SUMMARY

Rapid growth of pharmaceutical industry results in numerous formulations in medical science. The analytical assets deal with quality standards and efficacy of the drug products. Modern analytical techniques are playing important role in measuring quality standards of drug products. Analytical techniques are basically required for setting standards of drug products and its regular checking. Presently Chromatography is a very popular analytical technique which is used in pharmaceutical industry around the world to check the quality and efficacy of drug products.

The quality and safety of formulated drug products are ensured by monitoring quantity of API i.e. also called as Assay and related substances present in dosage forms. The related substances present in drug product possess unwanted pharmacological side effects.

A validated analytical method should be developed for the estimation of the active pharmaceutical ingredient (API) and related substances in the drug dosage forms to confirm drug efficacy. Related components are designated as impurities present in pharmaceutical products which are unwanted chemicals that remain in trace level quantity with the active pharmaceutical ingredients (APIs) during synthesis pathway, or formed during stability study of the drug product. Even a trace level quantity of impurities may influence the efficacy of formulated dosage form. A validated stability indicating analytical procedures should be used to evaluate the forced degradation studies and pharmaceutical product stability studies.

Regulatory guidance is available to establish the limit of impurities in drug products and API’s.
Stability studies during product development provide the information to method development scientists about unspecified degradants of drug. All unspecified and specified degradation products are quantified and monitored in these studies. Stress studies are useful to establish the specific stability indicating capability of test method to evaluate the impact on drug product stability due to any accidental exposure to the unwanted environmental conditions except normal stage conditions.

The degradation products formed during stress testing are known as “potential” degradants. Six major categories are classified for the forced degradation studies namely thermolytic degradation, oxidative degradation, photolytic degradation.

Preformulation study need to be performed to understand the drug excipient interaction which is the initial step to finalize active and placebo ratio. Although excipients are pharmacologically inactive but may influence safety and efficacy of drug products by inducing chemical or physical reactions with drug substances. Physical interactions can influence the quality attributes of drug product e.g. dissolution rate, hardness, disintegration time and uniformity of dosage unit. Degradation products formed due to chemical interactions between excipients and drug could be detrimental to the patient.

Chromatography is defined as, “Chromatography is a technique for the separation of mixture of components between two phases, a stationary phase and a mobile phase. Those components strongly retained by the stationary phase move slowly with the flow of mobile phase and elutes later. In contrast, components that are weakly held by the stationary phase travel rapidly down the column and elutes first. This difference in migration rates make the sample components to separate and can be analyzed quantitatively and qualitatively”.

Liquid chromatography is a separation technique based on a solid stationary phase and liquid mobile phase. There are mainly two modes of separation in
HPLC viz. normal phase and reverse phase. In normal phase liquid chromatography the stationary phase is more polar than the mobile phase and vice versa in reverse phase liquid chromatography.

The plot of detector response as a function of time is called chromatogram. It gives qualitative and quantitative information of analyte.

Analytical method development and validation of veterinary drug products by using liquid chromatography has been selected as title of the thesis. Marbofloxacin tablets and Deracoxib chewable tablets have been selected for the work. The thesis contains four chapters and deals with Assay and Related substances RP-LC method development and validation of Marbofloxacin tablets and Deracoxib chewable tablets.

**Marbofloxacin tablets**

Marbofloxacin is a carboxylic acid derivative third generation fluoroquinolone antibiotic. It is a veterinary medicine used in treatment of dermal, respiratory & urinary tract infection borne from Gram positive bacteria, Gram negative bacteria and Mycoplasma in dogs and cats. Marbofloxacin’s mechanism of action is to inhibit the bacterial enzymes like DNA-gyrase & topoisomerase IV which eventually helps in bactericidal activity.

**Deracoxib Chewable tablets**

Deracoxib is non-narcotic, nonsteriodal anti-inflammatory drug (NSAID) of the coxib class. It is a veterinary medicine used in treatment of postoperative pain and inflammation associated with orthopaedic surgery and control of inflammation and pain associated with osteoarthritis in dogs. Deracoxib’s mechanism of action is to inhibit the synthesis of prostaglandins. The enzyme inhibited by NSAID is cyclo-oxygenase (COX) enzyme. The COX enzyme is in two isoforms COX-1 and COX-2. COX-1 is responsible for synthesis of
prostaglandins important for maintaining healthy gastrointestinal tract, platelet function and renal function. COX-2 is responsible for synthesizing prostaglandins that are important mediators of fever, inflammation and pain.

**Chapter 1** deals with Introduction, scope of research, instrumentation, drug products selected for research study and review of literature.

**Chapter 2** deals with method development design and its validation

**Analytical Method Development**

In HPLC method development design most important step is to collect the maximum information about drug with literature review, check availability of drug in the pharmacopeia and journals article related to active substance. After literature search, solubility study should be performed (at different pH, media, water and solvent) of “components of interest” to understand the characteristic of the drug. All possible degradation products/related substance/ key starting materials and by products should be identified before initiation of related substances method development.

**Analytical Method Validation**

Once we have developed the methods suitable to quantify the amount of drug present in the formulation (assay), and quantification of impurities in the formulation (Related Substances), their suitability for their respective intended purposes is to be established by means of Validation. Analytical method validation is the process of demonstrating that an analytical procedure is suitable for its intended purpose.
Chapter 3 deals with Experimental work and results of Deracoixb chewable tablets.

Deracoixb chewable tablet which is administered orally and extensively metabolized in liver. Its recommended maximum daily dose is 4 mg/kg per day. Tablets are available in beef-flavor and used as veterinary medicine in the treatment of osteoarthiritis as well as post operative pain and inflammation associated with orthopaedic and dental surgery in dogs.

Deracoixb is chemically described as

**IUPAC name and structure of Deracoixb**

4-[(3-(difluoromethyl)-5-(3-fluoro-4-methoxyphenyl)pyrazole-1-yl)benzenesulfonamide

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S
O
H3CO

F
F

N

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Structure of Deracoixb
Optimized Reverse phase liquid chromatographic conditions of Assay:

The method was developed using a Hypersil BDS C-18 (150 x 4.6) mm, 5 μm column with mobile phase containing a mixture of acetonitrile and water in the ratio of 55:45. The mobile phase was filtered through nylon 0.45 μm membrane filter and degassed before usage. The flow of mobile phase was 1.0 mL/min. The column temperature was maintained at 30°C and analyte was detected at wavelength of 254 nm in 8 minutes run time. The injection volume was 5μL.

Optimized Reverse phase liquid chromatographic conditions of Related substances:

The method was developed using a YMC-ODS-AQ (150 x 3.0) mm, 5 μm column with mobile phase containing a mixture of acetonitrile and water in the ratio of 38:62. The mobile phase was filtered through nylon 0.45 μm membrane filter and degassed before usage. The flow of mobile phase was 0.8 mL/min. The column temperature was maintained at 40°C and analyte was detected at wavelength of 254 nm. The injection volume was 10 μL.

Both Assay and Related substances methods were validated as per the VICH guideline and all results were found satisfactory.

Based on the experimental results, it was concluded that analytical method can be used in quality control laboratory to check the quality and efficacy of drug products.
Chapter 4 deals with Experimental work and results of Marbofloxacin tablets

Marbofloxacin tablet is a third generation fluoroquinolone antibiotic for veterinary use. The antimicrobial activity of antibiotic depends upon its inhibition of DNA gyrase & topoisomerase IV. Antibiotic shows broad spectrum bactericidal activity. Tablets are used in dermatological, respiratory & urinary tract infection resulting from Gram positive, negative bacteria and mycoplasma.

Marbofloxacin is chemically described as

**IUPAC name and structure of Marbofloxacin**

9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyridol(3,2,1-ij)(4,2,1)benzoxadiazin-6 carboxylic acid

![Structure of Marbofloxacin](image.png)

**Optimized Reverse phase liquid chromatographic conditions of Assay:**

The method was developed using a Zorbax SB C-18 (150 x 4.6) mm, 5 μm column with mobile phase containing a mixture of 0.1% v/v Trifluoroacetic acid in 1000 mL water and acetonitrile in the ratio of 83:17. The mobile phase was filtered through nylon 0.45 μm membrane filter and degassed before usage. The flow of mobile phase was 1.0 mL/min. The column temperature was maintained at
30°C and analyte was detected at wavelength of 298 nm in 10 minutes run time. The injection volume was 10 μL.

**Optimized Reverse phase liquid chromatographic conditions of Related substances:**

The method was developed using X-Terra RP-18 (150 x 4.6) mm, 3.5 μm column. The buffer is (2.7 g sodium dihydrogen orthophosphate monohydrate dissolved in 1000 mL water, adjusted the pH to 2.5 with orthophosphoric acid, added 3.5 g of 1-octane sulphonic acid sodium salt monohydrate and dissolved again). This buffer was filtered through nylon 0.45 μm membrane filter. Mixed buffer, methanol and glacial acetic acid in the ratio of 77:23:0.5 for mobile phase preparation and degassed before usage. The flow of mobile phase was at a rate of 1.2 mL/min. The column temperature was maintained at 40°C and analyte was detected at wavelength of 315 nm. The injection volume was 10 μL. Run time for standard solution was 50 min and for resolution, placebo and sample solution was 60 min.

Both Assay and Related substances methods were validated as per the VICH guideline and all results were found satisfactory.

Based on the experimental results, it was concluded that analytical method can be used in quality control laboratory to check the quality and efficacy of drug products.