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ORIGINAL ARTICLE

RP-HPLC Method Development and Validation for Simultaneous Estimation of Tramadol Hydrochloride and Celecoxib in Dosage Form and Bulk Drug

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ABSTRACT
Celecoxib and tramadol hydrochloride were revealed to be quantified in bulk drug and dosage form in a series of steps employing an intuitive, significantly productive, and specific reverse-phase high-performance liquid chromatographic approach. The separation occurred with the assistance of column Phenomenex ODS 3. Acetonitrile: 0.05% OPA in water (70:30) is the mobile phase used in isocratic mode. The maximum absorption for both medications was noted at 218 nm, and the flow rate was 1.0 ml/min. The retention intervals for celecoxib and tramadol hydrochloride were 5.62 and 3.03 minutes, respectively. The chromatographic approach can be used for bulk, formulation, and medication quality control. The following parameters proved to be competent for concurrently investigation of celecoxib and tramadol hydrochloride in the bulk formulation and medication form: system appropriateness, linearity, reliability, precision, LOD and LOQ, robustness, and stability examinations.
Keywords: *Tramadol Hydrochloride, Celecoxib, RP-HPLC, Accuracy, Precision, Isocratic mode*

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INTRODUCTION

Celecoxib serves as a cyclooxygenase 2 blocker and non-steroidal anti-inflammatory medicine [1]. It has been put forward for several types of complications, namely ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis, painful and menstrual juvenile rheumatoid arthritis, osteoarthritis pain and inflammation, and acute pain in adults [1]. Individuals suffering from familial adenomatous polyposis (FAP), it is further employed to lessen colon precancerous malignancies. [1] Celecoxib, a 1H-pyrazole, corresponds to the pyrazole category. (Fig 1) [2].

A remarkably distinct reversible inhibitor of the COX-2 isoform of cyclooxygenase, celecoxib restricts arachidonic acid via being transformed into prostaglandin precursors. Consequently, it possesses analgesic and anti-inflammatory properties. Inflammatory cells generate excessive COX-2 in response to growth factors, cytokines, bacterial lipopolysaccharides, and neoplasm promoters [3, 4, 5]. Celecoxib is probably 10–20 times more addressed than COX-1 when it pertains to COX-2 inhibition [6, 7]. Benzene sulphonamide as it connects to hydrophilic lateral compartment zone close to the functional cyclooxygenase 2 receptor location [8], despite having polar sulphonamide side chain. Celecoxib's IUPAC exemption is 4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl] [9]. Celecoxib is dissolved in

organic solvents, consisting of DMSO, ethanol, and dimethyl formamide (DMF). As an illustration, it dissolves at a rate of 16.6 mg/mL in DMF as well as 25 mg/mL in ethanol.
The opioid analgesic Tramadol represents a group of the serotonin-norepinephrine reuptake inhibitors (SNRIs) intended for relief of moderately severe pain [10, 11]. When consumed orally in a rapid-onset form, pain relief frequently starts within an hour [10]. Tramadol can also be developed into an injection dosage and is most frequently utilized to treat minor to serious pain, both short and long term. [12, 13] Tramadol elicits analgesic effects across several kinds of positions on the noradrenergic, serotonergic, and opioid receptor systems. [14] Several antidepressants designated as serotonin-norepinephrine reuptake inhibitors (SNRIs) exhibit the identical consequence for serotonin and norepinephrine reuptake inhibition as performed by tramadol [15]. Tramadol is a racemic combination, one (+) enantiomer restricts serotonin reuptake while the other (-) enantiomer hinder noradrenaline uptake by attaching to and disables the transmitters. [16,17] 2-[(dimethylamino)methyl]-1-(3-methoxyphenyl) Cyclohexanol is the IUPAC exemption for tramadol. [18] Tramadol hydrochloride readily dissolves in water and methanol and is only merely dispersed in acetone, in accordance with the EP and USP. It further states that the medicinal component dissolves efficiently in water and ethanol. (Fig 2)
Literature reviews imply that a wide range of techniques, which may include RP-HPLC, LC-MS, impurity profiling checks, UV spectrophotometry, and HPTLC methods, have been conducted on both drugs. [19, 20, 21, 22, 23] Every approach has been reported to employ either a single medication or a combination of supplements. The main goal of the investigation is to examine the combination of celecoxib and tramadol hydrochloride, which was merely recently approved as the newly approved combination, in compliance with the Q2(R1) ICH guidelines. For the purpose of quantifying the required quantity of celecoxib and tramadol HCl in bulk and mixed-dose forms with more specificity, the proposed work attempts to provide a user-friendly, more precise, trustworthy, and specific study with a precise retention duration. The necessary chromatographic setups will guarantee optimal separation with high sensitivity, minimal retention time, and satisfactory resolution. The technique for the parameters under consideration will be developed and validated by implementing the ICH Guidelines.

STRUCTURE [2,18]

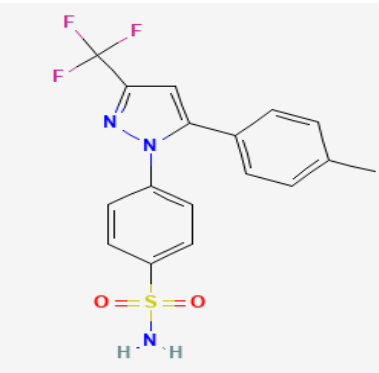

Fig no. 1 Celecoxib

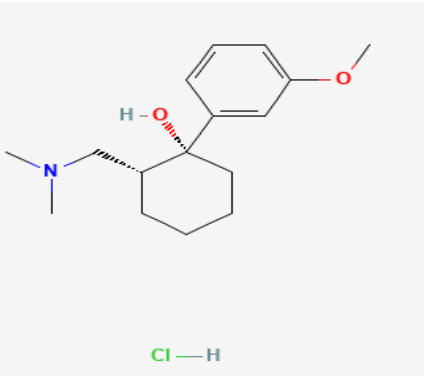

Fig no. 2 Tramadol Hydrochloride

MATERIAL AND METHOD

Chemicals and Reagents:

We acquired celecoxib and tramadol hydrochloride from Maxheal Pharmaceuticals in Nashik. From Rankem, India, we collected methanol, buffer, acetonitrile, and orthophosphoric acid, water. In a laboratory environment, tablets in pharmaceutical dosage forms have been produced.

Chromatographic Conditions:

An Agilent HPLC apparatus, OpenLab EZ Chrome software, and Phenomenex C 18 (250 mm x 4.6 mm, and 5µm) have been used. In water, acetonitrile:0.05% OPA (70:30) v/v renders the mobile phase. The injection volume was held at 20 µl, the λ max reading was recorded at 218 nm, and at 1.0 ml/min, this flow rate was retained.

Preparation of mobile phase:

Acetonitrile: 0.05% OPA in water (70:30) has been added to create the mobile phase, which then passed through a 0.45 µm membrane filter and was left to degas in an ultrasonic chamber. The final mobile phase eventually passed through the designated column and was held at a flow rate of 1.0 ml per minute.

Preparation of standard stock solution:

Celecoxib:

In order to fully dissociate the standard, 25 mg of celecoxib was carefully weighed, added to a volumetric flask with a volume of 50 ml and then mixed with 35 ml of methanol to create the stock solution, and then diluting it with methanol to the desired concentration. (500 PPM) Furthermore, each trial dilutes 2 ml to 10 ml with the mobile phase (100 PPM).

Tramadol Hydrochloride:

With the aim to derive the stock solution, 25 mg of Tramadol Hydrochloride were carefully quantified, moved into a 50 ml volumetric flask, adjusted with 35 ml of methanol, sonicated to completely dissociate the standard, and then merged to an adequate amount with methanol (500 PPM). In addition, the mobile phase has been diluted in each trial between 2 to 10 millilitres.

Preparation of Working Concentration:

A working range of 30 µg/ml of tramadol hydrochloride and nearly 38.10 µg/ml of celecoxib are being manufactured.

Sample preparation of Physical lab mixture sample:

A physical lab combination of 50 mg of Tramadol Hydrochloride and 63.5 mg of Celecoxib (397.73 mg of powder material) was measured. After cleansing and drying, place it in a volumetric flask with a volume of 100 ml and add 70 ml of methanol, and sonicate for 15 minutes with frequent stirring. After 15 minutes, let the solution cool to ambient temperature and employ adequate methanol to maintain the desired concentration. Filter the solution using a relevant 0.45 µ syringe filter, withdrawing 3–5 mL of the filtrate. The mobile phase was subsequently implemented in order to dilute 1.2 ml of the filtrate to 20 ml. Tramadol hydrochloride (30 PPM) with celecoxib (38.1 PPM).

Method Validation: [24,25,26]

The formulated approach for the concurrent quantification of Celecoxib and Tramadol Hydrochloride was validated to comply with the Q2(R1) ICH specifications for the following parameters.

System Suitability:

System suitability is a pharmacopoeial specification used to make sure the chromatographic system will be appropriate for the analysis that will be executed. Data for the tests was acquired utilizing five replicate injections of a standard drug solution, and conclusions have been reported.

Linearity:

Tramadol's linearity extends from 3.0 to 45.0 µg/ml, whereas celecoxib's covers from 3.81 to 57.15 µg/ml. Five linearity levels, comprising 10% to 150% of working concentration, were analysed.

Specificity:

A solution containing Blank (mobile phase as a diluent) and Placebo should be made and injected in order to illustrate the specificity of the entire process. It remains fully unknown what excipients serve to evaluate commercial test specimens.

LOD and LOQ:

The residue standard variation of the extrapolation line that results via the calibration curve is the method used to compute LOD and LOQ.

Accuracy:

Three replicas of each accuracy level's solution were generated, and the overall recovery's percentage RSD was then calculated. Between 50% and 150% of working concentrations will be achieved in terms of accuracy.

Precision:

For repeatability tests following the assay findings for Celecoxib and Tramadol Hydrochloride, six replicas were generated. The conclusions are presented as a percentage RSD.

Robustness:

Standard solutions were injected under diverse chromatographic situations, as stated here.

1. Adjustments in flow rate ± 10% (± 0.1 ml/min)
2. Adjustments in column oven temperature. (± 2 ºC)
3. Adjustments in Wavelength (± 3 nm)

Stability Studies:

For stability tests, the solution was put in a prevalent, adequately lit lab setting, and it was inspected after 12 and 24 hours. The stability evaluation of the standard and test outcomes has been executed by detecting and comparing the results of the test solution at each stability time point and the initial findings.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions:

Several chromatographic factors have been established, such as using an orth-phosphoric acid buffer in order to ensure pH, setting the column temperature at 40°C, and introducing 20 ` via the mobile phase acetonitrile: 0.05% OPA in water (70:30) v/v to gain a flow rate of 1.0 ml/min and an estimation of maximum absorption at 218 nm. (Fig 3)

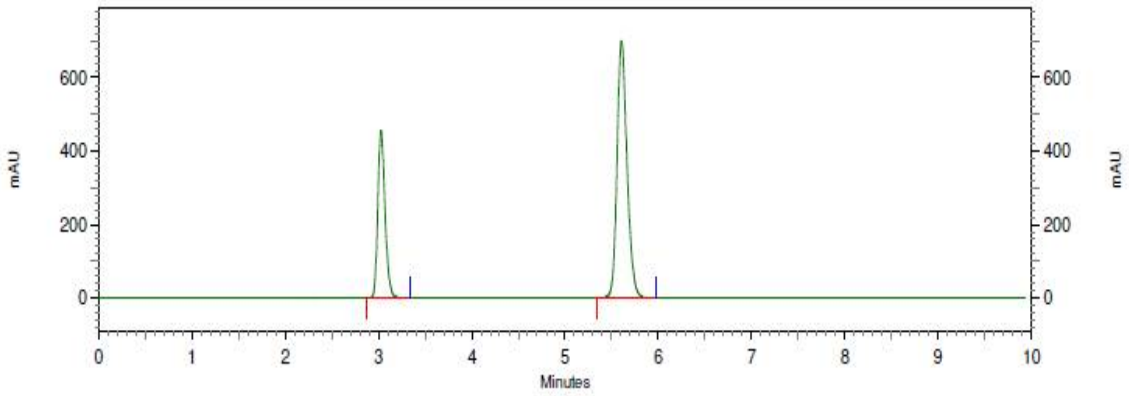

Figure 3 chromatogram of Mixture (Tramadol HCl & Celecoxib)

System Suitability:

Five replicates of celecoxib and tramadol hydrochloride were given in order to inspect the system's suitability. Parameters like theoretical plate (N) 13443 and 8516, the retention time, which turned out to be 5.63 and 3.03 min for Celecoxib and Tramadol Hydrochloride , and the tailing factor, which was detected to be 1.22 and 1.20, respectively, were all monitored.

Linearity:

Celecoxib and Tramadol Hydrochloride exhibit calibration curves that range from 3.81 to 57.15 µg/ml for Celecoxib and 3.0 to 45.0 µg/ml for Tramadol. For Celecoxib and Tramadol Hydrochloride, the regression equation and correlation coefficient were identified to be Y = 391232.78 x + 45453.96, R^2 = 0.99999, and Y = 992479.74 x + 285764.53, R^2 = 0.99997.

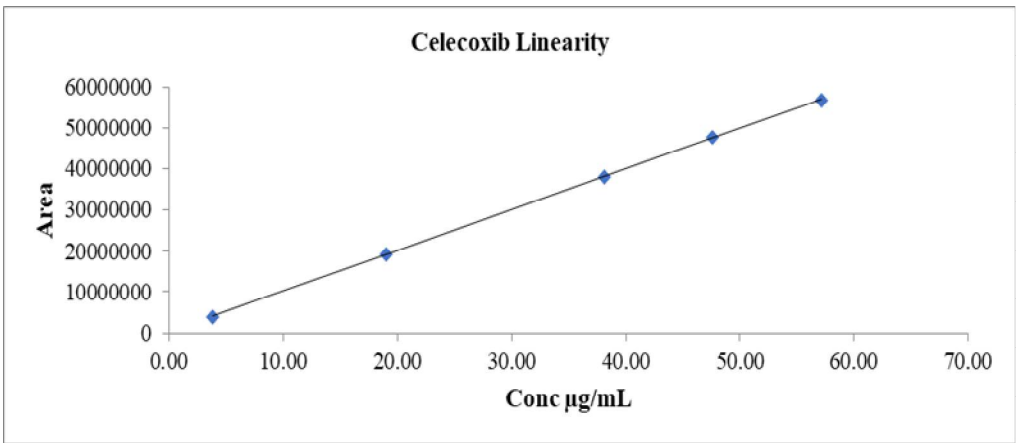

Figure 4 Calibration curve of Celecoxib

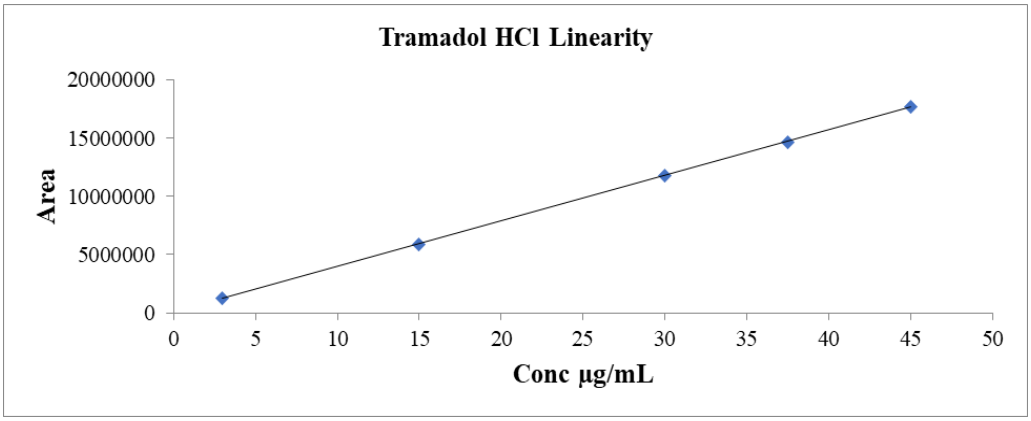

Figure 5 Calibration curve of Tramadol hydrochloride

Specificity:

Validate the blank and placebo solutions have been formulated and provided to determine whether interference at R.T. of Celecoxib and Tramadol Hydrochloride was experienced. Due to the blank and placebo, celecoxib and tramadol hydrochloride had no influence on R.T.

LOD and LOQ :

The LOD and LOQ of celecoxib were evaluated to be 0.533 µg/ml and 1.616 µg/ml, subsequently, while those of tramadol were evaluated to 0.225 µg/ml and 0.681 µg/ml.

Accuracy:

Recovery from the analytical technique appears to be within the compliance of standards at all three phases; recovery was not influenced by alterations in analyte concentration.

Table.1: Results of Accuracy

Parameter	Celecoxib		Tramadol Hydrochloride	
	Amount Added µg/ml	Mean % Recovery	Amount Added mg/ml	Mean % Recovery
50 % Level	19.08	99.37	15.12	99.45
	19.08		15.06	
	19.14		15.12	
100 % Level	38.16	99.68	30.18	99.70
	38.22		30.06	
	38.16		30.12	
150 % Level	57.30	99.30	45.06	99.21
	57.18		45.00	
	57.24		45.12	

Precision:

Standard deviation or relative standard deviation is frequently utilized to show how reliable the technique is. Because the assay and RSD percent have been shown to be well within the approved standard, which is equivalent to NMT 2 % RSD, the method can be said to be accurate.

Table 2: Results of precision

Drugs	Mean % Assay	Intra - day	Mean % Assay	Inter- Day
Celecoxib	99.37	1.210	99.36	1.313
Tramadol Hyrochloride	99.68	1.290	98.79	1.393

Robustness:

Table 3: Result of Celecoxib

	Alteration in parameters					
	Wavelength (± 3) nm		Flow Rate (±10) %		Column oven temp(±2) ºC	
	221	215	1.1	0.9	44	36
R.T	3.04	3.04	2.76	3.37	3.05	3.04
Standard Area	12260537	11840945	10730003	13107786	11560692	11647196
Asymmetry	1.23	1.25	1.19	1.24	1.20	1.23
Theoretical plates	8597	8519	8059	8863	8636	8461

Table 4: Result of Tramadol

	Alteration in parameters					
	Wavelength (± 3) nm		Flow Rate (± 10) %		Column oven temp (±2) ºC	
	221	215	1.1	0.9	42	38
R.T	3.04	3.04	2.76	3.37	3.05	3.04
Standard Area	12260537	11840945	10730003	13107786	11560692	11647196
Asymmetry	1.23	1.25	1.19	1.24	1.20	1.23
Theoretical plates	8597	8519	8059	8863	8636	8461

Assay of tablet dosage form:
In accordance to the data, the assay percentages for tramadol hydrochloride and celecoxib should probably be in the range of 90 and 110 percent, respectively.

Table 5: Assay of marketed formulation:

Drug	Sample	% Assay	Mean Assay
Celecoxib	Sample 1	98.37	99.49
	Sample 2	100.25	
Tramadol Hydrochloride	Sample 1	98.51	99.02
	Sample 2	99.53	

CONCLUSION:
Celecoxib and Tramadol Hydrochloride were simultaneously evaluated in bulk drug and dosage form employing a straightforward, reliable, and accurate reverse-phase high-performance liquid chromatographic technique. The system appropriateness criteria were found to be achieved by tailing factor, theoretical plates, retention duration, and % RSD. The regression value was found to be significantly within the range, and the calibration curve concluded that Celecoxib and Tramadol Hydrochloride exhibit linear responses within the preferred range. The process is likely to be accurate (reproducible), as demonstrated by the test and RSD percentages lying well within the acceptable range. The analyte concentration alteration had no influence on the analytical procedure's recovery; the recovery of analytical methodology seemed to appear well within the limit set by regulation. Findings mentioned above led to the conclusion that the analytical method was resilient and that the system suitability test result appears to be in compliance with the standards. The method is straightforward, reliable, and robust and can be used in laboratories for the estimation of combined drugs.

CONFLICT OF INTEREST
No potential disputes of interest have been identified by the author.

FUNDING SOURCE
We do not have any kind of funding sources.

ETHICAL STATEMENT:
There was no human subjects or animals utilized in this study. Standard laboratory protocols and regulatory requirements were followed in the execution of all analytical tests. The building and confirmation of an analytical technique for a medicinal substance was the only goal of the investigation. For this kind of investigation, ethical approval was not required.

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