An-Najah National University

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Pharmaceutical Dosage Form Preparation of a Synthesized Celecoxib Salt and Development of a Validated Method for its Analysis

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Dedication

For standing by me through thick and thin for not giving upon me when I didn't win, for your patience, love and caring, thank you my MOM.

For believing on me, being my solid back and support, thank you my DAD.

For being there for me through everything, for all your love and protection, for being my greatest treasure, thank you my brothers and sisters.

For making everything possible, for all the smiles and the great times we had, thank you my dear friends.

For being my soul mate, my best friend, my brother from another mother, thank you Hussein.

I dedicate this work….

Acknowledgement

First I am thankful to ALLAH ALMIGHTY, who gives me courage to perform and complete my research in very tough time I pass through, and without help of ALLAH I was not able to do my research completely.

I would like to express my special thanks to my supervisor Dr. Murad Abualhasan who gave me gold opportunity to do this wonderful research, and being so patient with me, also special thanks to my co-supervisor Dr. NidalZatar who help me in completing my research.

I would also like to thank my parents and friends who help me a lot in finalizing this research.

KefahRasmi Abu Shehab

الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Pharmaceutical Dosage Form Preparation of a Synthesized Celecoxib Salt and Development of a Validated Method for its Analysis

أقر بأن ما اشتملت عليه الرسالة هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

<u>Declaration</u>

The work provide in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name   **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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List of Abbreviations

Abbreviations	Full Name
NSAID	non-steroidal anti-inflammatory drug
CXB	Celecoxib
COX-2	Cyclooxygenase-2
DMSO	Dimethyl sulfoxide
API	Active pharmaceutical ingredient
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
RP-HPLC	Reversed phase high performance liquid chromatography
C18	Octadecylsilane
AUC	Area under the curve
ICH	The international conference on harmonization
LOD	Limit of detection
LOQ	Limit of quantification
M	Molarity
MCC	Microcrystalline cellulose
Mcg/ml	Microgram per milliliter
Mg	Milligram
mg/ml	Milligram per milliliter
RPM	Round per minuet
Mmol	Millimoles
R	Resolution
T	Tailing factor
N	Theoretical plate
RSD	Relative Standard Deviation
SD	Standard deviation
USP	United states pharmacopeia
UV/Vis	Ultraviolet/Visible
UV	Ultraviolet
λmax	Wavelength of maximum absorption
µL	Microliter

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Abstract

Background: Celecoxib is a non-steroidal anti-inflammatory drug (NSAID) and cyclooxygenase-2 (COX-2) inhibitor. It is used in the treatment of rheumatoid arthritis, osteoarthritis, juvenile arthritis, and acute pain. Celecoxib suffer of low systemic bioavailability due to its low water solubility. This study aimed to improve water solubility and dissolution profile by synthesizing a suitable celecoxib salt.

Method: A library of celecoxib salts was synthesized, and it is water solubility was determined using UV/Visible spectrophotometric. One of the synthesized salts was chosen for tablet formulation. A simple and feasible reverse phase high performance liquid chromatography (HPLC) method was developed for the analysis of the tablet formulation. The developed method was then validated according to international guidelines. The dissolution profile, the shelf life and accelerated stability studies were performed on the formulated tablet.

Results: Celecoxib-K salt showed an increase in water solubility by more than 140 folds (0.464mg/ml) compare to celecoxib. This salt was chosen to be formulated in tablet dosage form. The in vitro dissolution profile of the formulated celecoxib-K salt tablet was totally dissolved and reached

plateau after 10 minutes. The developed analytical HPLC method was reliable and valid method with good linearity, accuracy and precision. Also the validated method was sensitive, the LOD and LOQ value of 0.001mg/L and 0.1mg/L respectively. The formulated celecoxib-K salt tablet was stable under room temperature and accelerated condition for 60 days.

Conclusion: The solubility of celecoxib was improved by converting it to potassium salt form. The formulated tablet of celecoxib-K salt showeda good dissolution profile in water. The developed HPLC method was valid and reliable for analysis and quantification of the formulated tablet.The formulated tablet was stable at both room temperature and stress conditions.

1. Introduction

1.1 Celecoxib

Celecoxib is a non-steroidal anti-inflammatory drug (NSAID), it is cyclooxygenase-2 (COX-2) inhibitor. It is used to treat rheumatoid arthritis, osteoarthritis, juvenile arthritis, and acute pain [1-3].Celecoxib is a pyrazole derivative, Celecoxib is 4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrzol-1-yl] benzene sulfonamide, it has a chemical formula $C_{17}H_{14}F_3N_3O_2S$ with structure as in **Figure 1.1[2, 4].**
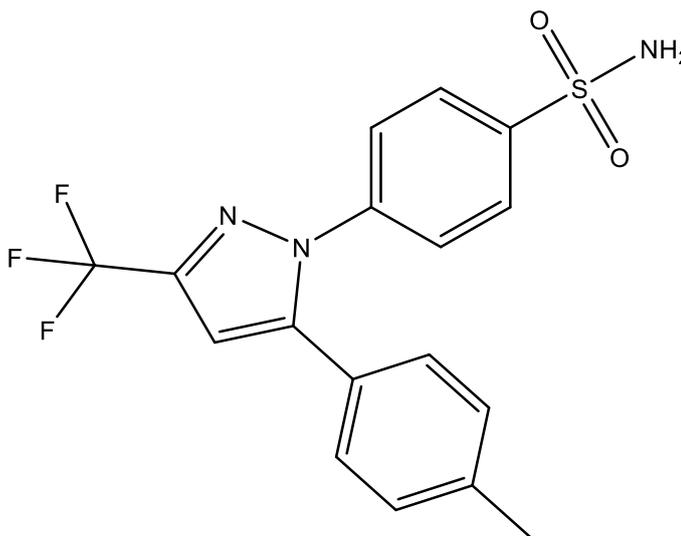
Figure1.1: Chemical structure of celecoxib

Celecoxib is yellow crystalline powder, with molecular weight of 381.372 g/mol[5], it has a melting point 157-159°C[4, 6], it is soluble in DMSO and ethanol, and its water solubility is very low (3.3 mg/L)[4, 7]. However, celecoxibsoluble in organic solvent but with increase in the solvent polarity celecoxib solubility will decreased [8].

All COX-2 inhibitors including (celecoxib, rofecoxib, and valdecoxib) are diaryl-5-membered heterocycle. Celecoxib has central pyrazole and two adjacent phenyl substituent's, one containing methyl group and the other polar sulfonamide binds to a distinct hydrophilic region that present in COX-2but not COX-1[1].

Celecoxib is very weak acid, and its source of acidity comes from the presence of sulfonamide group [9]. It has a pKa value of 11.1, and this explains its low solubility in water[10, 11], but its solubility increase in water at high alkaline pH due to formation of sulfonamide salt[12].

According to Biopharmaceutics Classification System (BCS); celecoxib belongs to class 2, which means it has low solubility in water, and high membrane permeability[13].

Many of the NSIAD is including celecoxib are poorly water soluble, and this is rate limiting step for absorption, and this is the cause of low bioavailability for these drugs [14].

The marketed celecoxib is a crystalline form, which has low solubility. Many researchers showed an improve celecoxib solubility by different techniques including (co-solvent, metastable polymorphs, amorphous dispersion, solution in organic vehicles) than the crystalline form[13, 15, 16]. Improvement of the oral drug absorption celecoxib can be achieved by crystalline hydrates and propylene glycol solvates of celecoxib, than the marked celecoxib crystalline form [17].

1.2 Salt formation

It has been estimated that almost 50% of the marketed drugs are in a form of salt, improvement in the physicochemical properties of the basic and acidic drugs by converting these drugs into salt form[18]. The benefits of formation drug products in salt form are increasing its solubility and dissolution rate and this will increase the efficacy of the drug[19]. The formation of the drug in salt form need ionisable functional group in the drug molecules, and appropriate counter ion, an ionic intermolecular forces attract the two parts of the molecules[20, 21].

Selection the salt form for the drugs depends on several factors like, dosage form of the formulated drug, for example injects, solutions, solid dosage form and immediate release formulation require highly soluble hydrochloride, sodium, potassium and mesylated salt form but for suspension and sustained release formulation insoluble counter ion can be used like tosylate and estylate salt [22]. The molecular weight of the counterion is also important factor; in high dose, low molecular weight counterion must be chosen, but in low dose drug the molecular weight of the counterion is not important[21, 23]. Some counterions have therapeutic interaction which may have affect the selection of type of salts for example sodium is restricted with hypertension patient and lithium has potential toxicity[23].

Degree of ionization (pKa) is an important parameter in salt preparation, for basic drug the counterion pKa should be at least 2 pH less than pKa of the drug, and for acidic drug the counter ion should be at least 2 pH higher

than pKa of the drug, difference in pKa value is important for proton transfer [22, 23].

The common method for drug synthesis at salt form is performed by the combination of free acid or base of drug molecules with its basic or acidic counterion, and performed in appropriate solvent system with specific molar ratio, and after that the salt will precipitated and recrystallized[22].

Formation of the drug in salt form increases the solubility and the dissolution rate, so converting weakly acidic drugs to sodium or potassium salt form and for weakly basic drugs converted to hydrochloride or other strong acid salt form.

The increase in dissolution rate can be explained by, that the weakly acidic drug in low pH like stomach is unionized, but the formation of drug in sodium or potassium salt form which is strong base, and exert neutralizing effect, the pH of the microenvironment will increased to 5-6 while the pH of the bulk media in stomach 1-2, and dissolution of the acidic drug will localized in this microenvironment, when the dissolved drug diffused into bulk media, the weakly acidic drug molecule start to precipitate, but this free acid particles are wetted, and have large surface area compare with free acid particles administrated in acid form, and this increase the dissolution rate [24, 25].

There are many examples of NSAID salt in the form of sodium which have higher solubility than the corresponding acid form; like diclofenac sodium [26], naproxen sodium [27, 28], ibuprofen sodium[29-31], and now we can find these sodium salt formulations in the market. Sometimes more than

one form of salt for NSAID was performed for example; diclofenac was formulated as a potassium and sodium salt. The diclofenac potassium salt showed a higher water solubility comparing to the diclofenac sodium salt, and higher absorption rate, so shorter time to start analgesic activity, and hence used for diclofenac immediate release dosage form[32, 33]. However, the main problem with pharmaceutical formulations containing potassium salt, it is hygroscopicity.

1.3 Tablet formulation

Tablets are the most common dosage form that is available in the market, and the wide spread of this dosage form is due to many reasons like; high stability, large scale production at low coast, the cheapest and most convenient dosage form in packaging and shipping process, and have high dosage precision, and low content variability[34].

Tablets contain the active ingredients and also the inactive ingredients identified as excipients or additives. Every excipients in tablet formulation used for specific purpose, diluents used to enlarge the formulation volume and weight, until reach a specific size of the tablet (e.g. lactose, microcrystalline cellulose and dextrin)[35, 36].

Binders give the adhesion proprieties between the formulation components, which is very important in granules formulation, and keeping the integrity of the tablet (e.g. starch, hydroxypropyl methylcellulose(HPMC) and polyvinyl pyrrolidone)[35, 36].

Disintegrants used in tablet formulation to facilitate the breaking down of the aggregation of tablet contents when the tablet reach and contact with gastrointestinal tract fluid, and there are many mechanisms for disintegrants like, increase the wettability and porosity in tablet matrix (e.g. microcrystalline cellulose, sodium starch and gelatine), or increase the table internal pressure by absorbing and swelling water (e.g. sodium starch glycolate and croscarmellose sodium), or facilitating disintegration by gas formation, which mainly used in effervescent tablet (e.g. sodium bicarbonate, citric acid and tartric acid)[37].

Lubricant used to prevent fraction of the tablet, by coating the surface of the tablet contents, and reduce the adhesion of the tablet surface with dies and punches, and within die wall, lubricant should be used in very small amount (e.g. Mg stearate, silion dioxide and talc)[38].

Glidants enhance the flow ability of the powder mixture of tablet formulation in the hopper when injected to tablet machine, and reduce the friction, glidants have hydrophobic properties, so very small amount should be used in the tablet formula (e.g. talc, colloidal starch and corn starch)[38]. Other excipients can also be used like, colouring agents, sweeting agent and flavorants.

In tableting process different methods are available, direct compression used when the drug formulation has good compressibility and flow ability, and used for drugs sensitive to heat and moisture, but when the drug formulation have low flow ability and compressability which affectein the uniformity of the tablet content, wet granulation used to overcome this

problem, but if the drug is sensitive to moisture and heat, dry granulation is main method for tablet formulation[39].

1.4 Dissolution test

Dissolution testing is an important and critical in the drug development stages and for stability testing for solid oral dosage form. Dissolution determine the rate and the extent of drug absorption, the drug must be released from the dosage form and dissolved in gastrointestinal fluid, and then absorption of the drug into blood circulation and reach to the site of action.

Dissolution test is the major step in quality control procedure and predicts the dissolution in vivo[40, 41]. It is essential for bioavailability evaluation, and to ensure uniformity and consistency of the drug product from batch to batch or in post approval changes[42].

There are many factors that affect dissolution of the dosage form including: physicochemical properties of the active pharmaceutical ingredient (API) like solubility, surface area, particle size, wettability, and polymophys. Also the hardness of dosage form, excipients and manufacturing process variables such as coating, drying, compression force, blending time of lubricate, and addition order of excipients with active pharmaceutical ingredient. All these factors can affect in dissolution profile [43].

There are seven different dissolution apparatus according to USP, and the selection of specific apparatus in dissolution test development will depend on drug product characteristics and the route of administration. USP

Apparatus 1(basket) and apparatus 2 (paddle) are widely used in dissolution test for tablets, capsules, enteric coated tablets, modified release tablet and extended release tablets, but in floating tablets and capsules USP 1(basket) mainly used [42, 44, 45].

USP 3 (reciprocating cylinder) used for extended release drug products; in this apparatus it is easy to perform changes in pH to simulate the condition of fed or fast state. USP 4(flow through the cell) used for implants, powder and suspension[46].

USP 5(paddle over the disk) and USP 6 (cylinder) used for transdermal drug delivery system. USP 7(reciprocating disk) used for extended release drug product[44, 47].

Selection of proper dissolution media is based on physicochemical properties of the drug and the purpose of the dissolution test. In dissolution media sink condition should be applied, which depends on drug solubility, and the dose of the drug product. To achieve sink condition in dissolution test; the volume of the fluid media should be three times greater than the volume needed to have saturated solution of the API[48].

Dissolution media required to mimic in vivo conditions and simulate the site of dissolution in-vivo (e.g. pH 1.2-6.8.for immediate release (IR) tablet, and pH 1.2-7.5 for sustained release tablet) so to establish in-vivo in-vitro correlation[49]. Simulated gastric fluid without enzymes and simulated intestinal fluid used in class 1 and class 3 as dissolution media, and for class 2 and class 4 drugs using biorelevant media, which is

simulated gastric fluid with surfactant, or milk with fat (3.5%) to mimic fed condition[50].

1.5 Shelf life stability and accelerated stability studies

Stability of drug product defined as the ability of drug product remains within its chemical, therapeutically, physical, toxicological specification throughout shelf life. Shelf life is the time required to decrease the drug concentration to 90% of its initial concentration under specific handling conditions. The importance of stability testing is to determine the shelf life of the product, and provides recommendation of storage condition, and container closure system suitability, so regulators insist in conducting stability test, and ICH has published guideline for this subject.

 Loss of stability and degradation in drug product, defined as decrease in activity and performance, and the analytical method must be able to separate, detect and quantify drug degradation products[51].

Shelf life can be determined by two methods of stability testing, real time stability test (long term stability) and accelerated stability test. In real time stability test drug product stored at the recommended storage conditions, but in accelerated stability test the product is stored in stress condition (temperature, humidity, and pH)[52, 53].

In accelerated stability test, temperature is the main factor used, due to its relationship with degradation rate according to Arrhenius equation, degradation rate can be calculated by Arrhenius equation[54].Testing frequency at real time storage condition conduct once every three months

during the first year, once every six months during the second year, and after two years sampling performed once a year, and for accelerated storage condition three time points at minimum (0, 3, 6 months) for 6 months study[55].

1.6 Analytical method development and validation

Analytical method development and validation are the main fundamental process in new drug formulation program. Analytical method development is required to test specific characteristics of drug against the proposed acceptance criteria for these characteristics, and the selection of the analytical instrument harmonized with purpose and the scope of the analytical method[56, 57].

Validation process of the analytical method must be performed by evaluating specific parameters which include the followings: accuracy, range, precision, linearity, specificity, limit of detection (LOD), limit of quantitation (LOQ), robustness[58].

Method validation develop gradually from analytical method development, and so the two process are connected with each other, and in the validation process the techniques where carrying out as determined in the method development.

Method validation has taken extreme attention from international regulatory agencies like, World Health Organization (WHO), United States Food and Drug Administration (US FDA), International Conference for Harmonization (ICH), Quality Manual ISO/IEC 17025,Good Laboratory

Practice (GLP), and Pharmaceutical Inspection Cooperation Scheme (PIC/S) [59, 60]. These agencies established protocol and set of standards to get an approval and registration of the product.

1.6.1 Analytical method development

Many important factors must be taken into account when considering HPLC method development, like keeping the method simple, trying the most popular column and stationary phase, using two compounds mobile phase rather using ternary, and searching of the factors that may enhance the resolution[61].

Mobile phase composition plays major rule in selectivity; and the pH of the mobile phase effect on the retention time of weak acids and weak bases, also the temperature has minor effect on selectivity[61, 62].

When developing an HPLC method many factors has to be considered including the followings: analyte properties like chemical structure, physical and chemical properties (pKa, sample solubility, stability, molecular size, weight, and electrical charge)[63].

The most common used chromatography in pharmaceutical analysis is reverse phase and used for weak acids and weak bases. Analysis of ionic, strong basic and strong acidic compounds is performed by reversed phase ion paring. Octadecylsilane(C18) is the most common used stationary phase in reversed phase chromatography. Normal phase HPLC is usually used for high polarity analytes, ion exchange chromatograph used for

inorganic analytes, and for high molecular weight analytes size exclusion chromatography is the proper HPLC choice[61, 64].

Mobile phase composition: acetonitril, methanol and tetrahydrofuran are common solvents used in RP-HPLC, these solvents are miscible with water, and have low cut off in UV. In HPLC method development gradient setting is required, mainly when the sample has more than one component, higher resolution and constant peak width when using gradient than isotonic setting, in isotonic setting the width of the peak increase with retention time, so for analytes with long retention time gradient setting is preferable[65]. Using buffer in mobile phase is widely used specially when the analytes are ionisable, and present in two form (HA/A-), so split peak or two peaks were existed, so to have one peak; one form of the analyte should be existed by using buffer in mobile phase and change the pH in to acidic or basic. most common pH used in HPLC(2-4), because basic pH increase column hydrolysis[66].

The most common column used has a length of 100-150 mm, this to reduce the analysis time, column particle size usually be between 3-5 µm, and flow rate can vary in the range between 1-1.5 ml/min[61].

 UV-Vis detector is the most commonly used when the analytes contains chromophores, and the measurements are usually performed at λ max absorption of the analytes. UV wavelength should be above 200nm to avoid noise increasing. Other detectors that are commonly used include: electrochemical fluorescence which is mainly used for trace analysis, and for high concentration analytes using refractive index detectors[63].

1.6.2 Analytical method validation

The purpose of validation of the developed analytical method, is to make assurance that the analytical method will always produce result, which is precise, accurate and specific[58].

Validation conducted according ICH recommendations; ICH Q2 (R1) is the main source for recommendations and characterisation of validation process for analytical method. FDA guidance for validation of chromatographic method is another valuable source for validation requirements[56].

Validation characteristics that should be considered in all analytical methods include specificity, linearity, accuracy, precision (repeatability, intermediated precision, reproducibility), range, quantitation limit (LOQ) and detection limit (LOD).

Accuracy of the developed analytical method is achieved by the closeness of agreement between the test results and the reference or accepted results. Accuracy validation parameter is usually determined by testing three different concentration, that cover the range of 50%-150% of the target concentration and three replicates for each concentration, which covering specified range[67, 68]. The recovery value is calculated by dividing the calculated concentration of the analyte to the true value using the developed analytical procedure, this value should be in control limit and the acceptance criteria of recovery will be between 95% -105% [68].

Linearity is the ability to have the a proportional relationship between test results and concentration of the analyte in the tested samples in specific

range[68, 69]. Linearity is determined by six concentrations around the target concentration which are prepared from stock solution. The analysis of each concentration is usually performed in triplicate [67]. The mean response for each concentration is plotted on y–axis versus.

The concentration on the x-axis and the regression equation and coefficient of determination R^2 are calculated[67]. When linear regression equation is calculated, the origin should not force as (0,0) in calculation, which may distort the best fit of the slop[69].

Range is the interval between the highest and lowest concentration of the analyte that exhibit linearity, accuracy and precision in the analytical method procedure[58, 69].

Specificity is the ability of analytical method to measure accurately the analyte in the presence of excepients, impurities, and degradation products[69]. Specificity should approve that the excepients must not interfere with analyte in the analysis procedure[58, 68].

Precision is the degree of agreement in test results when the analysis process repeated, precision usually measured by standard deviation, or relative standard deviation[67, 69].

Precision consist of repeatability, intermediated precision and reproducibility.

Repeatability or intra-assay precision is a precision over short time interval, without making any changes on the operation conditions[69].

Repeatability could determine by testing target concentration, and repeated at least six times by the same operator on the same equipment, or three

concentration on three replicates and calculation of the mean, standard deviation and relative standard deviation were performed[58, 68] . Intermediated precision is determined within laboratory variation, as the procedure is performed by different analysts, different days, or using column with different batch[58]. Reproducibility expressed by performing the analysis of the same sample in different laboratory[69].

Detection limit is the lowest concentration of the analyte can be detected by subsequent dilution but not necessarily quantitated [58, 67]. ICH documents express the detection limit in common approach for analytical method which has baseline noise, by measuring the signal of low concentration of the analyte and the noise of blank sample, and atypical signal to noise ratio is 3:1 [68].

Quantitation limit is the lowest amount of analyte can be measured with accuracy and precision of the analytical method[58, 67]. For analytical method which has baseline noise, quantitation limit determined by comparison of the signal of low concentration of the analyte to the signal of blank sample, and acceptable signal to noise ratio is 9:1[68].

 Robustness is the ability of the analytical method to remain unaffected by small changes on the method by carrying out small changes on pH, flow rate, and absorption at λ max[69].

System suitability test for HPLC is an integral parts of HPLC method development, it is very critical and important, according to USP and ICH because it's used to verify the effectiveness, reliability and suitability of HPLC system, and must be performed prior experiment and throughout

routine analysis[70, 71]. The reason behind the need of system suitability test that there is no guarantee that the system performance will behave properly all the time[70]. System suitability parameters include the followings: resolution (R), tailing factor (T), column efficiency (N), and repeatability (%RSD of peak response and retention time) , these parameter must be calculated and be with the system suitability limits to be accepted[68,72].

1.7 Main objectives of the research

1. Synthesis range of celecoxib salts (sodium, potassium…etc) to increase the solubility of celecoxib.

2. Determine the solubility of the formed salt in water.

3. Formulation of the celecoxib salt in a suitable dosage form depending on the solubility and stability of the synthesized salt. The targeted dosage form is tablet.

4. Development and validation of analytical method for quantification of celecoxib in the formulation and as well as in raw material.

5. Testing the dissolution of celecoxibsaltin a suitable dissolution media.

1.8 Significance of the research

1. Novelty of the project: To our knowledge there is no pharmaceutical dosage form of celecoxib potassium salt available in the market.

2. The formulated salt into tablet probably will increase the bioavailability of the drug and may enhance its onset of action.

3. The increase in solubility of the synthesized celecoxib salt will make it possible to formulate it in an injectable pharmaceutical dosage form.

4. The project will give a whole package of newly developed drug that can be adapted by pharmaceutical industry.

5. The project will give a valuable experience of different stages followed in the drug development field.

2. Methodology

2.1 Instrumentation and reagents

2.1.1 Instrumentation

The method development and validation was performed on a Waters Breeze HPLC System consisting of: Waters binary HPLC pump (model 5CH), Waters Photodiode Array Detector (model 2998), with a Rheodyne injection valve with a 20 μL loop. The reverse phase Isocratic Chromatographic separations were carried out using a stainless steel columns including: XTERRA MS RP-18 column (250 mm × 4.6 mm, 5 μm particle size), SeQuant ® ZIC ® HILIC column (150 × 4.6 mm, 5μm, 200 A, MERCK K, germany), and ACE 3 SIL column (150 ×3 mm, HICHCROM, UK). The absorption of the test solutions was measured by Ultaviolet-visible spectrophotometer (JENWAY model 7315, Bibbyscientific, UK), the pH was measured by pH meter (JENWAY model 3510, Bibby scientific, UK). The dissolution test was performed by a Dissolution tester (BTC model 9100, Hsiang Taimachinery Industry, Taiwan). Melting point apparatus (GALLENKAMPG, model SG96, UK) was used to determine the melting point for synthesized Celecoxib-K salt. Oven (ARILEVY)was used in drying process and was used in salt accelerated stability studies of the formulated Celecoxib-K tablet. Centrifugate (UNIVERSAL model 320, Hettich, Germany) and thermo shaker (BOECO TS model TS-100, Germany) were used in preparation and solubility testing of celecoxib salts. Pressure Gauge (SHIMADZU

corporation 5 TON, Japan) was used in tablet compression process. Disintegration test of the formulated Celecoxib-K tablet was performed by Disintegration test apparatus (model 190). The hardness, thickness and diameter of the formulated tablets was done by Multicheck tested (Erweka model 5.1). Friability test apparatus (PHARMA TEST, model D-63512, Hainburg) used to perform friability test on the formulated Celecoxib-K tablet. Freeze drier (Ttivac, model D 2,5E, Oerlikon, Germany) was used in the final drying step of the synthesized celecoxib salt. Karl Fisher titration system (KFT Titrino 795-Ti stand 703, Metrohm, Switzerland) was used to determine the water content in the synthesized celecoxib- K salt, and degree of hydration of the salt. All of the solutions were prepared using ultra-pure water obtained by (ELGA®, model PF3XXX, Veolia, UK).

2.1.2 Chemicals and reagents

The entire chemical reagents used in the study were of the analytical grade, and were purchased form reliable sources; all the chemicals used in the study are listed in **Table 2.1**.

Table2.1: Chemical reagentsused throughout the research

ITEMS	SOURCE
Celecoxib	Jerusalem pharmaceutical company
Acetonitrile supra gradient grade for chromatography HPLC	Industrial estate
Methanol for HPLC 99.9%	Sigma Aldrich
Methanol 99%	Sun Pharm Ltd
Acetone 99%	Sun Pharm Ltd
Sodium methoxide 95%	Sigma Aldrich
Sodium ethoxide 96%	Aldrich Chemical Company
Potasiumtert-butoxide	Alfa Aesar
Sodium methoxide 98%	Alfa Aesar
Lithium hydroxide anhydrous 98%	Alfa Aesar
Sodium dihydrogen phosphate	Sigma Aldrich
NaOH	Sigma Aldrich
KOH	Alfa Aesar
Diethyl ether	Industrial estate
Isopropanol	Frutarom Ltd
Propylene glycol	Industrial estate
THF	Alfa Aesar
Hexan	Chen Samuel Chemicals
Magnesium stearate	Jerusalem Pharmaceutical Company
Aerosil	Jerusalem Pharmaceutical Company
Acdisol	Jerusalem Pharmaceutical Company
$CaCl_2$	Alfa Aesar

2.2 Synthesis of celecoxib salts

A series of celecoxib salts were synthesized in order to test their solubility and assign one of them as a suitable salt for tablet formulation. The synthesized celecoxib salts were performed according to synthetic procedure of Remenar et al[73].

2.2.1 Synthesis of celecoxib potassium salt

Celecoxib (1 g, 2.6187mmoles) was added to KOH aqueous solution (11.5 ml, 0.35M). The dissolution of the mixture was accelerated using a water path (30°C) with continuous swirling for five minutes, the mixture was then dried using freeze drying. A white dry powder was produced of Celecoxib-K salt, 0.9869g.

2.2.2 Synthesis of celecoxib calcium salt

Celecoxib (300mg, 0.786 mmoles) of was added to NaOH in methanol (0.87ml, 1M), the celecoxib solid was dissolved and became clear solution by gentle heating and continuous swirling. Calcium chloride ($CaCl_2$) solution in methanol (0.393 ml of 3M) was added to the previous solution. Celecoxib Calcium salt was then precipitated in minutes. The precipitate was then filtrated and the produced salt was then dried overnight in oven at 40℃ to give celecoxib Ca salt, 0.386 mg.

2.2.3 Synthesis of celecoxib sodium salt

Celecoxib (2.513 g, 6.58 mmoles) was added to 6.59ml of 1M NaOH aqueous solution; aclear solution was produced through gentle heating using water path at 60 ℃ to dissolvethe remaining solid. The mixture was allowed to cool at room temperature, and after that was cooled in ice path for a 1 hour, a crystal was produced as a precipitate. The suspension was filtered; the filtered powder was then dried in oven at 70℃ for an hour to give celecoxib Na salt; 1.97g.

2.2.4 Synthesis of isopropyl alcohol solvate of celecoxib sodium salt

Celecoxib (0.204g, 0.535 mmoles) were dissolved in 6 ml diethyl ether. Isopropanol (6 ml) was added to the previous solution to obtaincolorless solution. Methanolic solution of sodium methoxide (0.5M) a volume of (2.52 ml) was added. To the whole mixture hexane (3ml) was added and the mixture was dried using nitrogen gas. The salt formed was isopropanol: celecoxib (1.5:1), 0.279 g.

2.2.5 Synthesis of celecoxib lithium salt

Celecoxib (101.4 mg 0.266 mmoles) was added to 1.05 ml of 0.35M LiOH aqueous solution. The dissolution was enhanced by gentle heating and continuous swirling. The solution was dried to eliminate the water and obtain the Lithium salt using freeze drying to give celecoxib Li salt; 0.386 g.

2.2.6 Synthesis of propylene glycol solvate of Celecoxib-K salt

Celecoxib (0.506g; 1.328 mmol) was added to 12 ml diethyl ether; to this solution   propylene glycol (0.075 ml) was added to get a clear solution. Potassium- t-butoxide in THF (1.32 ml) was then added to the clear solution. In 5 minutes' crystals startedto form, and were collected by filtration. The filtered crystals were then dried by air drying to give 1:1 propylene glycol solvate of Celecoxib-K salt, 0.7024 g.

2.3 Determination of water solubility of the synthesized celecoxib salts

At firstUV absorbance spectrum in the rage (200-400) of celecoxib and celecoxib salts were generated by UV spectrophotometer to determine the wavelength of maximal absorption (λ max) of these compounds, methanol and water were used as solvent for the tested celecoxib salts.

Calibration curve of celecoxib salts were constructed in methanol and were used to determine the solubility of these salts. A stock solution of 1mg/ml celecoxib and celecoxib salts were prepared. Serial dilutions from stock were performed having the following concentrations (0.015, 0.02, 0.025, 0.03 and 0.04mg/ml).

The solubility test of celecoxib salts in water was performed by addition of 1 ml of water to celecoxib salts in a super saturated quantity. A Thermo shaker at 37°Ctemperature and speed of 300 rpm was used. The solubility was determined after 24 hours and 48 hours. The tested samples were centrifuged for 15 minutes in 15000rpm, and the absorbance of the supernatant for the samples was examined after performing a suitable dilution if necessary. And the solubility of celecoxib salts was calculated from calibration curve which was performed for each salt.

2.4 Physicochemical properties of the synthesised Celecoxib-K

2.4.1 Melting point determination of Celecoxib-K

Melting point (Mpt) was determined on Gallenkamp melting point apparatus and the melting point was recorded in degrees Celsius (°C).

Melting point determination was performed by introducing a small amount of powdered Celecoxib-K (5-10mg) into a capillary tube, and was exposed to a monitored gradual heating. The melting point range was recorded when the solid powder started to convert into a liquid.

2.4.2 Water content determination of Celecoxib-K

Before determination of water content in celecoxib–K, drying process was performed to eliminate any humidity in the salt by using an oven; Celecoxib-K was placed in the oven at 40℃ for overnight.

Water content in Celecoxib-K was determined by using Karl Fischer titration device. One component reagent for volumetric Karl Fischer titration was used, the instrument was set to Default titration settings.

Celecoxib potassium salt (100mg) was added into titration vessel. To make sure a complete dissolution of the sample was achieved the mixture was continuously stirred. The test was done in triplicate, and the average of water content results was recorded.

2.5 Method development

2.5.1 Prepared Solution and mobile phases

I. Preparation of Phosphate buffer (pH 3)

The buffer was prepared by dissolving 2.7g of potassium dihydrogen phosphate in one liter of water, and the pH was adjusted to 3.0 with

phosphoric acid, filtration and sonication of the buffer solution were performed before used in HPLC[74].

II. Mobile phase composition

Different mobile phase composition was prepared and tried in throughout the method development. Four main mobile phase compositions with different organic solvent percentages were prepared: The compositions and the percentages of these mobile phases are summarized in **Table2.2.**

Table2.2: Composition and percentage of tried mobile phases in method development.

Mobile phase 1		Mobile phase 2		Mobile phase 3		Mobile phase 4		
Methanol	Water	ACN	Water	Methanol	Buffer	Methanol	ACN	Buffer
50%	50%	50%	50%	50%	50%	10%	30%	60%
60%	40%	60%	40%	60%	40%	30%	20%	50%
70%	30%	70%	30%	70%	30%	40%	10%	50%
80%	20%	80%	20%	80%	20%	50%	10%	40%
90%	10%			85%	15%			
				90%	10%			

III. Preparation of working Celecoxib-K solutions

A Stock solution of Celecoxib-K 1mg/ml was prepared by accurately weighing 100mg of Celecoxib-K and dissolved in methanol HPLC in 100 ml volumetric flask. A serialof working standard solutions were prepared by making a suitable dilution to have the following concentration 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.8mg/ml.

2.5.2 HPLC chromatographic condition

2.5.2.1 Determination of λ max

The Celecoxib-K salt was scanned in the UV/visible range (200-600 nm) to determine the maximum absorption and the λ max was adapted as measuring wave length in the HPLC method.

2.5.2.2 Determination of stationary phase

Three different column were used in HPLC method development (steel XTERRA MS RP-18 column (250 mm × 4.6 mm, 5 μm particle size), SeQuant®ZIC®HILIC column (150 × 4.6 mm, 5μm particle size, 200 A pore size, MERCK K, Germany), ACE 3 SIL column(150 ×3 mm, HICHCROM, UK). The choice of the stationary phase to be used in the analytical method depended on the peak shape and theoretical plates of the generated chromatography. The column temperature in all the tested stationary phases wasat room temperature 25℃.

2.5.2.3 Determination of flow rate

The flow rate affects on system pressure, analysis time, and chromatographic quality. Different flow rates were tried throughout the method development (0.7, 1, 1.5 ml/min).

2.5.3 HPLC method suitability

System suitability parameters were determined by using six replicates of standard solution of Celecoxib-K (0.5mg/ml), and calculating of tailing factor, theoretical plates (column efficiency) and reproducibility (%RSD of the area under the curve and retention time) and comparing it with acceptance limit.

Tailing factor is coefficient to define the degree of peak symmetry, and calculated by the following equation:

$$T = \frac{a+b}{2a} \quad \ldots\ldots\ldots\text{Equation} \qquad (1)$$

Where, a and b are the peak half width at 5% of the peak height, a is the front half width, b is the back half width.

Theoretical plates (column efficiency) which represent the number of theoretical plates per column, the larger the number of theoretical plates per column the sharper the peak, and calculated by the following equation:

$$N = 2\pi \times \left(\frac{t_r\,H}{A}\right)^2 \ldots\ldots\ldots \text{Equation} \qquad (2)$$

Where, t_r is the retention time, A represent the peak area, and H represent the peak height.

2.6 Method validation

2.6.1 Linearity and range

Linearity and range of the developed method was tested by preparing 8 test concentrations; 20%, 30%, 40%, 60%, 80%, 100%, 120%, and 140% of the

target concentration (0.5 mg/ml), and each test concentration was then placed in the sonicater for 5 minutes to complete dissolution and eliminate air bubble. Every test concentration was analyzed for 3 times under the same condition. Area under the curve was plotted versus the concentration. The generated curve was checked for linearity by examining the value of R^2; the curve is considered linear if $R^2 < 2$[67].

2.6.2 Accuracy:

Accuracy was performed on three concentrations (80%, 100%, and 120%) around the test concentration 0.570 mg/ml celecoxib potassium monohydrate (equivalent to 0.5mg/ml of celecoxib). Each concentration was run in triplicate and the area under the curve (AUC) for each concentration peak was used in the calculation [75].

A stock solution (1.14mg/ml) was prepared by dissolving 114mg of Celecoxib-K monhydrate salt (equivalent to 100mg celecoxib) in methanol HPLC in 100 ml volumetric flask. A suitable dilution of stock solution was done to prepare 0.456, 0.570 and 0.684 mg/ml solution which is equivalent to (80%, 100% and 120%) of the test solution.

Accuracy was performed by testing the three test solution (80%, 100% and 120%) mixed with formulation excipients. Stock solution of celecoxib-K (1.14mg/ml) was prepared by dissolving of celecoxib K (114mg), microcrystalline cellulose (265 mg), crosscarmellose sodium (16mg), magnesium stearate (5 mg) , and the mixture was completed with methanol HPLC up to 100ml[60, 75].

HPLC measurements were taken three times for each concentration, and the percentage recoveryand % RSD were calculated.

$$\% \ Recovery = \frac{Recovered \ conc.}{Injected \ conc.} \times 100\% \ \text{..............} \ \text{Equation} \qquad (3)$$

The method is considered accurate if the percentage recovery is not more than 100±5% and the developed analytical method is considered precise if the % RSD is less than 2[67].

2.6.3 Precision

2.6.3.1Intermediated precision

Intermediated precision is expressed within laboratory variation and this validation parameter was performed by carrying the HPLC analysis by two different analysts in three different days. The analysis of the sample by HPLC was done at the same condition and repeated in triplicate [76].

I. Intermediated precision (between days repeatability):
The intermediate precision (between days repeatability) was also done simultaneously on the prepared solution of the accuracy measurements. All the measurements for the three different concentrations were repeated for three consecutive days. The percentage RSD was calculated and the parameter is fulfilled if the % RSD is less than 2[67].

II. Intermediated precision (between analysts repeatability): The intermediate precision (between analysts repeatability) was also performed to determine within laboratory variation. The results of 0.5 mg/ml celecoxib–K prepared by two different analysts at the same conditioning

was analysed in triplicate. The percentage RSD was calculated and the parameter is fulfilled if the % RSD is less than 2[67].

2.6.3.2 *System precision*

Instrument precision was performed by doing ten replications of one prepared sample containing celecoxib–K (0.5mg/ml). The percentage RSD was calculated and the parameter instrument repeatability is fulfilled if the %RSD is less than 2 [67].

2.6.4 Reproducibility

Reproducibility which refers to the degree of agreement between the results of experiments conducted by different individuals, at different locations, with different instruments was performed by analysing 0.5 mg/ml solution of celecoxib- K in two different laboratories; one analysis was done in the chemistry department and the other was in the pharmacy department. The samples were prepared by two different analysts using two different instruments. The percentage RSD was calculated and the parameter is fulfilled if the %RSD is less than 2[67].

2.6.5 Robustness

The robustness of the method was examined using some minor modifications to the experimental parameters, like measuring the absorbanceat small varied wave length 250±2, small change in flow rate 1.5±0.2 and also small change in the pH of the mobile phase 3± 0.2 The

results of % recovery and % RSD indicated that minor modifications to the experimental parameters did not affect the assay and its ability to accurately and precisely detect/quantify the active ingredients.

2.6.6 Limit of detection and limit of quantitation

Limit of detection and limit of quantitation was determined manually after testing a serial dilution and measuring the signal: noise ratio. Signal: noise ratio 3:1 was considered as LOD value and signal to noise ration 9:1 was considered as the LOQ value.

2.7 Celecoxib-KTablet

2.7.1 Tablet formulation

Tablets containing celecoxib–K salt were prepared in our research laboratory. The quantity of each component of the tablet formulation is listed in **Table 2.1**

Table2.3: Quantities and components of the formulated tablets:

Component	Weight per tablet (mg)
Celecoxib-Kmonohydrate	114 mg
Micocrystaline cellulose (avicel pH101)	265 mg
Crosscarmellose sodium(acdisol)	16 mg
Mg stearate	5 mg
Total weight	400 mg

Tablet was prepared by direct compression after weighing each component separately. Celecoxib-K was added to microcrystalline cellulose (MCC) and was mixed for 5 minutes, and then addition of acdisol to the mixture by

geometric dilution with continues mixing for 10 minutes, finally addition of magnesium stearate and mixing for 2 minutes. This mixture was compressed into tablet, by using gauge pressure of 5 tons to have tablet with final weight of 400 mg ± 5% [77].

2.7.2 Tablet Weight variation test

Weight variation test was performed to make assurance of drug content uniformity in the tablets. Weight variation test is performed for the tablet that contain 50 mg or more of active ingredient, or when the active ingredient is 50% or more of the table weight.

20 tablet of the formulated celecoxib–K was randomly selected and was weighed individually, and then the average weigh was calculated.

The weights of 20 tablets were compared individually to the upper and the lower limit. The tablet pass the test if two tablet or less weigh different from the average weight by more 5%, and none of the tablet weigh different from the average weight by more 10% [78].

2.7.3 Tablet Content uniformity test

Content uniformity test was performed according to USP, 10 tablets were randomly selected. Each tablet was grinded using pestle and mortar. The grinded tablet powder was then dissolved in methanol and the volume was completed to 100ml, then filtration was performed for each solution.

The sample was then diluted twice by taking 5ml from each solution and the volume was completed to 10 ml in volumetric flask with methanol. The

sample was then put in a sonicater for 5 minutes to complete dissolution and eliminate air bubble. The examination the amount of Celecoxib-K in each tablet was performed by HPLC using the chromatographic condition of the developed method. Tablet will be accepted if 9 of 10 tablets contain not less than 85% and not more than 115% of the label amount [78].

2.7.4 Friability, hardness, diameter and thickness test of the tablet

Friability test was done using friability apparatus (PHARMA TEST, model D-63512,Hainbrug), according to USP when the tablet weight equal or less 650 mg, the total weight of the tablet sample should be corresponded to 6.5 gUSP, so 20 tablets were weighed, and placed on the drum of the apparatus and treated with rolling and repeated shocked, the drum was rotated 100 times in 4 minutes, after that loose dust was removed from the tablet, and the tablet were weighted, and the percentage friability was calculated by the following equation:

$$\text{Percentage Friability} = \frac{W1 - W2}{W1} \ X \ 100 \ldots\ldots\ldots\text{Equation} \qquad (4)$$

Where $W1$ isthe weight of tablets before friability test, and $W2$ represent the weight of tablet after the friability test.

The acceptance limit for percentage of friability according to USP should be not more than 1% [79].

Tablet physical parameters like the hardness, thickness and diameter were determined usingErweka model 5.1. Ten tablets were put in the machine the reading for the hardness, diameter and thickness was recorded

automatically by the machine. The average reading of hardness, thickness and diameter was then assigned as the in the specification certificated of the formulated celecoxib –K tablets tablet.

2.7.5 Dissolution test

A USP Paddle dissolution tester BTC-9100 was used for dissolution test profile. The dissolution tester was run at 100 rpm and 37℃for 60 minutes. One tablet was put in each of the six 900ml apparatus beaker.

The dissolution test was done in water dissolution media. Six tablets were tested; one tablet was placed in each dissolution beaker. 10ml sample dissolution vessel was withdrawn by syringes from each beaker and substituted with 10ml water. The testing was performed at time interval of 5, 10, 20, 30, 40, 50 and 60 minutes. The samples were run on HPLC; each run was done in triplicate. A dissolution curve was then constructed using Microsoft Excel program putting dissolution time on x-axis and the calculated percentage of dissolved celecoxib -K on the y- axis.

2.7.6 Tablet stability study

The formulated Celecoxib-K salt tablet was stored at room temperature as well as at 40 ℃ and periodically analyzed by our validated method and the percentage assay was checked periodically up to almost 60 days. The testing was done using the developed HPLC analytical method by taking one tablet and was then dissolved in methanol in 100ml volumetric flask, the solution was then diluted twice to have a test concentration of 0.570 mg.

3. Results

3.1 Solubility of the synthesized celecoxib salts

The solubility was calculated based on the calibration curve. The calibration curve of the synthesized potassium, sodium, lithium,calcium celecoxib, propylene glycol solvate celecoxib potassium and isopropyl alcohol solvate celecoxib sodium salt were generated and curves were found to be linear with R^2 more than 0.98. The curve and their regression equations are shown in **Figure 3.1.**
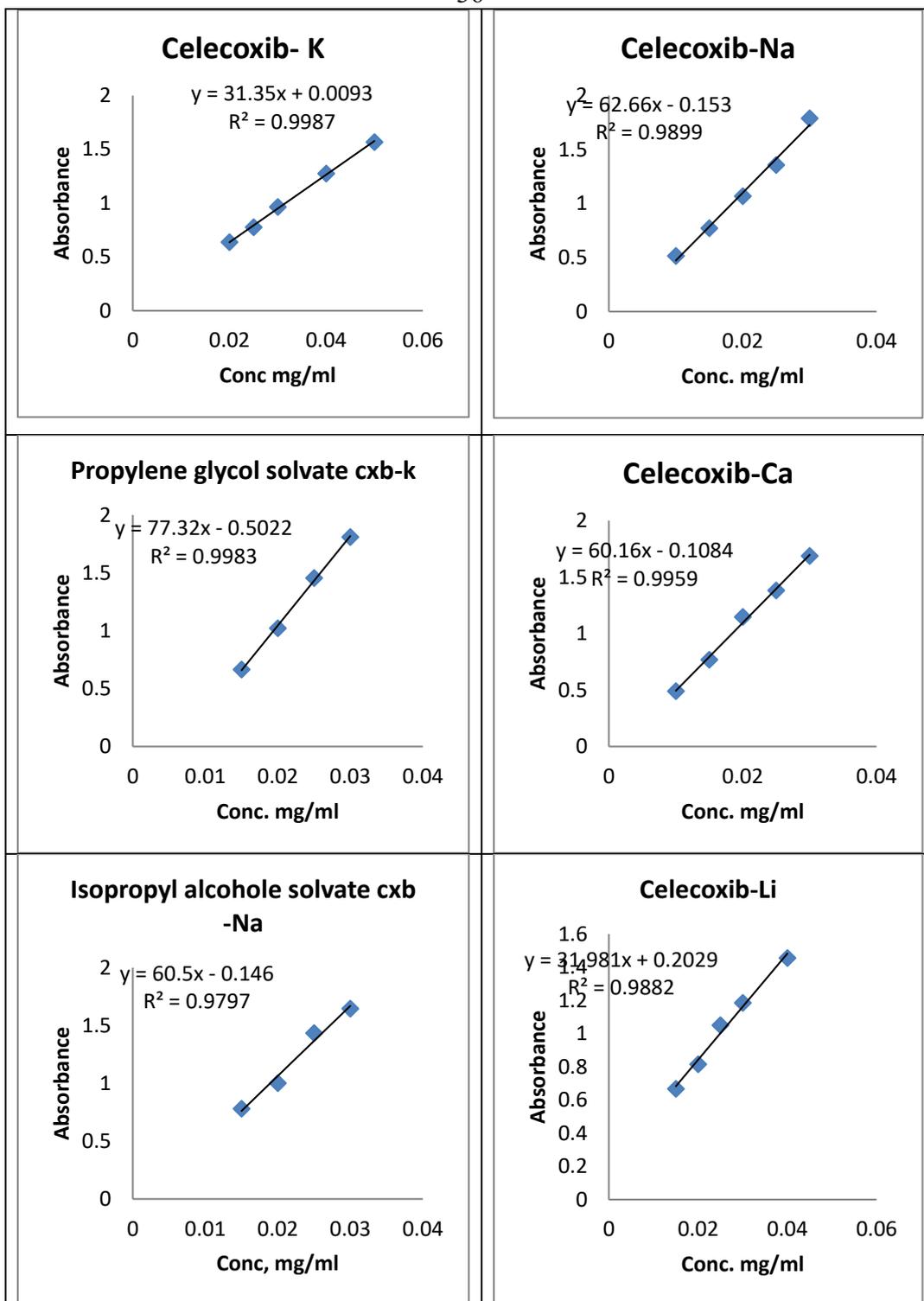
Figure3.1: The generated curves of the synthesized salts ant its regression

The calculated solubility for each salt after 48 houris summarized in **Table3.1**. The table shows that the potassium salt showed more solubility

than the other synthesized salts except in the case of lithium salt. Due to safety issue of formulating lithium ion in tablet it was decided to formulate a celecoxib as a potassium salt.

Table3.1: The solubility of the synthesized salt

Salt	Solubility (mg/ml)
Celecoxib-Na	0.440
Celecoxib-K	0.464
Celecoxib- Li	1.027
Celecoxib- Ca	0.350
Propylene glycol solvate celecoxib- K	0.433
Isopropyl alcohol solvate celecoxib- Na	0.358

3.2 Physicochemical properties of the synthesised Celecoxib potassium salt:

3.2.1 Melting point of Celecoxib-K

Melting point of celecoxib-K was determined by using melting point apparatus, and it was found to be between287-289℃ compared to celecoxib which was 157-159℃[80].

3.2.2 Water content determination in synthesized Celecoxib-K salt

The synthesized celecoxib potassium salt was dried in oven at 40℃for overnight before the determination of water content by Karl Fischer.

The average of water content in Celecoxib-K salt was determined using Karl Fischer volumetric titration in triplicate. The result showed that the formulated Celecoxib-K salt always has a water content equivalent to

4.21% of its total weight which conclude that the synthesized, so celecoxib K salt is formed as monohydrated.

3.3 HPLC analytical method development

3.3.1 Determination of the maximum absorption

The Celecoxib-K salt was scanned in the UV/visible range (200-600 nm) in methanol and water to determine the maximum absorption. The UV/visible scan showed a maximum absorption at 250nm, thus this specific λ was adapted as measuring wave length in the developed HLPLC method. The UV/Vis scan is shown in the **Figure3.2**.

+
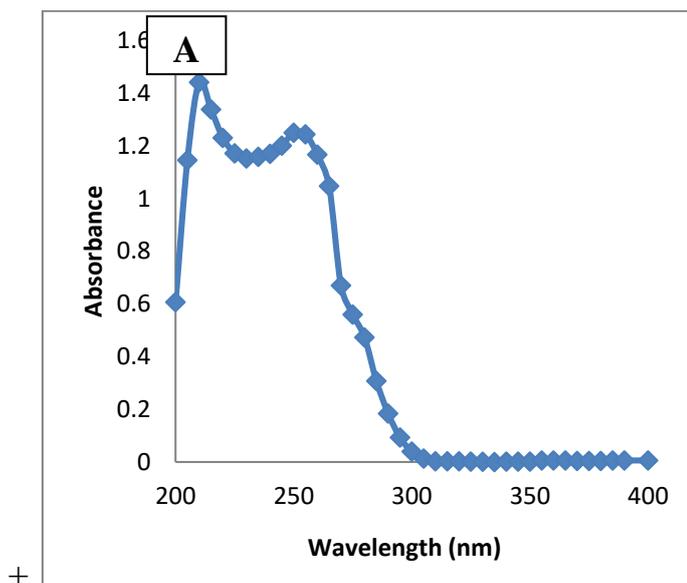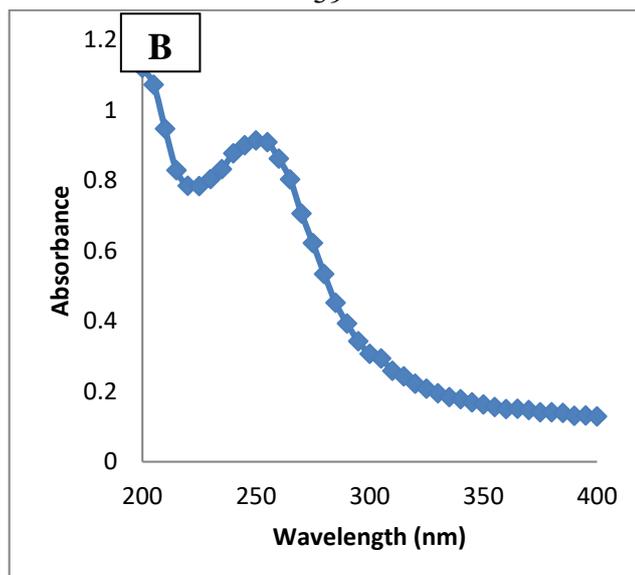
Figure3.2:(A)UV/ visible scan for celecoxib-K in water, **(B)** UV/visible scan for celecoxib-K in methanol.

3.3.2 Mobile phase composition

Different mobilephases with increasing the percentage of organic phase as stated earlier in the methodology section were run on the HPLC instrument. The compositions of the mobile phase of different organic solvent mainly include the following: methanol: water, ACN: water and dihydrogen phosphate (pH 3.0) with either methanol and CAN in different percentages. The synthesized celecoxib salt was run on HPLC and the generated peak was compared and based on the tailing factor, capacity factor, asymmetry, theoretical plates of the peak. The proper composition was adapted as a future composition of the developed analytical method. The results showed that the most appropriate mobile phase was methanol: buffer (90:10). The peak eluted after approximately 2minute and the peak was symmetrical with high theoretical plate number >than 2000 (**Figure 3.3**). However,

using other composition likes and ACN: water (80:20) and methanol: water (90:10) the peaks were tailed and in case of ACN the peak eluted early (1minute) as expected.
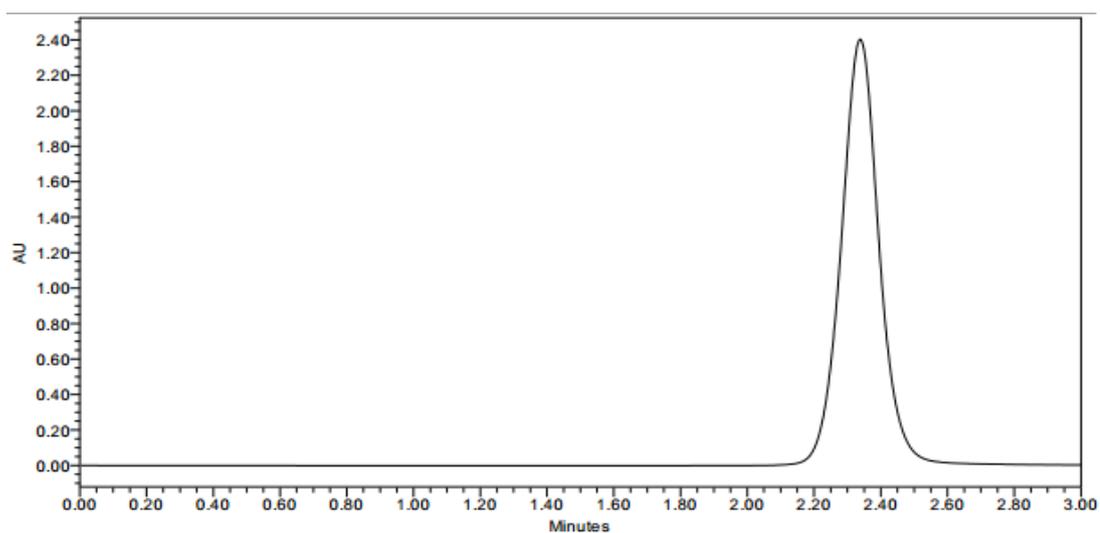
Figure 3.3:analysis celecoxib-K using mobile phase methanol: buffer (90:10).
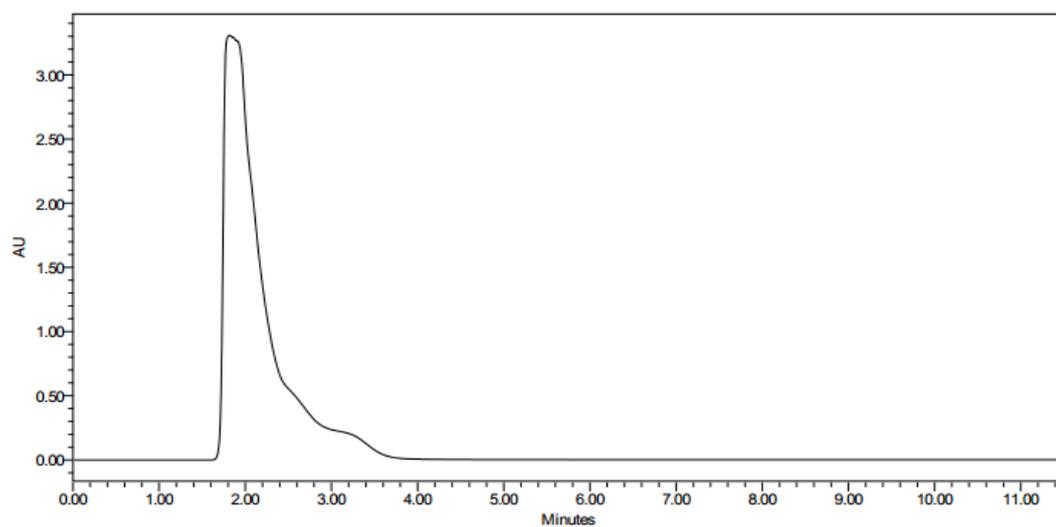
Figure3.4: analysis celecoxib-K using mobile phase CAN: water (80:20).
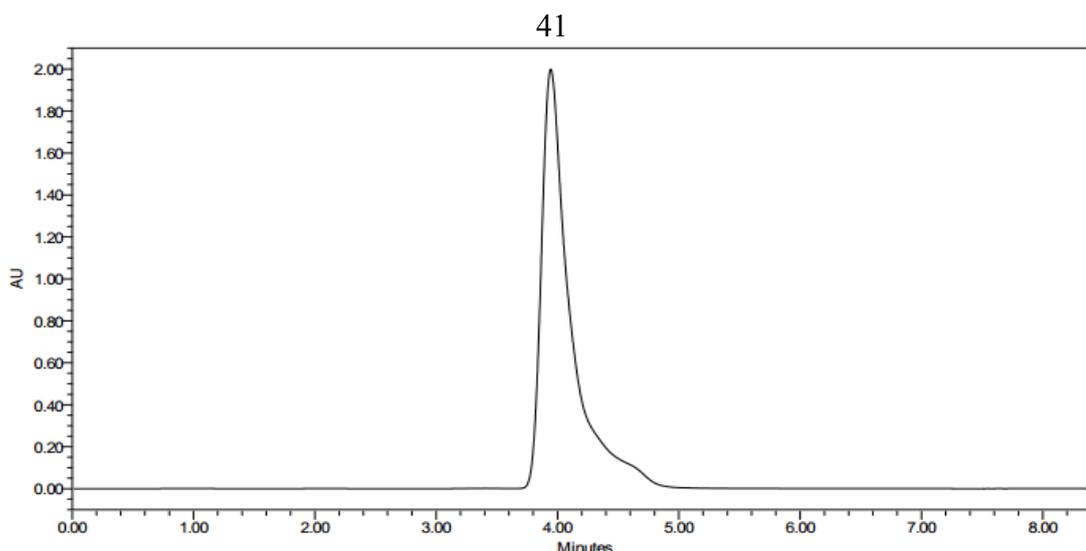
Figure3.5: Analysis celecoxib-K using mobile phase methanol: water(90:10)

3.3.3 Chromatographic condition of the developed method

The final chromatographic condition of the HPLC developed analytical method is summarized in the following**Table 3.2.**

Table3.2: HPLC chromatographic conditions

Mobile phase	methanol: phosphate buffer pH (3± 0.01 (90:10)
Flow rate	1.5ml/min
Detection wave length	250 λ
Stationary phase	C18(XTERRA® MS)4.6×250 mm
Column temperature	25°C
Injection volume	20μL
Run time	3 min

3.3.4 System suitability test

System suitability parameters were used to confirm method and column performance. Column efficiency verified by the number of theoretical plates, according to FDA , the number of theoretical plates should be not less than 2000[56, 70]. The tailing factor represent the symmetry of the peak, the peak should have the minimal broadening or fronting, and

according to FDA the tailing factor should be less than 2.0[56, 70]and also measuring the reproducibility (%RSD of area under the curve and retention time)and should be less than 1%[56, 70].

The values of HPLC system suitability parameters were within the acceptable limits. Theoretical plate was2215, tailing factor 0.8,% RSD of the area under the peak 0.42 and % RSD of the retention time 0.41.

3.4 Validation parameters

3.4.1 Linearity and range

Linearity and range was performed by measuring 8 test concentrations in the range of20% - 140% of the target concentration 0.5mg/ml. All the tested concentrations were analyzed in triplicate. The average result for the area under the curve (AUC) for each concentration was plotted against the concentration. **Table 3.3** and **Figure 3.2** showed a linear relationship between concentration and AUC. The measure of goodness-of-fit (R^2) for the regression line showed linearity with a value of $R^2 = 0.999$ and the regression line equation$y = 6 \times 10^7 x + 42219$ this result showed a linearity was achieved in the range of 0.1- 0.8 g/ml which equivalent to 20-140% of the target concentration 0.5 mg/ml which represent 100%.

Table3.3: Linearity and range results

Concentration of test solution (mg/ml)	AUC	Average
0.1	3379024 3423113 3483034	3428390
0.15	5058485 4964602 4924008	4982365
0.2	6762756 6803995 6766615	6777789
0.3	9794828 10015602 10002942	9937791
0.4	13104405 13128115 13137775	13123432
0.5	16174713 16188611 16206101	16189808
0.6	19381402 19383710 19404003	19389705
0.8	25508590 25327638 25164880	25333703
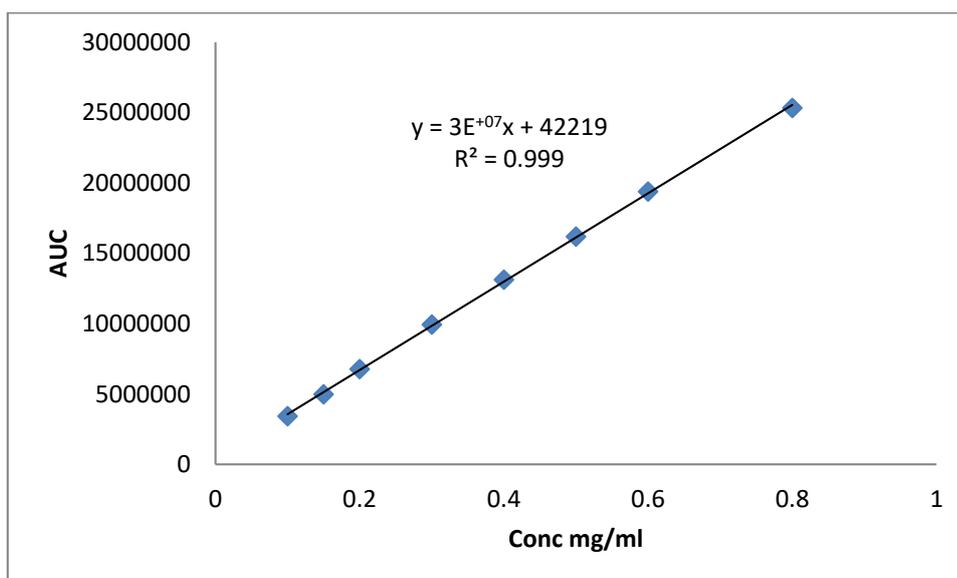
Figure3.6: Linearity curve of celecoxib-K

3.4.2 Accuracy

Accuracy was performed on three concentrations of celecoxib–K with formulation excipients (80%, 100%, and 120%) around the test concentration 0.57mg/ml (which is equivalent to 0.5mg/ml of celecoxib). The AUC for each concentration was used in recovery calculation and was stated as percentage recovery. The results in **Table 3.4** showed that the method has high level of accuracy within the desired rang; the percentage recovery was in range (100% ±5).

Table3.4:Accuracy and intermediate precision(*between days repeatability)* results:

sample peak area			Standard peak area			Assay%
	Inj #1	Inj #2	Average	Inj #1	Inj #2	Average	
80%						
1	14964773	14657785	14811279				97.55%
2	14997884	14934229	14966056	15085398	15278359	15181879	98.57%
3	15239212	14997884	15118548				99.58%
100%						
1	18496185	18261477	18378831				99.42%
2	18266609	18307894	18287251	18572494	18396930	18484712	98.93%
3	18459483	18249027	18354255				99.29%
120%						
1	21971462	21993318	21982390				99.32%
2	21852523	21949606	21901064	21933589	22331627	22132608	98.95%
3	21758263	21700862	21729562				98.17%
Mean				99.02			
SD				0.47			
RSD					0.47		

3.4.3 Precision

I. Intermediated precision (between days' repeatability)

The intermediate precision (between days' repeatability) was also done simultaneously on the prepared solution of the accuracy measurements. All the measurements for the three different concentrations were repeated for three days. The result of %RSD of intermediate precision was 0.47 which indicate that the method has high level of precision in the desired range of acceptable RSD < 2 (**Table 3.4**).

II. Intermediated precision (between analyst repeatability)

The intermediate precision (between analyst repeatability) was also performed to determine within laboratory variation. The results of 0.5mg/ml Celecoxib-K prepared by two different analysts at the same condition triplicate, showed the method is repeatable at the intermediate. The calculated RSD was 1.28.

Table3.5:Intermediated precision*(between analyst repeatability)* **results**

	AUC	% assay
Analysis 1	16237586	100.3
	15875328	98
	15983647	98.7
Analysis 2	16327937	100.8
	16286658	100.6
	16386557	101.2
Mean	**99.9**	
RSD	**1.28**	

III. System precision:

The system precision of the HPLC Instrument precision was performed by injecting ten replications of one prepared sample containing the target

concentration (0.5mg/ml). The results showed that %RSD was 0.43and was within the accepted range (**Table 3.6**).

Table3.6: System precision results

No	AUC of test solution(5mg/ml)
1	16174713
2	16188611
3	16206101
4	16123258
5	16155277
6	16101337
7	16131240
8	16080376
9	15999407
10	16249341
Mean	**16146887**
SD	**70104.93**
RSD	**0.43**

3.4.4 Reproducibility

Reproducibility which refers to the degree of agreement between the results of experiments conducted by different individuals, at different locations, with different instruments was performed by analysing 0.5 mg/ml solution of celecoxib -K in two different laboratories; one analysis was done in the chemistry department and the other was in the pharmacy department. The samples were prepared by two different analysts using two different instruments. The results illustrated in **Table 3.7** showed high degree of agreement and the RSD was less than 2 which proved the reproducibility of the method.

Table3.7: reproducibility results

	AUC	% Assay
Lab 1	15735903	97.19
	16283694	100.57
	16047628	99.12
Lab2	16482647	101.80
	16395799	101.27
	16125576	99.60
Mean	16178541	99.925
SD	**1.67**	
%RSD	**1.67**	

3.4.5 Robustness

The robustness of the method was examined using some minor modifications to the experimental parameters, like measuring the absorbance of in small varied wave length 250±2, and small change in flow rate 1.5±0.2 and the pH of the mobile phase 3±0.2. The results of %recovery and %RSD indicated that minor modifications to the experimental parameters did not affect the assay and its ability to accurately and precisely detect and quantify the active ingredients (**Table 3.8**).

Table3.8: Robustness results

Robustness parameter	Condition checked	AUC	%assay
Flow rate	**1.3ml/minute**	16715850	103.5
	1.7 ml/minute	15760268	97.6
Wave length	**248nm**	16369198	101.3
	252nm	16288144	100.8
Mobile phase pH	**2.8pH**	16347848	101.2
	3.2 pH	16287451	100.7
%RSD			**1.8**

3.4.6 Limit of detection and limit of quantitation

Limit of detection and limit of quantitation was determined manually after testing a serial dilution and measuring the signal: noise ratio. The results showed that the LOD and LOQ of the developed method was 0.001mg/L, 0.01mg/L respectively.
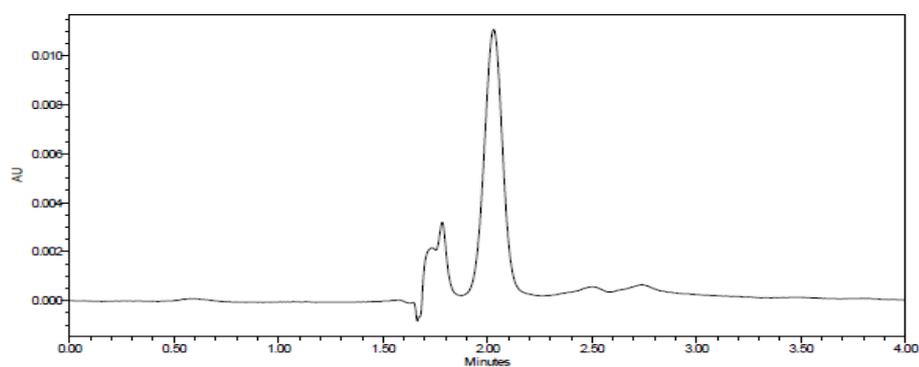

Figure3.7: determination of LOD
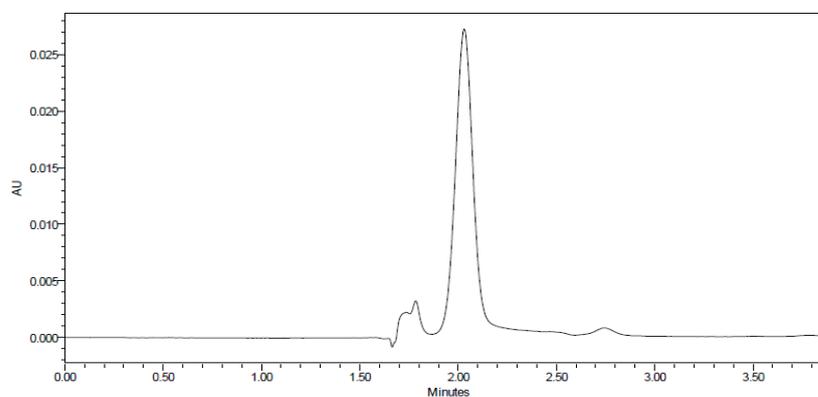
Figure3.8: determination of LOQ

3.5 Tests done on tablet formulation:

3.5.1 Weight variation test

Weight variation test was performed by randomly selecting 20 tablets. The tablets were weighed individually. The results in **Table 3.9**showed that the average weight of the tablet was 0.3925 gm the calculated allowable upper limit and lower limit was 0.4121 gm and 0.3728 gm respectively. **Table3:7** lists all the weighed tablets and it clearly show that none of the tablet was above or below the allowed limit.

Table3.9: Weight variation results of celecoxib-K tablets

No	Tablet weight(mg)	% variation	No	Tablet weight (mg)	% variation
1	0.3967	0.180	11	0.3925	1.875
2	0.3905	2.375	12	0.3897	2.575
3	0.3943	1.425	13	0.3892	2.700
4	0.3972	0.700	14	0.3915	2.125
5	0.3950	1.250	15	0.3921	2.125
6	0.3957	1.075	16	0.3953	1.175
7	0.3948	1.300	17	0.3928	1.800
8	0.3951	1.225	18	0.3905	2.375
9	0.3861	3.475	19	0.3884	2.900
10	0.3886	2.850	20	0.3955	1.125

3.5.2 Content uniformity test

This test was performed by selecting ten tablets randomly, and their content for celecoxib–K was calculated. The tablet content was stated as percent content of the labeled amount. According to USP the content uniformity test will pass if the % RSD <6 and no value out the accepted range 85%-

115%. The results of percentage assay content of the tested tablets showed that all the tablets were in the accepted range of 85% to 115% of the label amount, and the % RSD was 3.22 (**Table 3.10**).

Table3.10: Content uniformity test results of celecoxib–K tablet

No. of the sample	Weight(g)	% assay
1	0.3982	101.1
2	0.3871	95.79
3	0.3976	102.41
4	0.3890	95.47
5	0.3930	101.63
6	0.3889	95.75
7	0.3959	101.38
8	0.3988	99.52
9	0.3957	104.06
10	0.3905	96.36
Mean	99.34	
RSD	3.22	

3.5.3 Friability, hardness and thickness of the tested tablets

Friability test was performed according USP by using PHARMA TEST apparatus, and the test result of 20 randomly chosen tabletswere within the accepted range, the percentage friability was 0.25%. Hardness, thickness, and diameter were test on 10 tablets by Erwecka multi check instrument, the average value of these parameters was considered as our tablet specification, as shown in **Table 3.11.**

Table3.11: Hardness and thickness results

No	Weight (mg)	Thickness (mm)	Hardness (N)	Diameter (mm)
1	0.3946	2.22	120	13.11
2	0.3947	2.20	106	13.19
3	0.3963	2.23	115	13.17
4	0.3974	2.25	113	13.14
5	0.3959	2.25	118	13.11
6	0.3957	2.21	107	13.14
7	0.3978	2.22	102	13.20
8	0.3859	2.24	107	13.16
9	0.3868	2.22	110	13.21
10	0.3986	2.25	111	13.19
Average	0.39437	2.229	110.9	13.162
Min	0.3859	2.20	102	13.11
Max	0.3978	2.25	120	13.21

3.5.4 Tablet dissolution results

Dissolution test is an important test during tablet drug development, which performed to simulate in vivo dissolution profile. The test performed according USP, and the results indicate high solubility of the salt. The salt was totally dissolved in 10 minutes' time and reached the plateau solubility for the remaining time of 60 minutes as shown in **Figure3: 9and Table 3.12.**

Table3.12: Tablet dissolution results

| Time (min)	%Dissolved of the celecoxib-K
0	0
5	50
10	90
20	98
30	101
40	102
50	100
60	99.1
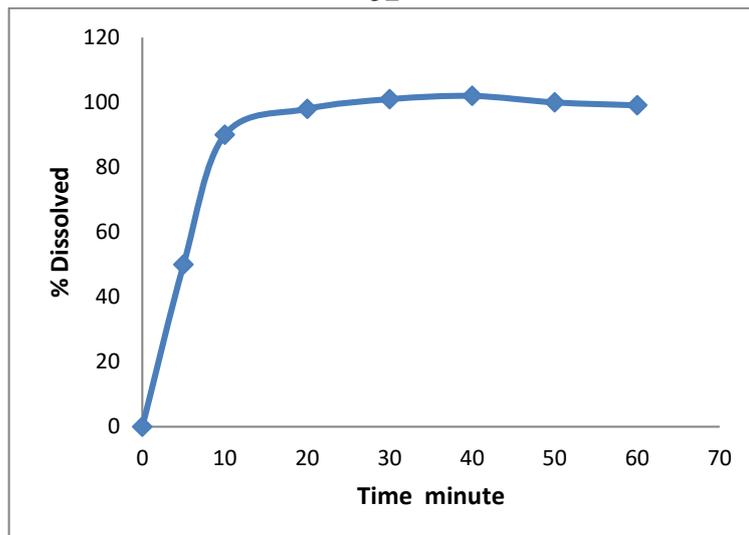
Figure3.9: Tablet dissolution results

3.5.4 Tablet stability study

Stability test was performed on the formulated celecoxib-K tablet under room temperature, and under accelerated condition of high temperature (40℃)for a period of 60 days and the percentage assay of the tested tablet was calculated.The results for both tablets under accelerated condition and shelf life conditions are shown in **Table 3.13.**

Table3.13: Percentage assay of celecoxib-K tablets after different storage conditions.

Storage period (days)	Percentage assay of tablet	
	At room temperature	At 40℃temperature
0	98.2%	97.5%
30	97.4%	98.2%
45	98.9%	97.2%
60	97.9%	96.6%

4. Conclusion

In this study, we increased the celecoxib water solubility by converting it to potassium salt and formulated in a tablet dosage form in our research labs. We developed a validated HPLC method for analysis of our formulated tablet.

Shelf life and accelerated stability were conducted on the formulated tablet and the results showed that the formulated tablet was stable at room temperature and at 40℃ for 60 days.Content uniformity test and weight variation test were performed on the formulated tablet, and the results were within the acceptance limit of USP.This study is noveland there is no so far any pharmaceutical dosage form of celecoxib potassium salt available in the market.Furthermore the increase in solubility of the synthesized celecoxib salt will make it possible to formulate it in an injectable pharmaceutical dosage form. This project will give a valuable experience of different stages followed in the drug development field.

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جامعة النجاح الوطنية

كلية الدراسات العليا

تكوين أملاح سيليكوكسيب وتركيب شكل صيدلاني والتحقق من طريقة تحليلها

إعداد

كفاح رسمي أبو شهاب

إشراف

د. مراد أبو الحسن

د.نضال زعتر

ب

تركيب شكل صيدلاني لملح السيليكوكسب المصنع وتطوير طريقة محققة لتحليله

إعداد
كفاح رسمي محفوظ أبو شهاب

إشراف
د. مراد أبو الحسن
د. نضال زعتر

الملخص

الخلفية: السيليكوكسب هو دواء مضاد للالتهاب غير الستيرويدية (NSAID)، ومثبط انزيمات الأكسدة الحلقية –2 (COX-2). يتم استخدامه في علاج التهاب المفاصل الروماتويد، هشاشة العظام، التهاب المفاصل، والألم الحاد. تعاني السيليكوكسب من انخفاض التوافر الحيوي (bioavialblity) بسبب انخفاض قابليتها للذوبان في الماء. في هذه الدراسة، نهدف إلى تحسين قابلية الذوبان في الماء والتحلل عن طريق تصنيع ملح السيليكوكسب مناسب.

الطريقة: تم تصنيع عدد من أملاح السيليكوكسب، وتم تحديد قابلية الذوبان في الماء باستخدام جهاز إمتصاص الأشعة فوق البنفسجية / المرئية الطيفية. تم اختيار واحد من الملح المركب لصياغة قرص. تم تطوير طريقة تحليل بإستخدام جهاز الHPLC. ثم تم التحقق من صحة الطريقة المتقدمة وفقا للمبادئ التوجيهية الدولية. كما تم إجراء تحليل الذوبان وفترة الصلاحية ودراسات الثبات المعجل على الحبوب المصنعه.

النتائج: أظهرت النتائج أن ملح سيليكوكسب-بوتاسيوم عمل زيادة في الذوبانسيلكوكسيب في الماء بأكثر من 10 مرات (0.464 ملغم/مل) مقارنة مع سيلكوكسيب الأصلي. تم اختيار هذا الملح ليتم صياغته في شكل جرعة أقراص. تم فحص الذوبان للأقراصوأظهرت النتائج أنأقراص سيليكوكسببوتاسيوم المصاغ تذوب تماما بعد 10 دقيقة. كانت طريقة HPLC التحليلية المطورة طريقة موثوقة وصالحة مع خطية ودقة جيدة. كما كانت الطريقة التي تم التحقق منها حساسة مع

LOQ وLOD بقيمة 0.001 ملغم/لترو 0.1 ملغم/لتر على التوالي. كان قرص الملح سيليكوكسبملغم/لتر المصمم ثابتا تحت درجة حرارة الغرفة والظروف المسرعة لمدة 60 يومًا.

الخلاصة: تم تحسين قابلية الذوبان للسيليكوكسب عن طريق تحويله إلى شكل ملح البوتاسيوم. وأظهرت النتائج ان ملح سيلكوكسيبالبوتاسيوم له انحلال جيد في الماء. كانت طريقة HPLC المطورة صالحة وموثوق بها لتحليل الأقراص المصنعه. كما أظهرت النتائج أن الأقراص المصنعه ثابته تحت الظروف العاديه وظروف الإجهاد.