Ind. J. Pharm. Edu. Res., 2024; 58(3s):s805-s814.
https://www.ijper.org

Original Article

Smart Manipulated UV Spectroscopic Methods for Resolving the Overlapped Spectra for Quality Control of Two Analgesic Binary Combination Formulations

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ABSTRACT

Background: The combination of two drugs celecoxib + tramadol and diclofenac sodium + tramadol with different mechanisms of action is better for achieving effective pain control. **Materials and Methods:** The simple reproducible mathematically modified UV spectroscopic methods were established for concomitant evaluation of the binary combination of celecoxib + tramadol and diclofenac sodium + tramadol formulations. The first technique is predicated on separating the pure zero-order spectra of TDL and CCB from the mixture spectra and quantification at their lambda max. The second and third method involves the ratio absorption difference and ratio first derivative spectroscopic method for quantification of DFS and TDL. Additionally, we followed ICH guidelines to validate these approaches for specificity, accuracy, and precision. **Results:** Both formulations were effectively analyzed using the proposed methods, with results falling into the series of 2-50 µg/mL for TDL, and 1-30 µg/mL for DFS and CCB. The good recovery of 98.56% -101.48% with low relative error and percentage relative standard deviation verified the methodologies' correctness and repeatability. Finally, proposed mathematically modified spectroscopic procedures were exploited for quality control of analytes from formulation and manually prepared mixtures. The determination of % of retrieval of an added known quantity of authentic medications of CCB, TDL, and DFS to the powdered pill served as additional evidence of correctness. **Conclusion:** The established UV spectroscopic techniques are simple, rapid, and perfect for simultaneous quantification of CCB, DSF, and TDL from the solid dosage forms. The assay results also confirmed the nonexistence of tablet adjuvants intervention in the quantification of medicines in the tablets Therefore, these approaches can be utilized for systematic quantification of these drugs in the binary formulations without chemical separation.

Keywords: Celecoxib, Tramadol, Diclofenac sodium, Derivative spectroscopy, Validation, Formulation.

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Received: 25-01-2024;
Revised: 11-04-2024;
Accepted: 24-06-2024.

INTRODUCTION

Pain is a most common health problem due to multifactorial origin affecting all age populations.[1] It is difficult to monitor acute pain with monotherapy, hence blending two or more drugs with diverse mechanisms of action is better to achieve effective pain control.[2,3] Further, combination therapy works in different mechanisms and relieves pain with lower doses and fewer side effects compared to monotherapy. The binary combinations having celecoxib + tramadol and diclofenac sodium + tramadol are used for controlling the different types of acute pain. These combinations have better pain control due to both peripheral and central pain inhibition mechanisms hence used in the management of different kinds of pains such as musculoskeletal pain, post-operative pain, acute pancreatitis, osteo-arthritis, dental pain, and rheumatoid arthritis.[4,5]

Tramadol (TDL, Figure 1A), a synthetic opioid-related centrally acting analgesic drug, after its administration (+) tramadol will metabolize into (+) O-desmethyl tramadol and both forms show analgesic activity by binding to mu-opioid receptor. In addition, tramadol has an inhibition effect on the uptake of serotonin and non-epinephrine, which reduces pain transmission in the spinal cord. Further tramadol is safer than other opioids in terms of constipation and addiction properties.[6]

Diclofenac Sodium (DFS, Figure 1B), a propionic acid derivative non-steroidal anti-inflammatory drug, inhibits pain and inflammation by acting on the cyclooxygenase enzyme in addition to its novel mechanism of action. It is a nonspecific

DOI: 10.5530/ijper.58.3s.82

inhibitor of COX-1 and COX-2. However, a blend of diclofenac and tramadol is prescribed for the management of acute pain It is a more efficient and less disruptive combinatorial analgesic for the interim management of intense pain, and dental pain.[7]

Celecoxib (CCB, Figure 1C) is a powerful safer analgesic, antipyretic, and anti-inflammatory drug due to its selective inhibition of COX-2, which prevents the formation of only pain and inflammation mediator prostaglandins from the arachidonic acid in the peripheral region.[8]

DFS was estimated using the UV spectroscopic method, HPLC, GCMS, infrared spectroscopic, potentiometric, and fluorimetric methods in formulations and biological samples.[9-15] A simultaneous determination of DFS and TDL was described by means of first derivative spectroscopic, and HPLC methods.[16-18] Perman *et al.*[19] reported the stability indication HPLC technique for the contemporary estimation of diclofenac and tramadol. However, derivative UV spectroscopic procedures were not reported for the concomitant quantification of DFS and TDL, hence in the present work ratio difference absorption and ratio first derivative spectroscopic methods were demonstrated. Since the suggested techniques were straightforward, precise, and repeatable, they can be applied to standard quality assurance of both formulations.

Recently, a co-crystal of the equimolar quantity of celecoxib and tramadol in an easily dissociable form has been developed for the treatment of pain. The weak intermolecular interactions and other physicochemical properties of the co-crystal show better clinical applications of both the analytes compared to the effect of the individual drugs. The co-crystal shows better solubility and dissolution properties compared to the traditional binary combination formulation, which in turn improves the pharmacokinetic properties of the drugs. Furthermore, the co-crystal helps the patients to stick to the dosage, is easy to administer, and has low chances of medication error.[5]

The different analytical techniques are industrialized for the study of DFS, CCB, and TDL individually and in combination with other drugs. Furthermore, the quantification of both CCB and TDL was established using derivative spectroscopic, HPLC, HPTLC, and LCMS techniques individually and with other NSAIDs.[20-29] In addition the simultaneous estimation of both the analytes in co-crystal form was carried out using spectroscopic, HPLC, and HPTLC methods.[30-34] The reported UV spectroscopic procedures included a concurrent equation system, induced dual-wavelength process, first and second derivative methods, ratio absorption difference, and ratio derivative methods. However, the isolation of independent UV absorption spectra from the mixture spectra and measuring the absorption at their lambda max is a fingerprinting technique, that provides more accurate, reliable, and precise results. Hence, in this particular research, the individual spectra of CCB and TDL have been extracted from the mixture spectrum of celecoxib and tramadol and quantified without chemical separation.

MATERIALS AND METHODS

Materials and Reagents

The authentic standards of DFS (99.2%), TDL (99.5%), and CCB (98.7%) have been purchased from Sigma Aldrich. Analytical rating ethyl alcohol (99.8%) was supplied by Scharlau (Sentmenat, Spain). All solutions and dilutions were made with Millipore water purifier-prepared deionized fresh water.

Apparatus and software

UV-vis spectrophotometer (Shimadzu 1600) coupled with a laptop has been utilized for scanning the absorption spectra. Two undistinguishable quartz cuvettes (1 cm) supplied by Hellma were used as standard and sample solutions. The absorption spectra were recorded with fast scan speed, using single scan mode, along with a sampling interval of 0.1 nm, and the slit width was adjusted to 2 nm. UV probe (Shimadzu, Ver 2.2) software has been exploited for manipulation of stored UV absorption spectra, and wherever required spectra were smoothened using 4 nm as delta lambda to reduce the noise.

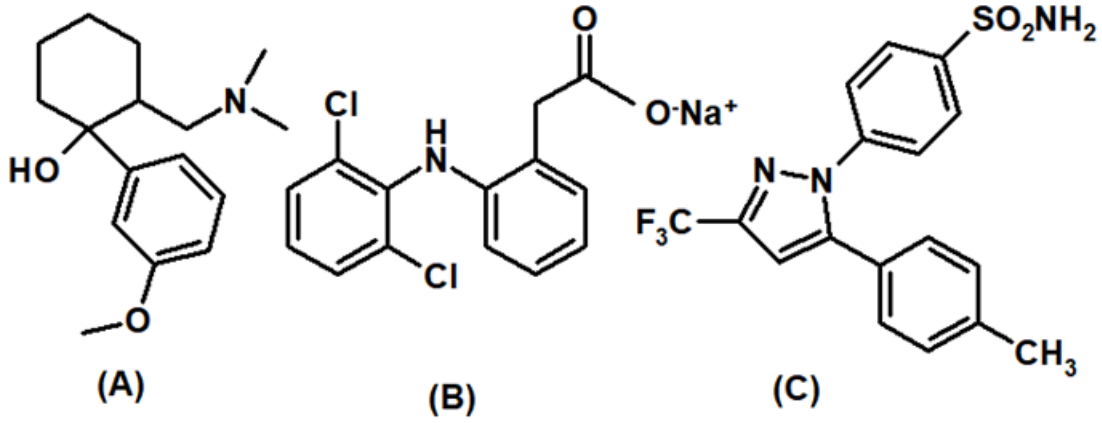

Figure 1: Structural formula of tramadol (A) diclofenac sodium (B) and celecoxib (C).

Preparation of authentic standards

The authentic analytically pure DFS, TDL, and CCB were weighed (50 mg) and dissolved in ethyl alcohol to get 1000 µg/mL solution. To get the proper concentrations of all three analytes for the creation of authentic working standards, the aforementioned solutions have been mixed with water.

Calibration curves

Procedure for isolation of zero order (^{0}D) spectra of TDL and CCB

The sufficient quantity of authentic working solutions of TDL and CCB were conveyed separately to 5 mL graduated flasks to get 2, 5, 10, 20, 30, 40, and 50 µg/mL and 1, 5, 10, 15, 20, 25, and 30 µg/mL final concentration respectively. After adjusting the final volume using fresh water, all solutions were processed between 200-400 nm, and spectra were deposited on the desktop. The absorption has been recorded at 270.5 nm and 248.9 nm for TDL and CCB respectively and a straight line was constructed against the corresponding concentration.

Ratio Absorption Difference method (RBD) for TDL and DFS

A suitable amount of authentic working solutions for TDL and DFS were transferred individually to a 5 mL volumetric flask in order to get final concentrations of 2, 5, 10, 20, 30, 40, and 50 µg/mL and 1, 5, 10, 15, 20, 25, and 30 µg/mL, respectively. All calibration standards have been scanned between 200 and 400 nm, and spectra were deposited in the laptop after the ultimate capacity was attuned with deionized water. TDL spectra were divided using 15 µg/mL solution spectrum of DFS to creäte ratio spectra of TDL. For each spectrum, the peak amplitude at 241.1 nm was subtracted from 220.9 nm to get the absorbance variance, which was displayed over the associated TDL quantity. Alternatively, the linearity equation and correlation coefficient were generated. Similarly, DFS spectra were divided using a 10 µg/mL spectrum of TDL to get the ratio spectra of DFS. The peak height at 339.6 nm was subtracted from 289.4 nm to find the peak magnitude divergence and plotted against the corresponding concentration of DFS. The straight-line equation and relationship coefficient generated were used for the determination of the concentration of DFS from the sample.

Ratio First Derivative method (RFD) for TDL and DFS

The first derivative spectra of the previously computed ratio spectra of TDL and DFS have been obtained by setting the delta lambda at 2 nm. The spectra were smoothened with 4 nm as delta lambda. The apex amplitudes at 230.7 nm and 284.6 nm were determined for TDL and DFS and plotted independently in relation to respective quantities. The straight line and correlation coefficients were generated and used for the determination of the concentration of TDL and DFS from the formulation.

Procedure for Preparation of manually mixed solutions and analysis

Different ratios of TDL+CCB and TDL+DFS were prepared separately by mixing the authentic working solutions to get final concentrations of 10:15, 5:15, 10:20, 20:10, and 30:10 respectively. Every solution was measured for UV absorption at a wavelength of 200–400 nm and then downloaded to a software program. The above-mentioned UV spectra manipulation technique was utilized to generate ratio and ratio first derivatization spectra of TDL and DFS from the respective mixture spectra. To conclude, the quantity of analytes in the combination has been determined by means of the relevant linearity calculation. For generating the normal spectra of TDL and CCB from the mixture spectra, the mixture spectra were divided by 10 µg/mL spectrum of TDL and CCB independently to generate ratio spectra of CCB and TDL separately. To derive the zero-order spectra of CCB, a constant value from the ratio spectra of CCB was arithmetically removed, brought to the baseline at 317.6 nm, and multiplied by the divisor spectra of TDL. Similarly, from the ratio spectra of TDL, a constant value was subtracted arithmetically to bring the ratio spectra of TDL to the baseline at 242.2 nm and multiplied by the divisor spectrum of CCB to create the normal spectra of TDL. The concentration was computed using the associated linear equation after the absorption was determined at 248.9 nm and 270.5 nm, respectively, from the normal spectra of CCB and TDL.

Procedure for Sample preparation and analysis

The average weight of twenty tablets consisting of TDL (44 mg) and CCB (56 mg) was determined and the pills were pulverized with the help of mortar and pastel. After precisely weighing the fine particles of the pill, which was equal to 11 mg of TDL and 14 mg of CCB, it was added to a 10 mL volumetric flask holding 5 mL of ethyl alcohol. After 15 min of sonication to dissolve the two analytes, the mixture was filtered into a second volumetric flask with a capacity of 10 mL. The remainder has been rinsed with additional ethyl alcohol and the solution was completed to the 10 mL mark with ethyl alcohol. In order to maintain the concentrations of both analytes within their linear value scale, an adequate volume of water has been added to the mixture. The UV absorption spectra were documented and stored in the computer and the normal spectra of TDL and CCB were isolated using the above-mentioned method. Since the neighbourhood pharmacies did not have the TDL and DFS tablets, ten tablets each of the DFS 75 mg and TDL 50 mg were collected and crushed. The substance containing 5.0 mg of TDL and 7.5 mg of DFS was transferred to a 10 mL graduated flask having 5 mL of ethyl alcohol. After 15 min of sonication, the mixture was filtered into a second 10 mL graduated container. After using ethanol to wash the leftovers, the final amount was adjusted to the 10 mL threshold. To get the concentration within the linearity line, the necessary amount of mixture was adjusted with water. The solution was scanned for recording the UV absorption spectra and kept in the laptop. The

ratio spectra and ratio first derivative spectra were generated following the above-mentioned methods. Finally, concentration of DFS and TDL was computed with the help of the respective linearity formula.

RESULTS AND DISCUSSION

CCB, TDL, and DFS are aromatic compounds with polar function groups, hence show very noble UV absorption. Analysis of these compounds using UV spectroscopic technique is easy, accurate, and reproducible if they are present alone. Further, UV spectrophotometric methods are fast, easy to perform, economical, and eco-friendly due to the use of safe and less amount of solvents. However, the multi-component formulation of these analytes showed completely overlapped UV absorption spectra, producing a difficult situation to measure without physical separation (Figure 2). However, different manipulation techniques of UV absorption spectra for isolation of the pure normal spectra of one of the components or removing the effect of one of the components by generation of ratio spectra or differentiation techniques are established.[35-39]

Hence, in the present work, CCB and TDL were quantified by isolating pure spectra of CCB and TDL from mixture spectra. Whereas DFS and TDL were quantified by the ratio difference absorption method and ratio first derivative method without the physical separation of both the analytes from the mixture.

Isolation of normal (^{0}D) spectra method

Segregation of normal spectra of pure analyte from the mixture spectra involves the division of mixture spectra with one of the analytes, to generate the ratio spectra.[39] The effect of another analyte in the ratio spectra could be excluded by bringing the ratio spectra to the baseline by deducting a fixed value arithmetically, thereafter multiplying by the divisor spectrum. Different concentration spectra of CCB and TDL were envisaged to select appropriate concentration spectra as a divisor spectrum, based on the good reproducibility and sensitivity 10 µg/mL spectra of CCB and TDL were selected. The ratio spectrum of pure CCB showed zero absorption at 317.6 nm, (Figure 3A) whereas the ratio spectra obtained from the mixture showed some absorption at 317.6 nm due to the presence of TDL. The TDL effect was eliminated by subtracting the constant at 317.6 nm to fetch the ratio spectra to baseline at 317.6 nm. The normal spectra of the CCB were produced by multiplying the fetched ratio spectra by the divisor spectrum. (Figure 3B). Similarly, the mixture spectra were divided with CCB spectrum, then deducting a fixed value from the ratio spectra a constant value at 242.2 nm (Figure 3C), and multiplication with divisor spectra generated the normal spectra of TDL (Figure 3D). The absorption was measured at the lambda max of CCB and TDL at 248.9 nm and 270.5 nm respectively and straight lines were generated separately.

Ratio Absorption Difference method (RAD)

The RAD technique is a well-established procedure for the estimation of one of the components from the mixture spectra.[37,38] The process entails creating ratio spectra by partitioning the blended spectra with one of the analytes. By determining the absorption discrepancy at two chosen wavelengths at the peak and the trough, one may eliminate the effect of one of the analytes. In the present work, the peak and the trough of ratio spectra of

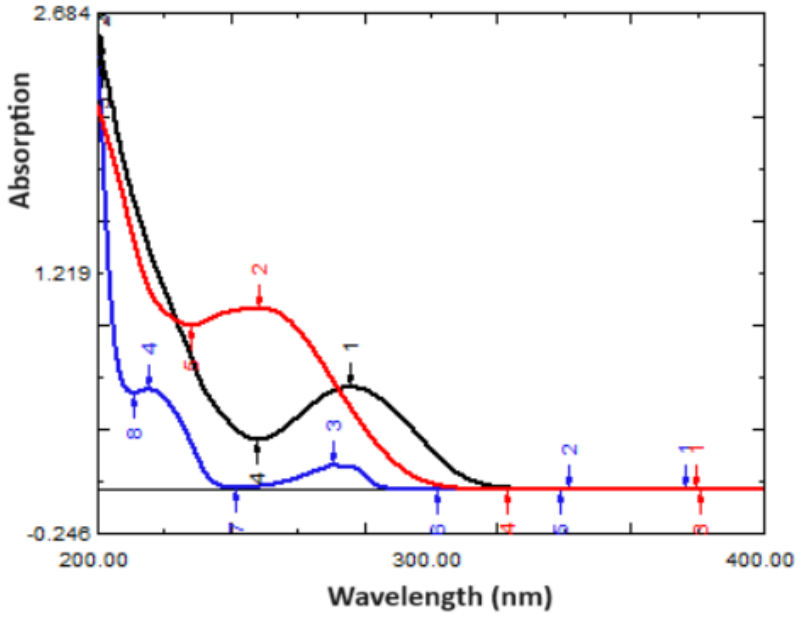

Figure 2: UV absorption spectra of tramadol (blue), diclofenac sodium (red) and celecoxib (black).

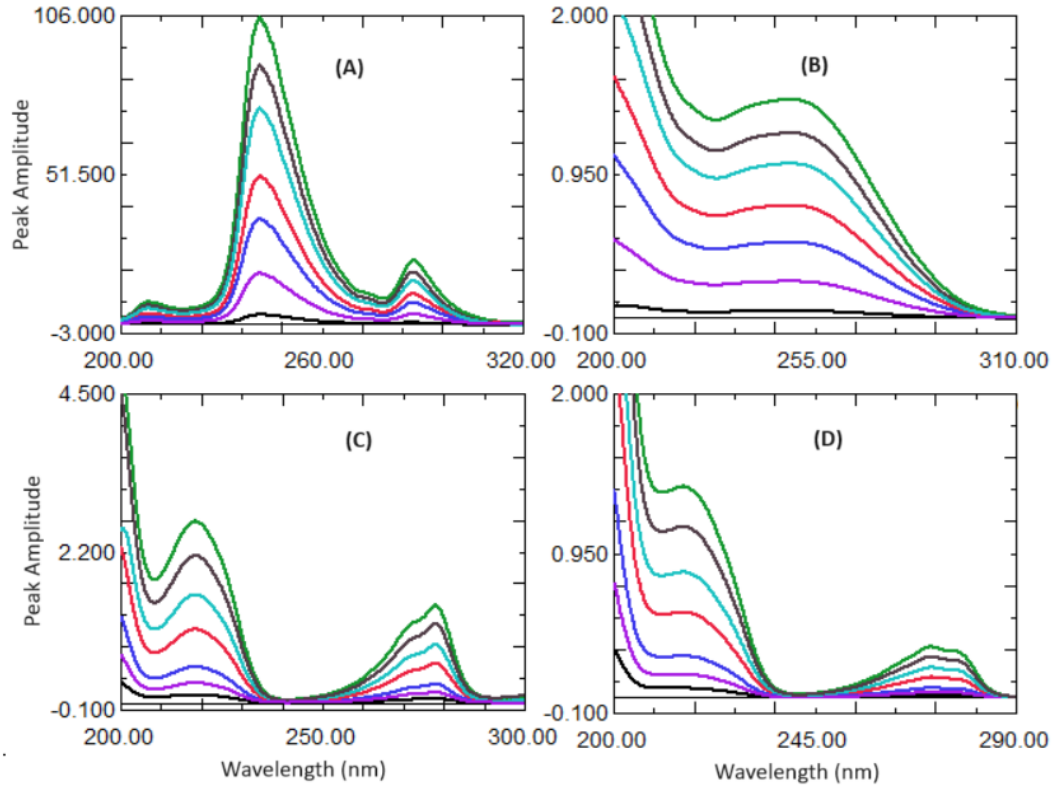

Figure 3: Ratio absorption spectra of CCB using TDL as divisor (A), Ratio absorption spectra of TDL using CCB as divisor (C), normal spectra of CCB (B) and TDL (D).

DFS (Figure 4A) were at 289.4 nm and 339.6 nm whereas for TDL (Figure 4B) they were 220.9 nm and 241.1 nm correspondingly. A series of DFS and TDL in the series of 1 to 30 µg/mL and 2-50 µg/mL were scanned in the wavelength 200 – 400 nm followed by division with 10 µg/mL TDL and 15 µg/mL DSF spectra to produce ratio spectra of DFS and TDL correspondingly.

Additionally, the ratio spectra's peak amplitude difference between the pure and combination analytes revealed the same discrepancy. (Figures 4C and 4D) Using the relevant linearity equation, the concentration was determined using the peak amplitude difference computed from the ratio spectra.

Ratio First Derivative method (RFD)

Alternatively, the effect of one of the components in the ratio spectra was eliminated by the derivation technique.[37,38] The derivation of the number is null, which removes the constant value, which we subtracted in the above method. Further, derivation spectra showed many peaks and valleys, which can be used for quantification of compounds for the current situation objective, first derivative spectra have been created from the ratio spectra. The first derivative spectra of DFS showed several peaks and valleys, (Figure 5A) however, the apex height at 284.6 nm was high, and reproducible hence, 284.6 nm was designated for further studies. The first derivative spectra of TDL disclosed two apex and three minima, (Figure 5B) however a minimum at 230.8 nm showed good sensitivity and reproducibility, therefore 230.8 nm was designated for further investigation. In addition, the first derivative spectra obtained from pure analyte and from the mixture spectra showed identical peak amplitudes. The apex height of the first derivative spectra of DFS (Figure 5C) and TDL (Figure 5D) was documented at 284.6 nm and 230.8 nm respectively and the concentration of DFS and TDL were computed using the corresponding linearity equation.

Validation of UV-spectrophotometric methods

The proposed manipulated UV-spectrometric approaches have been authenticated to ascertain the straight-line range, limit of detection and quantification, accuracy, precision, and stability following the standard procedure described in the International Conference on Harmonization (ICH).

Linearity

The normal absorption spectra of CCB and TDL in the 1-30 µg/mL and 2-50 µg/mL showed a straight line by plotting the absorption at 248.9 nm and 270.5 nm respectively against the corresponding concentrations. It became apparent that the relationship coefficients for TDL and CCB were, 0.9995 and 0.9997 respectively, the linearity curves for DFS and TDL were created between levels of 1–30 µg/mL and 2–50 µg/mL, respectively. In the RAD method apex height variations have been determined

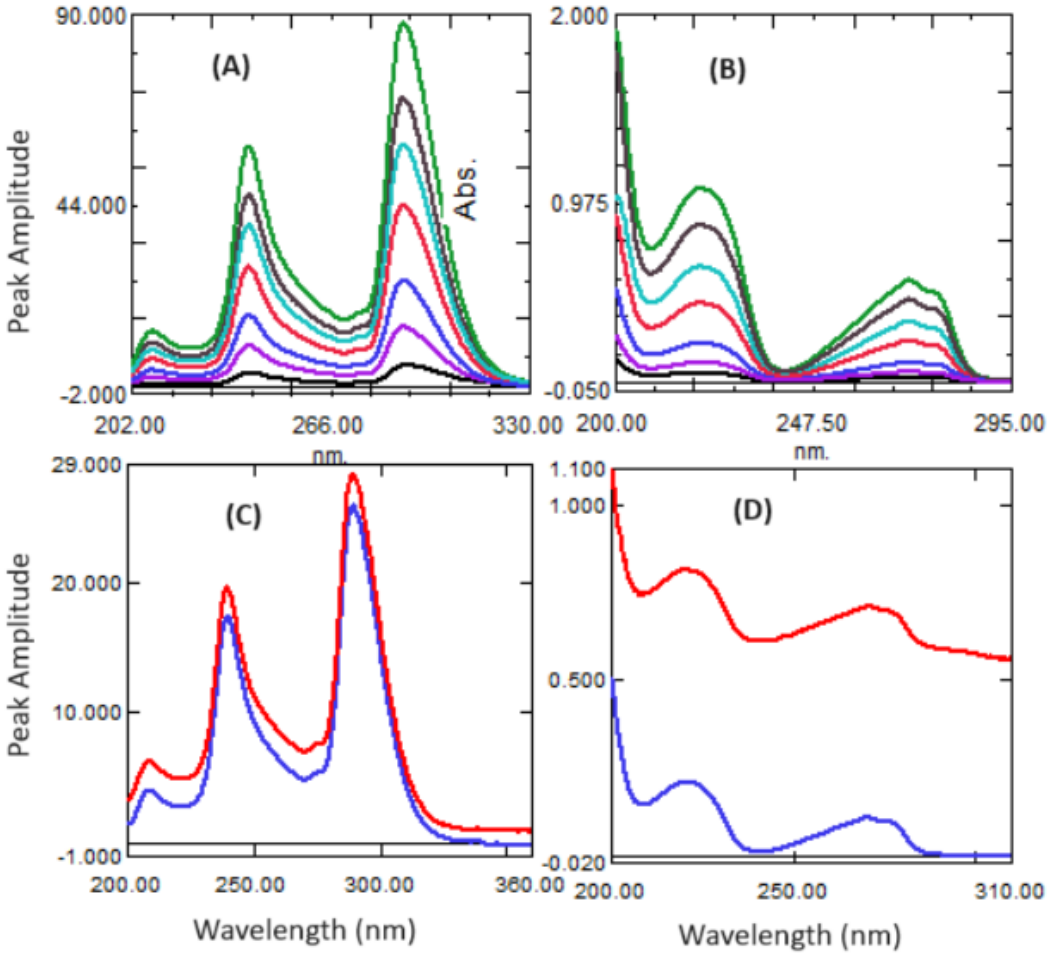

Figure 4: Ratio absorption spectra of DFS using TDL as divisor (A), Ratio absorption spectra of TDL using DFS as divisor (B). Overlay of ratio spectra of mixture and pure DFS (C) and TDL (D).

at 289.4 nm and 339.6 nm from DFS ratio spectra and at 241.1 nm and 220.9 nm from TDL ratio spectra and plotted against respective quantities of DFS and TDL correspondingly. Similarly, for the RFD methods, the apex amplitudes of first derivative spectra of DFS and TDL in the quantity range between 1-30 µg/mL and 2-50 µg/mL at 284.6 nm and 230.8 nm were recorded and represented against the associated concentrations of DSF and TDL respectively. The linearity equations and the best relationship coefficient (r2>0.998) for both analytes by the two methods are tabulated (Table 1).

Sensitivity

The suggested techniques' sensitivity was assessed by utilizing slope (s) and the intercept's standard deviation (θ) to calculate the Limits of Detection (LOD) and Quantification (LOQ). The formula 3.3 θ/s was utilized to determine the LOD, while the formula 10 θ/s was employed to estimate the LOQ. The low LOD and LOQ outcomes verified that the technique is sufficiently sensitive to measure the analytes in the formulations (Table 1).

Accuracy and precision

The correctness of the anticipated method was assessed by examining the three distinct levels of analytic compounds in the linearity range (Low, intermediate, and high) and expressed as the percent recovery and percent relative error (Table 1). All three samples in three different concentrations were analyzed six times and mean percentage recovery and %RE were recorded. The extent of the mean percentage recovery was 98.56% to 101.48%. By examining the aforementioned three distinct concentrations in six duplicates, the precision of the procedure was ascertained on the basis of repeatability and intermediate precision. The precision was expressed as %RSD (Table 1) and the %RSD for the repeatability ranged from 0.86 – 1.54%. For the within-day precision all three levels of analytic compounds were investigated three times on the same day and for between-day precision three concentration solutions have been analyzed for three successive days.

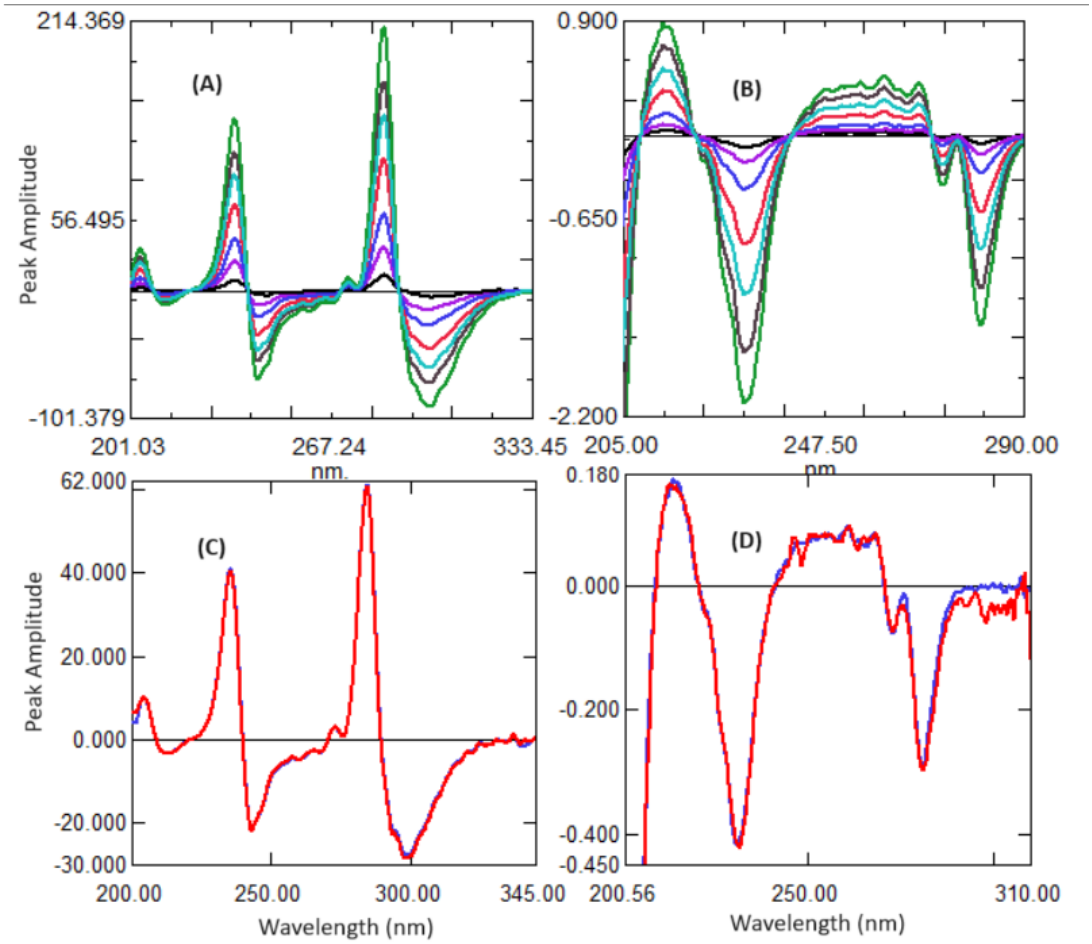

Figure 5: First derivative ratio absorption spectra of DFS (A) and TDL (B). Overlay of first derivative ratio spectra of mixture (blue) and pure (red) DFS (C) and TDL (D).

Table 1: Outcomes of Validation features for CCB, TDL, and DFS.

Factors	Zero order spectra Method		RAD		RFD	
Analytes	**CCB**	**TDL**	**DFS**	**TDL**	**DFS**	**TDL**
Wavelength [nm]	248.9	270.5	289.4-339.6	220.9-241.1	284.6	230.7
Linearity [µg/mL]	1 - 30	2 - 50	1 - 30	2 - 50	1 - 30	2 - 50
LOD [µg/mL]	0.24	0.52	0.19	0.48	0.28	0.61
LOQ [µg/mL]	0.75	1.58	0.58	1.25	0.81	1.84
Slop	0.0485	0.0067	2.7768	0.0198	6.6502	0.0419
Intercept	0.007	0.001	0.629	0.002	1.091	0.003
Correlation Coefficient [r^2]	0.9995	0.9997	0.9993	0.9999	0.9994	0.9997
Accuracy (Mean % ± RSE)	99.24±1.45	101.48±0.97	100.93±1.07	99.18±1.35	98.56±1.37	99.07±1.49
Precision (%RSD)						
Repeatability	0.98	1.24	0.86	1.54	1.49	1.22
Within day	1.34	1.08	0.91	1.05	1.38	0.79
Between Days	1.62	1.83	1.76	0.92	1.56	1.36

Table 2: Analysis results of the manually mixed solutions

Manually Mixed Ratio (µg mL^{-1})	Zero order method (% Recovery)		Manually Mixed Ratio (µg mL^{-1})	RAD (% Recovery)		RFD (% Recovery)	
CCB:TDL	CCB	TDL	DFS:TDL	DFS	TDL	DFS	TDL
10:15	99.85	99.76	10:15	100.39	100.75	101.42	98.67
15:10	100.49	101.37	15:10	101.08	101.44	98.75	98.28
10:20	101.07	101.49	10:20	99.07	99.05	99.61	100.83
20:10	98.67	98.62	20:10	99.82	99.34	100.66	100.76
30:10	99.22	99.72	30:10	98.79	101.76	98.34	101.04
Across Mean	99.86	100.19	Across Mean	99.83	100.46	99.75	99.91
%RSD	0.96	1.21	%RSD	0.94	1.22	1.28	1.32

%RSD: %Relative Standard Deviation.

Table 3: Assay results of formulations and authentic drug addition technique.

Formulation (mg Tablet^{-1})	Zero order method (Mean % ± SD)		Formulation (mg Tablet^{-1})	RAD (Mean % ± SD)		RFD (Mean % ± SD)	
CCB:TDL	CCB	TDL	DFS:TDL	DFS	TDL	DFS	TDL
56:44	99.46 ±1.25	98.81 ±0.94	75:50	99.04 ±1.48	98.55 ±0.86	100.47 ±0.69	100.92±1.07
Authentic drug Addition technique							
Amount Added (µg mL^{-1})	Zero order method (% Recovery)		Amount Added (µg mL^{-1})	RDM (% Recovery)		RFD (% Recovery)	
CCB:TDL	CCB	TDL	DFS:TDL	DFS	TDL	DFS	TDL
5.6:4.4	98.43	100.95	7.5:5.0	98.07	101.09	98.98	100.54
11.2:8.8	100.45	101.78	15.0:10.0	101.41	98.77	99.35	101.57
16.8:13.2	98.67	99.07	22.5:15.0	99.28	101.85	98.07	100.76
Across mean	99.18	100.60	Across mean	99.59	100.57	98.80	100.96
%RSD	1.10	1.39	%RSD	1.69	1.60	0.66	0.54

Analysis of manually mixed solutions

Five different ratios of analytes having different concentrations of analytes were prepared based on the formulation concentrations. The manually mixed solutions of different ratios were scanned and manipulated utilizing the recommended UV spectrophotometric approaches. The amount of medicines determined adopting respective straight line formulae, showed 99.83% – 100.46 with low %RSD (Table 2). The specificity of the anticipated spectroscopic approaches was confirmed with the excellent recovery along low % RSD.

Analysis of formulation

The analyte concentration in the generated sample mixtures was brought within the desired linear range and determined using the proposed UV spectroscopic methods. The consistency between the amount of analytical substances in the tablets and the labeled amount was verified by the data shown in Table 3. Further, the accuracy was confirmed by the authentic drug addition technique. It became apparent that the additional amount's recovery percentage ranged from 98.80% to 100.96%. This attested to the lack of tablet excipient interference with analyte measurement in the preparations.

CONCLUSION

The mathematically filtered UV spectroscopic technique development resulted in an easy, reproducible, and rapid analytical approach for concurrent quantification CCB+TDL and DFS+TDL in tablets. The manipulation of spectra for generation of ratio spectra and first derivative ratio spectra of DFS and TDL, and isolation of pure normal spectra of CCB and TDL was carried out with simple steps using the software provided with the instrument. The linearity, specificity, accuracy, and precision of the resulting spectroscopic techniques harmonized with ICH requirements. The proposed method can replace the existing HPLC methods for the analysis of CCB+TDL and DFS+TDL in formulations due to ease and fast investigation and can be used regularly in pharmaceutical laboratories for quality control of formulations.

FUNDING

This work was supported through the Annual Funding track by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Al Ahsa, Saudi Arabia [Project No. GrantA223].

ACKNOWLEDGEMENT

The authors are thankful to the Deanship of Scientific Research, King Faisal University, Al-Ahsa, Saudi Arabia for the support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPLC: High Performance Liquid Chromatography; **UV:** Ultra violet; **CVD:** Cardiovascular disease; **^{0}D:** Zero-order absorption spectra; **RSD:** Relative Standard deviation; **RE:** Relative error; **LDL:** Low density lipoprotein; **COX:** Cyclooxygenase; **GCMS:** Gas Chromatography Mass spectrometry.

SUMMARY

The simple reproducible mathematically modified UV spectroscopic methods were established for concomitant evaluation of binary combination of celecoxib + tramadol and diclofenac sodium + tramadol formulations. Both the formulations were effectively analyzed using the separating the pure zero order spectra of TDL and CCB, and the ratio absorption difference and ratio first derivative spectroscopic method for quantification of DFS and TDL with results falling into the series of 2-50 µg/mL for TDL, and 1-30 µg/mL for DFS and CCB. The good recovery of 98.56% -101.48% with low relative error and percentage relative standard deviation verified the methodologies' correctness and repeatability. Finally, proposed mathematically modified spectroscopic procedures were exploited for quality control of analytes from formulation and manually prepared mixtures. The assay results also confirmed the nonexistence of tablet adjuvant intervention in the quantification of medicines in the tablets. Therefore, these approaches can be utilized for the systematic quantification of these drugs in binary formulations without chemical separation.

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Cite this article: Attimarad M, Aldhubiab B, Nagaraja S, Pottathil S. Smart Manipulated UV Spectroscopic Methods for Resolving the Overlapped Spectra for Quality Control of Two Analgesic Binary Combination Formulations. Indian J of Pharmaceutical Education and Research. 2024;58(3s):s805-s814.