Ind. J. Pharm. Edu. Res., 2023; 57(3s):s787-s797.
https://www.ijper.org

Original Article

Different Innovative UV-spectroscopic Approaches for Simultaneous Assessment of Celecoxib and Tramadol Hydrochloride in Binary Mixture

Ashim Kumar Sen*, Preksha Darji, Dhanya B. Sen, Aarti S. Zanwar, Chintan J. Aundhia, Rajesh A. Maheshwari

Department of Pharmacy, Sumandeep Vidyapeeth (Deemed to be University), Piparia, Vadodara, Gujarat, INDIA.

ABSTRACT

Background and Aim: Tablets containing celecoxib and tramadol hydrochloride that are sold under the brand name Seglentis® have given approval by the "Food and Drug Administration" (FDA) for the treatment of adults suffering from acute pain. Simultaneous assessment of celecoxib and tramadol hydrochloride in synthetic mixture was performed by five new UV-spectrophotometric approaches. **Materials and Methods:** The quantification of proposed medication was performed using the simultaneous equation approach by measuring absorbance at 217.2 and 272.4 nm. The second approach, known as the dual wavelength method, relies on computing the absorbance difference at two different wavelengths where the other drug has same absorbance. The first derivative zero crossing approach relied on the conversion of UV-spectra into first derivative spectra and subsequently measuring the amplitude at 272.4 and 229.6 nm for celecoxib and tramadol hydrochloride, respectively. The ratio difference approach was used to measure the variation in the amplitudes of ratio spectra, and the regression equation was employed to determine the amounts of pharmaceuticals. In first derivative ratio spectra approach, the UV-spectra were transformed into their ratio spectra and first derivatives, and the first-derivative signal was gauged at 236.4 and 229.8 nm for celecoxib and tramadol hydrochloride, sequentially. **Results and Discussion:** All the parameters were evaluated during validation of the new methods in accordance with ICH guidelines. For both medicines, a linear response was observed in all five methods over the concentration series of 1-18 µg/mL. Method validation parameters were found to be within the permitted limits set forth by the ICH. **Conclusion:** Assay results were compared using Repeated Measures ANOVA, IBM SPSS, version 20.0. The findings of the statistical analysis demonstrated that none of the projected methods differed significantly from others. Thus, the anticipated approaches have the potential to be productively utilized for the simultaneous evaluation of celecoxib and tramadol hydrochloride in a combination mixture.

Keywords: Celecoxib, Tramadol hydrochloride, Simultaneous equation, Dual wavelength, First derivative zero crossing, Ratio difference and the First derivative of ratio spectra spectroscopic methods, Binary mixture.

Correspondence:
Prof. (Dr.) Ashim Kumar Sen
Department of Pharmacy, Sumandeep Vidyapeeth (Deemed to be University), Piparia, Vadodara-391760, Gujarat, INDIA.
Email: ashims01@gmail.com
ORCID: 0000-0003-2587-5623

Received: 10-08-2022;
Revised: 25-01-2023;
Accepted: 23-06-2023.

INTRODUCTION

Patients generally require many medications to attain satisfactory pain relief, despite the fact that pain is the leading reason for frequent medical attention.[1] Research shows that many people experiencing severe pain may not receive the level of analgesia they require.[1-4] Multimodal analgesia, which involves the use of numerous classes of pain-relieving medicines with distinct mechanism of action, is one approach to address this unmet medical need with the ultimate goal of enhancing patient-reported outcomes.[5] When two or more dissociable constituents create a crystal lattice, the result is called a co-crystal. With their unusual molecular architectures, co-crystals containing multiple APIs present an exciting new direction in the field of poly pharmacology. API-API co-crystals exhibit improved physicochemical properties compared to their individual pharmaceutical components due to the presence of weak intermolecular interactions among the drugs within the crystal lattice. This characteristic has the potential to result in clinical advantages. These may be observable as improved solubility or dissolving properties, which, in turn, may result in improved Pharmacokinetics (PK) in comparison with open or standard Fixed Dose Combinations (FDCs).[6] In addition, the preparation procedures for co-crystals are not nearly as complicated as those for FDCs, and they do not share a significant portion of the formulation challenges that FDCs

DOI: 10.5530/ijper.57.3s.89

are known to have.[7] The Food and Drug Administration (FDA) has given its approval for the use of Seglentis® tablets in the treatment of individuals who are experiencing acute pain that is severe enough to call for the use of an opioid analgesic and for which alternative treatments are insufficient. Tablets of Seglentis® [Celecoxib (CXB) and Tramadol Hydrochloride (TMD)] contain a co-crystal with a molecular weight of 681.2. This co-crystal is made up of TMD, which is an opioid agonist and analgesic, and CXB, which is a nonsteroidal anti-inflammatory drug, in a molecular ratio of 1:1. TMD is chemically known as (1*RS*,2*RS*)-2-[(dimethylamino)methyl]-1-(3methoxyphenyl) cyclohexanol hydrochloride. TMD is a synthetic opioid that is not produced from opioids. It is an analgesic that acts on the central nervous system, but it may also act, at least in part, by binding to opioid mu receptors and inducing suppression of ascending pain pathways. CXB is a diaryl-substituted pyrazole and has the chemical name 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide. Because CXB inhibits Cyclooxygenase (COX)-2 but has no effect on cyclooxygenase 1 (at therapeutic dosages), the production of prostaglandins is reduced.[8,9] Chemical construction of both the analytes are given in Figure 1.

A study of the relevant literature revealed a plethora of analytical methods for the evaluation of CXB and TMD both on their own and in combination dosage forms with other medications. These methodologies include UV Spectrophotometry,[10-16] HPLC,[15,17-28] HPTLC,[18,29-32] and LC-MS/MS.[33] On the other hand, the simultaneous measurement of CXB and TMD in the binary combination has not been reported by any analytical methods until this point. This study is the foremost to discuss the development and validation of five straightforward, replicable, responsive, cost-effective, and accurate UV-spectroscopic methodologies for quantifying CXB and TMD in a binary combination. As a result, this paper is the first of its kind. The proposed methods have many benefits, some of which are as follows: they have a broad concentration range while maintaining a high level of sensitivity; they have been validated in accordance with the recommendations made by ICH and they provide a relatively simple method for preparing standards and samples. Analytical methodologies that make use of UV-spectroscopy are regarded as being straightforward, speedy, and economical methods for evaluating the quality of medications that are utilized on a consistent basis. However, the most significant disadvantage of direct UV-spectroscopic techniques are the effect of multicomponent formulations as well as formulation additives. As a consequence of this, besides simultaneous equation method, derivative and ratio derivative UV-spectroscopic methodologies were also developed in order to circumvent these effects.[34] These methodologies were determined to be apt for the simultaneous evaluation of CXB and TMD without any observed interference.

MATERIALS AND METHODS

Chemicals and Reagents

Reference standard of CXB and TMD were generously provided by Dalton PharmaChem (Vadodara, Gujarat, India) and Supriya Lifescience Ltd. (Mumbai, Maharashtra, India), respectively. All of the AR grade solvents, chemicals, and excipients (specificity study) were ordered from Loba Chemie Pvt. Ltd., Mumbai, India.

Instruments

For the experiment, a Shimadzu UV-visible spectrophotometer (double beam) with a matched quartz cell having 1 cm path length (UV-1800, UV Probe, Shimadzu Corporation, Kyoto Japan) was utilized. Weighing was carried out using the Ohaus Corporation's Adventurer Pro AVG264C electronic balance.

Preparation of Standard Solution

Both CXB and TMD (10 mg each) were weighed accurately and shifted to separate 10 mL volumetric flasks for stock solution preparation. Methanol was used to dilute standard medicines to 10 mL to attain a concentration of 1000 µg/mL. To achieve the desired concentration, further dilutions in methanol were performed.

Preparation of Synthetic mixture

The synthetic mixture of CXB and TMD was formulated in 56:44 w/w proportion. Excipients such as sodium lauryl sulphate 15 mg, crospovidone 15 mg, mannitol 6 mg, sodium stearyl fumarate 3 mg, talc 3 mg, cellulose microcrystalline 178 mg and copovidone 15 mg have been accurately measured; kept into a mortar, and mixed with pure CXB and TMD medicines. (Above mentioned calculation is for synthetic mixture generated in a lab that is equivalent to one tablet).[8,9,35]

Reference stock solutions of CXB and TMD Mixture

In a sequence of 10 volumetric flasks, CXB and TMD standard solutions were blended together to produce mixture of desired concentrations and the volume was brought up to the level with methanol. With methanol, additional dilutions were carried out in order to acquire the necessary concentration.

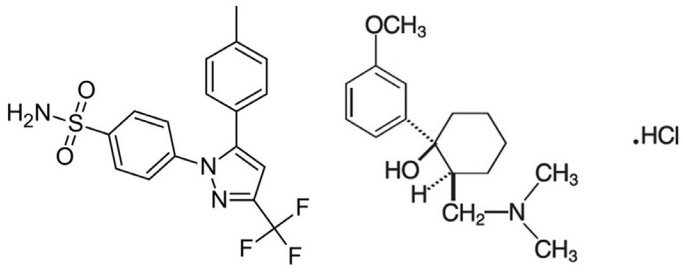

Figure 1: Chemical constructions of CXB and TMD.

Preparation of sample solution

Equivalent amounts of a synthetic mixture made in a lab (CXB: 14 mg; TMD: 11 mg) was weighed and placed in a 100 mL standard flask. 50 mL of methanol was added to this standard flask and agitated for 10 min. The volume was filled up to 100 mL using methanol and then filtered by means of Whatman filter paper no. 41. 1 mL of the aforementioned solution was transferred to a 10 mL volumetric bottle, and the volume was adjusted with methanol. Then, 1 mL of the resulting solution was transferred to a 10 mL volumetric bottle, and methanol was added to reach the desired concentration (CXB: 14 µg/mL; TMD: 11 µg/mL).

Procedure

Simultaneous Equation Method (SEM)

In order to estimate CXB and TMD in a binary mixture, the simultaneous equation method was used. All of the standard analytes in this investigation had their UV-spectra recorded between 200 and 400 nm. The estimation of the anticipated analytes in the synthetic mixture required choosing a suitable wavelength from the overlapping UV-spectra. Significant absorbance was seen at 217.2 and 272.4 nm in the combined zero-order spectra of CXB and TMD (Figure 2). As a next step, we computed the absorptivity of analytes. The below mentioned formulas were used to calculate how much drugs were present in the synthetic mixture.

$$Cx = \frac{A_2\, a_{y1} - A_1\, a_{y2}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

$$Cy = \frac{A_1\, a_{x2} - A_2\, a_{x1}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

In the above equation, Cx and Cy are the amount of CXB and TMD, consecutively in the sample solutions.

A1 and A2 are the absorbances of the sample at 217.2 and 272.4 nm. ax1 (643.29) and ax2 (346.87) are the absorptivity of CXB at 217.2 and 272.4 nm, sequentially. ay1 (366.90) and ay2 (101.58) are the absorptivity of TMD at 217.2 and 272.4 nm, sequentially.[14,36-38]

Dual Wavelength Method (DW)

The DW method is established on the idea that "the variation in absorbance between two positions on the mixture spectrum is directly proportional to the concentration of the factor of interest." The requirement for the DW approach is selecting two wavelengths at which the intrusive component exhibits the same absorbance and the analyte of interest exhibits a substantial dissimilarity in absorbance with concentration. The overlain spectrum of CXB and TMD proposed that a DW spectrophotometric method is suitable for simultaneous assessment of CXB and TMD. The wavelengths, 270.2 and 278.2 nm were utilized for the estimation of CXB, where the absorbance difference was zero for TMD. The wavelengths 228 and 260 nm were chosen for TMD, where the absorbance difference was zero for CXB. Then, calibration graphs for CXB and TMD in the concentration series of 1-18 µg/mL were plotted. Outcomes were subjected to regression study using the technique of least squares to set up slope, intercept, and correlation coefficient values.[11,39,40]

1st Derivative Zero Crossing Method (^{1}DR)

For the assessment of CXB and TMD by First derivative (Zero Crossing) Method, the stored UV-spectra of both the analytes were converted into their respective first derivative spectra. Then the zero crossing points of CXB and TMD were traced at 229.6 and 272.4 nm, sequentially utilizing 2 nm as wavelength interval (Δλ) and 1 as scaling factor. With the help of traced zero crossing points of both the analytes, estimation was performed at 272.4 and 229.6 nm for CXB and TMD, correspondingly. Further calibration curve was put up utilizing the amplitudes of first derivative spectra and concentration of analytes. Outcomes were subjected to regression study using the technique of least squares to set up slope, intercept, and correlation coefficient values.[37-39]

Ratio Difference Spectroscopic Method (RD)

In the RD approach, the formerly scanned absorption spectrum of the mixture (CXB and TMD: 1-18 µg/mL) was divided by the spectrum of 9 µg/mL of TMD and 3 µg/mL CXB individually to achieve the ratio spectra of CXB and TMD, sequentially. The resulting ratio spectra were traced. Calibration curve was put up for CXB by making use of amplitude difference of attained ratio spectra at 241 and 257 nm; for TMD, amplitude difference of attained ratio spectra at 220 and 237 nm against concentrations were plotted and the regression equations were computed.[12,40-46]

First Derivative of Ratio Spectra Method (DR^1)

In the DR^1 approach, the formerly scanned absorption spectrum of the mixture (CXB and TMD: 1-18 µg/mL) was divided by the spectrum of 9 µg/mL of TMD and 3 µg/mL CXB individually to achieve the ratio spectra of CXB and TMD, sequentially. The first derivative ratio spectra were then documented. Using the first derivative signal at 236.4 nm, the quantity of CXB was calculated. For TMD at 229.8 nm, a similar technique was utilized. The first derivative signal's amplitudes were plotted against their respective concentrations, and regression equations were produced.[11,12,43-46]

Analysis of sample solution

The procedure for making and diluting the sample solution was described earlier. Standard absorbivities and absorbances of test solutions at their corresponding wavelengths were used to solve

a simultaneous equation to determine the amount of analyte for SEM. Absorbance was measured using the dual wavelength method, and analyte concentrations were calculated with regression equations. Whereas peak amplitude was recorded and analytes were quantified using regression equations in the other three procedures (^{1}DR, RD, DR^1).

Validation of Spectroscopic Approaches

The established methodologies were validated as given in the recommendations of the "International Conference on Harmonization."[12,37,38,42,47-49]

Specificity

A specificity study was performed to examine the interaction between tablet formulation excipients and drug ingredients. All tablet excipients (according to the commercial formulation) were mixed ratio wise, diluted with methanol, and passed through a whatman filter paper no 41. Later scanning of placebo and reference solutions were performed and analyzed in the UV area to determine the interference between excipients and medicines.

Linearity and Range

Analysis of all the standard solutions consisting of CXB and TMD (1, 3, 6, 9, 12, 15 and 18 µg/mL) in methanol was performed separately, for assessing the linearity and range of all five approaches. For SEM, absorbance was measured at 217.2 and 272.4 nm, whereas the DW method used 270.2 and 278.2 nm for CXB; 228 and 260 nm for TMD. For ^{1}DR, amplitude was measured at 272.4 and 229.6 nm for CXB and TMD, sequentially. Differences in amplitude were measured at 241 and 257 nm for CXB, 220 and 237 nm for TMD in RD method. However, DR^1 method utilized 236.4 and 229.8 nm for CXB and TMD, respectively. Using absorbance versus concentration in SEM and DW approach, amplitude difference versus concentration in RD and amplitude of first derivative spectra versus concentration in ^{1}DR and DR^1 method, calibration graphs were created. Using the least-squares method, the slope, intercept, and correlation coefficient for CXB and TMD at their respective wavelengths were calculated for regression analysis.

Precision

Repeatability and intermediate precision (intra and inter-day, respectively) were measured and represented as percentage RSD for the acquired data so as to evaluate the precision of the procedures. Repeatability of measurement was performed at two concentration level (CXB and TMD: 6 and 12 µg/mL) 6 times for both medicines and computing the % RSD of the response. Inside the linearity range, intra and inter-day precision experiments were conducted at two distinct concentration levels (CXB and TMD: 6 and 12 µg/mL) for both medicines 3 times on the same day and on three different days, correspondingly, and the percent RSD of response was estimated.

Accuracy

To make sure the applicability and dependability of the proposed procedures, recovery analysis was conducted by standard addition technique. To a pre-examined test solution (CXB: 7 and TMD: 5.5 µg/mL), known concentrations of reference CXB and TMD were supplemented at the 50, 100, and 150% level, and the obtained solutions were re-examined using the proposed procedures and the percent recoveries were computed. Using the following formula, accuracy of the proposed approaches were evaluated on the basis of proportion of standard CXB and TMD recuperated from the formulation.

$$\% \text{ Recovery} = (\text{Quantity of analyte found after adding standard drug} - \text{Quantity of analyte adding before addition of standard drug}) / (\text{Quantity of standard analyte added}) \times 100$$

LOD and LOQ

For determining the sensitivity of the anticipated approaches in terms of LOD and LOQ in accordance with ICH recommendations, the limit of detection and limit of quantification of CXB and TMD were computed with the help of the below mentioned equation.

$$LOD = 3.3 \times \frac{\sigma}{S}$$

$$LOQ = 10 \times \frac{\sigma}{S}$$

Where σ = The SD of the response, S = The slope of the linear graph.

Stability of the Solution

By storing the solutions at room and refrigerated temperature and assessing them at regular time periods, the stability of the solutions was determined by monitoring any differences in absorbance/amplitude and spectral pattern as compared to newly created solutions.

Statistical comparison by Repeated Measures ANOVA

Comparison of assay results with the help of Repeated Measures ANOVA, IBM SPSS, version 20.0. was done.

RESULTS AND DISCUSSION

It is expected that the proposed spectrophotometric methods would find widespread application in quality control departments, where both affordability and rapid evaluation are paramount. Regular research of pharmaceutical preparation often employs UV-spectroscopic methods due to their simplicity, speed, low cost, and reproducible results. These spectrophotometric methods are superior to other analytical

methods and have many benefits. It is challenging to analyze all analytes in a multi-component formulation whose UV-spectra overlap without first separating them. To analyze CXB and TMD simultaneously in binary mixtures with overlapping spectra, this work proposes a straightforward and cost-effective method.

Simultaneous Equation Method (SEM)

This method was developed and demonstrated to be sensitive and selective enough for the detection of CXB and TMD in a synthetic combination. Absorption maxima were seen at 217.2 and 272.4 nm in the zero-order UV-spectra of CXB and TMD, respectively. As can be seen in Figure 2, there is spectral overlap between the UV-spectra of CXB and TMD, which makes it possible to estimate both components of the binary combination at the same time. A simultaneous equation was used to assess the total drug content of the formulation. Where A1 and A2 are the absorbances of the sample at 217.2 and 272.4 nm. ax1 (643.29) and ax2 (346.87) are the absorptivity of CXB at 217.2 and 272.4 nm, sequentially. ay1 (366.90) and ay2 (101.58) are the absorptivity of TMD at 217.2 and 272.4 nm, sequentially. Table 2 displays the results of the method validation parameters.

Dual Wavelength Method (DW)

The third technique evaluated CXB at wavelengths of 270.2 and 278.2 nm, where the absorbance difference for TMD was zero. The selected wavelengths for TMD were 228 and 260 nm, where the absorbance difference for CXB was zero (Figure 2). Then, calibration curves for CXB and TMD in the concentration series of 1-18 µg/mL were plotted. Results were put through regression analysis with the help of technique of least squares to establish slope, intercept, and correlation coefficient values.

Derivatization of UV-spectra

It is widely recognized that derivatization of UV-spectra increases the specificity and selectivity of pharmaceuticals in marketed preparation by enhancing the resolution of spectra. Additionally, derivatization eliminates the excipient effects and permits the computation of one analyte in the presence of another analyte. To obtain the ratio spectra, which are free of divisor analyte and excipient interferences, the mixture spectra are divided using one of the analyte spectra. When divided by an optimal spectrum, interferences and errors in the research are reduced. The ratio spectra method also boasts improved precision, sensitivity, and specificity because measurements are made in proportion to the peaks. Ratio spectroscopic methods were created as a result, and they produce superior results than other spectroscopic approaches.

1st Derivative Zero Crossing Method (^{1}DR)

Zero order UV-spectra of the analytes (CXB and TMD) were converted into their corresponding first derivative spectra and subsequently, first derivative signal at 272.4 and 229.6 nm was traced sequentially using 2 nm as wavelength interval (Δλ) and 1 as scaling factor. The 1st derivative UV-spectra of CXB and TMD which exhibit overlapping of spectra, zero-crossing point and detection wavelength has displayed in Figure 3, which enables simultaneous assessment of CXB and TMD in the binary admixture. The quantity of drugs exist in the formulation was computed by means of regression formula.

Optimization of divisor and scaling factor for first derivative of ratio spectra

Numerous instrumental variable settings were adjusted in order to attain the optimal curve for the first derivative of ratio spectra. The most crucial of these were scaling factor and divisor optimization. Different CXB and TMD concentrations were attempted in order to choose a divisor of the right concentration. The final divisor for the RD and DR^1 method of quantifying CXB

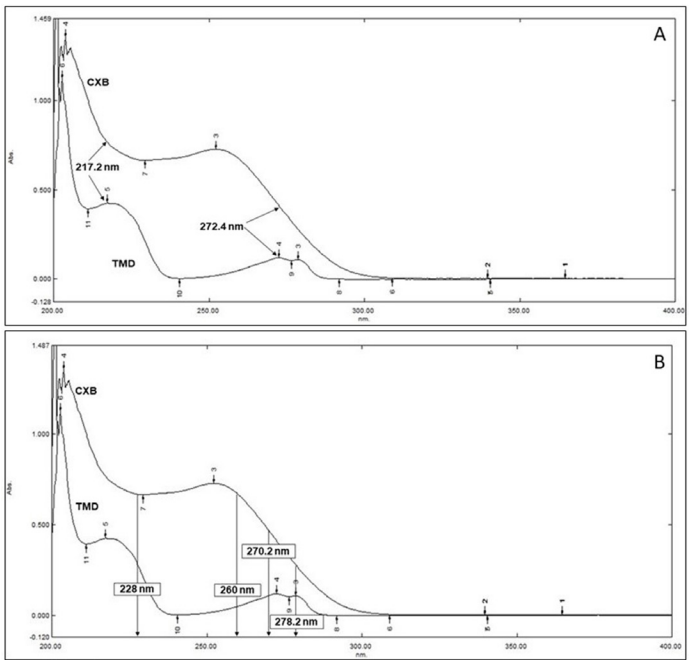

Figure 2: Overlain UV spectra of CXB and TMD (12 µg/mL) (A); Overlain UV spectra of CXB and MTD showing wavelengths of CXB at 270.2 and 278.2 nm, where the absorbance difference for TMD is zero and TMD at 228 and 260 nm, where the absorbance difference for CXB is zero (B).

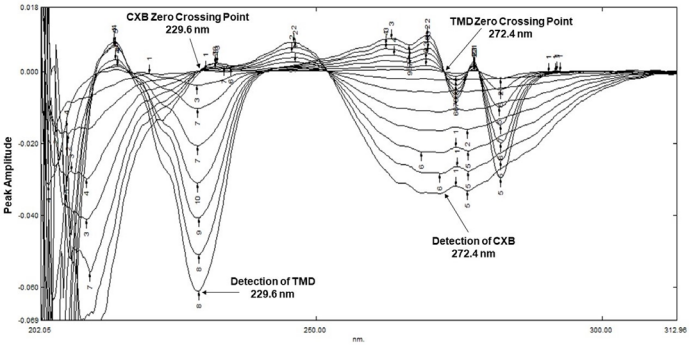

Figure 3: Overlain 1st derivative UV spectra of CXB and TMD (1-18 µg/mL) showing zero crossing points and detection wavelengths.

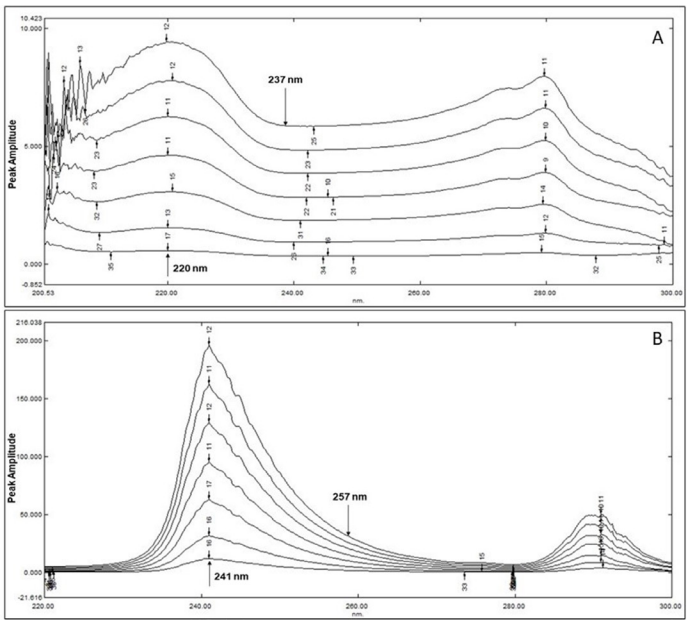

Figure 4: Overlain ratio spectra of CXB utilizing 9 µg/mL TMD as divisor (A); Overlain ratio spectra of TMD utilizing 3 µg/mL CXB as divisor (B).

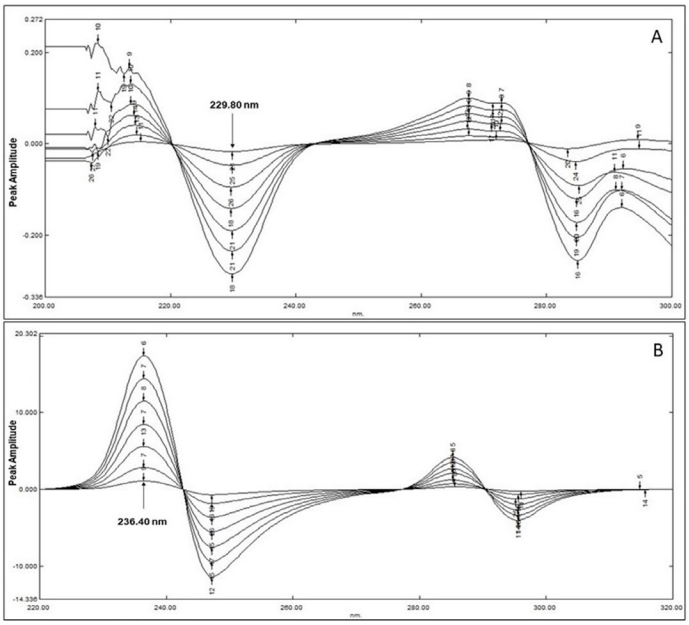

Figure 5: Overlain 1st derivative ratio spectra of CXB utilizing 9 µg/mL TMD as divisor (A) and Overlain ratio spectra of TMD utilizing 3 µg/mL CXB as divisor (B) at Δλ=4.

and TMD in their binary mixture was 9 µg/mL TMD and 3 µg/mL CXB. The first derivative of the ratio spectra was also attained by fixing the scaling factor at one, which was found to be optimal. The optimal wavelength for the first derivative spectra was tested at 2, 4, 8, and 10 nm. Based on the findings, a wavelength of 4 nm was selected and implemented using a scaling factor of 1.

Ratio Difference Spectroscopic Method (RD)

The wavelengths chosen for this procedure were 241 and 257 nm for determining CXB, whereas 220 and 237 nm for determining TMD. The amplitude difference at the given wavelength was then calculated for both CXB and TMD. The concentration of CXB was estimated utilizing the linear regression equation derived by graphing the difference between the amplitude values at 241 and 257 nm ($\Delta P_{241\text{-}257}$) of the ratio spectra presented in Figure 4 against their respective concentrations. The concentration of TMD was computed by means of linear regression equation achieved by graphing the difference in amplitude values at 220 and 237 nm ($\Delta P_{220\text{-}237}$) of the ratio spectra displayed in Figure 4 against their respective concentrations.

1st Derivative of Ratio Spectra Method (DR^1)

CXB was successfully determined using this method by dividing the spectra of mixed standard solutions by 9 µg/mL of TMD. Using 1 as a scaling factor and 4 as Δλ, the resultant ratio spectra were transformed to their first-order spectra. Using the first derivative signal at 236.4 nm, the quantity of CXB was calculated. For TMD, a similar technique was utilized, with 3 µg/mL CXB serving as the divisor, 1 as the scaling factor, and 4 as Δλ. The TMD concentration was then calculated by detecting the first derivative signal at 229.8 nm. The first derivative signal's amplitudes were plotted against their respective concentrations, and regression equations were produced. Within the concentration series of 1-18 µg/mL, a linear relationship between CXB and TMD was observed. Figure 5 depicts the overlaying first derivatives of the ratio spectra of CXB and TMD.

Method Validation

All of the proposed methods were evaluated based on the "International Conference on Harmonization" criteria for reliability. The subsequent part talks about the results of different validation parameters.

Specificity

It was confirmed that there was no interaction between excipients and standard pharmaceuticals by looking at the overlapping spectra of drug solutions and placebos. A placebo is a mixture of common excipients that are used in the marketed formulation. This information was presented in the preceding section.

Linearity and Range

In order to evaluate linear correlation and range, absorbance was measured at predetermined wavelengths for the SEM and DW approaches, the amplitude difference of ratio spectra for the RD approach, and the amplitude of first derivative spectra for the ^1DR and DR^1 method. Linear correlation was noticed for both the analytes between 1-18 µg/mL for all five methods. Using the least-squares method, the slope, intercept, and correlation coefficient for CXB and TMD at their respective wavelengths were calculated for regression analysis. The value of the correlation

Table 1: Summary of linear regression and method validation data for the projected approaches.

Parameters	SEM		DW		^{1}DR		RD		DR^1	
	CXB	TMD	CXB	TMD	CXB	TMD	CXB	TMD	CXB	TMD
Wavelengths (nm)	272.4	217.2	270.2-278.2	228-260	272.4	229.6	241-257	220-237	236.4	229.8
Linearity range (µg/mL)	1-18									
Correlation coefficient	0.9998	0.9991	0.9995	0.9997	0.9996	0.9999	0.9996	0.9999	0.9997	0.9998
Regression equation:	y = 0.0346x - 0.0006	y = 0.036x + 0.0028	y = 0.0146x - 0.0003	y = 0.0185x - 0.0003	y = 0.0019x + 0.0001	y = 0.0034x + 0.0003	y = 8.7692x - 0.5852	y = 0.1925x + 0.0179	y = 0.9647x - 0.051	y = 0.0157x + 0.0015
LOD (µg/mL)	0.2133	0.0504	0.1172	0.1383	0.2375	0.1650	0.0726	0.1318	0.0588	0.1490
LOQ (µg/mL)	0.6465	0.1529	0.3551	0.4192	0.7198	0.4999	0.2201	0.3995	0.1781	0.4516
Specificity	No interferences									
Precision (% RSD) Repeatability of measurement (n=6)*	0.6234	0.6886	1.7987	1.6630	1.7207	0.8012	1.6362	0.4320	0.7790	1.5541
Intra-day (n=3)*	0.1314	1.0943	1.3679	1.5491	1.7538	0.6831	0.5977	0.9258	1.1008	1.3164
Inter-day (n=3)*	0.1706	0.7630	1.6431	1.2209	1.4942	0.8671	0.9768	1.1498	0.7442	1.7768

*n = number of estimations, % RSD (% Relative standard deviation).

Table 2: Recovery information of the projected approaches.

Drugs	Recovery Level (%)	Recovery (%)*					RSD (%)				
		SEM	DW	^{1}DR	RD	DR^1	SEM	DW	^{1}DR	RD	DR^1
CXB	50	98.72 ± 1.37	98.37 ± 0.85	98.40 ± 0.84	98.05 ± 0.43	99.41 ± 1.06	1.39	0.87	0.85	0.44	1.07
	100	99.02 ± 0.69	100.68 ± 0.49	98.20 ± 0.49	98.43 ± 1.22	98.11 ± 1.24	0.70	0.48	0.50	1.23	1.27
	150	99.70 ± 0.52	97.83 ± 0.45	99.38 ± 1.61	97.80 ± 0.50	97.89 ± 0.58	0.52	0.46	1.62	0.51	0.60
TMD	50	98.20 ± 0.66	98.04 ± 0.35	98.75 ± 0.44	98.73 ± 0.53	98.36 ± 0.91	0.67	0.37	0.45	0.48	0.93
	100	98.12 ± 0.49	100.35 ± 0.93	99.62 ± 1.09	99.69 ± 0.97	99.45 ± 0.93	0.50	0.93	1.10	0.98	0.94
	150	98.69 ± 0.60	98.99 ± 0.59	99.85 ± 0.59	97.69 ± 1.19	99.66 ± 1.52	0.61	0.60	0.60	1.22	1.52

*Mean ± SD (n = 3), SD (Standard deviation), % RSD (% Relative standard deviation).

Table 3: Results of formulation analysis utilizing different approaches.

Drugs	Labelled Amount (mg/tab)	Amount Found (mg/tab)					Amount Found (%)*					RSD (%)				
		SEM	DW	^{1}DR	RD	DR^1	SEM	DW	^{1}DR	RD	DR^1	SEM	DW	^{1}DR	RD	DR^1
CXB	56	55.33	55.25	55.06	54.97	55.18	98.81±0.93	98.67±1.57	98.33±0.70	98.15±1.15	98.54±0.63	0.94	1.60	0.71	1.17	0.64
TMD	44	43.63	43.05	43.20	42.89	43.89	99.17±1.39	97.85±0.77	98.19±1.15	97.48±0.93	99.75±1.30	1.40	0.79	1.17	0.95	1.30

*Mean ± SD (n = 6), SD (Standard deviation), % RSD (% Relative standard deviation).

coefficient argues in favor of the linearity of every method that has been established (Table 1). Each response reflected an average of the results of six separate investigations.

Precision

Results of precision trials (repeatability, intra and inter-day variation) expressed in % RSD meet ICH suggested limits (<2), confirming the outstanding repeatability and low intra and inter-day variation of all the proposed methods (Table 1).

Accuracy

The accurateness of the anticipated methodologies were determined by calculating it on the basis of the retrieval of drugs using the standard addition technique. The results of the recovery trials showed that all of the established techniques were accurate, as they ranged from 96 to 102% for each drug (Table 2).

LOD and LOQ

The extent of responsiveness of the anticipated approaches were demonstrated by extremely low values of LOD and LOQ for all five methods (Table 1).

Stability of the Solution

The stability of the solution was tested in both room temperature and a cooled environment (6°C), and it was found to be unaffected for up to two days in a room temperature and ten days in a chilled environment.

Determination of CXB and TMD in Binary Mixture

The anticipated methods were successfully utilized to evaluate CXB and TMD. Six separate measurements were conducted to obtain a statistically reliable dataset, with results ranging from 97% to 102% for both the substances. Consequently, these established techniques can be used to simultaneously assess CXB and TMD in combination (Table 3).

Statistical comparison by Repeated Measures ANOVA

Statistical methods were utilized to analyze the data obtained from the assays, aiming to determine the influence of the five proposed approaches. The statistical software IBM SPSS version 20.0 was employed to conduct a Repeated Measures ANOVA to compare the significance of the five distinct approaches. A significance level of $p<0.05$ was established for all tests. According to Table 4, the Repeated Measures ANOVA demonstrated that there was minimal variation among the developed methods.

CONCLUSION

Five distinct spectroscopic approaches, SEM, DW, ^{1}DR, RD and DR^1 were developed for the simultaneous assessment of CXB and TMD in combined synthetic mixture. All approaches were validated in accordance with ICH recommendations. It was

Table 4: Statistical comparison of assay results utilizing Repeated Measures ANOVA.

Groups	n	Method	Mean (% Assay)	SD	Mean Rank	*p* value
CXB	6	SEM	99.17	0.983	3.920	0.429
	6	DW	98.83	1.602	2.920	
	6	^{1}DR	98.33	0.816	2.670	
	6	RD	98.17	1.329	2.420	
	6	DR^1	98.67	0.816	3.080	
TMD	6	SEM	99.00	1.265	3.750	0.061
	6	DW	98.17	0.753	2.500	
	6	^{1}DR	98.33	1.033	2.670	
	6	RD	97.50	1.049	2.000	
	6	DR^1	99.67	1.366	4.080	

n = 6 (Number of determinations); SD (Standard deviation), p value (significant if $p < 0.05$).

confirmed that the proposed approaches are economical, easy, sensitive, precise and accurate. In addition, the UV-spectrophotometric approaches developed need minimal sample preparation and have a broad concentration range and good sensitivity. There is no statistically significant variation among the five approaches. Therefore, all the described methodologies are suitable for regular quality control examination of CXB and TMD in binary mixture or tablet dosage form.

ACKNOWLEDGEMENT

The authors are sincerely grateful to Sumandeep Vidyapeeth Deemed to be University, Piparia, Waghodia, Vadodara, Gujarat, India for offering financial support and all the resources for the smooth conduct of the study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

API: Active Pharmaceutical Ingredient; **PK:** Pharmacokinetics; **FDCs:** Fixed dose combinations; **FDA:** Food and Drug Administration; **COX-2:** Cyclooxygenase-2; **CXB:** Celecoxib; **TMD:** Tramadol hydrochloride; **USFDA:** United State Food and Drug Administration; **ICH:** International Conference on Harmonization; **SEM:** Simultaneous Equation Method; **DW:** Dual Wavelength Method; **^{1}DR:** 1st Derivative Zero Crossing Method; **RD:** Ratio Difference Spectroscopic Method; **DR^1:** First Derivative of Ratio Spectra Method.

SUMMARY

Seglentis® tablets containing Celecoxib (CXB) and Tramadol Hydrochloride (TMD) have given approval by the Food and Drug Administration (FDA) for the treatment of adults suffering from acute pain. This paper proposes five quick, easy, accurate, and reproducible spectrophotometric procedures for assessing binary mixtures simultaneously. The quantification of proposed medications were performed using the simultaneous equation approach by measuring absorbance at 217.2 and 272.4 nm. The second approach, known as the dual wavelength method, relies on computing the absorbance difference at two different wavelengths where the other drug has same absorbance. The first derivative zero crossing approach relied on the conversion of UV-spectra into first derivative spectra, which was then followed by the measurement of amplitude at 272.4 and 229.6 nm for CXB and TMD, respectively. The ratio difference approach was used to measure the difference in the amplitudes of ratio spectra, and the regression equation was employed to determine the amounts of pharmaceuticals. In first derivative ratio spectra method, the UV-spectra were transformed into their ratio spectra and first derivatives, and the first-derivative signal was gauged at 236.4 and 229.8 nm for CXB and TMD, sequentially. Both the medications showed excellent linear correlation in the concentration series of 1-18 µg/mL for all the approaches. The proposed methodologies were validated according to ICH strategies and showed good precision, accuracy, and sensitivity. Because they do not require expensive solvents or specialised instruments, the new spectrophotometric techniques are considered cheaper than conventional analytical procedures. Thus, the proposed approaches might be used to assess CXB and TMD in binary mixture.

REFERENCES

1. Moore RA, Derry S, Aldington D, Wiffen PJ. Single dose oral analgesics for acute postoperative pain in adults-an overview of Cochrane reviews. Cochrane Database Syst Rev. 2015;9(9):CD008659. doi: 10.1002/14651858.CD008659.pub3, PMID 26414123.
2. Correll DJ, Vlassakov KV, Kissin I. No evidence of real progress in treatment of acute pain, 1993-2012: Scientometric analysis. J Pain Res. 2014;7:199-210. doi: 10.2147/JPR.S60842, PMID 24748816.
3. Sommer M, De Rijke JM, Van Kleef M, Kessels AG, Peters ML, Geurts JW, *et al*. The prevalence of postoperative pain in a sample of 1490 surgical inpatients. Eur J Anaesthesiol. 2008;25(4):267-74. doi: 10.1017/S0265021507003031, PMID 18053314.
4. Wu CL, Raja SN. Treatment of acute postoperative pain. Lancet. 2011;377(9784):2215-25. doi: 10.1016/S0140-6736(11)60245-6, PMID 21704871.

5. Chou R, Gordon DB, de Leon-Casasola OA, Rosenberg JM, Bickler S, Brennan T, *et al*. Management of postoperative Pain: A clinical practice guideline from the American Pain Society, the American Society of Regional Anesthesia and Pain Medicine, and the American Society of Anesthesiologists' Committee on Regional Anesthesia, Executive Committee, and Administrative Council. J Pain. 2016;17(2):131-57. doi: 10.1016/j.jpain.2015.12.008, PMID 26827847.
6. Thipparaboina R, Kumar D, Chavan RB, Shastri NR. Multidrug co-crystals: Towards the development of effective therapeutic hybrids. Drug Discov Today. 2016;21(3):481-90. doi: 10.1016/j.drudis.2016.02.001, PMID 26869329.
7. Desai D, Wang J, Wen H, Li X, Timmins P. Formulation design, challenges, and development considerations for fixed dose combination (FDC) of oral solid dosage forms. Pharm Dev Technol. 2013;18(6):1265-76. doi: 10.3109/10837450.2012.660699, PMID 22339230.
8. Available from: https://www.rxlist.com/seglentis-drug.htm#description.
9. Available from: https://reference.medscape.com/drug/seglentis-celecoxib-tramadol-4000087#10.
10. Attala K, Elsonbaty A. Smart UV-spectrophotometric methods based on simple mathematical filtration for the simultaneous determination of celecoxib and ramipril in their pharmaceutical mixtures with amlodipine: A comparative statistical study. Spectrochim Acta A Mol Biomol Spectrosc. 2021;244:118853. doi: 10.1016/j.saa.2020.118853, PMID 32882657.
11. Nakhla DS, Hussein LA, Magdy N, Abdallah IA. Comparative study of different spectrophotometric methods for simultaneous determination of tramadol and paracetamol in pharmaceutical formulations. J Appl Spectrosc. 2021;88(4):894-900. doi: 10.1007/s10812-021-01256-7.
12. Attimarad M, Narayanswamy VK, Aldhubaib BE, Sreeharsha N, Nair AB. Development of UV-spectrophotometry methods for concurrent quantification of amlodipine and celecoxib by manipulation of ratio spectra in pure and pharmaceutical formulation. PLOS ONE. 2019;14(9):e0222526. doi: 10.1371/journal.pone.0222526, PMID 31525229.
13. Attimarad M, Venugopala KN, Aldhubiab BE, Nair AB, SreeHarsha N, Pottathil S, et al. Development of UV-spectrophotometric procedures for determination of amlodipine and celecoxib in formulation: Use of scaling factor to improve the sensitivity. J Spectrosc. 2019;2019:1-10. doi: 10.1155/2019/8202160.
14. Puranik M, Hirudkar A, Wadher SJ, Yeole PG. Development and validation of spectrophotometric methods for simultaneous estimation of tramadol hydrochloride and chlorzoxazone in tablet dosage form. Indian J Pharm Sci. 2006;68(6):737-9. doi: 10.4103/0250-474X.31005.
15. Saha RN, Sajeev C, Jadhav PR, Patil SP, Srinivasan N. Determination of celecoxib in pharmaceutical formulations using UV-spectrophotometry and liquid chromatography. J Pharm Biomed Anal. 2002;28(3-4):741-51. doi: 10.1016/s0731-7085(01)00678-1, PMID 12008154.
16. Murthy TK, Reddy MN, Sankar DG. UV-spectrophotometric methods for the determination of celecoxib and tizanidine hydrochloride. Indian J Pharm Sci. 2001;63(6):521-3.
17. Attimarad M, Venugopala KN, SreeHarsha N, Aldhubiab BE, Nair AB. Validation of rapid RP-HPLC method for concurrent quantification of amlodipine and celecoxib in pure and formulation using an experimental design. Microchem J. 2020;152:104365. doi: 10.1016/j.microc.2019.104365.
18. Ahmed HM, Elshamy YS, Talaat W, Labib HF, Belal TS. Simultaneous analysis of chlorzoxazone, diclofenac sodium and tramadol hydrochloride in presence of three potential impurities using validated HPLC-DAD and HPTLC methods. Microchem J. 2020;153:104505. doi: 10.1016/j.microc.2019.104505.
19. Abdel Hamid MA, Mabrouk MM, Michael MA. A fast and green reversed-phase HPLC method with fluorescence detection for simultaneous determination of amlodipine and celecoxib in their newly approved fixed-dose combination tablets. J Sep Sci. 2020;43(16):3197-205. doi: 10.1002/jssc.202000345, PMID 32506818.
20. Tome T, Časar Z, Obreza A. Development of a unified reversed-phase HPLC method for efficient determination of EP and USP process-related impurities in celecoxib using analytical quality by design principles. Molecules. 2020;25(4):809-26. doi: 10.3390/molecules25040809, PMID 32069880.
21. Nagamani P, Manjunath SY, Kumar TH. Development and validation of RP-HPLC method for estimation of amlodipine besylate and celecoxib in pharmaceutical formulation. J Drug Deliv Ther. 2020;10(6):31-6. doi: 10.22270/jddt.v10i6.4521.
22. Donda ST, Baviskar VB, Bari SB, Deshmukh PK, Deore DS, Girase NM, *et al*. Development and validation of RP-HPLC method for the simultaneous estimation of tramadol hydrochloride and dicyclomine in bulk and pharmaceutical formulation. J Chil Chem Soc. 2016;61(2):2852-5. doi: 10.4067/S0717-97072016000200001.
23. Bapatu HR, Maram RK, Murthy RS. Stability-indicating HPLC method for quantification of celecoxib and diacerein along with its impurities in capsule dosage form. J Chromatogr Sci. 2015;53(1):144-53. doi: 10.1093/chromsci/bmu031, PMID 24837233.
24. Jadhav PS, Jamkar PM, Avachat AM. Stability indicating method development and validation for simultaneous estimation of atorvastatin calcium and celecoxib in bulk and niosomal formulation by RP-HPLC. Braz J Pharm Sci. 2015;51(3):653-61. doi: 10.1590/S1984-82502015000300017.
25. Baboota S, Faiyaz S, Ahuja A, Ali J, Shafiq S, Ahmad S. Development and validation of a stability indicating HPLC method for analysis of celecoxib (CXB) in bulk drug and microemulsion formulations. Acta Chromatogr. 2007;18:116-29.
26. Hamama AK, Ray J, Day RO, Brien JA. Simultaneous determination of rofecoxib and celecoxib in human plasma by high-performance liquid chromatography. J Chromatogr Sci. 2005;43(7):351-4. doi: 10.1093/chromsci/43.7.351, PMID 16176646.
27. Kartinasari WF, Palupi T, Indrayanto G. HPLC determination and validation of tramadol hydrochloride in capsules. J Liq Chromatogr Relat Technol. 2004;27(4):737-44. doi: 10.1081/JLC-120028261.
28. Dhabu PM, Akamanchi KG. A stability indicating HPLC method to determine celecoxib in capsule formulations. Drug Dev Ind Pharm. 2002;28(7):815-21. doi: 10.1081/ddc-120005627, PMID 12236067.
29. Naguib IA, Ali NA, Elroby FA, El Ghobashy MR, Abdallah FF. Ecologically evaluated and FDA-validated HPTLC method for assay of pregabalin and tramadol in human biological fluids. Biomed Chromatogr. 2021;35(4):e5023. doi: 10.1002/bmc.5023, PMID 33169415.
30. Attala K, Eissa MS, El-Henawee MM, Abd El-Hay SS. Application of quality by design approach for HPTLC simultaneous determination of amlodipine and celecoxib in presence of process-related impurity. Microchem J. 2021;162:105857. doi: 10.1016/j.microc.2020.105857.
31. Dhumal BR, Bhusari KP, Patra A, Thareja S, Jain NS. Stability indicating high performance thin layer chromatographic method for the determination of tramadol hydrochloride in pharmaceutical formulation. J Liq Chromatogr Relat Technol. 2015;38(10):1088-93. doi: 10.1080/10826076.2015.1020167.
32. Roosewelt C, Harihrishnan N, Gunasekaran V, Chandrasekaran S, Haribaskar V, Prathap B. Simultaneous estimation and validation of tramadol and paracetamol by HPTLC in pure and pharmaceutical dosage form. Asian J Chem. 2010;22(2):850-4.
33. Park MS, Shim WS, Yim SV, Lee KT. Development of simple and rapid LC–MS/MS method for determination of celecoxib in human plasma and its application to bioequivalence study. J Chromatogr B Analyt Technol Biomed Life Sci. 2012;902:137-41. doi: 10.1016/j.jchromb.2012.06.016, PMID 22771234.
34. Attimarad M, Al-Dhubiab BE, Alhaider IA, Nair AB, Sree HN, Mueen AK. Simultaneous determination of moxifloxacin and cefixime by first and ratio first derivative ultraviolet spectrophotometry. Chem Cent J. 2012;6(1):105. doi: 10.1186/1752-153X-6-105, PMID 22995678.
35. Patel IM, Chhalotiya UK, Jani HD, Kansara D, Kachhiya HM, Shah DA. Simultaneous quantification of empagliflozin, linagliptin and metformin hydrochloride in bulk and synthetic mixture by RP–LC method. Future J Pharm Sci. 2021;7(1):182-91. doi: 10.1186/s43094-021-00332-1.
36. Sen AK, Hinsu DN, Sen DB, Zanwar AS, Maheshwari RA, Chandrakar VR. Analytical method development and validation for simultaneous estimation of Teneligliptin hydrobromide hydrate and metformin hydrochloride from its pharmaceutical dosage form by three different UV-spectrophotometric methods. J Appl Pharm Sci. 2016;6(9):157-65.
37. Sen AK, Sen DB, Maheshwari RA, Balaraman R, Seth AK. Simultaneous estimation of aliskiren hemifumarate and hydrochlorothiazide in combined Tablet Formulation by Simultaneous equation, Absorbance ratio and First derivative Spectroscopic Methods. J App Pharm Sci. 2016;6(7):164-70. doi: 10.7324/JAPS.2016.60724.
38. Beckett AH, Stenlake JB. Instrumental methods in the development and use of medicines. In: Practical Pharmaceutical Chemistry Part 2. 4th ed. New Delhi, CBS Publishers and Distributors. 2005;1(3):275-99.
39. Eissa MS, Abou Al Alamein AM. Innovative spectrophotometric methods for simultaneous estimation of the novel two-drug combination: Sacubitril/valsartan through two manipulation approaches and a comparative statistical study. Spectrochim Acta A Mol Biomol Spectrosc. 2018;193:365-74. doi: 10.1016/j.saa.2017.12.050, PMID 29272807.
40. Darwish HW, Bakheit AH, Naguib IA. Comparative study of novel ratio spectra and isoabsorptive point based spectrophotometric methods: Application on a binary mixture of ascorbic acid and rutin. J Anal Methods Chem. 2016;2016:2828647. doi: 10.1155/2016/2828647, PMID 26885440.
41. Zaazaa HE, Elzanfaly ES, Soudi AT, Salem MY. Application of the ratio difference spectrophotometry to the determination of ibuprofen and famotidine in their combined dosage form; Comparison with previously published spectrophotometric methods. Spectrochim Acta A Mol Biomol Spectrosc. 2015;143:251-5. doi: 10.1016/j.saa.2015.02.050, PMID 25733252.
42. Lotfy HM, Hassan NY, Elgizawy SM, Saleh SS. Comparative study of new spectrophotometric methods; An application on pharmaceutical binary mixture of ciprofloxacin hydrochloride and hydrocortisone. J Chil Chem Soc. 2013;58(3):1892-8. doi: 10.4067/S0717-97072013000300022.
43. Millership JS, Parker C, Donnelly D. Ratio spectra derivative spectrophotometry for the determination of furosemide and spironolactone in a capsule formulation. Farmaco. 2005;60(4):333-8. doi: 10.1016/j.farmac.2005.02.001, PMID 15848209.
44. Erk N. Derivative ratio spectrophotometry and differential derivative spectrophotometric determination of isoniazid and pyridoxine hydrochloride in dosage forms. Spectrosc Lett. 2001;34(6):745-61. doi: 10.1081/SL-100107897.

45. Sen DB, Sen AK, Zanwar AS, Pandey H, Maheshwari RA. UV-spectrophotometric methods to quantify alogliptin benzoate and pioglitazone hydrochloride. J Pharm Res Int. 2021;33(37B):31-41. doi: 10.9734/jpri/2021/v33i37B32017.
46. Alvi SN, Patel MN, Kathiriya PB, Patel BA, Parmar SJ. Simultaneous determination of prasugrel and aspirin by second order and ratio first order derivative ultraviolet spectrophotometry. J Spectrosc. 2013;2013:1-7. doi: 10.1155/2013/705363.
47. International Conference on Harmonization (ICH). Validation of Analytical Procedures: Text and Methodology Q2(R1). Switzerland, Geneva; 2005.
48. Sen AK, Pandey H, Maheshwari RA, Zanwar AS, Velmurugan R, Sen DB. Novel UV Spectroscopic Methods for Simultaneous Assessment of Empagliflozin, Linagliptin and Metformin in Ternary Mixture. Indian J Pharm Edu Res. 2022;56(4s):s669-s681. doi: 10.5530/ijper.56.4s.213.
49. Sen DB, Jatu S, Maheshwari RA, Zanwar AS, Velmurugan R, Sen AK. New Eco-friendly UV-spectroscopic Methods for Simultaneous Assessment of Dapagliflozin, Saxagliptin and Metformin in Ternary Mixture. Indian J Pharm Edu Res. 2023;57(2):559-69. doi: 10.5530/ijper.57.2.69.

Cite this article: Sen AK, Darji P, Sen DB, Zanwar AS, Aundhia CJ, Maheshwari RA. Different Innovative UV-Spectroscopic Approaches for Simultaneous Assessment of Celecoxib and Tramadol Hydrochloride in Binary Mixture. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s787-s797.