ASM Sc. J., 19, 2024

https://doi.org/10.32802/asmscj.2023.1587

Simultaneous Quantitation Tramadol Hydrochloride and Diclofenac Sodium by RP-HPLC in Fixed-dose Combination(FDC)

S. Rajasree[1], T. Hemant Kumar[2*], V. Shushma[1] , B. Sunitha[3] and B. Ravindar[4]

[1]*Department of Pharmaceutical Analysis, Gyana Jyothi College of Pharmacy, Hyderabad – 500098, Telangana, India*

[2]*Department of Quality Assurance, School of Pharmacy and Technology Management, NMIMS, Shirpur, India*

[3]*Department of Pharmaceutical Analysis, Vision College of Pharmaceutical Sciences and Research, Hyderabad – 500092, Telangana, India*

[4]*Department of Pharmaceutical Analysis, Srikrupa Institute of Pharmaceutical Sciences, Siddipet-502277, Telangana, India*

The concurrent detection of tramadol hydrochloride and diclofenac sodium in their mixed dosage form as tablet was developed and validated using a simple, accurate, inexpensive, and precise RP-HPLC approach with a UV-Visible detector. Enable C18 G analytical column (250 x 4.6 mm, i.d., 5 µm) was used for the separation. The mobile phase was made of a mixture of 80 volumes of acetonitrile and 20 volumes of 1 % v/v glacial acetic acid. Isocratic elution was utilised at a flow rate of 1 mL/min during the chromatographic process. Tramadol hydrochloride and diclofenac sodium were identified at UV detector wavelength of 264 nm and established linear calibration curves with concentration ranges of 20–100 µg/ml and 15–75 µg/ml, respectively. Tramadol hydrochloride had recoveries of 99.62–99.86% and diclofenac sodium had a recoveries of 99.48-100.22%. The approach was validated in the context of the International Conference of Harmonisation's requirements for accuracy, precision, specificity, robustness, limits of detection and quantification. The precision studies for both drugs were found to be < 2% (%RSD). The limits of detection for tramadol hydrochloride and diclofenac sodium were found to be 0.12 µg/ml and 0.07 µg/ml, respectively. The limits of quantification for tramadol hydrochloride and diclofenac sodium were found to be 0.45 µg/ml and 0.21 µg/ml, respectively. Both tramadol hydrochloride and diclofenac sodium, two commercial pharmaceutical drugs, were effectively analysed using high-performance liquid chromatography using the described approach.

Keywords: Tramadol hydrochloride; Diclofenac sodium; RP-HPLC

I. INTRODUCTION

Diclofenac Sodium (DIC) is chemically (Figure 1), "sodium; 2-[2-(2,6-dichloroanilino)phenyl]acetate, used as non-steroidal anti-inflammatory agent" and is official in BP, USP. It prevents leukocyte migration and cyclooxygenase (COX-1 and COX-2) enzyme activity, which inhibits prostaglandin synthesis in the periphery. Tramadol HCl (TRA) is chemically (Figure 1), "*cis*-2-[(dimethyl amino) methyl]-1-(3-methoxy phenyl) cyclohexanol HCl, used mainly as analgesic and narcotic" and is official in IP, BP & USPNF. It functions as a pure agonist at the µ opiate receptors, just like morphine, and has a centrally acting opioid-like effect. Its mode of action is to elevate the concentrations of serotonin and nor-adrenaline in the spinal cord through reuptake. A survey of published works on DIC analysis uncovered the existence of some UV Spectrophotometric (Sirajuddin *et al.,* 2009), and simultaneous HPLC assay methods (Gowramma *et al.*, 2010), Bioanalytical LC method (Brunner & Luders, 1991; Demircan *et al.*, 2005; Yilmaz *et al.*, 2011; Degwy *et al.*,

*Corresponding author's e-mail: hemkar_pharma@yahoo.co.in

2013) and HPTLC methods (Liawruangrath *et al.,* 2006) reported either singly or in combination with other medications such as "paracetamol, camylofin, Chlorzoxazone, Methocarbamol, Ibuprofen, Metaxalone, Thiocolchicoside and Drotaverine". Analytical strategies for TRA have been reported to show that an UV Spectrophotometric (Kucuk & Kadıoglu, 2005; Rani *et al*., 2011; Hosny & Ismaiel, 2012), HPLC assay methods (El-Sayed *et al*., 2011; Chandra *et al*., 2012; Kumar *et al*., 2012; Kumar *et al*., 2019) and stability indicating HPLC method (Rajesh & Shrawan, 2012) either by alone or in combination with medications like Domperidone, Aceclofenac, and Paracetamol.

For the relief of severe, sudden pain, a fixed-dose combination (FDC) of DIC and TRA is currently on the market (DIC 75 mg and TRA 50 mg). The use of both of these medications together greatly lowers the required opioid dose and lessens the occurrence of adverse effects such as nausea, vomiting, and respiratory depression. A search of the scientific literature showed scant reports of UV (Anantha *et al*., 2009; Akhtar *et al*., 2013) and HPLC methods (Deepthi *et al*., 2013; Peraman *et al*., 2017) for simultaneous assessment of DIC and TRA in their dose forms. For simultaneous quantification, HPLC (Kumar *et al*., 2019; Kumar *et al*., 2019; Kumar & Asha, 2019) clearly outperforms spectrophotometric techniques. Many limitations are noticed in the reported HPLC methods that include: prolonged retention times, more mobile phase consumption, long analysis time and costly approaches. As a result, there is a need to develop new HPLC technique with shorted retention time with improved method parameters.

The goal of this research was to create an RP-HPLC method that is quick, easy, precise, and accurate enough for concurrent quantification of these medicines in a combination tablet dosage form.

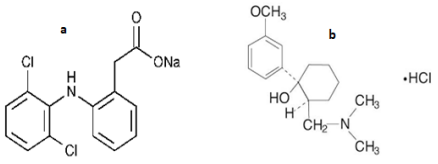

Figure 1. Structure of (a) Diclofenac Sodium and (b) Tramadol hydrochloride

II. MATERIALS AND METHOD

A. Materials and Reagents

Reference standards of TRA and DIC were provided by RA Chem Ltd, Hyderabad Pvt. Ltd. (India). DURAPAIN tablet formulation containing Tramadol HCl (50 mg) and Diclofenac Sodium (75 mg) was obtained from the commercial market. On the day of preparations, all of the solutions were shielded from light and analysed. Glassware used in each step was properly cleaned with double-distilled water before being dried in a hot air oven after being soaked overnight in a solution of chromic and sulfuric acids. All reagents, unless otherwise specified, were of analytical-reagent (AR) grade. Merck, India, supplied Millipore water, HPLC grade methanol, and glacial acetic acid solution. Borosilicate (Class - A) glassware was used.

B. Instrumentation

HPLC analysis was carried out using a high-performance liquid chromatography method that involved the use of a reversed-phase column. The system was comprised of a Shimadzu LC-20 AD binary pump and a programmable, variable-wavelength SPD-20A detector. Using a Rheodyne injector connected with a 20 µL loop and LC solutions software, version 5.0, to record and analyse data. The pH was determined using a systronics pH meter and a Digital Microbalance (CX-265) manufactured by Citizen Scale(I) Pvt. Ltd. for weighing.

C. Chromatographic Conditions

On an enabled C18 G column (250 x 4.6 mm i.d., 5-µm particle), the chromatographic separation was conducted. Methanol and 1 % glacial acetic acid were the components that made up the mobile phase in the proportion of 80:20 v/v. The volume of injection was 20 µL, and eluents were detected at 275 nm at a flowrate of 1 ml/min. The HPLC system was set to run at 30°C room temperature.

D. Preparation of Standard Solutions

25 mg of each TRA and DIC were weighed individually and transferred into 25 ml volumetric flask and added 15 ml of acetonitrile, and sonicated for 15 minutes. Acetonitrile is

added to the volume to bring it up to the desired concentration, which is 1000 µg/ml of each drug. From this 2.5 ml was taken from the standard stock solutions and poured to 25 ml volumetric flasks, where the volume is then raised up to the required level with diluent to create 100 µg/ml working standard solutions individually.

E. Preparation of Sample Solution

Utilising the current chromatographic conditions, which have proven to be more precise and reliable, Commercial tablets' assay was established. Twenty tablets were weighed, and their mean weight was determined to determine how much TRA and DIC are present in a typical tablet (50mg TRA/75mg DIC), and they were finely pulverised. Weighed tablet powder containing 20 mg TRA and appropriate amounts of DIC were added to a 100ml volumetric flask, extracted with methanol for 30 minutes, and the volume was adjusted to 100ml with diluents (methanol). The resultant solution (20 µg TRA, 30 µg DIC/ml) was passed through a 0.45 millipore filter before being analysed using an HPLC system.

F. Method Validation

The proposed technique was validated in accordance with the ICH recommendation (ICH, 2005). The validation parameters for the method included specificity, linearity, accuracy, precision, and robustness, as well as stability and system suitability testing.

III. RESULT AND DISCUSSION

A. Optimisation of Chromatographic Condition

It was found from the UV spectra that TRA and DIC have a significant absorption at 264 nm. Therefore, 264 nm was chosen as the detection wavelength. acetonitrile, methanol, and water in a variety of combinations, with or without glacial acetic acid and orthophosphoric acid. According to preliminary investigations, using various ratios of acetonitrile or methanol with water did not separate the TRA and DIC peaks or produce the desired retention times and peak symmetry. Aqueous mobile phase was swapped out for one with an acidic pH in order to obtain satisfactory peak symmetry and separation with good resolution in the form of 0.1 percent phosphoric acid and 1 percent glacial acetic acid make up the mobile phase. In order to achieve adequate resolution and satisfactory peak symmetry, a mobile phase made up of methanol and 1 % glacial acetic acid in an 80:20 ratio and an Enable C18 G column (250 x 4.6 mm; 5 µm) were chosen. The flow rates tested ranged from 0.5 to 1.2 ml/min. It was found that a flow rate of 1.0 ml/min was sufficient to elute both medicines in less than 10 minutes. Figure 2 displays the optimised chromatogram, and Figure 3 presents the overlay UV spectra of TRA and DIC.

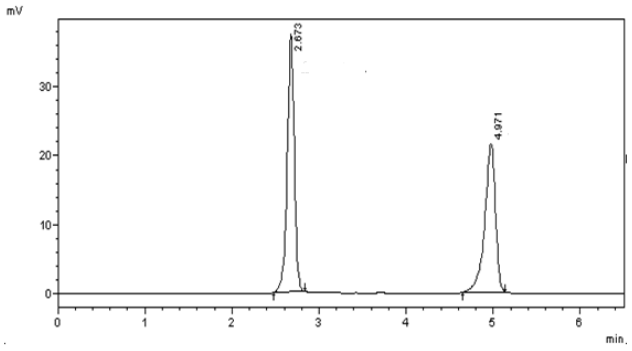

Figure 2. Chromatogram of TRA and DIC from standard solution

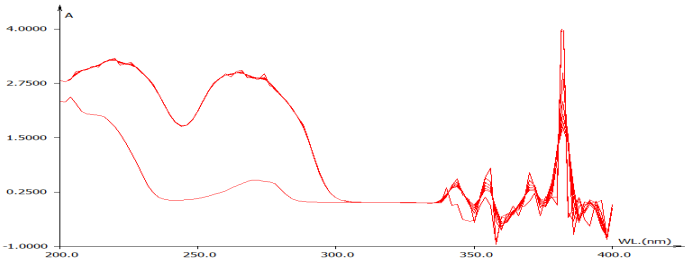

Figure 3. UV spectra of TRA and DIC in overlay mode

B. Method Validation

1. System suitability test

The system suitability parameters for the created approach were established after the ideal conditions had been established, and they were compared to the suggested limits. Table 1 presents the method's system suitability parameters. The analysis was judged to be appropriate for the procedure, and every system suitability criterion was within the suggested ranges.

Table 1. Results of system suitability test (n = 6)

Parameter	Criteria	TRA	DIC
Capacity factor(k')	k'> 2	2.824	4.721
Tailing factor (T)	T < 2	1.747	1.384
Theoretical plates (N)	N> 2000	2534	3694
Resolution (Rs)	Rs > 2	-	3.02
% RSD (peak area)	% RSD ≤ 1	0.74	0.52

2. Stability of sample solution

The identical solution was injected at 0, 12, 24, and 48 hours, the stability of the sample solution was examined. The created method did not see an identical shift. The results, which are presented in Table 2, were also found to be within acceptable ranges (RSD 2).

Table 2. Stability data of TRA and DIC (standard solutions)

Time (hr)	Assay (%)		% Difference	
	TRA	DIC	TRA	DIC
Initial	100.08	99.62	---	---
After 12 hr	99.96	99.16	0.05	0.92
After 24 hr	100.02	99.03	0.04	0.93
After 36 hr	99.92	98.32	0.21	1.76
After 48 hr	99.87	98.11	0.34	1.85

3. Linearity

It was discovered that the linearity ranges for TRA and DIC were 20-100 µg/ml and 15-75 µg/ml, respectively. With a correlation coefficient of 0.9997, the linear regression equation for TRA was discovered to be 6505x - 4001.9. The correlation coefficient was found to be 0.9998, and the linear regression equation for DIC was found to be 72797 x-0.4. Table 3 displays the TRA and DIC linearity data. Figure 4 depicted the calibration curve for TRA and DIC. Data from the calibration curve that were subjected to linear regression showed a linear response over the range of both medications' concentrations. As a result, the curve can be used to calculate TRA and DIC in pharmaceutical formulations.

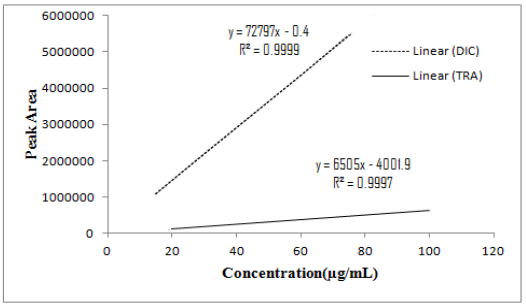

Figure 4 . Linearity graph for TRA and DIC

Table 3. Spectral and statistical data for determination of Tramadol HCl and diclofenac sodium by proposed RP-HPLC method

TRA
Detection wavelength (nm)- 264
Linearity range (µg/ml) - 20-100
Coefficient of determination (r^2) - 0.9997
Regression equation (Y^a) Y= 6505x – 4001.9
Slope (m)- 6505
Intercept (c)- -4001.9
Limit of detection, LOD (µg/ml) -0.12
Limit of quantitation, LOQ (µg/ml) -0.45
DIC
Detection wavelength (nm)- 264
Linearity range (µg/ml) - 15-75
Coefficient of determination (r^2) -0.9990
Regression equation (Y^a)- Y= 72797x -0.4
Slope (m) -7072.8
Intercept (c)- -0.4
Limit of detection, LOD (µg/ml)- 0.07
Limit of quantitation, LOQ (µg/ml)- 0.21

[a]$Y = mx + c$, where x is the concentration (µg/ml).

4. Sensitivity

Limits of detection (LOD) and quantitation were used to assess the analytical method's sensitivity (LOQ). Table 3 lists the LOD and LOQ values for TRA and DIC. The sensitivity of the technique is indicated by the low values of LOD and LOQ.

5. Precision

Within the linearity range, three distinct concentrations (40, 60, and 80µg/ml for TRA and 30, 45, and 60µg/ml forDICs) were used to calculate the percent RSD for intraday and interday precision experiments. According to Table 4, the approach was sufficiently precise because the intraday and interday RSD values were both less than 1.5 percent.

Table 4. Precision Studies

Drug	Con. (µg/mL)	Intraday(n=3)		Interday(n=3)	
		Amount found		Amount found	
		Mean±SD	%RSD	Mean±SD	%RSD
TRA	40	39.65± 0.48	1.23	39.88 ± 0.56	1.40
	60	60.12 ± 0.53	0.89	59.87 ± 0.98	1.63
	80	79.87 ± 0.75	0.94	79.46 ± 0.91	1.14
DIC	30	30.12 ± 0.12	0.39	29.74 ± 0.29	0.97
	45	44.86 ± 0.51	1.13	44.79 ± 0.33	0.73
	60	59.77 ± 0.78	1.30	59.63 ± 0.51	0.85

6. Accuracy

Recovery studies were used to perform accuracy using the standard addition approach. The sample solution had standard drugs added at concentrations of 80, 100, and 120% of the sample concentration. In triplicate, each concentration was examined. According to the findings of the recovery investigations, both TRA and DIC had recovery rates between 98 and 102%, as indicated in Table 5.

Table 5. Recovery studies (n = 3)

Recovery level	TRA		
	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery
80 %	32	31.88	99.62
100 %	40	39.92	99.80
120 %	48	47.84	99.66
Recovery level	**DIC**		
	Amount Added (µg/ml)	**Amount Recovered (µg/ml)**	**% Recovery**
80 %	48	48.11	100.22
100 %	60	59.69	99.48
120 %	72	71.82	99.75

7. Robustness

A robustness analysis was conducted by making minor adjustments to the method's parameters to see if they had an impact on the response. The study's technique parameters included flow rate variation (0.2 ml/min), mobile phase organic content (5 % v/v), and analytical wavelength (2 nm). Recovery values for analyte solutions were used to evaluate the results. According to a robustness investigation, changes in flow rate, mobile phase composition, and analytical wavelength do not significantly affect the analyte response. The data is presented in Table 6.

Table 6. Chromatographic parameter setting applied in the robustness investigation

Parameter	Modification	% Recovery		% RSD	
		TRD	DIC	TRD	DIC
Flow Rate (ml/min)	0.9	100.20	99.64	0.67	0.88
	1.0	99.92	99.38	1.21	0.94
	1.2	99.45	99.82	0.82	0.55
Mobile Phase (acetonitile:glacial acetic acid)	85 : 15 v/v	99.74	99.34	0.84	0.71
	80:20 v/v	99.49	99.66	0.91	0.86
	75:25 v/v	99.68	99.49	0.72	0.75
Wavelength (nm)	262	99.84	99.63	0.87	1.32
	264	99.92	99.71	1.25	1.01
	266	99.89	99.85	1.41	0.46

8. Sample Analysis

The tablet dosage form containing TRD and DIC in combination was analysed using the established and validated method. The designed technique was applied to three batches of commercial tablets for analysis. Results were satisfactory in that the label claims and the mean percentages for TDR and DIC were both in good accord. The RSD values and mean percentages observed in Table 7 suggested that the suggested approach might be used to measure TDR and DIC in commercially available tablets. Chromatograms were provided in Figure 6, Figure 7 and Figure 8.

Table 7. Assay results of TRD and DIC in marketed tablets

Marketed Brand	Found[a] (%)		% RSD	
	TRD	DIC	TRD	DIC
M1	100.52	99.64	1.21	0.89
M2	99.01	98.33	0.96	0.81
M3	99.21	98.48	1.02	1.16

[a]Average of six determinations

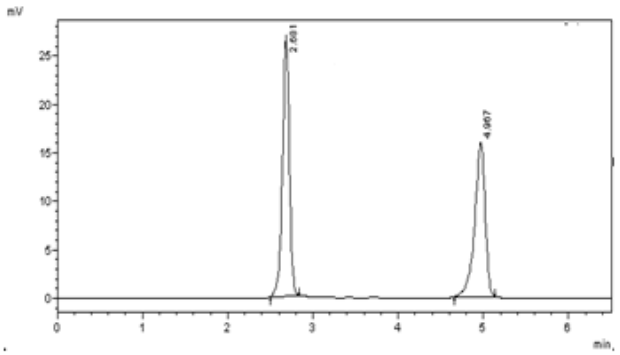

Figure 6. Chromatogram of TRA and DIC from sample solution(M1)

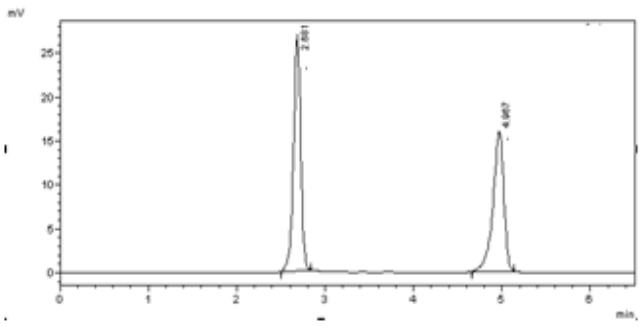

Figure 7. Chromatogram of TRA and DIC from sample solution(M2)

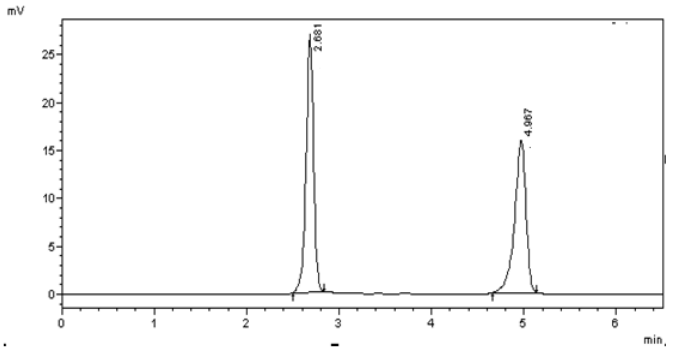

Figure 8. Chromatogram of TRA and DIC from sample solution(M2)

IV. CONCLUSION

A quick and easy HPLC approach that may be used to measure TRA and DIC simultaneously was rationally developed. The suggested approach was completely validated, showing that it was accurate and robust in determining TRA and DIC in pharmaceutical dose form as well as sensitive, precise, linear, and robust in the concentration range under study. The method's capacity to measure small amounts of medicines was confirmed by the LOQ values for the two drugs. For routine quality control of mixtures, the proposed HPLC approach might be useful.

V. ACKNOWLEDGEMENT

The authors no conflict of interest. We are thankful management of NMIMS university for providing necessary facilities to carry out the research.

VI. REFERENCES

Akhtar, J, Pasha, TY, Padaria, M, Shah, B, Mansuri, N & Prajapati, K 2013, 'Spectrophotometric Methods for Simultaneous Estimation of Tramadol Hydrochloride and Diclofenac Sodium in Combined Dosage Form', Research Journal of Pharmacy and Technology, vol. 6, no. 3, pp. 227-231.

Anantha, KD, Sujan, DP, Vijayasree, V & Seshagirirao, JVLN 2009, 'Development and validation of dual wavelength spectrophotometric method for simultaneous estimation of tramadol hydrochloride and diclofenac sodium in their combined dosage form', European Journal of Chemistry, vol. 6, pp. 541-554.

Brunner, LA & Luders, RC 1991, 'An automated method for the determination of diclofenac sodium in human plasma', Journal of Chromatographic Science, vol. 29, no. 2, pp. 287-291.

Chandra, P, Rathore, AS, Lohidasan, S & Mahadik, KR 2012, 'Application of HPLC for the simultaneous determination of aceclofenac, paracetamol and tramadol hydrochloride in pharmaceutical dosage form', Science Pharma, vol. 80, pp. 337–351.

Deepthi, MT, Ramalingam, P & Reddy, JR 2013, 'A New Validated RP-HPLC Method for Simultaneous Determination of Tramadol Hydrochloride and Diclofenac Sodium in Pharmaceutical Dosage Form', Inventi Rapid: Pharmaceutical Analysis and Qualilty Assurance, vol. 2, pp. 1-8.

Degwy, MA, Aly, M & Mamdouh, SE 2013, 'Development and Validation of a HPLC Method for In-Vivo Study of Diclofenac Potassium', International Journal of Pharma Sciences and Research, vol. 4, pp. 622-627.

El-sayed, AY, Mohamed, KM, Hilal, MA, Mohamed, SA, Aboul-hagag, KE & Nasser, AY 2011, 'Development and validation of high-performance liquid chromatography–diode array detector method for the determination of tramadol in human saliva', Journal of Chromatography and Separation Techniques, vol. 2, no. 4, pp. 2-6.

Gowramma, B, Rajan, S, Muralidharan, S, Meyyanathan, SN & Suresh, BA 2010, 'Validated RP-HPLC Method for Simultaneous Estimation of Paracetamol and Diclofenac Potassium in Pharmaceutical Formulation', International Journal of ChemTech Research, vol. 2, pp. 676-680.

Hosny, MM, Ismaiel, O 2012, 'A Development and Validation of a Spectrophotometric Method for the Determination of Tramadol in Human Urine Using Liquid-Liquid Extraction and Ion Pair Formation', International Journal of Instrumentation Science, vol. 1, no. 3, pp. 34-40.

ICH Harmonized Tripartite Guidelines, Validation of analytical procedures: Text and Methodology, Q2 (R1), Geneva, 2005.

Kumar, PSN, Gowda, BDG, Mantelingu, K, Rangappa, K 2012, 'Development and validation of HPLC method for determination of tramadol hydrochloride in solid dosage form', Journal of Pharmacy Research, vol. 5, no. 3, pp. 1438-1440.

Kumar, TH, Prajna, CKK, Rao, KV & Rao, YS 2019, 'RP-HPLC method for estimation of tramadol hydrochloride and paracetamol in pharmaceutical formulation', GSC Biological and Pharmaceutical Sciences, vol. 8, no. 1, pp. 89-97.

Kumar, TH, Begum, A & Sankar, DG 2019, 'Determination of azilsartan medoximil and chlorthalidone in tablets exposed to forced degradation by using RP-HPLC', Biomedical Research, vol. 30, pp. 775-785.

Kumar, TH, Asha, C & Sankar, DG 2019 'Estimation of amlodipine besylate and irbesartan in pharmaceutical formulation by RP-HPLC with forced degradation studies', International Journal of Applied Pharmaceutical Sciences, vol. 11, pp. 159-167.

Kumar, TH & Asha, C 2019, 'New Validated Stability Indicating RP-HPLC Method For Simultaneous Estimation Of Amlodipine Besylate And Valsartan In Pharmaceutical Formulation', International Journal of Pharmaceutical Sciences and Research, vol. 10, pp. 2633-43.

Kucuk, A & Kadioglu, Y 2005, 'Determination of tramadol hydrochloride in ampoule dosage forms by using uv spectrophotometric and HPLC-DAD methods in methanol and water media', Il farmaco, vol. 60, no. 2, pp. 163–169.

Liawruangrath, B, Thongchai, W, Thongpoon, C & Machan, T 2006, 'High performance thin layer chromatographic method for the determination of diclofenac sodium in pharmaceutical formulations', Chiang Mai Journal of Science, vol. 33, no. 1, pp. 123-128.

Peraman, R, Rao, DS, Kadiri, RR & Bommireddy, AR 2017, 'Stability-indicating RP-HPLC-DAD method for the simultaneous estimation of tramadol HCl and diclofenac

sodium', Journal of Applied Pharmaceutical Science, vol. 7, no. 9, pp. 085-093.

Rajesh, MK & Shrawan, GS 2012, 'Stability-Indicating RP-HPLC Method for Analysis of Paracetamol and Tramadol in a Pharmaceutical Dosage Form', E-J Chem, vol. 9, pp. 1347-1356.

Rani, GT, Rajitha, B, Prashanthi, S & Reddy, K 2011, 'Extractive spectrophotometric determination of tramadol hydrochloride in pure and pharmaceutical dosage forms', International Journal of Pharmtech Research, vol. 3, no. 1, pp. 114-117.

Sirajuddin, AR, Khaskheli, Abrol, K, Sherazil, STH, Afridi, HI, Mahesar, SA & Saeed, M 2009, 'Simpler and faster spectrophotometric determination of diclofenac sodium in tablets, serum and urine samples', Pakistan Journal of Analytical & Environmental Chemistry, vol. 10, no. 2, pp. 53-58.

Yilmaz, B, Ali, A & Palabiyik, SS 2011, 'HPLC method for determination of diclofenac in human plasma and its application to a pharmacokinetic study in turkey', Journal of Chromatographic Science, vol. 49, pp. 422-427.