conventional HPLC. The results in general show that both approaches would be applicable to the routine assay analysis of this fixed-dose combination and the UPLC technique may have an added value in terms of effectiveness and solvent usage [17,18].

CONCLUSION

The suitability of high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) techniques in simultaneous determination of aceclofenac, paracetamol and chlorzoxazone in KAPNAC MR Tablets formulations was verified. The accuracy, precision, specificity and robustness of both techniques met the requirements of ICH Q2(R2), therefore indicating their suitability in the routine use of the assay. Despite similar performance in the two chromatographic strategies, the UPLC protocol was significantly superior in terms of lowering the duration of the analysis and reduce solvent consumption, which makes it preferable to use in large-scale routine quality-control procedures. These procedures meet the analytical standards of this fixed-dose combination, even in the absence of an official pharmacopeial monograph, and can be used in regular quality-adjustment assessments of similar non-compendial preparations.

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Author Contributions: Janorious Winka J contributed to conceptualization, experimental work, method development, validation studies, data collection, statistical analysis, and drafting of the manuscript; S. P. Dhanabal contributed to supervision, study design, critical review of methodology, validation strategy oversight, manuscript revision, and final approval; Nalin D contributed to data analysis, statistical evaluation, method transfer assessment, interpretation of validation results, and manuscript editing; Archana Anebarassane contributed to experimental assistance, forced degradation studies, validation documentation, and literature review support; Veera Venkata Satyanarayana Reddy Karri contributed to

technical guidance on chromatographic optimization, comparative evaluation between HPLC and UPLC systems, critical scientific review, and final manuscript approval.

Ramesh Jagadeesan contributed to technical guidance on chromatographic optimization, comparative evaluation between HPLC and UPLC systems, critical scientific review, and final manuscript approval.

REFERENCES

1. Zhang Q, Chan DXH, Ho KY. Efficacy and Safety of Fixed-Dose Combinations for Pain in Older Adults. Drugs Aging. 2024;41(11):873-879.
2. Trows S, Wuchner K, Spycher R, Steckel H. Analytical challenges and regulatory requirements for nasal drug products in europe and the u.s. Pharmaceutics. 2014;6(2):195-219.
3. Pereira FJ, Rodríguez-Cordero A, López R, Robles LC, Aller AJ. Development and Validation of an RP-HPLC-PDA Method for Determination of Paracetamol, Caffeine and Tramadol Hydrochloride in Pharmaceutical Formulations. Pharmaceuticals (Basel). 2021;14(5):466.
4. Joshi, R., and R. Sharma. "Development and Validation of RP-HPLC Method for Simultaneous Estimation of Three-Component Tablet Formulation Containing Acetaminophen, Chlorzoxazone, and Aceclofenac. Analytical Letters. 2008; 41 (18): 3297–3308.
5. Hameedat F, Hawamdeh S, Alnabulsi S, Zayed A. High Performance Liquid Chromatography (HPLC) with Fluorescence Detection for Quantification of Steroids in Clinical, Pharmaceutical, and Environmental Samples: A Review. Molecules. 2022;27(6):1807.
6. Chandrasekhar K, Manikandan A. A new rp-hplc method development and validation of paracetamol and aceclofenac in tablets by separating diclofenac. Research Journal of Pharmacy and Technology. 2020;13(9):4334-8.
7. El-Yazbi AF, Guirguis KM, Bedair MM, Belal TS. Validated specific HPLC-DAD method for simultaneous estimation of paracetamol and chlorzoxazone in the presence of five of their degradation products and toxic impurities. Drug Dev Ind Pharm. 2020;46(11):1853-1861.
8. Shaikh KA, Devkhile AB. Simultaneous determination of aceclofenac, paracetamol, and chlorzoxazone by RP-HPLC in pharmaceutical dosage form. J Chromatogr Sci. 2008;46(7):649-52.
9. Zawilla NH, Mohammad MA, El Kousy NM, El-Moghazy Aly SM. Determination of aceclofenac in bulk and pharmaceutical formulations. J Pharm Biomed Anal. 2002;27(1-2):243-51.
10. Bouhsain Z, Garrigues S, de la Guardia M. PLS-UV spectrophotometric method for the simultaneous determination of paracetamol, acetylsalicylic acid and caffeine in pharmaceutical formulations. Fresenius' journal of analytical chemistry. 1997;357(7):973-6.
11. Creteanu A, Lisa G, Vasile C, Popescu MC, Pamfil D, Lungu CN, Panainte AD, Tantaru G. New Hydrophilic Matrix Tablets for the Controlled Released of Chlorzoxazone. Int J Mol Sci. 2024 ;25(10): 5137.
12. Yin L, Yu L, Guo Y, Wang C, Ge Y, Zheng X, Zhang N, You J, Zhang Y, Shi M. Green analytical chemistry metrics for evaluating the greenness of analytical procedures. J Pharm Anal. 2024 ;14(11):101013.
13. Swartz, Michael E.. "UPLC™: An Introduction and Review. Journal of Liquid Chromatography & Related Technologies 2005; 28 (7–8): 1253–63.
14. Cassidy B, Bloomingdale T, Carmody J. Navigating ICH Q2(R2) compliance in analytical method validation: A gap analysis toolkit to streamline risk assessment and change management. J Pharm Sci. 2025;114(6):103749.
15. Coskun O, Akbarzadeh S, Guncer B. Different chromatographic techniques and recent advancements for biomedical and pharmaceutical applications. *North Clin Istanb*. 2025;12(6):739-752.
16. Holčapek M, Jirásko R, Lísa M. Recent developments in liquid chromatography-mass spectrometry and related techniques. J Chromatogr A. 2012; 1259:3-15.
17. Desai PP, Patel NR, Sherikar OD, Mehta PJ. Development and validation of packed column supercritical fluid chromatographic technique for quantification of chlorzoxazone, paracetamol and aceclofenac in their individual and combined dosage forms. J Chromatogr Sci. 2012 ;50(9):769-74.
18. Bawazeer S, Badr El-Din KM, Abdel-Megied AM. Development and Validation of a Versatile UPLC-PDA Method for Simultaneous Determination of Paracetamol, Tizanidine, Aceclofenac, and Nimesulide in Their New Combinations. J Anal Methods Chem. 2018; 2018:7463914.