International Journal of Pharmaceutical Chemistry and Analysis 2023;10(4):237–242

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International Journal of Pharmaceutical Chemistry and Analysis

Journal homepage: https://www.ijpca.org/

Review Article

Analytical and bio-analytical methods of rofecoxib: A comprehensive review

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ARTICLE INFO

Article history:
Received 25-10-2023
Accepted 30-10-2023
Available online 21-12-2023

Keywords:
Rofecoxib
Analytical method
Nonsteroidal
Highperformance liquid chromatography
Bioanalytical methods
Liquid chromatographymass spectroscopy

ABSTRACT

A selective COX-2 inhibitor Rofecoxib it is a non-steroidal anti-inflammatory (NSAIDs) medicines work by inhibiting the COX enzyme, which is a mediator of inflammation. Rofecoxib is used to treat rheumatoid arthritis, osteoarthritis, and primary dysmenorrhea. The main objective of this study is to examine Rofecoxib in pharmaceutical and biological formulations both qualitative and quantitative. In this review paper, we have outlined the approaches based on UV/Vis spectroscopy, High-performance liquid chromatography (HPLC), High-performance thin layer chromatography (HPTLC) and Liquid chromatography-mass spectrometry (LC-MS) for estimating rofecoxib. We have also discussed the bioanalytical methods used to analyse RFX. In conclusion, this review paper will aid researchers in developing new techniques for drug estimation in biological fluids and pharmaceutical dosage forms.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat pain and inflammation in rheumatoid arthritis. Their analgesic and anti-inflammatory effects, as well as some of their chemo preventive effects, are attributed to their inhibition of cyclooxygenase (COX) enzymes, which turn arachidonic acid into prostaglandins.[1] Rofecoxib is chemically 3-phenyl-4-(p-methylsulphonyl)-phenyl-(5H)-furan–2-one is a highly selective cyclooxygenase–2 (COX-2) inhibitor.[2] Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) are the two types of the enzyme. Normal physiological processes mediated by prostaglandins, such as platelet aggregation and gastric cytoprotection, are controlled by COX-1. Gastric damage and platelet inhibition have been linked to nonselective NSAID's COX-1 inhibition. It has been established that COX-2 plays a key role in the production of prostanoid mediators of pain and inflammation.[2]

In addition to treating acute migraine episodes with or without auras, rofecoxib is also used to treat adult cases of primary dysmenorrhea, rheumatoid arthritis, osteoarthritis, and acute pain.[3]

2. Mechanism of Action

The suppression of prostaglandin production appears to be the cause of the anti-inflammatory, analgesic and antipyretic actions of NSAIDs. These effects appear to be achieved by inhibiting the COX-2 isoenzyme at the sites of inflammation, which then results in a decrease in the manufacture of certain prostaglandins from their arachidonic acid precursors, however the precise mechanism of action has not yet been established. The COX-2 enzyme, which is crucial for the regulation of pain

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https://doi.org/10.18231/j.ijpca.2023.040

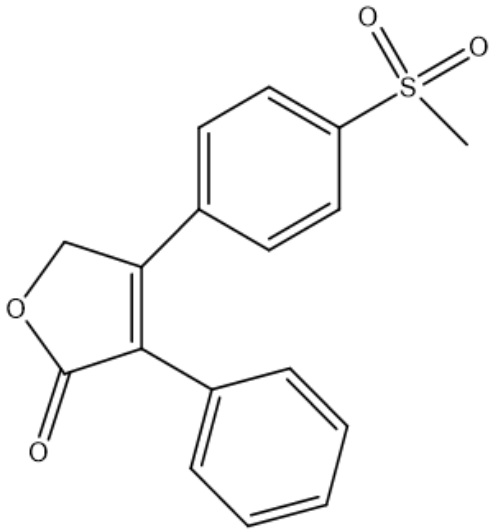

Figure 1: Depict the chemical structure of rofecoxib

and inflammation, is specifically inhibited by rofecoxib. Rofecoxib does not prevent platelet aggregation, unlike non-selective NSAIDs. Affinity for COX-1 is also negligible to non-existent.[4,5]

2.1. *Pharmacokinetics*

1. ***Absorption:*** At clinically advised dosages of 12.5, 25, and 50 mg respectively rofecoxib had a mean oral bioavailability of 93%.[4]
2. ***Protein binding:*** 87%
3. ***Metabolism:***Rofecoxib is predominantly metabolized by cytosolic enzymes by reduction. The cis-dihydro and trans-dihydro derivatives of rofecoxib, which make up about 56% of the radioactivity collected in the urine, are the main metabolic products. 8.8% more of the dosage was recovered as the hydroxy derivative's glucuronide, which is a by-product of oxidative metabolism. In humans, rofecoxib's biotransformation into this metabolite can be partially reversed (5%). As COX-1 or COX-2 inhibitors, these metabolites are ineffective. Cytochrome P450 has a small impact on how rofecoxib is metabolized.[4]
4. ***Pharmacodynamics:*** In contrast to celecoxib, rofecoxib lacks a sulfonamide chain and does not require CYP450 enzymes for metabolism. Like other NSAIDs, rofecoxib exhibits anti-inflammatory, analgesic, and antipyretic activity. NSAIDs appear to inhibit prostaglandin synthesis by inhibiting cyclooxygenase (COX), which is responsible for catalyzing the formation of prostanoids.[4]

3. Analytical Account of RFX

An extensive literature search revealed a variety of analytical methods, including UV/Visible Spectrophotometry, High-performance liquid chromatography (HPLC), High-performance thin layer chromatography (HPTLC), Liquid chromatography-mass spectrometry (LC-MS) and bioanalytical approaches, for the determination of RFX in bulk and pharmaceutical formulations. Celecoxib (CXB), Paracetamol (PCT), Diclofenac (DIC), Niflumic Acid (NIF), Mosapride Citrate (MSPC), and Tizanidine (TNZ) are all evaluated alone as well as in combination with RFX.

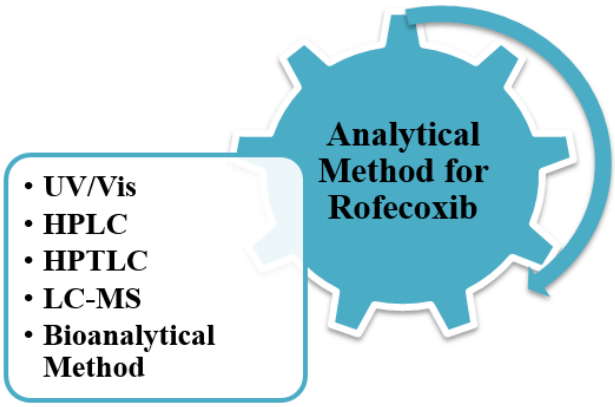

Figure 2: Different analytical methods implemented for the estimation of RFX in a bulk and pharmaceutical dosage form

3.1. *Bio-analytical method for RFX*

A branch of analytical chemistry known as "bio-analysis" deals with the quantitative measurement of biotics (macromolecules, proteins, DNA, large-molecule drugs, metabolites) and xenobiotics (drugs and their metabolites) in biological systems.[6] The summary of the reported bioanalytical methods is shown in Table 1 **.**

3.2. *UV-Visible spectroscopy method for RFX*

The spectrophotometric methods have been accounted for the determination of RFX. The details of Spectrophotometry determination of basic principle, sample matrix, lambda max, solvent linearity range and the correlation coefficient are summarized in Table 2.

4. Liquid-Chromatography-Mass Spectroscopy Methods (LC-MS) for RFX

The LC/MS combo has drawn a lot of attention recently for its enhanced performance in the detection of important analytes in challenging samples.[21–23] A detailed analysis resulted in the separation of LC/MS interfaces into two categories: interfaces for indirect and direct input of column

Table 1: Summary of bioanalytical methods for the determination of RFX in a single and combined dosage form

Sr. No.	Drug	Sample Matrix	Method	Column	Detection	Internal Standard	Ref
1	RFX	Human serum	HPLC	Novapak-C_{18} analytical column	254 nm	Diazepam	7
2	RFX	Bovine serum albumin microsphere	HPLC	C_{18} column	272 nm	***	8
3	RFX	Rat and Human Plasma	HPLC	C_{18} analytical column	272 nm	***	9
4	RFX	Bulk Drug, Tablets and Human Plasma	RP-HPLC	Spherisorb ODSI column	244 nm	Etodolac	10
5	RFX	Human Plasma	HPLC	BDS-Hypersil C_{18} analytical column	250 nm	***	11
6	RFX and CXB	Human plasma	HPLC	Zorbax SB-CN analytical column	254 nm	4-n-pentyl-phenyl-acetic acid	12
7	CEL, RFX DIC and NIF	Human serum	HPLC	C_{18} bonded silica column	254, 261, 282 and 288 nm	***	13
8	RFX	Human plasma	HPLC-MS	Nucleosil C_8 guard column	***	Celecoxib	14
9	RFX	Human plasma	HPLC	Symmetry C_{18} column	250 to 375 nm	***	15
10	RFX	Human plasma	Solid-phase extraction	Waters Symmetry C_{18} analytical column	250 nm	***	16
11	RFX	Human plasma	HPLC-MS	C_{18} analytical column	***	***	17
12	RFX	Human plasma	HPLC-MS	C_{18} analytical column	***	***	18

Table 2: Spectrophotometric methods used for determination of RFX in a single and combined dosage form

Sr. No.	Drug	Matrix	Solvent	Lambda Max (nm)	Linearity (μg/mL)	Correlation coefficient (R^2)	Ref.
1	RFX	bulk and pharmaceutical formulations	Methanol	279 nm	2.5–30.0 ng/ml	0.9985	19
2	RFX and MSPC	Indivisual dosage form	Methanol	282 nm and 331 nm	10-50 ng/ml 2-10 ng/ml	0.9990 0.9996	20

effluent. The column effluent is transferred mechanically from the indirect introduction contact to the MS vacuum. The transportation system is a prime example of an indirect introduction type of interface. The mass spectrometric vacuum system receives the column effluent directly through a tube in the direct introduction system. In general, the direct introduction seems to be the easiest way to connect LC and MS.[24] In this section, we have discussed the LC-MS methods for the determination of RFX in a dosage form Table 3.

4.1. High-performance liquid chromatography (HPLC) method for RFX

The specificity of the HPLC method is excellent and simultaneously sufficient precision is also attainable. However, it has to be stated that the astonishing specificity, precision, and accuracy are attainable only if wide-ranging system suitability tests are carried before the HPLC analysis. For this reason, the expense to be paid for the high specificity, precision, and accuracy is also high. The summary of the reported HPLC methods is shown in Table 4.

4.2. High-performance thin layer chromatography (HPTLC) method for RFX

Thin-layer chromatography is a popular technique for the analysis of a wide variety of organic and inorganic materials, because of its distinctive advantages such as minimal sample clean-up, a wide choice of mobile phases, flexibility in sample distinction, high sample loading capacity and

Table 3: Summary of LC-MS methods for the determination of RFX in a single and combined dosage form

Sr. No	Drug	Matrix	Stationary Phase	Mobile Phase	Internal Standard	Linearity (mg/mL)	Ref.
1	RFX	***	Shimpak ods C^{20} column	Acetonitrile/0.05% phosphoric acid (35:65)	***	2–36 mg/ml	25
2	RFX	Bulk and pharmaceutical dosage forms	Symmetry C_{18} analytical Column	Acetonitrile-water (50:50, v/v)	Chlorophenyl methyl sulphone	125 to 500 mg/ml	26
3	TZN and RFX	Tablets	Spherisorb ODS column	Triethylamine (pH adjusted to 2.5 using dilute orthophosphoric acid): acetonitrile 55:45% (v/v)	Nimesulide	0.1–0.5 mg/ml 1.2–6.0 mg/ml	27

Table 4: 4. Summary of HPLC methods for the determination of RFX in a single and combined dosage form

Sr. No.	Drug name	Column	Mobile phase	Lambda max(nm)	Linearity (μg/mL)	Retention time (min)	Flow rate (mL/min)	Detector	Ref.
1	RFX	C_{18} analytical column	Water: Acetonitrile (55:45 v/v)	366 nm	10-350 ng/ml	7.5 to 8 min	1 ml/min	Fluorescence	28
2	RFX	Column Apollo C_{18} column	Methanol and water (45:55 % v/v)	260 nm	24-120 mg/ml	2.379 ±0.02 min	0.8 ml/min	PDA	29
3	RFX	ODS C_{-18} column	Methanol: Water (50:50)	230 nm	2-40 mg/ml	7.79–8.00 min	1 ml/min	UV-Vis	30
4	RFX and TNZ	Luna C_{-18} column	Methanol: Phosphate buffer pH 3.5 (55:45 v/v)	240 nm	7.5-17.5 mg/ml and 0.6-1.4 mg/ml	4.53 min and 5.92 min	1 ml/min	UV-Vis	5
5	RFX and TNZ	Wakosil C-18 column	Acetonitrile: phosphate buffer pH 5.0 (50:50 v/v)	240 nm	50-200 mg/ml and 10-80 mg/ml	4.9 min and 12.2 min	0.5 ml/min	UV-Vis	31
6	PCT and RFX	Hypersil C_{-18} column	20mM phosphate buffer (pH 7.0±0.1): Acetonitrile (55:45 v/v)	254 nm	7-13 mg/ml and 0.35-0.65 mg/ml	2.61 min and 10.49 min	1 ml/min	UV-Vis	32
7	TNZ and RFX	Kromasil C_{-18} column	Phosphate buffer ph 5.5 and methanol (45:55 v/v)	235 nm	10-200 g/ml and 100-2000 g/ml	3.199 min and 7.109 min	1 ml/min	UV-Vis	33

Table 5: Summary of HPTLC methods for the determination of RFX in a single and combined dosage form

Sr. No.	Drug	Stationary Phase	Mobile Phase	Detection	Linearity	Ref.
1	RFX and TZN	Precoated with silica gel $60F_{254}$ on aluminium sheets	Toluene: ethyl acetate: methanol: triethyl amine 6:3:0.5:0.1 (v/v/v/v)	235 nm	3.75 to 11.25 μg/spot 0.30 to 0.90 μg/spot	34
2	TZN and RFX	Merck HPTLC aluminium sheets of silica gel 60 F_{254}	Toluene: methanol: acetone (7.5:2.5:1.0, v/v/v)	311 nm	10–100 ng/spot 100–1500 ng/spot	35
3	TZN and RFX	Precoated silica Gel G 60 F_{254} TLC plate	N- butyl acetate: formic acid: chloroform (6:4:2 v/v/v)	315 nm	2-10 mg/spot 16-80 mg/spot	36

low cost. The summary of the reported HPTLC methods is shown in Table 5.

5. Conclusion

The current review paper provides in-depth knowledge of the several analytical and bioanalytical methods developed for Rofecoxib, both individually and in combination. For analysis purposes, a variety of unique analytical procedures, including HPLC, HPTLC, LC-MS and UV spectroscopy, etc., have been reported. For the advantage of the researchers, the approach has been laid out in tabular form and includes details about the mobile phase, stationary phase, retention time, etc. The gathered information can be used to create future analytical methods for the bio-analysis of rofecoxib in pharmaceutical and biological formulations. Finally, it provides a chance to learn more about what has previously been accomplished as well as potential future plans and adjustments to further our knowledge of rofecoxib.

6. Abbreviations

1. UV/VIS **-** Ultra violet/visible spectroscopy
2. HPLC - High-performance liquid chromatography
3. HPTLC - High-performance thin layer chromatography
4. LC-MS - Liquid chromatography-mass spectroscopy
5. RP - Reverse phase
6. nm - Nanometer
7. μg/mL - Micro gram per Milliliter
8. PDA - Photo diode array
9. CXB – Celecoxib
10. RFX – Rofecoxib
11. DIC – Diclofenac
12. NIF – Niflumic Acid
13. MSPC – Mosapride Citrate
14. TNZ – Tizanidine
15. PCT – Paracetamol

7. Source of Funding

None.

8. Conflict of Interest

The authors declare that no conflict of interest.

Acknowledgments

Authors are thankful to Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda: 425107, Jalgaon (MH), India for providing necessary library facilities.

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Cite this article: Patil VR, Vadnere GP, Baviskar KD, Sarode VV, Nimbalkar JT. Analytical and bio-analytical methods of rofecoxib: A comprehensive review. *Int J Pharm Chem Anal* 2023;10(4):237-242.