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ACECLOFENAC AND DICLOFENAC: A REVIEW ON LIQUID CHROMATOGRAPHY TECHNIQUES

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Abstract

The literature entitles the various analytical techniques like, High Performance Liquid Chromatography, Liquid Chromatography-Mass spectrometry. In this literature we reviewed the various analytical, stability studies, impurity profiling and bio-analytical methods used for the estimation of selected drugs. This review gives the concise and collective information about the analytical validative parameters like Limit of detection (LOD), Limit of Quantification (LOQ), Standard Curve, Accuracy & Precision for the analysis of Aceclofenac and diclofenac alone or combination with other drugs. This review helps to carry out further analytical and Bioanalytical studies on the mentioned drugs.

Keywords: Aceclofenac, Diclofenac, RP-HPLC, Bioanalytical.

Introduction

Arthritis is a painful condition of joints, the tissue which is nearby it, and the connective tissue. Various types of dosage forms are existing to cure arthritis, especially NSAIDS, among the maximum are based on the unit dosage for which they must be administered at a fixed time interval. Aceclofenac [(2-{2,6-dichloro-phenyl) amino} phenylacetoxy acetic acid] is a widely used (NSAID) nonsteroidal anti-inflammatory drug, taken to relieve pain and inflammatory conditions with fewer side effects, particularly related to GIT as noticed with NSAID treatment [1]. Diclofenac sodium [N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3- pyrazole-carboxamide] is one of the analgesic-antipyretic-nonsteroidal anti-inflammatory drug. Diclofenac is a widely used for the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis osteoarthritis, musculoskeletal injuries, and post surgery analgesia in human and veterinary medicine . The molecule is practically water insoluble, but it is readily absorbed from the gastrointestinal tract as the salt form. Various analytical techniques have been reported for the quantification of diclofenac sodium (DS) and Diclofenac in different matrices. High-pressure liquid chromatography detection (HPLC) is the most common used method for the determination of DS in biological sample or dosage forms [2]. The literature entitles the various analytical techniques like High Performance Liquid

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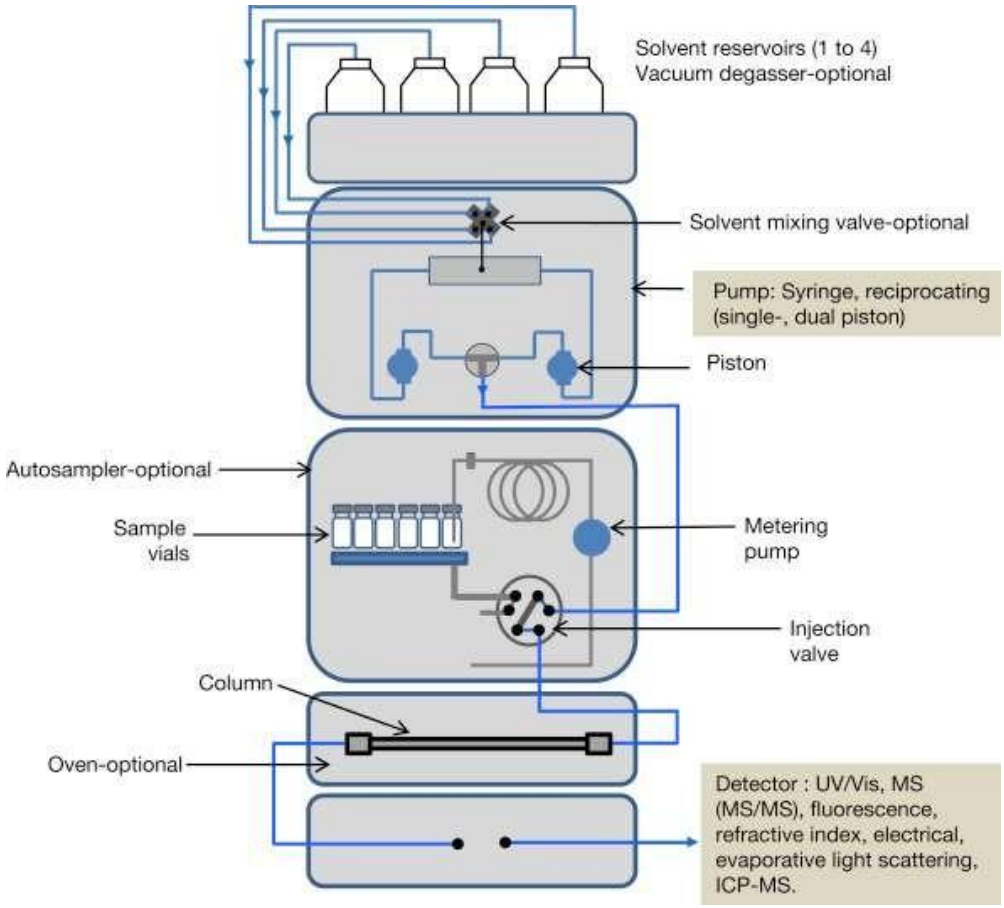
Chromatography, Liquid Chromatography-Mass spectrometry.

Methodology:

HPLC Methods:

Liquid chromatographic (LC) methods have the benefit of high separation capacity, hence their repeated use for the analysis of antihypertensive drugs. HPLC is the advanced form of LC employed for separating specific molecules in complex mixtures such as biological fluids. Owing to its remarkable selectivity and sufficient precision, HPLC is the most applied LC method for the analysis of drugs; reversed-phase HPLC (RP-HPLC) is certainly the most common technique in pharmaceutical drug development such as analysis of drug substances in biological samples. Of note, most of the methods reviewed in this study employed reversed-phase (RP) columns such as C_{18} or C_8. Furthermore, HPLC columns are usually filled with 3-5 μm particles.



Analytical Methods:

In analytical method development all the reported methods carried out by the reverse phase technique for the LC method development and these methods helps in estimation of the drugs and selection of mobile phase for the estimation of the drugs and methods are validatedaccording to the ICH Q2 R1 guidelines.

Chandrasekhar K *et al.*, (2020) has developed a new simple precise, accurate and selective RP- HPLC method for the simultaneous estimation of Paracetamol and Aceclofenac in their mixed formulation by separating Diclofenac. All the three drugs were separated on Kromasil C18 (150x4.6, 5μ) with reverse phase elution of the mobile phase compose of 0.05M potassium dihydrogen phosphate and Acetonitrile in the ratio 40:60 v/v at a flow rate of 1.0mL/min. The detection was made at 275nm. The retention times were 2.36 min for Paracetamol, 3.23 min for Aceclofeanc and 6.38 min for Diclofenac. The linearity ranges for paracetamol and aceclofenac were 16.25 to 48.75 and 5 to 15 mcg/ml respectively with correlation coefficients 0.999 and 1.0 [3]. Sharmin S *et al.*, (2020) has developed a reverse phase liquid chromatographicmethod for estimation of Aceclofenac in bulk drug and tablet dosage form was developed and validated. The chromatographic conditions to achieve the highest performance parameters using octylsilyl column with guard filter were optimized. The

separation was carried out usinga mobile phase containing 10 mM Phosphate Buffer, pH 2.1 and methanol (30:70% v/v) pumped at a flow rate of 1.0 mL/min with detection at 272 nm. The method was shown to be linear in 19.8–148.5 µg/mL concentration range (regression coefficient of 0.999). The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.0692 µg/mL and 0.2076 µg/mL, respectively. The percentage mean recoveries were 97.91% to 100.39% with %RSD values of 0.64–0.79. The method was found to be precise with %RSD value of 1.13 and 1.60 for intraday and interday precision study, respectively [4]. Kolekar AK et *al.,* (2019) has developed a simple, precise and reproducible Reverse Phase High Performance Liquid Chromatography method was developed and validated for simultaneous estimation of Pregabalin and Aceclofenac in tablet dosage form.Chromatographic separation was achieved by Grace C18 (250 mm x 4.6 ID , Particle size- 5 micron) column and methanol : water pH3 (60:40v/v) as mobile phase, at a flow rate of 1 ml/min (millilitre per minute) using UV detectionat 216nm. The method has been validated for linearity, accuracy, precision, LOD, and LOQ. The retention time for Aceclofenac and Pregabalin were obtained as 4.296 min and 5.955 min respectively. Linearity of Aceclofenac and Pregabalin were found to be in range 20-100µg/ml and7.5-37.5µg/ml.(R2 =0.998) respectively. The accuracy of present method was evaluated at 50%,100%,150%. Recovery was found to be in a range from 99.80%-100.42% for both of the drugs. Intermediate precision studies were carried out and the RSD values were less than 2%. Lower values of LOD (0.35µg/ml for ACF and 0.18 µg/ml for PRE) and LOQ (1.08µg/ml for ACF and 0.56 µg/ml for PRE) indicated good sensitivity of the method [5]. Gousuddin M *et al.,* has developed a stability-indicating reverse-phase high-performance liquid chromatography (HPLC) method for simultaneous quantitation of tramadol and aceclofenac in presence of their degradation products. The drugs were subjected to various International Conference on Harmonization recommended stress conditions, such as acid hydrolysis, alkaline hydrolysis, peroxide oxidation, thermolysis, and photolysis. The major degradation products got well resoluted from the analytes in HPLC analysis with a mobile phase composed of a mixture of 0.01 M ammonium acetate buffer (pH 6.5) and acetonitrile (65:35, v/v) through a Phenomenex Gemini C18 (250 mm × 4.6 mm, 5 µm particle size) column. The method was linear over a range of 15–60 µg/mL for tramadol and 40–160 µg/mL for aceclofenac concentration. The analytes were detected at a wavelength of 270 nm. The method was validated and found to be specific, accurate, precise, stable, and robust for its intended use [6]. Paul K *et al.,* has developeda rapid and sensitivity RP-HPLC method was proposed for the qualitative and quantitative estimation of Aceclofenac in dosage form. Aceclofenac had been chromatographed on a C18 column with a mobile phase buffer pH 5.0 and Acetonitrile in the ratio of 60:40v/v. The mobile phase was pumped at a flow rate 1ml/min. Etoricoxib was used as internal standard and elutentswere monitored at 275nm. The retention time of the drug was 7-10 min. With this method linearity was observed between area under curve and concentration of Aceclofenac in the injected solution, in the range of 25-125µg/ml [7]. Joshi R has developed a simple reversed-phase high-performance liquid chromatographic method and validated for simultaneous estimation of acetaminophen, chlorzoxazone, and aceclofenac in tablet dosage form. The estimation was carried out on an Luna C18 (5 µm × 25 cm × 4.6 mm i.d.) column using a mixture of buffer, methanol, and acetonitrile in the ratio 215:130:155 with final pH of 6.5 as a mobile phase, at a flow rate of 1.5 ml/min. Ultraviolet (UV) detection was performed at 275 nm. Total run time was 10 min; these three drugs (acetaminophen, chlorzoxazone, and aceclofenac) were eluted at the retention times of 2.055, 5.096, and 7.605 min respectively. From the validation study it was found that the method is specific, rapid, accurate, precise, and reproducible. Calibration curves were linear over the concentration ranges of 5–50 µg/ml for acetaminophen and chlorzoxazone, and 5–30 µg/ml for aceclofenac. The limit of detection (LOD) values were 16.2, 14.6, and 4.8 ng/ml, and LOQ values were 49.0, 46.5, and 14.5 ng/ml for acetaminophen, chlorzoxazone, and aceclofenac respectively [8]. Alquadeib BT *et al* ., has developed A new selective and sensitive high-performance liquid chromatography (HPLC) method was developed for the quantification of diclofenac sodium (DS) in pharmaceutical dosage form using lidocaine as internal standard (IS). Chromatographic separation was

achieved on a symmetry C18 column (4.6 mm × 150 mm, 3 µm spherical particles) using 0.05 M orthophosporic (pH 2.0) 35% and acetonitrile as 65%, as the mobile phase at a flow rate of 2.0 mL/min and monitored at 210 nm. The run time was 2 min. The calibration curve was linearover the concentration range from 10 to 200 µg/ml, and lower limit of detection of 12.5 ng/ml. The accuracy and precision of the method were within the acceptable limit of ±20% at the lower limit of quantitation and ±15% at other concentrations. Diclofenac was unstable at room temperature it showed more than 25% loss after 24 h. While, DS is very stable at refrigerator4 °C auto-sampler, freeze/thaw cycles and 30 days storage in a freezer at -35 ± 2 °C [9]. Rodríguez-Basso ÁG *et al.*, has developed a reversed-phase high-performance liquid chromatography method was developed and validated for the simultaneous determination of pridinol, diclofenac and diclofenac-related compounds in tablet formulations. Separation was achieved on a base-deactivated silica C8 column, using 50 mM phosphate buffer (pH 2.5) and methanol (40:60 v/v) as mobile phase, at a flow rate of 1.0 mL/min and column temperature of 40°C. Ultraviolet detection was made at 225 nm. The method showed specificity and linearity (R2: 0.999 for the three analytes) over the assessed concentration range (diclofenac: 2.5-75.0 µg/mL, pridinol: 2.0-60.0 µg/mL and impurity A: 1.25-5.0 µg/mL) and demonstrated good precision as reflected by the low coefficient of variation in all cases. Recovery rates obtained were 99.81, 100.58 and 100.96% for diclofenac, pridinol respectively [10]. El Kacemi M *et al.*, Monitoring impurities in drug products is a principal requirement of pharmaceutical regulatory authorities all over the world to ensure drug safety. For this reason, there is a great need for analytical QC of dugs products.In this study, a simple, efficient, and direct HPLC method was developed for the determination of three impurities of diclofenac. The HPLC method was developed using a mobile phase which consisted of an HPLC grade mixture, acetonitrile-0.01M phosphoric acid adjusted to pH 2.3 (1 + 3, by volume). The separation was performed in 15 min. The calibration curves of the three impurities were linear; the correlation coefficients were 0.999 at concentrations of 0.00015-0.003 µg/mL. The validation of this method shows that it meets all validation criteria. This shows the reliability of this method for the routine control of diclofenac impurities. The validation of a robust HPLC method for the determination of diclofenac impurities is of great importance for the pharmaceutical industry to control its Products [11]. Faseeh AS *et al.*, has delevloped a high-pressure liquid chromatography (HPLC- UV) based simple and specific method for simultaneous quantitative determination of Ofloxacin, Fexofenadine HCl and Diclofenac Potassium has been developed and validated according to ICH guidelines. Chromatographic separation of the three drugs was carried out on 4.6 x 250 mm x 5 µ Licrospher RP Select B Column, using mobile phase constituted of methanol and phosphate buffer pH 3.5 (650: 350), pH adjusted to 3.5 ± 0.05 with dilute ortho- phosphoric acid and delivered at a flow rate of 1 ml/min. The eluents were detected at UV wavelength of 220 nm and the retention times for Ofloxacin, Fexofenadine HCl and Diclofenac Potassium were 2.5 minutes, 4 minutes and 11.5 minutes, respectively. This method is suitable and specific for the three drugs and was found to be linear ($R^2 > 0.996$), accurate, specific, reproducible and robust over a concentration range of 0.05 to 0.15 mg/ml for Ofloxacin, 0.015to 0.045 mg/ml for Fexofenadine HCl and 0.0125 to 0.0375 mg/ml for Diclofenac Potassium [12]. Mulgund SV *et al.*, has developed a simple, specific, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of mephenesin and diclofenac diethylamine, using a Spheri-5-RP- 18 column and a mobile phase composed of methanol: water (70:30, v/v), pH 3.0 adjusted with o-phosphoric acid. The retention times of mephenesin and diclofenac diethylamine were found to be 3.9 min and 14.5 min, respectively. Linearity was established for mephenesin and diclofenac diethylamine in the range of 50-300 mug/ml and 10-60 mug/ml, respectively. This method can be successfully employed for simultaneous quantitative analysis of mephenesin and diclofenac diethylamine in bulk drugs and formulations [13]. Korodi T *et al.*, has developed a fast and reproducible high performance liquid chromatography method has been developedfor the determination of diclofenac sodium and its degradation products in commercial and in-house produced ointments. The method employs a RP-LiChrospher select B

(C8) column with a mobile phase containing methanol/water (63:37, v/v) and detection at 220 nm. This rapid and simple HPLC assay was used for QA/QC of large scale in-house produced diclofenac gel. The validation protocol was designed following international guidelines, e. g. ICH Q2(R1). Selectivity tests also included the separation of synthesis related by-products like 1-(2,6- dichlorphenyl)indoline-2-one (impurity A) and indoline-2-one (impurity E), and in addition selectivity with regard to several photodegradation products produced by both UV and simulated sunlight irradiation has been shown [14]. Kasperek R *et al.*, has developed a HPLC method for simultaneous determination of diclofenac sodium and papaverine hydrochloride in tablets was developed and validated. The determination was performed with a Zorbax SB-C18 column, mobile phase: methanol-water (60:40, v/v), flow rate: 1 mL min (-1) and UV detection at 278 nm [15]. Azougagh M *et al.*, has developed a innovative simple, fast, precise and accurateultra-high performance liquid chromatography (UPLC) method was developed for the determination of diclofenac (Dic) along with its impurities including the new dimer impurityin various pharmaceutical dosage forms. An Acquity HSS T3 (C18, 100×2.1mm, 1.8μm)column in gradient mode was used with mobile phase comprising of phosphoric acid, whichhas a pH value of 2.3 and methanol. The flow rate and the injection volume were set at 0.35ml·min(-1) and 1μl, respectively, and the UV detection was carried out at 254nm by using photodiode array detector. The relative percentage of standard deviation obtained for the repeatability and intermediate precision experiments was less than 3% and LOQ was less than 0.5μg·ml(-1) for all compounds [16]. Panda SS *et al* ., has developed a simple, precise and accurate isocratic RP-HPLC stability-indicating assay method has been developed to determine diclofenac potassium and metaxalone in their combined dosage forms. Isocratic separation was achieved on a Hibar-C(18), Lichrosphere-100(®) (250 mm × 4.6 mm i.d., particle size 5 μm) column at room temperature in isocratic mode, the mobile phase consists of methanol: water (80:20, v/v) at a flow rate of 1.0 ml/min, the injection volume was 20 μl and UV detection was carried out at 280nm. The drug was subjected to acid and alkali hydrolysis, oxidation, photolysis and heat as stress conditions. The method was validated for specificity, linearity, precision, accuracy, robustness and system suitability. The method was linear in the drug concentration range of 2.5-30 μg/ml and 20-240 μg/ml for diclofenac potassium and metaxalone, respectively [17]. Shaalan RA *et al.*, A simple, rapid, and highly selective HPLC- DAD method was developed for the simultaneous determination of diclofenac sodium (DIC) and diflunisal (DIF) in pure form and in their combined formulation. Effective chromatographic separation was achieved using a Zorbax SB-C8 (4.6×250 mm, 5 μm particle size) column with a mobile phase composed of 0.05 M phosphoric acid, acetonitrile, and methanol in the ratio of 40:48:12 (by volume). The mobile phase was pumped isocratically ata flow rate of 1 mL/min, and quantification of the analytes was based on measuring their peak areas at 228 nm. The retention times for diflunisal and diclofenac were about 7.9 and 9.5 min, respectively. Calibration curves were linear in the ranges of 5-100 μg/mL for both drugs with correlation coefficients >0.9998 [18]. Fayed AS has developed a new, simple, highly sensitive, precise, and accurate gradient reversed-phase chromatographic methods were developed using HPLC and ultra-HPLC (UPLC) systems for the determination of five components, namely thiamine, pyridoxine, cyanocobalamin, benfotiamine, and diclofenac in tablets and capsules. The methods were compared for their efficiency in the separation and determination of these five compounds using two different C18 columns (250 × 4.6 mm, 5 μm; and 100 × 4.6 mm, 2.6 μm) for HPLC and UPLC, respectively. Chromatographic separation was performed with a mobile phase containing acetonitrile and 0.025 M phosphate buffer (pH 3.5), with a gradient program and a flow rate of 1.5 and 1.0 mL/min for both methods, respectively. The methods were validated according to ICH guidelines. Linearity was achieved in the range of 5.00 to 150.00 μg/mL for each of the five compounds [19]. Gohel NR has developed a Chemometrics-assisted UV spectrophotometric and RP-HPLC methods are presented for the simultaneous determination of tolperisone hydrochloride (TOL) and diclofenac sodium (DIC) from their combined pharmaceutical dosage form. In addition, an HPLC method was developed using a reversed-phase C18 column at ambient temperature with a mobile phase consisting of methanol:acetonitrile:water (60:30:10 v/v/v), pH-adjusted to 3.0, with UV

detection at 275 nm. The methods were validated in terms of linearity, accuracy, precision, sensitivity, specificity, and robustness in the range of 3-30 µg/mL for TOL and 1-10 µg/mL for DIC [20]. Elkady E has developed a An isocratic HPLC method has been developed and validated for estimating paracetamol and diclofenac sodium simultaneously with three skeletal muscle relaxants, namely, methocarbamol, tizanidine hydrochloride and chlorzoxazone in their pure standard mixtures and in different multi-component dosage forms in a single chromatographic run. HPLC separation was achieved on a C18 Inertsil ODS-3V 5 µm column (250 × 4.6 mm) usinga mobile phase mixture containing acetonitrile and 25 mM phosphate buffer (pH 7.4 adjusted with NaOH) in the proportion of (39.7:60.3, v/v) pumped at 1.2 mL/min flow rate with UV detection at 220 nm. An experimental design was used by applying Plackett-Burman design for screening the most critical predictors affecting the chromatographic separation and Box- Behnken design for optimizing the selected predictors and creating the response surface between the selected predictors and the interested responses. ICH recommendations were applied for validating the proposed method with regard to linearity, precision, accuracy, selectivity, limits of detection and quantitation, and robustness. There are many applications for the optimized method that can be applied for routine estimation of the cited drugs in laboratories of quality control and pharmaceutical industries to save money and time and to reduce material waste and effort [20].

Bio Analytical Methods

There are only few methods are reported in the bio analysis of the drugs. Bio analytical methods gives the estimation or analysis of the samples from the blood, serum, plasma, urineand other biological fluids this helps in the clinical and preclinical studies of the drugs.

Sl No	Drugs	Method	Chromatographic conditions	Author
1	Aceclofenac	LC MS/MS	Stationary Phase: C18 (4.6 X 50mm, 50µm, 60AO) Mobile Phase:Wavelength: Flow Rate: 0.350ml/min Biological Fluid: human plasma	REDDY R[21]
2	Aceclofenac	RP- HPLC	Stationary Phase: C18 HPLC column (5 µm, 4.6 × 250 mm) Mobile Phase: 0.1% formic acid and acetonitrile in a ratio of 40 : 60 (v/v) Wavelength:275nm Flow Rate: 1.2 mL/min Biological Fluid:Human Serum	Moni F
3	Paracetamol, Chlorzoxazone and Aceclofenac	LC MS/MS	Stationary Phase: C18 HPLC column (5 µm, 4.6 × 50 mm) Mobile Phase: acetonitrile–10 mM ammonium formate pH 3.0 (65:35, *v*/v) Biological Fluid: Human Plasma	Mohamed D

4	Diclofenac Diethylamine, Methyl Salicylate, And Capsaicin	RP-HPLC	Stationary Phase: C18 column (Waltham, MA, USA, 4.6 mm × 150 mm, 5 µm) Mobile Phase: phosphoric acid mixed with acetonitrile in a 35:65% (v/v) Wavelength: 205 nm Flow Rate: 0.7 mL/min Biological Fluid: rabbit skin samples	Mohamed D[25]
5	Timolol Maleate (TM), Moxifloxacin Hydrochloride (MOXI), Diclofenac Sodium (DS) And Dexamethasone (DEXA)	RP-HPLC	Stationary Phase: C18 column Mobile Phase: 0.05 M monobasic phosphate buffer: acetonitrile (65:35v/v) Wavelength: 265 nm Flow Rate: 1 mL/minute Biological Fluid: Human Plasma	Shahzad A[26]
6	diclofenac and resveratrol	UPLC/MS-MS	Stationary Phase: ACQUITY UPLCC18 Column (2.1 × 100 mm; 1.7 µm) Mobile Phase: ammonium acetate (5 mM) in water and methanol (50, 50 v/v) Flow Rate: 0.4 mL/min Biological Fluid: mice skin samples	Alqahtani SM[27]
7	diclofenac sodium (DS)	RP-HPLC	Stationary Phase: Hypersil BDS, C18 column (250 mm × 4.6 mm; 5 m) Mobile Phase: acetonitrile and methanol (70:30, v/v) Wavelength: 276 nm Flow Rate:1.0 ml/min Biological Fluid: rabbit Plasma	Bhattacharya SS[28]

8	diclofenac	RP-HPLC	Stationary Phase: Ace C(18) column Mobile Phase: 20 mM phosphate buffer (pH 7) 0.1% trifluoroacetic acid-acetonitrile (65:35, v/v) Flow Rate: 1 ml/min Biological Fluid: human plasma	Yilmaz B[29]
9	diclofenac sodium	UPLC- MS-MS	Stationary Phase: C18 1.7 µm, 2.1 × 50 mm column Mobile Phase: acetonitrile (0.1% glacial acetic) and water (pH 3.5) Flow Rate: 0.2 mL/min Biological Fluid: rabbit plasma	Alam MA [30]
10	febuxostat (FEB) and diclofenac (DIC)	HPLC-DAD	Stationary Phase: C18 column Mobile Phase: methanol-formic acid pH 2.1 (76:24, v/v) Biological Fluid: Rabbit Plasma	El-Yazbi [31]

Conclusion

The above-noted records is concise collective statistics approximately the analysis of the Aceclofenac and Diclofenac by myself or in mixture with different tablets. All the techniques noted are verified in step with the ICH/USFDA guidelines and are usefull inside the analysis of the mentioned drugs. The distinguished method for the analysis of the medication is carried out by using RP-HPLC in these strategies the majorly used solvents are Acetonitrile with potassium dihydrogen phosphate buffer and methanol with phosphate buffer. the lamda max for Aceclofenac and Diclofenac changed into determined to be 250-270 nm. LC-MS methods with MRM techniques in high quality and poor mode below electro spray ionisation method had been pronounced for the evaluation. In the Bio-analysis the extraction of the drugs is finished through the Protein precipitation and Solid segment extraction, inside the protein precipitation technique Acetonitrile is majorly used because the precipitating agent. Insolid segment extraction method MCX sorbent supply the nice consequences as compared to HLB and WCX sorbents.

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Conflict of Interest

No Conflict of Interest

Abbreviations

AM: AmlodipineDC: Diclofenac

TM: Timolol Maleate
MOXI: Moxifloxacin Hydrochloride
DS: Diclofenac Sodium
DEXA: Dexamethasone ACN: Acetonitrile
MRM: Multiple Reaction monitoring

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