Advances in Bioresearch
Adv. Biores., Vol 16 (6) November 2025: 413-424
©2025 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.6.413424

Advances in Bioresearch

REVIEW ARTICLE

Analytical Method Development and Validation for The Simultaneous Estimation of Celecoxib and Tramadol: A Comprehensive Review

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ABSTRACT

Tramadol, a centrally acting analgesic, and celecoxib, a selective COX-2 inhibitor, are commonly prescribed together to alleviate moderate to severe pain. Due to their different chemical structures, solubility profiles, and detection desires, these two pharmacologically distinct medicines give out analytical problems if examined concurrently. The multiple methods devised for the determination of Celecoxib and Tramadol in bulk medications. They are pharmaceutical formulations, and biological matrices were extensively reviewed and evaluated in this review. With an emphasis on their methodological parameters, sensitivity, specificity, and regulatory compliance, techniques like UV-visible spectrophotometry, high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), and hyphenated methods like LC-MS have been thoroughly reviewed. In line with ICH suggestions. the research additionally analyzes method validation criteria and illustrates recent advances which include quality-by-design (QbD) techniques and green analytical chemistry. The intent is to offer analysts and researchers an integrated source of data to guide the development novel approaches, improve present methods, and ensure reliable and efficient the drug analysis.

Keywords : *Celecoxib, Tramadol, Analytical method development, Simultaneous estimation, HPLC*

Received 19.09.2024 Revised 11.10.2024 Accepted 23.11.2025

How to cite this article:
Rutuja Bharat A, Deepali K K ,Shraddha K, Laxmikant. B. B. Analytical Method Development and Validation for The Simultaneous Estimation of Celecoxib and Tramadol: A Comprehensive Review. Adv. Biores., Vol 16 (6) November 2025: 413-424.

INTRODUCTION

Celecoxib relates to a group of pyrazoles that includes 1*H*-pyrazole, and it has 4-sulfamoylphenyl, trifluoromethyl, and *p*-tolyl groups substituted at positions 1, 3 and 5, respectively.[1] It acts as a non-steroidal anti-inflammatory drugs, a geroprotector, a cyclooxygenase 2 inhibitor, and a non-narcotic analgesic.[2] It is used to treat arthritis and has been implicated for minimizing the risk of other NSAIDS-related gastrointestinal bleeding.[2] It is used in familial adenomatous polyposis (FAP) to decrease precancerous polyps in the colon and to alleviate the symptoms of a variety of arthritis circumstances, notably osteoarthritis, rheumatoid arthritis, acute pain, painful menstruation, and cramps during menstruation.[2]

Celecoxib is a highly targeted reversible inhibitor of the COX-2 isoform of cyclooxygenase which hinders the metabolism of arachidonic acid to precursors of prostaglandins. It therefore contains anti-inflammatory and analgesic properties. Various cells involved in inflammation express COX-2, that also gets elevated by growth-promoting cytokines, bacterial lipopolysaccharides, and neoplasm promoters.[3,4,5] For effective inhibition of COX-2, celecoxib is probably 10–20 times more selective over COX-1. In close vicinity their active COX-2 binding site, it generates a hydrophilic side pocket region with its polar sulfonamide side chain

Drug Interaction: -

1) Considering P450 2C9 inhibitors, which involves fluconazole, may drastically increase celecoxib serum scales, celecoxib must be utilized with caution when combined with those drugs.[6]
2) Celecoxib enhances lithium plasma levels when paired with lithium.[6]
3) The simultaneous use of celecoxib and warfarin could elevate the risk of bleeding related issue.[6]

Tramadol is a serotonin-norepinephrine reuptake inhibitor (SNRI) and opioid pain reducer used to treat moderate to severe pain.[7,8] When an immediate release formulation is administered by mouth, pain relief typically begins to take action within an hour [7]. It is additionally accessible as an injectable form. Acute and relentless mild to severe pain were the most common conditions in which tramadol is indicated.

Tramadol operates via activating multiple noradrenergic, serotonergic, and opioid receptor systems to generate analgesic effects.[9] Similar to different antidepressants termed serotonin-norepinephrine reuptake inhibitors (SNRI), tramadol additionally impacts the inhibition of serotonin and norepinephrine reuptake. Tramadol is a racemic mixture; the negative enantiomer adheres to and disables the transporters which inhibit noradrenaline reuptake, whereas the positive enantiomer inhibits serotonin reuptake.[10,11]

Drug Interaction:-

1) Enzymes termed CYP2D6 metabolize tramadol, assisting in the metabolism of around 25 percent of all medicines.[12]
2) Tramadol may interact with medications which possess a tendency to lessen or stimulate these enzymes. Analgesics, tamoxifen, anti-emetics, antidepressants, antipsychotics, and common anti-arrhythmics belong to them.[13]
3) When used with other analgesics and opioid-containing analgesics, tramadol might raise the risk of negative reactions due to its serves as an opioid agonist.[15]
4) Having 5 HT3 antagonists such ondansetron, dolasetron, and palonosetron during the identical interval might reduce the effectiveness of every tablet.[14]

Table No. 1: Physicochemical and Pharmacological Profile. [16,17]

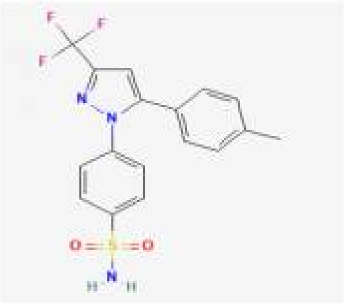
Sr. No.	Parameters	Celecoxib	Tramadol Hydrochloride
1	The Molecular Formula	$C_{17}H_{14}F_3N_3O_2$	$C_{16}H_{26}ClNO_2$
	Structure		
2	Molecular Weight	381.4 g/mol	263.37g/mol
3	IUPAC Name	4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide	2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexan-1-ol hydrochloride
4	Pka	11.1	9.4
5	Log P	3.5	2.35

Table No. 2: Analytical Techniques of Celecoxib

Sr. No.	Method	Method Details	References
1	RP-HPLC	**Mobile phase**: acetonitrile, water, triethylamine, orthophosphoric acid **Flow rate:** 1.0 ml/min **λ max**: 220 nm **Retention time**: 9.5	20
2	HPLC	**Mobile phase:** monobasic potassium phosphate buffer [pH 3.0], methanol and acetonitrile in the ratio of 60: 30 : 10 v/v/v. **Flow rate:** 2.0 ml/min **λ max:** 215nm **Column:** column L11 (4.6 x 250mm, 5 µm) **correlation coefficient:** 0.9999. **% RSD :** 0.3%	21
3	Spectrophotometric	**Detection wavelength**: 230.95nm-254,25nm in phosphate buffer & 231.07nm-254.76 nm in borate buffer **Linearity range:** 1-30 µg/ml Co-relation Co-efficient: 0.9999 in phosphate buffer and 0.999 in borate buffer **% Recovery range:** 99.7-99.88 & 99.46-99.91 **%RSD:** <2%	22
4	RP-HPLC	**Detection wavelength**: 300nm **Linearity range:** 20-60 µg/ml **Co-relation Co-efficient:** 0.999 **% Recovery range:** 98.90-100.87 **%RSD:** 0.14%(intra-day), 0.18%(inter-day), 0.46% (intra-day)	23
5	UV Spectrophotometric	**λ max**: 254 nm **Linearity range**: 0.2 µg/ml-1 µg/ml. **Precision :** Intra-day- 0.410% Inter- day - 0.328%	24
6	RP-HPLC	Applying the theories of Analytical Quality by Design (AQbD), this article describes the development of a reversed-phase (RP) high-performance liquid chromatographic (HPLC) method for evaluating process-related contaminants in a celecoxib medicinal substance.	25
7	UV Spectroscopy	**λ max:** 253nm **Linearity:** 2µg-28µg/ml **correlation coefficient**: 0.0342, 0.0228, and 0.9995 **% Drug recovery:** 100.28%	26
8	HPLC	**Column:** Monolithic silica column **λ max:** 254 nm **Assay**: 100.5 ± 1.3% **Linearity:** 10–800 ng ml^{-1}	27
9	RP-HPLC	**Column:** C18 column (250x4.6 mm i.d 5 µm) **Mobile Phase** : Methanol and acetonitrile (70:30) *v/v* **λ max:** 254nm **Flow rate** :1 ml/min **Retention time** : 3.2 min **LOD :** 0.69 µg/ml **LOQ** : 2.12 µg/ml **Run time**: 10 min **% Drug recovery**: 99.99 to 100.41	28
10	RP-LC	**Column**: Symmetry RP-18 **Mobile phase** : Column and Potassium dihydrogen phosphate monohydrate buffer (pH adjusted to 3.0 with diluted orthophosphoric acid): methanol: acetonitrile (400:400:200) v/v **λ max**: 225nm **Linearity:** 25 µg/mL to 75 µg/mL	29
11	Human Plasma-HPLC	**Column**: Luna HILIC column (50 mm × 2.0 mm, 3 µm, Phenomenex, USA) **Mobile phase**: Formate buffer (pH3.0):methanol (5:95, v/v) **Detector:** MS/MS **Linearity:** 10-2000 ng/mL **LOD:** 3.03 ng/mL	30

12	Human Plasma-HPLC	**Column**: Shim Pack GLCCN, C18 column (150 mm×6 mm, 5 µm) **Mobile phase**: Acetonitrile:1% acetic acid (4:1) **Detector**: MS **Linearity**: 50-1000 ng/mL **LOD**: 20 ng/mL	31
13	Human and rat plasma-HPLC	**Column**: NucleosilRP C18 column (30 x 2 mm, 5 µm **Mobile phase**: Acetonitrile:water :ammonium hydroxide solution (65:35:0.1, v/v/v) **Detector**: MS/MS **Linearity:** 0.25-250 ng/mL **LOD:** 0.075 ng/mL	32
14	Human Plasma-HPLC	**Column**: ACE C8-300 column (50 × 4.0 mm, 3.0 µm) **Mobile Phase**: Methanol: ammonium acetate 80:20 (v/v) **Detector:** MS/MS **Linearity:** 10.0-4000 ng/mL **LOD:** 2.50 ng/mL	33
15	Porcine skin-HPLC	**Column:** LiChrospher RP-C18 column (10 cm, 5 µm, 4 mm) **Mobile phase:** Methanol:water(72:28, v/v) **Detector:** UV detector at 251 nm **Linearity:** 0.1-3.0 µg/mL in the AS layer 5.0-50.0 µg/mL inSC layer and [EP + D] **LOD:** 0.1 µg/mL	34
16	Human plasma-HPLC	**Column:** Nova Pak C8 column (3.8 × 150 mm, **Mobile phase:** Acetonitrile: tetrahydrofuran: 0.02M sodium acetate buffer (30:8:62) **Detector:** UV detector at 252 nm **Linearity :** 40–4000 ng/mL **LOD :** 12.12 ng/mL	35
17	Human Plasma-HPLC	**Column:** Nucleosil-NO2 column (150 x 4.6 mm, 5 µm **Mobile phase :** Hexane:methylenechloride:isopropyl alcohol (70:25:5, v/v). **Detector:** UV detector at 260 nm **Linearity :** 25-2000 ng/mL **LOD:** 7.57 ng/mL	36
18	Human serum-HPLC	**Column:** Prontosil C18 AQ column (150×3 mm, 3µm) **Mobile phase:** Water:acetonitrile (40:60, v/v) **Detector**: Fluorescence detector (λex240 and λem 380 nm) **Linearity:** 12.5-1500 ng/mL **LOD :** 3.78 ng/mL	37
19	Human plasma-HPLC	**Column:** Knauer C18 column (4.6 mm. x 250 mm, 5 µm) **Mobile phase:** Acetonitrile: water (75/25, v/v) **Detector :** UV detector at 250 nm **Linearity:** 0.2–2000 µg/L **LOD:** 0.08µg/L	38
20	HPLC	**Column:** Aqua C18 (75 mm × 4.6 mm 5 µm) **Mobile phase:** Acetonitrile: phosphate buffer pH 3.5 (50:50, v/v) containing 0.1% trimethylamine **Detector:** UV detector at 254 nm **Linearity:** 10-2000 µg/L **LOD:** 3.03 µg/L	39
21	HPTLC	**Stationary phase:** Silica gel 60F254 plates **Mobile phase:** n-hexane–ethyl acetate, 60 + 40 (v/v) **Detector:** UV at 262 nm **Linearity:** 200 - 2000 ng	40
22	HPLC and LC-MS	Four polar impurities (impurities I, II, III, and IV) and one non-polar impurity (impurity V) were detected by HPLC and LC-MS during the impurity profile of Celecoxib. Via synthesis and structural characterization with the range data, the structures of impurities I, II, III, and IV were determined.	41
23	RP-HPLC	The process proved to be straightforward, accurate, resilient, sensitive, and exact. This approach can therefore be applied to both regular testing and stability analysis of the medicinal ingredient celecoxib. The	42

		approval criteria were met by all statistical results (mean, percentage RSD, and recovery percentage).	
24	LC-MS	**Linearity:** 0.06 and 7.5 **Correlation Coefficient:** > 0.9998 **LOD:** 0.02 **LOQ:** 0.06	43
25	HPLC	The two pharmacopeial methods' drawbacks were removed by the development of a new HPLC approach that can effectively separate and identify all seven unwanted substances stated in the EP and the proposed USP monographs. The two pharmacopeial methods' drawbacks were removed by the development of a new HPLC approach that can effectively separate and identify all seven impurities stated in the EP and the proposed USP monographs.	44
26	RP-HPLC	**Mobile Phase:** Phosphate buffer : Acetonitrile (45/55) **Flow rate:** 1.0 mL/min **λ Max:** 250 nm **Column:** Inertsil ODS C18 column	45
27	UV spectrophotometry and LC	The linear regression equations obtained by least square regression method, were Abs=4.949×10−2 · Conc. (in _g/ml)+1.110×10−2 UV method and Area under the curve= 5.340× 101 · LC method - Conc. (in ng/ml)+3.144×102 The detection limit, as per the error propagation theory, was found to be 0.26 _g/ml and 25 ng/ml, respectively,	46

Table No. 3: Analytical Techniques of Tramadol Hydrochloride

Sr. No.	Method	Method Details	References
1	HPLC	A sample preparing technique had been invented that combined liquid-liquid extraction and protein precipitation. Using a BDSHypersil-C18 column (5μm, 250cm, 4.6μm), chromatographic separation occurred using a double gradient method. 6.7 ng/ml was the detection limit for both tramadol and ODT. The precision and reliability met ICH standards.	47
2	HPLC	A straightforward, quick, and verified HPLC technique has been created to measure tramadol hydrochloride in tablet formulations. Mobile phase - acetonitrile–ion pair solution. **λ max**: 275 nm	48
3	HPLC	**Mobile phase:** Acetonitrile : 0.015 M Na_2PO_4 (2:8) **Column:** C18 XTerra **Flow Rate:** 1.0 ml min **λ Max:** 218 **% Drug recovery:** 98.25% **RSD:** 1.80 % **Linearity:** 0.05–0.8 mg ml	49
4	UPLC	The approach recommended is distinctive as it is based on current chromatographic techniques such as UPLC and enables the estimation of tramadol HCI impurities. Using the Waters Acquity BEH C18 column and a gradient mixture for the mobile phase, an approach has been developed Solvent A - Trifluroacetic acid buffer Solvent B - Methanol: acetonitrile	50
5	RP-HPLC	**Column:** C-18 (50x4.6 mm, 3.5μ) **Mobile phase:** 5mM Ammonium acetate: ACN (50:50 v/v) of pH 6.5 **Flow rate:** 1 ml/min **λ Max:** 215nm	51
6	UV Spectroscopy	**λ Max:** 270 nm **Linearity:** 20-160μg/ml **Correlation (R^2):** 0.9998	52
7	Visible Spectroscopy	In conditions involving chronic pain, such as osteoarthritis and	53

		surgical situations, it is used to manage mild pain. When used as a racemic blend in treatment, the (+)- enantiomer primarily binds to the µ-opioid receptor, and both enantiomers inhibit the absorption of serotonin and norepinephrine.	
8	RP-HPLC	**Column:** Zorbax C18 (150 X 4.6 mm, 5µ) **Mobile phase:** 5mM ammonium acetate buffer (pH 4.0 ± 0.3): acetonitrile (15: 85 v/v) **Flow rate:** 0.8 mL/min **Column temperature:** 40°C **λ Max:** 270 nm **Correlation coefficient:** 0.9998 **% Drug recovery:** 98.5 and 100.8	54
9	HPLC	**Column:** RP-18e 50 mm × 4.6 mm **Mobile phase**: methanol:water (13:87, v/v) adjusted to pH 2.5 by phosphoric acid **Flow rate**: 2 ml/min. r^2 **:** > 0.997	55
10	UPLC	**Column:** C18 column (100 mm × 2.1 mm,1.7 µm) **Mobile phase :** potassium dihydrogen phosphate buffer of pH 2.8: acetonitrile (60 : 40 v/v **λ Max:** 226nm (UV Detector) **(r^2) value:** 0.9999 **LOD:** 0.08 µg mL^{-1} **LOQ:** 0.2 µg mL^{-1} .	56
11	UV- Spectroscopy	**λ Max:** 271nm **Linearity:** 30 to 150µg/ml **Correlation (R^2):** 0.999	57
12	Colorimetric Method	**λ Max:** 591nm **Linearity:** 5-30µg/ml **Correlation coefficient**: 0.999.	58
13	UV and Derivative Spectrophotometric	**Linearity:** 10-100 µg mL^{-1} **Wavelength ranges from:** 240-290 nm.	59
14	LC-MS	Column: Hyprity Cl8 column, Mobile phase: methanol: water (35:65) with 0.2% formic acid Linearity: 0.1 and 160 ng/mL R^2: 0.9981	60
15	RP-HPLC	**Column :** Phenomenax luna C-18 (250x4.6 mm,5µ) **Mobile Phase :** 20 mM potassium dihydrogen phosphate, 1.75mM 1-octane sulfonic acid sodium salt, 2% isopropanol: Methanol (25:75 v/v) of pH 4.0 adjusted with orthophosphoric acid. **Flow rate:** 1 ml/min. **λ max:** 272 nm	61

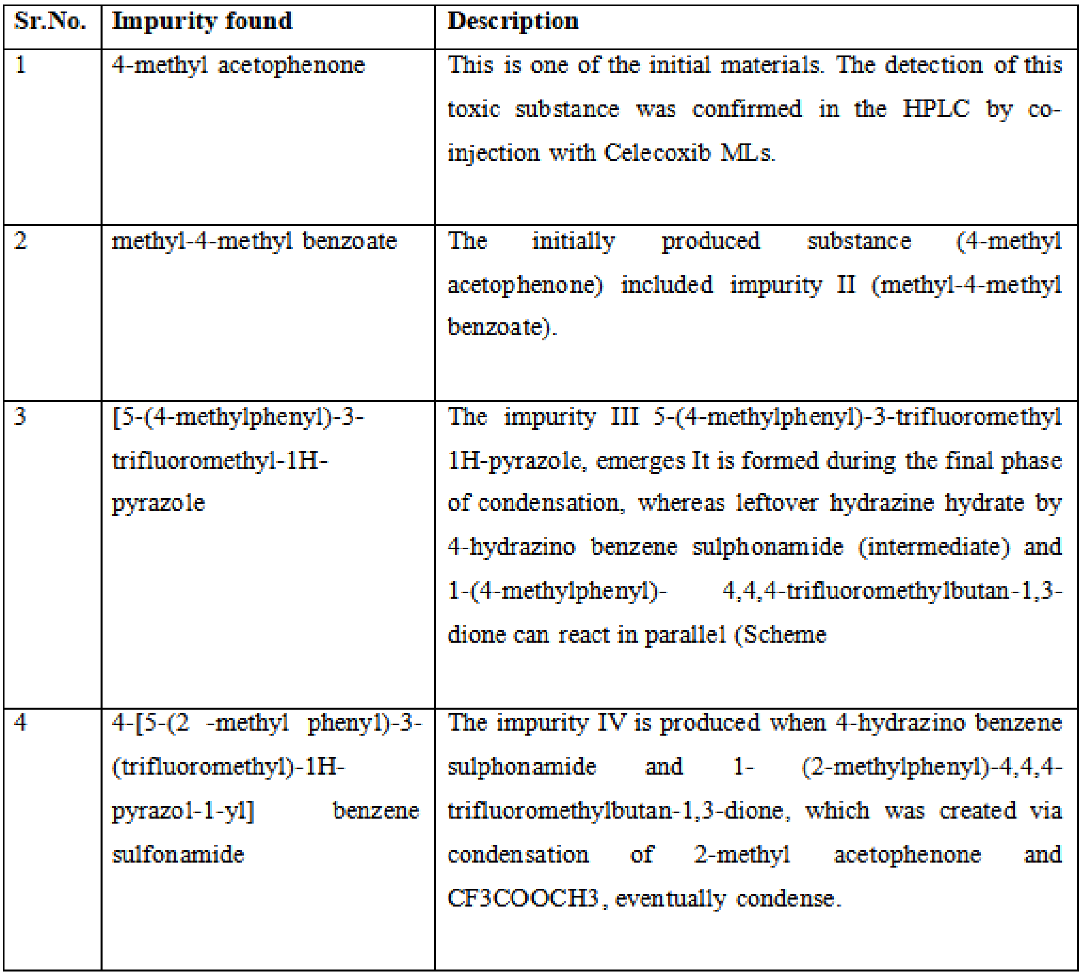
Table No. 4: Analytical Techniques of Combination of Celecoxib and Tramadol Hydrochloride:

S. N.	Method	Method details	References
1	RP-HPLC	**Mobile phase:** Methanol and Phosphate buffer **Flow rate:** 1.0 ml/min **λ max:** 330nm **Retention time:** Celecoxib - 2.131 Tramadol – 3.056	**1**
2	RP-HPLC	**Mobile phase:** Methanol **Flow rate:** 1 ml/min **λ max:** 225 nm **Retention time:** Celecoxib- 4.138 Tramadol- 8.870	**61**
3	HPTLC	Separation of CEX and TRM on silica gel 60 F254 sheets followed by	**62**

		densitometric scanning at 270 nm **Mobile phase:** Ethyl acetate–methanol–ammonia (5:5:0.05, v/v)	
4	Innovative Spectrophotometric	"Food and Drug Administration" (FDA)-approved tablets with celecoxib and tramadol hydrochloride under the Seglentis® brand have been approved for the treatment of acute pain in adults. A synthetic mixture consisting of celecoxib and tramadol hydrochloride have been assessed concurrently using five distinct UV-spectrophotometric approaches.	**63**
5	UV-Spectrophotometric	The FDA recently approved a co-formulated celecoxib and tramadol medication to treat acute pain in adults. Celecoxib and Tramadol were co-formulated, and their tablet dosages were efficiently determined via three spectrophotometric methodologies.	**64**

IMPURITY PROFILING [18]

Table No. 5: Impurity Profiling of Celecoxib



Sr.No.	Impurity found	Description
1	4-methyl acetophenone	This is one of the initial materials. The detection of this toxic substance was confirmed in the HPLC by co-injection with Celecoxib MLs.
2	methyl-4-methyl benzoate	The initially produced substance (4-methyl acetophenone) included impurity II (methyl-4-methyl benzoate).
3	[5-(4-methylphenyl)-3-trifluoromethyl-1H-pyrazole	The impurity III 5-(4-methylphenyl)-3-trifluoromethyl 1H-pyrazole, emerges It is formed during the final phase of condensation, whereas leftover hydrazine hydrate by 4-hydrazino benzene sulphonamide (intermediate) and 1-(4-methylphenyl)- 4,4,4-trifluoromethylbutan-1,3-dione can react in parallel (Scheme
4	4-[5-(2 -methyl phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzene sulfonamide	The impurity IV is produced when 4-hydrazino benzene sulphonamide and 1- (2-methylphenyl)-4,4,4-trifluoromethylbutan-1,3-dione, which was created via condensation of 2-methyl acetophenone and CF3COOCH3, eventually condense.

Tramadol Hydrochloride : [19]

1) Tramadol HCI's known impurities, especially the tramadol isomer, rac-{^R,2R -2-[(dimethylamino)methyl] One of the impurities is -1-(3-methoxyphenyl)cyclohexanol hydrochloride.
2) Dehydrated tramadol, rac-1-[(-2-(3-methoxyphenyl)cyclohex-2-en-1-yl] Impurity C contains dimethylmethanamine hydrochloride.
3) In besides extra undiscovered degraded contaminants that the proposed method may identify, O-hydroxybenzoic acid (salicylic acid) was investigated for its precise, accurate, linear, and specific determinations. Tramadol HCI and its three impurities' structures appear in Fig. 1

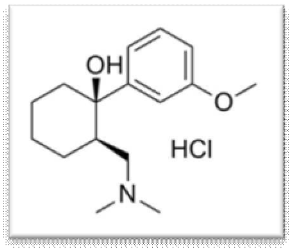


Fig. Fig 1Tramadol Isomer (Impurity A)

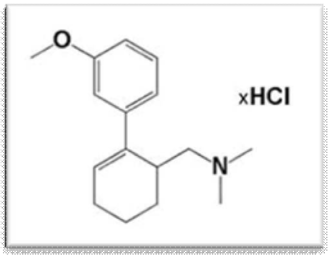


Fig. 2 Dehydrated Tramadol (Impurity C)

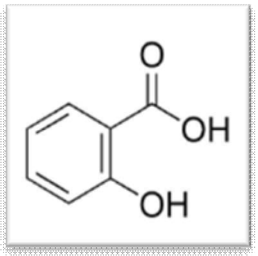


Fig. 3 Salicylic Acid

PROSPECTS FOR THE FUTURE:

1) Innovative Drug Delivery Methods:

For the therapy of localized pain, targeted distribution using liposomes, microspheres, or transdermal patches.

2) Pharmacogenomics and Individualized Therapy:

A patient's genetic makeup (CYP2D6 for Tramadol metabolism, for example) may assist to predict the right dosage to mitigate toxicity or negative effects.

Celecoxib's negative cardiovascular effects may be prevented with the use of predictive techniques.

3) Drug research using AI and machine learning:

Machine learning models are being used to anticipate synergistic effects and optimize drug combinations.

Artificial intelligence-driven drug toxicity and metabolism modelling could improve clinical trial design.

4) Dynamics of Regulation and the Market:

Because of the possibility of abuse, regulatory monitoring is increasing, especially with regard to opioid components like Tramadol.

Celecoxib has become more widely available as more biosimilar and generic versions are approved worldwide.

The market is headed toward multi-target treatments for pain and non-opioid analgesics.

5) Green Pharmacy and Sustainability:

Pay attention to drug production and analysis techniques that are environmentally friendly. Developments in production and packaging that use recyclable materials and greener solvents

CONCLUSION

Tramadol hydrochloride and celecoxib are frequently prescribed analgesics which serve significant functions in addressing symptoms of inflammation and pain. In addition, an outcome of their widespread clinical applications, pharmaceutical formulations' quality, efficacy, and safety rely on the development and validation of precise, accurate, trustworthy approaches to analysis. From spectrophotometry to proficient chromatographic and mass spectrometric techniques, this review aggregates and evaluates an extensive spectrum of analytical techniques applied for the estimation and characterization of this medicine. Considerable focus has been directed to impurity profiling, highlighting extremely crucial it is to recognize and analyze known and unknown harmful substances in a way to compliance via regulatory standards. Regarding adopting appropriate methods of analysis for medication development, quality assurance, and regulatory submission, the insight this review provides can be beneficial. For enhanced analytical sustainability and efficiency even further, future research should concentrate on integrating automation, high-throughput approaches, and green analytical chemistry.

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