An-Najah National University

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Analytical and Dissolution Method Development and Validation for a Home Formulated Rutin Tablet

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Dedication

After praise and thanks to God; this work is dedicated to my father, mother, husband, brothers, sister, relevant and friends. I also dedicate this work to my supervisors who have played a key role in helping me to complete this thesis and to all those who have supported me in this study.

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Jumana Saleh Mohamed Mansour

الإقرار

انا الموقع ادناه مقدم الرسالة التي تحمل عنوان:

Analytical and Dissolution Method Development and Validation for a Home Formulated Rutin Tablet

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Declaration

The work provided 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: جمانة صالح محمد منصور

Signature: التوقيع:

Date: التاريخ: 25-2-2015

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List of Abbreviations

AA	Ascorbic acid.
ACS	American chemical society
ASTM	American Society for Testing and Materials
BP	British pharmacopeia
CC_{50}	Cytotoxic concentration 50
FBS	Fasting blood sugar
GMP	Good Manufacturing Practices
HDL	High-density lipoprotein
HOG	8-hydroxy-2'-deoxyguanosine .
HPLC	High-performance liquid chromatography
HPMC	Hydroxypropyl Methyl Cellulose
ICH	The International Conference on Harmonisation
IP	Indian pharmacopeia
KBr disk	Potassium bromide disk
LDL	Low-density lipoprotein.
LFT	Liver function tests
LOD	Limit of detection
LOQ	Limit of quantification
MCC	Microcrystaline cellulose
MDA	Malondialdehyde
mg/ml	Milligram per milliliter
Mg-stearate	Magnesium stearate
mM	Millimolality
mm	Millimeter
MOH	Ministry of Health
N	Newton
nm	Nanometer
PEG	Polyethylene Glycol
PGS	Pregelatinized Starch
POC	Plasma antioxidant capacity
PVP	Polyvinyl Pyrrolidone
RSD	Relative Standard deviation
SD	Standard deviation
TC	Total cholesterol
TDDS	Transdermal drug delivery system
TGL	Triglycerides
THF	Tetra hydro furan
TP	Tocopherols
USFDA	The United States Food and Drug Administration.

USP	The United State Pharmacopeia.
UV	Ultraviolet
UV/IR	Ultraviolet/ infrared
VLDL	Very low-density lipoprotein.
w/w	Weight by weight
WHO	The World Health Organization
λ_{max}	maximum absorbance wave length

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Abstract

Rutin can be classified as flavone, colorant and vitamin. It is highly available in some foods, fruits, vegetables and plant-based beverages. Rutin is highly attracted to the researchers due to its variant beneficial medical effects making rutin used in the treatment of various ailments. Rutin is available in different oral dosage forms such as tablets or capsules either alone or in combination with other active ingredients. Rutin pharmaceutical preparations are widely available in international market as well as Palestinian market.

In this study we formulated a 250mg rutin tablet and we developed an easy and simple validated analytical method to quantify rutin in our formulated tablet as well as the internationally marketed Rutin® tablet of Solgar. The method was validated in accordance to international guidelines of the ICH and USP. The dissolution profile of our formulated tablet was also inspected. The shelf and the accelerated stability of the locally formulate tablet was studied. The results clearly show that our developed method was a valid method with a good linearity, precision and accuracy. The validated method was sensitive

with LOD and LOQ value of $4.34*10^{-3}$ and 0.013mg/ml respectively. The locally formulated rutin tablet was stable under accelerate as well as room temperature for 150 days, only with slight and tolerable drop in the %assay with no detrimental effect on the physical properties of the tablets.

The dissolution profile of our locally formulated tablet show slightly better dissolution in phosphate buffer compared with the internationally marketed Rutin® tablet of Solgar.

Our study encourages and helps companies that manufacture herbal products especially those present in Palestine to improve their formulated herbals and apply validation analytical methods to check their product quality.

In conclusion we succeeded in developing a validated analytical method to quantify rutin in our locally formulated rutin tablet as well as the available rutin formulations present in the local and international markets. Our formulated tablet showed a slight improvement in the dissolution profile and was stable in normal as well as under stress condition

Chapter One

1. Introduction:

1.1 Rutin:

Rutin also known (Rutoside, sophorin, and quercetin-3-rutinoside or 3,3',4',5,7-pentahydroxy flavones-3-rutinoside) is the yellow crystalline rhamnoglucoside of the flavonoid quercetin, and has a chemical formula $C_{27}H_{30}O_{16}$ with chemical structure as shown in **Figure 1.1** and molecular weight of 610.5175 g/mol [1].

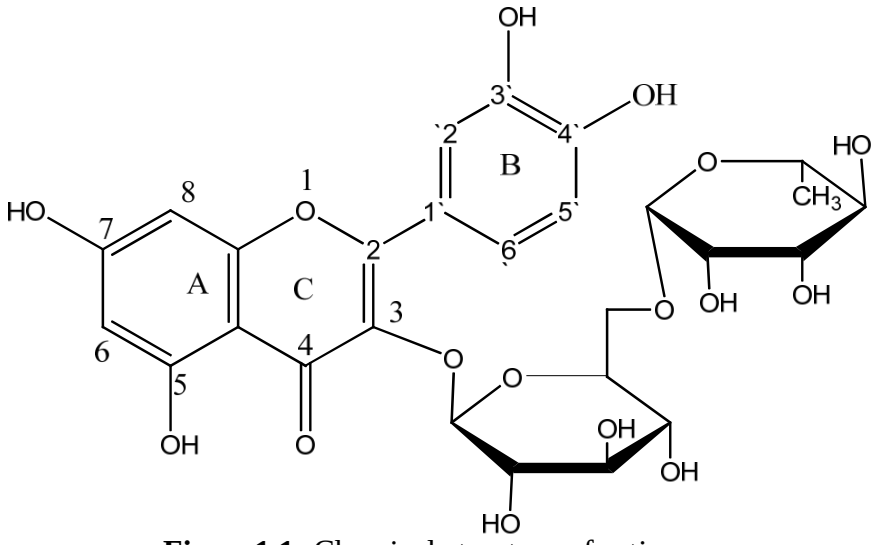


Figure1.1: Chemical structure of rutin

Upon hydrolysis of rutin by water it yields quercetin and rutinose [2].The pharmacokinetic parameters of rutin has extensively been studied including the metabolism, absorption and bioavailability. Rutin showed low bioavailability after studying it in animals and human volunteers; low bioavailability is due to its low water solubility but also there is another reason for low bioavailability which is due to low absorption of the hydrolyzed form of rutin by cecal micro flora [3].

Rutin is yellowish to green crystal powder or needles, it has a density of 1.82 g/cm^3, it has boiling point of 983.1 °C at 760 mm Hg and melting point 195 °C. Rutin is very slightly soluble in water; each 12.5 mg of rutin is soluble in 100 ml of water. However, it has a better solubility in organic solvent; one gram of rutin dissolves in 7 ml boiling methanol. It is soluble in pyridine, formamide and alkaline solution. Rutin is practically insoluble in petroleum.

The rutin should be stored in a tightly closed container in a cool, dry, well-ventilated area away from incompatible substances and protected from moisture or light [4]. Some of the physical and chemical properties of rutin are summarized in **Table 1.1**

Table1.1 0:Physical and chemical properties

Description	Yellow to greenish crystalline powder or needle
Melting point	195 °C
Boiling point	983.1 °C at 760 mmHg
Solubility in water	Slightly soluble in water, Soluble in pyridine and formamide solvent
Stability	Stable under ordinary conditions. It is hygroscopic and light sensitive

Rutin is found in some foods, fruits, vegetables and plant-based beverages such as buckwheat, onions, apples, berries, orange, grape fruit, lemon, tea and asparagus [3,5]. Buckwheat is the best-known food source of rutin; the rutin content of products derived from buckwheat seeds have been shown to range from 0.48mg/100g to 4.97mg/100g, with popped grains showing higher levels of rutin than boiled grains. The rutin

content of tea made from buckwheat flowers has been shown to contain even more rutin (up to 396mg/100g) [5]

Rutin can be classified as flavone , colorant and vitamin. It is highly attracted the researchers due to its variant beneficial medical effects. Studies show Rutin can be useful as an adjuvant in radioiodine therapy, since this flavonoid increased thyroid iodide uptake without greatly affecting thyroid function after experiment in rats [6,7]. Rutin also possesses antioxidant activity in isoproterenol-induced experimental myocardial infarction rats as shown in study of subcutaneous injection of isoproterenol to male Wistar rats at an interval of 24 h for two days showed a significant increase in the activity of serum cardiac marker enzymes and a significant decrease in the activity of these enzymes in the heart. Meanwhile; Lipid peroxidative products (thiobarbituric acid reactive substances and lipid hydroperoxides were significantly increased; antioxidants showed a significant decrease in isoproterenol-treated rats. In addition to that; Pretreatment with rutin (40 or 80 mg $kg^{(-1)}$) to isoproterenol-treated rats orally for a period of 42 days daily caused a significant effect [8]. Marjan nassiri-aslindicate et al. showed that rutin has potential anticonvulsant and antioxidative activities against oxidative stress in Kainic acid induced seizure in mice [9]. Some studies showed that Rutin has lipid lowering properties when given in a dose of 100 mg/kg alone or with lovastatin supplementation it resulted in lowering liver weight and enzymes as well as plasma total cholesterol and LDL in animal model [10].

Rutin and Hesperidin could protect the liver against Doxorubicin-induced liver toxicity [11]. Rutin also exerts stronger protection against nitrosative stress and hepatocellular damage however it has a lower antioxidant, anti-inflammatory activities and antifibrotic potential than quercetin [12]. Rutin proved to have Anti hyperglycemic, in addition to its antioxidant activity in streptozotocin-induced diabetic rats, rutin helped in lowering plasma glucose, glycosylated haemoglobin and increasing insulin, C-peptide, haemoglobin and protein levels [13]. in addition to that; results on forced swimming rat model indicates that rutin treatment ameliorates the various impairments associated with physical fatigue [14]. Results on animal model suggest that rutin has a gastro protective effect through its an anti-lipoperoxidant effect, and also by enhancement of the anti-oxidant enzymatic (GSH-Px) activity [15]. Keivan Zandi et al. show that rutin exhibits cytotoxicity effect against Vero cells *in vitro* with a value of Cytotoxic concentration 50(CC50) of 1000 µg/ml [16]. Sharma et al. reviewed some major clinical trials conducted using rutin as shown in **Table 1.2** and it clearly showed the anti-cancer effect and other curative effect of rutin [3].

Table 1. 2**: List of major clinical trials conducted using rutin [3]**

Activity studied	Human volunteers	Period of study (500mg rutin)	Study outcome
Anti oxidant	18 (female volunteers) Normo cholesterolaemic	6 weeks	Elevated plasma flavonoids, Decreased endogenous, oxidation of pyrimidines
Anti diabetic activity	40 patients with type II diabetes mellitus	120 days	Lowering of blood sugar, level in diabetic patients
Antihypertensive activity	40 patients with type II diabetes mellitus	120 days	Systolic, diastolic blood pressure
Anti lipidemic activity	50 volunteers with type-II diabetes mellitus	3months	Significantly increased the levels of high density lipoprotein whereas Low density lipoprotein level was attenuated

The medical and beneficial uses of rutin is not restricted only to what is mentioned above but it includes uses for hemorrhage and varicose for example; Venoruton forte® for Novartis which contains oxerutin as active ingredient has an effect on the smallest vessels (capillaries) so that it decreases leak of water and other substances through their walls. Venoruton® is indicated for patients with varicose veins and with some other diseases of lower limb veins, strong leak of substances through the capillary walls occurs, which leads to ankle oedemas. Venoruton forte® reduces these oedemas and the symptoms occurring along, such as the feeling of painful, tired and heavy legs, convulsions etc. A beneficial co-effect of Venoruton forte® has also been proved in haemorrhoids.

Taking in mind beneficial view of rutin use for treatment of various ailment; various dosage forms are available in international market with limits of dosage strength to give specific purpose and this includes oral dosage forms such as tablets or capsules either alone or in combination with other active ingredients and topical applications as gels. Some examples of dosage forms of rutin are shown in **Table 1.3**

Table1.3**: Some dosage forms of rutin in international market[3]**

Ingredient(s)	Brand Name	Trade Mark	picture
Rutin	Rutin Tablet	Solgar, UK	
Rutin and Quercetin	Rutin Tablet	Carlson labs, USA	
Rutin	Rutin Capsules	Now foods, USA	
Rutin, Panax Ginseng, Ecithin	Ciplaton Softgel Capsules	Cipla, India	
Rutin	Venoruton Gel	Novartis, USA	
Rutin, Bromelain, Blueberry extract and Aloe	Erbaven Gel	Esi, Italy	

1.2 Quantitative and qualitative analysis of rutin:

Liquid Chromatography methods are the most popular method of rutin analysis. The extracted rutin from stem bark of Ginkgo biloba was determined by HPLC technique and compared with a standard rutin by using reverse phase column chromatography; the mobile phase was a combination

of methanol: water (1:1 ratio) with a flow rate of 1 ml min^{-1} and detection wave length was 360 nm [17].

Capillary electrophoresis and UV spectrophotometer methods were also used in the analysis of Rutin. In 1999, Kreft, S., M. Knapp, and I. Kreft had extracted Rutin from buckwheat and analyzed it by capillary electrophoresis by using running buffer of 50 mM borate of pH 9.3 with 100 mM sodium dodecyl sulfate and a detection wave length of 380 nm [18]. Moreover; simple, rapid, accurate, precise, and economic spectrophotometric method for simultaneous estimation of rutin and galic acid in *Triphala churna* have been developed in 2013 and hence rutin and galic acid show absorbance maximum at 359 and 273 nm respectively [19]. For identification purposes the infrared spectrum of the rutin can be examined by using KBr disk methodology.

1.3 Analytical methods validation:

The validation of analytical methods is used to demonstrate that the method fits for its purpose. The validation process is a follow plan which includes scope, performance characteristics, and acceptance limits [20].

Rapid increase in laws, regulations and guidelines for reporting and evaluating the data on safety, quality and efficacy of new medicinal

products increase overtime; thus there was a need to become internationalized as much as possible, that is due to diversity of technical requirement between countries. This diversity creates extensive time for testing before introduction products to markets [21]. The urgent need to rationalize and harmonize regulation was through the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use that was held in April 1990 in Brussels, the aim of this conference was to bring together the regulatory authorities of Europe, Japan and the United States. The experts discussed the scientific and technical aspects of pharmaceutical product registration of three regions. The purpose of this organization was to reduce the need to duplicate the testing carried out during the research and development of new medicines by achieving greater harmonization in the application of technical guidelines and requirements for product registration. This eliminated the unnecessary delay in the global development and availability of new medicines while maintaining safeguards on quality, safety, and efficacy, and regulatory obligations to protect public health [22].

The ICH has harmonized requirements of validation of analytical procedure into two guidelines. The first one defines and summarizes the characteristics of validation required for test procedure and the second one includes experimental data required for validation and some statistical interpretation [23].

Many regulatory agencies such as the United States Food and Drug Administration (USFDA) require that the drug product be tested for its identity, strength, quality, purity and stability before it can be released

for use in order to enhance the effectiveness and safety of the drug product after approval. For this reason, pharmaceutical validation and process controls are important [24].

ICH divided the 'validation characteristics' somewhat differently to that stated in the USP. **Table 1.4** illustrates in details the validation requirements for each of them. The majority of the validation parameters required by the ICH and the USP are common for both of them, the common parameter include: specificity, accuracy, precision, intermediate precision, repeatability, linearity, limit of detection and limit of quantification.

However, the USP require two more parameter in the validation process namely the ruggedness and robustness. Reproducibility is a parameter included in the ICH paragraph but still is not a requirement. The FDA validation requirements require sample solution stability and system suitability in addition to the main validation parameters[25].

Table 1.4: The ICH, USP and FDA validation parameters [25]

ICH validation parameters	USP validation parameters	FDA validation requirements
Specificity	Specificity	Sensitivity
Accuracy	Accuracy	Recovery
Precision	Precision	Reproducibility
Repeatability	Repeatability	Robustness
Intermediate precision	Intermediate precision	Sample solution stability
Linearity	Linearity	System suitability
Limit of detection	Limit of detection	
Limit of quantitation	Limit of quantitation	
Range	Range	
Reproducibility	Ruggedness	
	Robustness	

According to ICH, the validation of analytical procedures must include the main types of analytical procedures. The validation method must involve the identification tests to ensure the identity of an analyte in a sample by compared to that of a reference standard. The identification can be performed by any of the identification test which includes UV/IR spectrum, chromatographic behavior or chemical identification test. The validation of methods must also include quantitative tests for impurities content that a limit tests for the control of impurities. The validation of the method must have a quantitative test of the active moiety in samples of drug substance or drug product or other selected components in the drug product by assaying procedures to measure the analyte present in a given sample and additional analytical tests such as dissolution test [26].

Employment of a fully validated analytical methods for reliable results in the laboratories during analyzing the registration batch and accelerated stability testing samples is the actual need of analytical method validation [27]. The typical validation parameters should be considered in the analytical validation procedure; these parameters include: Accuracy, Precision, Repeatability, Intermediate Precision, Specificity, Detection Limit, Quantitation Limit, Linearity and Range [28].

Linearity is defined as the ability within a given range to obtain test results, that are directly proportional to the concentration of analyte in the sample. It can be demonstrated by directly diluting the standard stock solution using the proposed procedure with a minimum of 5 concentrations is recommended. The range of an analytical procedure is the interval between

the upper and lower concentration of analyte in the sample including the concentrations for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

The WHO Manual in 2005 defined accuracy as the degree of correlation with the value achieved by the previous method [29]. Specificity is defined as the ability to assess unequivocally the analyte in the presence of components which may be expected to be present and typically these might include impurities, degradants, matrix, etc [28]. The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility and it should be investigated using homogeneous, authentic samples. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. Repeatability (intra-assay precision) expresses the precision under the same operating conditions over a short interval of time. Intermediate precision expresses within laboratories variations: different days, different analysts, different equipment, etc. Reproducibility expresses the precision between laboratories where collaborative studies usually applied to standardization of methodology. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The

quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy and this is a parameter of quantitative assays for low levels of compounds in sample matrices. It is used particularly for the determination of impurities and/or degradation products. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value [28].

1.4 Stability and stability indicating study:

USFDA define stability indicating assays as 'validated quantitative analytical methods that can detect the changes with time in the chemical, physical or microbiological properties of the drug substance. The stability indicating study is specific so that the contents of active ingredient, degradation products and other components of interest can be accurately measured without interference'[30]. The (ICH) guidelines clearly stated the requirement and establishment of stability-indicating assay method. It requires the conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, dry heat, etc. The drug must be separated from the degradation products and the method must be able to analyze each individual degradation product [31].

1.5 Formulation:

In all types of dosage formulations including tablet, capsule and topical application; usually consist of one or more active ingredient with other

excipients. Tablets are the most used dosage forms available in the market; this is due to many reasons as it is simple, economical in manufacturing and stable. It is also convenient in packaging, shipping and transportation. Tablets may be formulated to contain more than one therapeutic ingredient showing a combination thus reducing multiple tablets use. Moreover, tablets dosage forms can mask the taste of bitter active ingredients thus make it more convenience for patient [32].

Tablets can be prepared by three general methods: wet granulation, dry granulation (roll compaction or slugging), and direct compression. Although wet and dry granulation will improve flow of the mixture and enhance its compressibility; but direct compression is more simple, inexpensive and fast method [33]. The type and amount of excipients, that are pharmacologically inactive that are added to the mixture of tablet formulation are important factors, they affect appearance, hardness, friability , dissolution and other tablet properties. The excipients that are widely used in the tablet formulation include diluents/fillers, binders, disintegrants, lubricants, glidants and miscellaneous.

Diluents are used to increase dosage form volume, allow direct compression and enhance flow. They are used also to adjust weight of tablet according to the die capacity. They are can be used in a wide range of 5-80% in each tablet. The selection of diluent should take into consideration the physical and chemical properties including: compactibility, flowability, solubility,

disintegration, qualities, hygroscopicity, lubricity and stability. Some of the common diluents used in tablet dosage forms are shown in **Table1. 5** [34].

Table1.05: some examples of diluents used in tablets based on their solubility

Insoluble Tablet Filler or Diluents	Soluble Tablet Filler or Diluents
Starch	Lactose
Powdered Cellulose	Sucrose
Microcrystalline Cellulose	Mannitol
Calcium Phosphate	Sorbitol

Binders are used to impart cohesive qualities to the tablet ingredients together maintaining the final shape of the tablet and provide it with a mechanical strength. Binders are usually natural or synthetic polymers e.g. starches, sugars, sugar alcohols and cellulose derivatives [35] . Some of the common binders and their characteristics are shown in **Table 1.6** [36], [37].

Table1.6: Characteristics of Commonly Used Binders

Binder	Specified concentration
Starch Paste	5-25%w/w
Pregelatinized Starch (PGS)	5-20%w/w for (Direct Compression) 5-10%w/w for (Wet Granulation)
Hydroxypropyl Methyl Cellulose (HPMC)	2-5%w/w
Polyvinyl Pyrrolidone (PVP)	0.5-5%w/w
Polyethylene Glycol (PEG) 6000	10-15%w/w

Disintegrants are usually added to formulation in order to promote the breakup of the tablet into smaller fragments when placed in an aqueous environment. The disintegration of tablets result in an increasing of the available surface area and promoting a more rapid release of the drug substance. The disintegration mainly occur through swelling, porosity, capillary action and deformation of the tablets [38]. Some of the commonly

used disintegrants are starch (Amylum), pregelatinized starch (Starch 1500) and its optimum concentration is 5-10%. Sometimes, modified starch which consist of sodium starch glycolate (primogel, explotab) is used in a concentration of 4-6 %. Cellulose and its derivatives are also used as disintegrant these include microcrystalline cellulose (MCC). Acdisol (croscarmellose sodium) is also used as disintegrant in concentration up to 5%. Other miscellaneous disintegrants like surfactants, gas producing disintegrants and hydrous aluminium silicate may be used in tablet formulations [39].

Lubricants are added to the tablet formulation primarily to reduce friction between die wall and granules and ease the ejection of the tablet. Moreover, lubricants prevent sticking of granules to the tools and improve the granules flow property. Example of commonly used lubricants are magnesium stearate, talc, stearic acid and its derivatives, PEG, surfactants, waxes [40]. Miscellaneous excipients which are commonly used in tablet formulation include: adsorbents, coloring agents, and flavorants. Examples of adsorbents include magnesium oxide, kaolin/bentonite [41].

The choice of excipient affects the flowability, compressibility, hygroscopicity, palatability, dissolution, disintegration, sticking, and dust generation [42].

1.6 Dissolution of tablets:

The dissolution test as defined in the USP is used in judging the quality of pharmaceutical products. It is important for evaluation physiological availability that depends upon having the drug in a dissolved state [43].

Dissolution test used mainly in the development stage of drug product for optimization the therapeutic effectiveness and stability assessment, also for quality purpose in order to ensure uniformity between production lots. It is also important in bioavailability and bioequivalence studies of two manufactured products with the same active ingredient [44].

It is impossible to develop a single dissolution test method; due to significance difference in formulation design among novel drugs and physicochemical properties. Thus different apparatus with different properties and its suitability for intended dosage forms are used. The test is performed according to USP or IP and BP. Most common apparatus and their uses according to USP are listed in **Table 1.7** [45].

Table1.7: Types of USP apparatus, features and uses.

Name of apparatus	Main features of apparatus	Uses
USP apparatus 1	Basket	Tablets, capsules, Floating dosage forms
USP apparatus 2	Paddle	Tablets, capsules, enteric forms
USP apparatus 3	Reciprocating cylinder	Extended release drug product
USP apparatus 4	Flow through cell	Implants, powders, suspensions
USP apparatus 5	Paddle over disk	Transdermal drug delivery system(TDDS)
USP apparatus 6	cylinder	Transdermal drug delivery system(TDDS)
USP apparatus 7	Reciprocating disk	Extended release drug products

Similarity and difference factors are emphasized by USFDA for comparison of *in-vitro* dissolution. Similarity factor (f2) stresses on the comparison of closeness of two comparative formulations which

commonly used to establish similarity of two dissolution profiles. The formula to find similarity factor is as following:

$$f2= 50 \log\{[1+ (1\backslash n)S_{t=1}^{n} (R_t-T_t)^2] ^{-0.5} \times 100\} \quad (1)$$

where,

n: Number of dissolution sample times,

R_t and T_t : The individual or mean percent dissolved at each time point, t, for the reference and test dissolution profiles, respectively.

f2: The similarity factor should be between 50 and 100

However, dissimilarity factor (f1) focus on comparing the difference between percent drug dissolved per unit time for a test and a reference product. Dissimilarity factor (f1) is used to calculate the approximate % error in drug release profile; it should be between 0 and 15.

The dissimilarity factor (f1) is given as follows [46]:

$$f1= \{[S_{t=1}^{n} |R_t-T_t|] / [S_{t=1}^{n} R_t]\}x100 \text{.....(2)}$$

1.7. Disintegration test:

The purposes of disintegration test does not mean complete dissolution of the unit or its active constituent; even though complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the discs, if used, is a soft mass having no palpably firm core.

As described by WHO this test was done by adding one dosage unit in each of the six tubes of the basket and if specified add a disc then operate

the apparatus using water as the immersion fluid unless another liquid is specified and maintain its temperature at 35-39 °C. At the end of the specified time, lift the basket from the fluid and observe the dosage units. All the dosage units must have been disintegrated completely; but if one or two dosage units fail to disintegrate; the test must be repeated on 12 additional dosage units and the requirements of the test are met if not less than 16 of the 18 dosage units tested are disintegrated [47].

1.8. Hardness, thickness and diameter test:

Hardness test is sometimes called the breaking force testing. The machine used to test the hardness usually apply a force on in which the tablets are placed between two plates of the machine one of the plates moves towards the tablet applying a measurable force until the tablet is totally damaged. The hardness test is an important quality control parameter since parameter has an influences on many other tablet properties like the disintegration, dissolution and friability [48]. The thickness and diameter are specifications of the tablet that are usually specified by the company and are usually checked during in-process quality control and as a finished product to insure uniformity of the tablet dosage form.

1.9. Registration and quality control check of Herbal products in Palestine

Quality control is an essential operation in the pharmaceutical industry in order to ensure safe and therapeutically active formulations whose performance is consistent and predictable. However, there are several challenges facing the quality control of the herbal products as a result of

deliberated adulteration of plant material; some of these challenges are the standardization of the active ingredients as well as the poly ingredient of herbal products. Herbals are highly affected by wrong storage and transportation which have a diverse effect on the quality of herbal products. Standardization and quality control parameters for herbal formulations according to WHO guideline are based on following fundamental parameters: quality control of crude drugs material, plant preparations and finished products, stability assessment and shelf life, safety assessment; documentation of safety based on experience or toxicological studies, assessment of efficacy by ethno medical information and biological activity evaluations [49].

To our knowledge; there is still no systematic quality control check on herbal products in Palestine. However, the Palestinian Ministry of Health (MOH) has established requirements for importing and manufacturing of food supplements and herbal products.

The registration of herbal product is the responsibility of the registration department in the pharmacy directorate in the MOH. Up to date; there are only two functioning herbal manufacturing establishments. The total herbal products that are registered in Palestine are only 79 imported drugs and 12 local drugs registered. There are procedures of registering herbal products that are either imported or locally manufactured which goes into different staged.

For imported herbals at first a food supplement store must be licensed by the licensing unit in the MOH. The importing store must have an

authenticated letter from the manufacturer showing that the importing company is the sole agent of the producing company and authorized for registration in the Palestinian National Authority that is duly certified by the Ministry of Foreign Affairs and Embassy of Palestine. The company has to supply a free sale certificate issued from the health authorities clarifying that the product is sold freely in the country of origin, as a food supplement and not as a therapeutic agent under the same name, composition and directions mentioned in the application form for registration.

The Palestinian MOH has put requirements for the manufacturer of herbal products; the establishment must be licensed and approved to manufacture food supplement products by a specialized department. The manufacturing company must be implementing the Good Manufacturing Practices requirements (GMP) and is under the continuous supervision and control of the specialized authority. There are technical Requirements and documentation which are required to be submitted to the authority; these documents include: the master formula including all ingredients and their quantities, specification of the raw materials included in the manufacturing product including a copy of the references used and certificates of analysis from the manufacturer. The documents must also include the finished product specifications; certificate of analysis of the finished product; certificate prepared by the manufacturer including the shelf life and required storage conditions. Moreover, labels and packaging materials specifications of the internal and external packaging must be supplied. A

clear printing on the outer and internal packaging must be included a statement of “dietary supplement”. Samples from the finished product and the active ingredient (s) sufficient for at least three analyses [50].

2. Objectives and significance of the study

2.1 Objectives of the study:

To formulate a rutin tablet and compare its quality with that available in the local and international market using a home developed method, the developed analytical method for quantification of rutin both in raw material as well as in its final dosage. The method will be validated according to the international standards. The dissolution of our developed tablet formulation will evaluate and will be compared to the dissolution of already marketed rutin tablets. Moreover; a stability study under normal and stress condition will conducted for our formulated tablet.

2.2 Significance of the study:

To our knowledge there is no pharmacopeial method or a validated method to quantify rutin in its final dosage form. The validation of our methods will give it a chance to include it in one of the international pharmacopeial and make it a standard and applicable method for quality control labs. Moreover; quality control lab in the Ministry of Health as well as the private quality control labs will adapt out validated analytical and dissolution method in their quality control analysis procedures. The stability study on our formulated dosage form will be a guideline for the

local establishment as well as the Ministry of health on how to judge about the stability of the herbal and food supplement. There are only two establishment that are licensed for herbal and for supplement and it is a growing business in Palestine, thus our research project is taking into consideration different stages including the formulation, quality control and stability which can provide high experience and benefit to these establishments

Chapter Two

2. Methodology:

2.1 Materials:

All reagents used in this study full fill the minimum requirement set by ASTM international and American chemical society ACS specification for analytical reagents and were purchased form reliable resources; these chemicals and materials include the following as shown in **Table 2.1**:

Table 2.1:chemicals used in solubility of rutin

Item	Grade	Source
Acetone≥99%	ACS	Sun Pharm Ltd.
Acetonitrile	AR	Bio-lab ltd
Ethanol	AR	Sun Pharm Ltd
Hydrochloric Acid (HCl) 32%	ACS	Sun Pharm Ltd
Hydrogen peroxide 30%	AR	Merck
Isopropyl alchohol	ACS	Sun Pharm Ltd
Methanol	ACS	Sun Pharm Ltd
Potassium dihydrogen phosphate	AR	Sigma Aldrich
Rutin trihydrate	USP	MP Biomedicals
Rutin®500 tablets	Reference finished product	Solgar
Tetrahydrofuran	ACS	Carloerba reagenil

2.2 Instruments and Equipments:

Instruments: The instrumentations that were used during our research include the followings: Adventurer^{TM} (*Dhaus* ^{R}), Disintegration test apparatus *(model# 190),* Dissolution tester BTC -9100*(Hsiang tai machinery industry co , ltd),* Hotplate Stirrer (*Lab tech*^{R,} *daihasn lab*

tech co,ltd), Multi-check of Hardness, thickness and diameter (*Erweka 5.1)*, Oven (*Arilevy), 1*Pressure Gauge *(Simadzu corporation 5 TON), Rotavapor (*Heildolph VV2000) and UV-Visible Spectrophotometer-JEWAY 7315 (*Biobby Scientific ltd)*.

Glassware: All the glasswares used were of grade B,. They include the following: volumetric flasks (50ml, 150ml , 100ml , 25ml), Glass rod, cylinders (1000ml, 100ml ,50ml), pipettes , funnel , filter paper and mortar.

2.3 Solubility Determination:

Different solvents have been checked for best solubility of Rutin trihydrate the solvents used were namely: isopropyl alcohol, acetonitrile, water, methanol, ethanol, tetrahydrofuran and acetone with different solvent ratios and different rutin percentages.

The equilibrium solubility was judged at saturation point of rutin, all tests were done at room temperature 25°C. A summary of the solvent , co-solvent and exact weight of Rutin trihydrate is illustrated in **Table 2.2**

Table 2.2: solubility check of rutin active ingredient in different solvents and weights.

Solvent	**Ratio** (V/V)	**Rutin weight (g)**
Methanol	100%	0.117g
Methanol:water	9:1	0.107g
Methanol:water	8:2	0.119g
Methanol:water	4:6	0.108g
Ethanol	100%	0.108g
Ethanol:water	9:1	0.101g
acetonitrile	100%	0.101g
Acetonitrile:methanol	9:1	0.098g
Acetonitrile:water:methanol	1:1:8	0.975g
THF	100%	0.103g
THF:Methanol	4:6	0.103g
Isopropyl alcohol	100%	0.140g
Isopropyl alcohol:methanol	1:9	0.134g
Isopropyl alcohol:methanol	2:8	0.101g
Acetone	100%	0.110g

2.4 Determination of wave length of maximum absorption (λmax):

The spectrum of Rutin trihydrate was performed using UV spectrophotometer in the range of 200nm - 800nm. The solution was zeroed using blank solvent (Methanol: Water; 9:1). The spectrum was also run with serial dilution until reaching the optimum detection of absorption peak. The interfering effect on the maximum absorption (λ_{max}) of excipients used in formulation was studied by checking the spectrum of each excipient alone and all in combination with rutin.

2.5. Validation method parameters:

2.5.1 Linearity and range:

The linearity and range were checked by a series of solutions of rutin shown in **Table 2.3**. Astock solution of rutin was prepared by dissolving

112.4mg of rutin powder and dissolving it in 100ml diluent composed of Methanol: Water in a ratio of 9:1. The solution was prepared at room temperature using a magnetic bar stirrer and stirring for 30 minutes

Then 2ml of the stock was diluted with diluent up to 50ml to prepare solution making solution (One), after that 15ml and 10 ml of solution (One) is diluted in volumetric flask up to 20ml and up to 25ml to prepare solution (Two) and (Three) respectively .Solution (Four) was prepared by taking 5ml of stock solution and diluted with diluent in volumetric flask up to 50ml. Further 5ml of solution (Four) was diluted with same diluent in volumetric flask up to 50ml to prepare solution (Five). Moreover; 20ml of solution (Five) was diluted with diluent up to 25ml of volumetric flask to prepare solution (Six). Solution (Seven) was prepared by taking 20ml of solution (Four) and diluted with diluent up to 25ml volumetric flask and then 20 ml of this prepared solution was diluted in volumetric flask up to 25ml to prepare solution (Eight). Another 15ml of solution (Four) was diluted with diluent in volumetric flask up to 20ml to prepare solution (Nine).

Table 2.3: Serial dilutions of stock to make different concentrations to check linearity and range of the method

Name of solution	Dilution factor	Resultant concentration (mg/ml)
Solution (Six)	125	0.00899
Solution (Five)	100	0.01124
Solution (three)	62.5	0.01798
Solution (two)	33.33	0.03372
Solution (one)	25	0.04496
Solution (Eight)	15.65	0.07193
Solution (Nine)	13.33	0.08430
Solution (Seven)	12.5	0.08992

The UV- Spectrophotometer was zeroed using blank solution (Methanol: Water; 9:1), this solution will be the diluents for all the prepared solutions and were measured at λ_{max} of 360 nm; the absorption reading for each solution was plotted to construct the calibration curve in which the concentration of each solution (mg/ml) was in the X-axis versus its absorption on the (Y- axis). The calibration curve was constructed using Microsoft Excel 2007. The regression line equation of the plotted data was calculated and the range of lowest and highest concentration was determined. The linearity of the line was judged form the value of R^2

2.5.2 Accuracy and Precision:

The accuracy and precision were established on three concentrations around the test concentration (80%, 100% and 120%), three replicates of each concentration was prepared; the test was as per ICH guidelines.

The recovery and precision was performed on prepared working solution as well as a solution prepared form tablet formulation developed in our research lab.

The above concentration of the working solution was prepared as following: At first a stock solution was prepared by weighing and dissolving 0.209g of rutin powder in solvent (Methanol:Water; 9:1) in 100ml volumetric flask. Solution (A) was prepared by diluting the stock solution 62.5 times which makes a concentration of (0.0332 mg/ml) this constitute 80% of the test solution. Another solutions of 0.2506g and 0.3057g in two separate 100ml volumetric flasks, both were then diluted 62.5 times in order to get a final concentration of 0.040mg/ml and 0.0489mg/ml. These solution are 100% and 120% of the test concentration solution. The absorption of these solutions were tested at wave length of 360nm after blanking on the diluents.

The accuracy and precision test was also performed on our prepared formula and was done by recording the weight of one formulated tablet. The tablet was then grinded in mortar and dissolved by methanol-water solution and then was transferred to a volumetric flask and completed to 250ml. Three separate solutions were then prepared form stock solution each having a dilution factor of 31.25 , 25 and 20 that are equivalent to 80, 100and 125% of the test concentration.

The absorbances of the solutions were measured at 360nm and the percentage recovery and % RSD were calculated. All measurements were done on triplicate for each prepared solution and also repeated after three days.

2.5.3 Specificity and selectivity:

The specificity and selectivity of the method were carried out by measuring the absorbance of the excipients mixture without the active ingredient. The absorbance was measured in the range of 200 – 800 nm. The resulted Spectrum of the excipients was compared to that of rutin and was checked for any interference at λ_{max} of 360nm. The method specificity and selectivity were also checked for degradative substances. This was performed by subjecting the sample solution to forced degradation as outlined in **Section 2.6.**

2.5.4. Ruggedness and robustness:

Effect of slight changes on absorption and recovery at wave lengths 362nm and 358nm has been studied for the locally prepared formula of rutin tablet 250mg. Secondly, robustness has been checked by studying the effect of slight changes in solvent composition ratio of (Methanol:Water; 9:2) and (Methanol: Water;10:1) on absorption and recovery. Thirdly, the effect of changing personnel has been studied and this was done by preparing solution and reading absorption by another analyst in order to see the effect of these changes on the analytical method.

2.5.5 Limit of detection (LOD) and limit of quantification (LOQ):

The LOD and LOQ of Rutin was determined by using standard deviation of the response and slope approach as defined in ICH guidelines. LOD and LOQ were calculated using the relation:

LOD=3.3* SD/Slope

LOQ=10 SD /slope

Where, SD is the standard deviation of residuals from the curve.

The Standard deviation of the residuals was calculated at first, then multiplied by 3.3 and 10 the slope of the regression line to get both the LOD and the LOQ, respectively.

2.6. Forced degradation study:

The test solution (0.04mg/ml) of the formulated tablet was preserved for 24 hours at room temperature and analyzed on the following day to test for short-term stability of the test solution. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Intentional degradation was carried out by exposing the formulation solution of 0.04mg/ml final concentration to five stress conditions (0.1 N HCl), (0.1 N NaOH), (0.3% H_2O_2), UV light at 254nm for 3 hrs. The time and conditions are outlined in the **Table 2.4**. Stressed samples were analyzed periodically for 2 days.

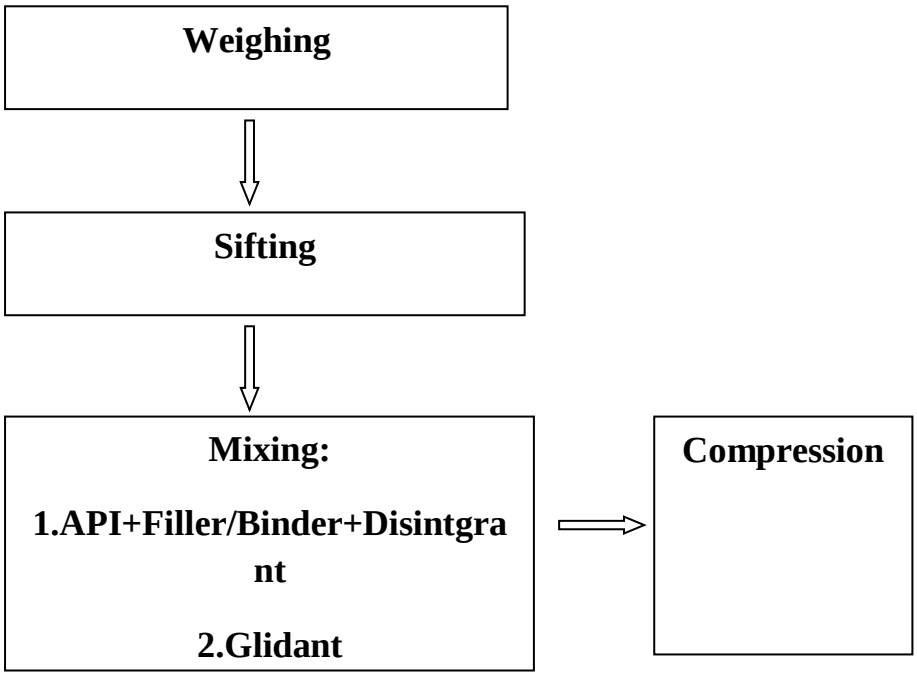
Table 2.4: Time periods for each force degradation conditions

0.1 N NaOH Time (hours)	**0.1 N HCl Time (hours)**	**in 0.3% H_2O_2 Time(hours)**	**254nm UV light Time(hours)**
Zero time	Zero time	At Zero time	0.92
one	One	One	
2.25	2.17	2.08	
3.25	3.17	3.17	
25.25	25.17	18.42	
26.25	26.17	19.42	
27.25	27.17	20.42	1.92
28.25	28.17		
47.92	34.75		
48.92	35.75	21.42	
49.92	36.75		
50.92	37.75		

The stress solution used in the forced degradation study was prepared as followings: The acidic stress solution 0.1N HCl stress stability solution was prepared by dilution of 9.7ml of 32%HCl in volumetric flask of distilled water up to 1000ml. The alkaline 0.1N NaOH solution was prepared by weighing 4g of NaOH crystals and dissolving it in distilled water, and the volume was completed to1000ml in a volumetric flask. The Solution of 0.3% H_2O_2 was prepared by diluting concentrated hydrogen peroxide (3%) ten times.

2.7 Formulation Development:

Three different formulae of rutin 250mg tablets were prepared in our research lab. The components used are: Rutin trihydrate, Magnesium stearate, Microcrystalline cellulose, Aerosil and Acdisol. The tablets were prepared by "Direct compression". Method according to the following **scheme 2.1**:



Scheme 2.1: Steps of formulation preparation

The detailed composition of the three tablet formulations are listed in **Table2.5**.

Table 2.5: Compositions of formulated tablets

Component	Weight/ tablet (mg)						Function
	Formula 1	**%**	**Formula 2**	**%**	**Formula 3**	**%**	
Rutin	251	55.78	250	55.56	250	55.56	API
MCC	180	4o	180	40	185	41.11	Filler/Binder
Magnesium stearate	5	1.11	5	1.11	5	1.11	Lubricant
Aerosil	5	1.11	5	1.11	5	1.11	Glidant
Acdisol	9	2.0	10	2.22	5	1.11	Disintegrant
Total weight	450		450		450		

Each component was weighed separately and labeled. Mixing of components was done in the following order: At first the active component Rutin powder and microcrystalline cellulose were mixed together for 5 minutes. Acdisol was then added by geometric dilution into previous mixture in order to ensure a well-mixed composition. The geometric dilution was done by addition of the same weight of acdisol into the same weight of mixture rutin and microcrystalline cellulose then the same weight of the resultant mixture is added from rutin and microcrystalline cellulose until finishing the mixture, the mixing time was continued for 10 minutes. Aerosil was then added to it and mixed well for 5 minutes. Finally Magnesium Stearate was added and mixed for 2-3 minutes. The final resulted mixture of each formula was compressed into

tablets of 450mg ±5% weight by using Pressure Gauge compressed at 5 tons pressure.

2.8 Weight variation of the formulated tablets:

Weight variation of the formulated tablets was performed in accordance to the USP method specified for the uncoated tablets [50].

The weight variation is done by weighing 20 tablets individually which were selected randomly and their average weight was calculated. The test will be considered pass if it meets the requirements set by the official pharmacopeia which states that: 'not more than two of the tablets differ from the average weight by more than the percentage listed in the **Table 2.6** and no tablet differs in weight by more than double the percentage'.

Table 2.6: Weight variation requirements set by the official pharmacopeia

IP\BP	Limit	USP
80mg or less	± 10%	130mg or less
More than 80mg and less than 250mg	±7.5%	130mg to 324mg
250mg or more	±5%	More than 324mg

2.9 Content Uniformity test:

The content uniformity test was done in accordance to USP. Ten tablets were chosen randomly and weighed individually, and each tablet was grinded into powder using a pestle and mortar. The powder was then dissolved by the diluent; the volume was completed to 250ml using a volumetric flask. This stock solution was diluted 5 times by taking 20ml

of the stock solution and the volume was completed to 100ml volumetric flask. Further dilution was done by taking 20ml of the second solution into a 100ml volumetric flask and the volume was completed to 100ml using the same diluent. The absorption of the final dilution was measured at the specified wave- length (360nm) and the rutin content in each was calculated relative to the label claim using the formula:.

%of Rutin= Absorbance of Sample/ Absorbance of Standard *100

2.10 Dissolution profile:

Dissolution was done according to USP using paddle dissolution apparatus. The dissolution test was performed in three different pH media of 1.2 (0.1N HCl), 4.5 and 6.8 phosphate buffer. The dissolution profiles were performed for the three prepared formulae in order to select the best similar formula among the three formulations in comparison to that of the reference Rutin® tablet marketed by Solgar Similarity (f2) and dissimilarity (f1) were calculated using the equations:

$$f2= 50 \log\{[1+ (1\backslash n)S_{t=1}^{n} (R_t-T_t)^2] ^{-0.5} \times 100\} \quad (1)$$

$$f1= \{[S_{t=1}^{n} |R_t-T_t|] / [S_{t=1}^{n} R_t]\}x100 \quad (2)$$

2.10.1 Preparation of dissolution media:

0.1N HCl dissolution medium was prepared by diluting 97ml of 32% HCl to 1000ml by distilled water. The phosphate buffer pH 4.5 was prepared by dissolving 13.61g of potassium dihydrogen phosphate (KH_2PO_4) powder in about 750ml distilled water. The pH was adjusted to 4.5 with freshly

prepared 0.1N NaOH, using a pH meter; distilled water was then added to make a final volume of 1000ml using volumetric flask.

The phosphate buffer pH 6.8was prepared by dissolving 6.8 g of KH_2PO_4 in 250ml of distilled water then adjusting the PH of solution to pH 6.8 by the addition of 0.1N NaOH solution, and the volume was completed with water to 1000ml.

2.10.2 Dissolution testing and dissolution profiles:

Dissolution testing is a way to study, under *in-vitro* conditions, the release of an API from tablet. During a dissolution test, the cumulative amount of API released into the dissolution medium is measured as a function of time.

A USP Paddle dissolution tester (BTC-9100) was used for dissolution test profile. The dissolution tester was run at 50rpm and 37° C for 45minutes. One tablet was placed into each of the six dissolution vessel containing 900ml of dissolution medium. The dissolution test profile was performed to compare the three locally prepared tablet formulas as well as the Rutin® tablet of solgar.

The dissolution test was done on three selected dissolution media namely:

0.1N HCl, Phosphate buffer pH 4.5 and phosphate buffer pH 6.8. Six tablets were tested in each run. The test was performed for all the developed formulae as well as the Rutin® of Solgar.

Samples of 10ml were withdrawn by syringes from each dissolution vessel at time intervals of 5, 10, 15, 20, 25, 30, 35, 40 and 45 minutes. The samples were filtered through a filter fitted on the end of syinge and their

UV absorbance was measured at wave length 360nm. The readings were done in triplicates. The actual amount of released Rutin was calculated using the regression line equation of the calibration curve. The percentage of dissolved rutin was calculated using the following formula:

% of dissolved Rutin in tested tablets= [Actual amount of released rutin/ theoretical amount of rutin in tablet]*100%

A dissolution curve was then constructed using Microsoft Excel 2007 putting time on x-axis and the percentage of dissolved amount on the y-axis. The dissolution profile of reference Rutin® 500mg Tablets was carried out first in the three dissolution media, to be a standard for further comparison. The dissolution profiles of three formulated tablets (F1, F2 and F3) were studied in phosphate buffer PH 6.8 as dissolution medium and the same dissolution parameters used in the reference product, in order to select the best similar formula.

The selected formula`s dissolution profiles were studied in two additional media, i.e. 0.1N HCl and phosphate buffer PH 4.5.

The similarity factors (f2) and dissimilarity factors (f1) were calculated and discussed in chapter three of the thesis.

2.11 Setting the specification of some tablet physical parameters

2.11.1 Disintegration test:

The disintegration time of our formulated tablet was performed according to USP for uncoated tablets, one tablet was placed in each of the six tubes of the basket and the specified disc was added. The apparatus

was operated using water and the temperature was maintained at 37 ± 2° C. The tablets were observed. When all the tablets have been completely disintegrated; this was recorded as disintegration time.

2.11.2 Hardness test, diameter, thickness of the tablet:

Tablet physical parameters like the hardness, thickness and diameter were determined using Multi-check (*Erweka 5.1)*. Ten tablets were put in the machine which automatically gives the reading for the hardness, thickness and diameter. The average reading was then assigned as the tablet hardness, thickness and diameter in the specifications of the formulated tablets.

2.12 Quality control check of the marketed Rutin® 500mg tablet:

The reference Rutin® 500mg tablet, marketed in Palestine , was tested for its rutin content following the developed assay method.

The tablets of Rutin® 500mg , were powdered , an amount of powder equivalent to one tablet(500mg rutin) was dissolved in diluent (containing Methanol ; water in a ratio of 9:1 respectively), the volume was completed to 250.0ml, then the stock solution was diluted 50 times to have the test concentration of 0.04mg/ml. The absorption of test solution was measured in triplicates for three consecutive days at λmax 360nm.

2.13 Stability of formulated Rutin 250mg Tablets:

The formulated tablet was stored at room temperature as well as at 40° C and analyzed periodically by using the developed analytical test method. The content of Rutin tablets was determined periodically through 150 days. The results are illustrated in chapter three**.**

Chapter Three

3. Results and Discussions:

3.1. Solubility of bulk rutin in different solvents:

The solubility of rutin of about 0.1g was dissolved in different solvent ratio. Rutin solubility was tested in absolute ethanol and methanol; but the solubility of ethanol was lower compared to methanol, the same result was also for acetone solvent and Isopropyl alcohol. Additionally, the best solubility of rutin was in methanol and also in THF, but the choice of THF solvent were excluded because of its high UV cutoff (220nm) compared by low UV cutoff for Methanol (205nm). The best solubility was in absolute methanol, it also shows high solubility in (methanol: water; 9:1) and we considered this aqueous ratio to reduce solvent volatility and to make solubility test applicable to dissolution test as the dissolution media will definitely have an aqueous media . Summary of results for equilibrium solubility of rutin in different weights and different solvents are shown in **(Table 3.1).**

Table 3.1: solubility of rutin in different ratios of methanol

Weight of bulk Rutin (g)	Solvents ratio Total volume (10ml)	Solubility
0.12	Methanol only	-
0.11	9 Methano:1 Water	+
0.12	8 Methanol:2 Water	++
0.11	7 Methanol:3 Water	+++
0.10	6 Methanol:4 Water	++++
0.11	5 Methanol:5Water	+++++
0.11	4 Methanol:6Water	++++++

Note: (-) No precipitation, (+) precipitation

3.2. Determination of maximum wave length absorption:

Figure (3.1) shows the spectrum of rutin solution of concentration 0.10mg/ml in the range of 200- 800nm. There are two absorption maxima, at 360 nm and at 260 nm. The absorption at near 200nm was excluded due to low accuracy at the range. Thus method development was mainly based on these two λ_{max}.

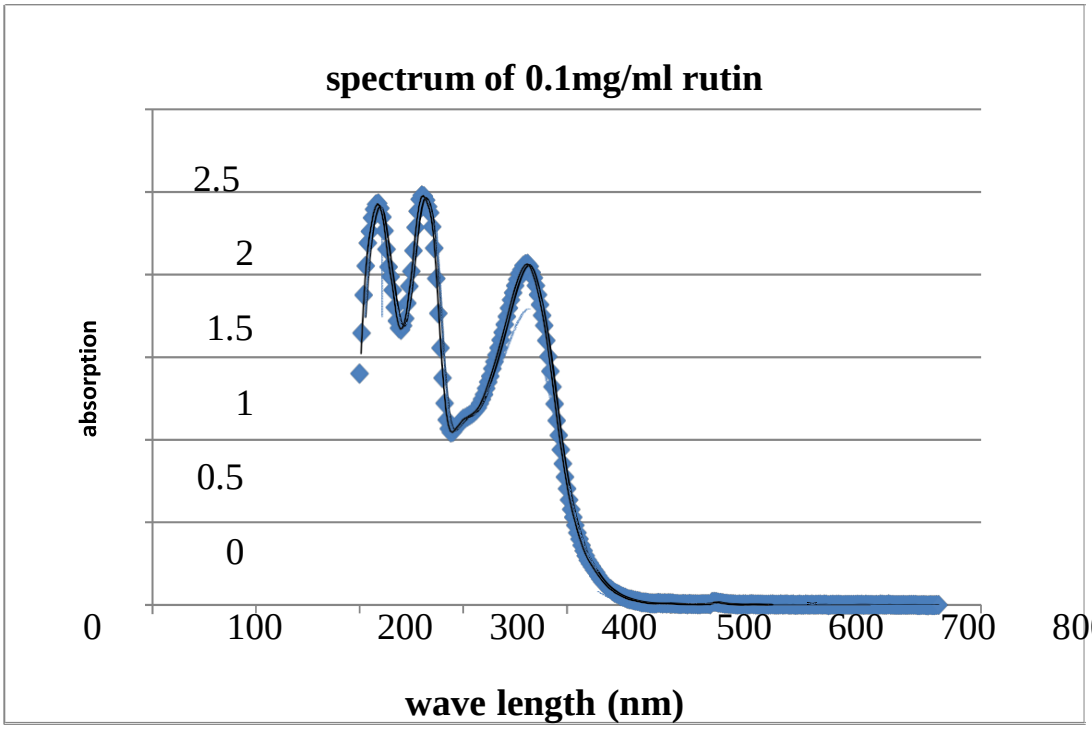


Figure 3.1: Spectrum of 0.10 mg/ml rutin in the range of 200-800 nm

The absorption maximum peaks were also tested using rutin dissolved in solution of different solvents. The peaks were then overlaid each other to see if any shift occur in the λ_{max}. The result clearly demonstrated that there is no shift in any of the detected λ_{max} as shown in **Figure 3.2**. These results demonstrate that the other solvent has no any effect on λ_{max}.

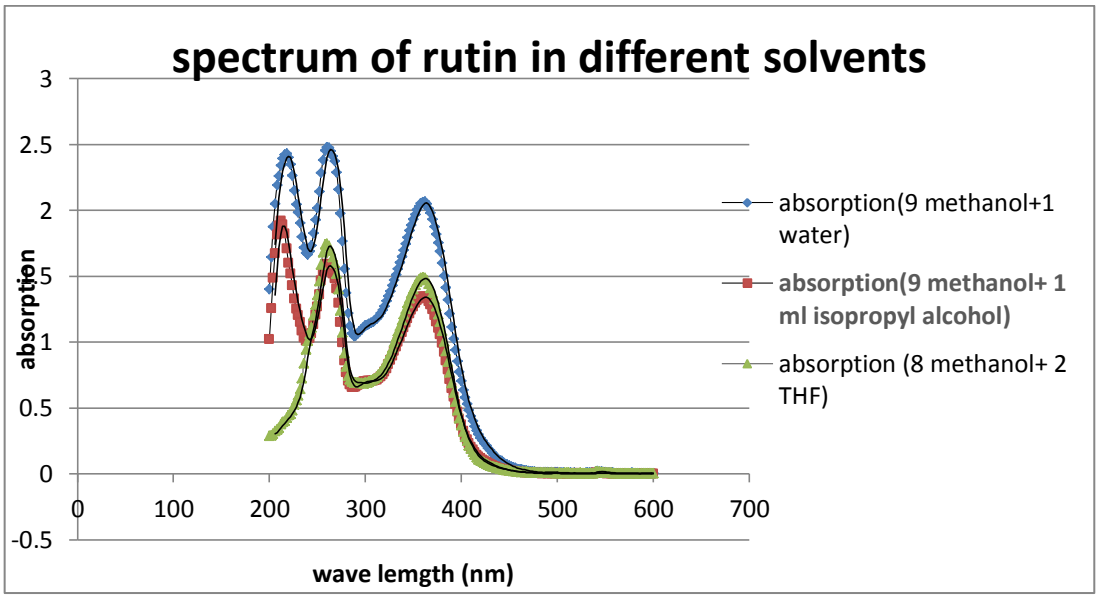


Figure 3. 2: Rutin spectrum in different solvents

To examine the probable effect of the excipients on the determined λ_{max}; the absorption of all expected excipients which will take part in the future formulation was mixed in (Methanol: Water; 9:1) and their absorbance was measured after filtration. The results show that the absorbance at the selected λ_{max} is negligible relative to rutin absorption at both λ_{max} namely 360nm & 260nm **(Table 3.2).**

Table3.2: absorption of excipients without rutin

Solution ingredient mixture	Solution concentration (gm/ml)	Absorption at 360nm	Absorption at 260nm
MCC	$7.2*10^{-6}$		
Acdisol	$4* 10^{-7}$		
Mg-stearate	$4* 10^{-7}$		
Aerosil	$4* 10^{-7}$	≈0.01	≈0.02

Moreover, the absorption of excipients along with rutin was measured at wave length of 360nm and 260nm and the absorption values are shown in **Table3.3**. The result clearly demonstrates that there is no interaction

between the excipients and rutin active ingredient nor it has any effect on the absorption λ_{max}.

Table 3.3: Absorption of rutin and excpients at 360 nm and 260 nm

Solution components	Solution concentration (gm/ml)	Absorption at 360nm	Absorption at 260nm
rutin,	$100*10^{-7}$		
MCC	$76*10^{-7}$		
Acdisol	$4*10^{-7}$		
Mg stearate	$4*10^{-7}$		
Aerosil	$4*10^{-7}$	0.26	0.14

3.3. Validation results:

3.3. 1. Linearity and range:

Eight serial solutions in the range of (0.008992- 0.08992 mg/ml) had been tested to study linearity and range of the method. The absorption of these serial solution at 360nm are shown in **Table 3.4** .

Table 3.4: The UV Absorption of the serial rutin solution

Concentration (mg/ml)	Absorption at 360nm
0.00899	0.27
0.01124	0.35
0.01798	0.50
0.03372	0.97
0.04496	1.18
0.07193	1.87
0.08430	2.16
0.08992	2.32

A calibration curve was plotted and examined for linearity over the concentration range (**Figure3.3).** The results demonstrate a linearity of the

method in the expected concentration range 0.00899-0.08992 mg/ml for the active ingredient rutin demonstrating its suitability for analysis. The curve was linear with a regression line equation of y = 25.035x + 0.0634. The goodness-of-fit (R^2) was also found to be 0.999 indicating a linear relationship between the concentration of analyte and its absorption.

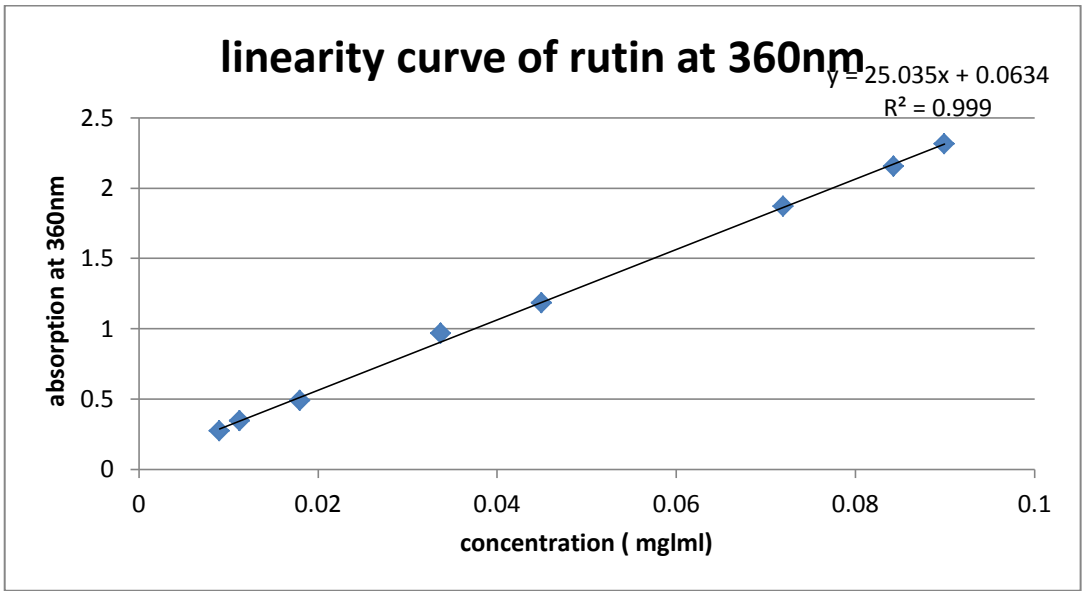


Figure 3.3: Linearity curve according to Beer`s Law

3.3.2 Accuracy and Precision:

The results of accuracy studies were done on both the formulated tablet and rutin active ingredient. The accuracy and precision were established on three concentrations around the test concentration (80%, 100% and 120%), three replicates test of each concentration was performed.

The results of the recovery of the rutin active ingredient when spiked at the concentration range (80-120%) around the test concentration are shown in **Table3. 5**. The results show that the recovery percentage with a good accuracy of (100±5%).

Table 3.5: Recovery for bulk rutin at 80, 100 and 120%

Weight of rutin powder (g)	Conc. of claimed solution (mg/ml)	Range around the test Conc.	Abs at 360nm	Recovered Conc. (mg/ml)	% Recovery
0.209	0.03344	80%	0.895	0.033	99.40
0.2506	0.040096	100%	1.12	0.0420	105.2
0.3057	0.048912	120%	1.192	0.047	96.3

The formulated tablets were prepared in a three concentration levels that are around the test concentration (80, 100 and 125%). The prepared solutions were tested for precision in three replicates and an intraday testing for three consecutive days. The results indicate that the method is precise and the % RSD was in the acceptable range (1.168%) and the accuracy of the prepared solution was 100±4% (**Tables 3.6**). The wider range of recovery can be attributed to the formulation rather than the analytical method but we believe that the recovery is of an acceptable range for our formulated food supplement which is allowed for a wider range compared to registered drugs.

Table 3.6: Recovery and intraday precision assay of home prepared formula of rutin250mg tablet

Day	Absorbance Sample No.			Average	%RSD	% Recovery
	1	2	3			
80%						
1	0.84	0.84	0.84	0.84	0	98.95
2	0.84	0.84	0.84	0.84	0	99.38
3	0.84	0.85	0.85	0.846	0.68	99.64
100%						
1	1.10	1.10	1.10	1.10	0	103.58
2	1.09	1.08	1.08	1.083	0.53	101.91
3	1.1	1.09	1.1	1.097	0.53	103.28
125%						
1	1.35	1.35	1.35	1.35	0	102.62
2	1.36	1.36	1.35	1.36	0.43	103.24
3	1.35	1.36	1.35	1.35	0.43	103
Mean					**101.73**	
SD					**1.188**	
RSD					**1.168**	

3.3.3. Method Specificity and selectivity:

The test for the specificity was carried out using only excipients. Spectra for placebo excipients blank, and sample were compared. The results showed the absorption of solution of placebo excipients as mentioned in **Table 3.2** at the wave length of 360 nm was 0.01. The specificity was also determined by subjecting the sample solution to forced degradation by keeping the sample under stress conditions for 2 days in order to verify that none of the degradation products interfered with the quantification of the drug. The results are shown in **Tables 3.11 (a-d)** in **Section 3.4.**

3.3.4. Ruggedness and robustness:

The ruggedness and robustness of the method were examined using some minor modifications listed in the table below. The result indicated that minor modifications to the experimental parameters did not affect the assay and its ability to accurately and precisely quantify rutin active ingredient. The robustness of the method was at first checked by performing the test by slight modification in the solvents composition. The results shown in (**Table 3.7)** demonstrate that after slight modification of the solvent composition and wave length still the method is valid and the assay of the rutin is within the acceptable range 100± 5

Table 3.7: Robustness with changes in analyst and solvent compositions

Variation on method	**Concentration (mg/ml)**	**Absorption at 360nm**	**%Assay**
9 ml Methanol:11 ml Water	0.04	S1: 1.01	94.59
		S2: 1.02	95.58
		S3: 1.02	95.58
		Average: 1.017	**95.29**
		RSD: 0.57%	
19 ml Methanol: 1 ml Water	0.04	S1: 1.01	94.59
		S2:1.02	95.58
		S3:1.03	96.58
		Average:1.02	**95.58**
		RSD:0.98%	

Moreover, the robustness of the method was checked by slightly changing the wave length ± 2 nm of the method measuring wavelength (360nm). The robustness was checked at three concentration levels (80%, 100% and

125%) of the method test concentration. The results in (**Table 3.8**) show good accuracy at the modified wave length

Table 3.8: Robustness at wave length 362 nm and 358 nm at recovery of 80,100 and 125%.

Wave length (nm)	Concentration (mg/ml)	Test Concentration level	Absorption	%Assay
362	0.03	80	0.83	97.93
	0.04	100	1.09	102.58
	0.05	125	1.33	101.40
358	0.03	80	0.84	98.70
	0.04	100	1.1	103.58
	0.05	125	1.34	102.36

3.3.5. Limit of detection (LOD) and limit of quantification (LOQ):

The LOD was calculated as stated in the methodology **Section 2.5.5**. The linear regression line equation was found to be $y = 25.035x + 0.0634$ with an ($R^2 = 0.999$) the standard deviation of the regression line was found to be 0.0329. The calculated LOD and LOQ as specified in the methodology section were $4.34*10^{-3}$ and 0.013 respectively **(Table 3.9**). Our assay test concentration was 0.04mg/ml and was above the LOQ.

Table 3.9: Results of LOD and LOQ.

Concentration mg/ml	Abs at 360nm	
0.045	1.182	**LOD=$4.34*10^{-3}$ LOQ=0.013**
0.034	0.965	
0.018	0.49	
0.011	0.345	
0.009	0.273	
0.090	2.315	
0.072	1.871	
0.084	2.155	

3.4 Stability of formulated rutin tablet and stability indicating study:

The formulated tablets that were stored in room temperature as well as those stored at 40°C were analyzed periodically through 150 days. The results indicate a stable tablet both under normal condition as well as under accelerated condition of 40°C. The result of the assay are shown in **(Table 3.10)**.

Table 3.10: Assay of rutin in Rutin 250 mg tablets stored at different storage condition versus time.

Storage period [Day]	Assay of Rutin [%]	
	At 40°C	At Room temperature
7	96.38	105.411
14	95.98	96.325
150	91.69	95.580

The stability of our formulated rutin tablet has been checked under different stress conditions these condition include the followings: 0.1N NaOH, 0.1N HCl, UV light (254 nm) and 0.3% H_2O_2. This stress test was also done to check the specificity and selectivity of the method. The result under each condition is shown separately in **Tables 3.11 (a-d)**.

Table 3.11 **-a: Stability of rutin under 0.1N NaOH.**

Time (hours)	Abs of 0.1N NaOH of 0.05 mg/ml at 360nm	% Assay
Zero time	0.92	63.68
one	0.87	66.88
2.25	0.88	65.28
3.25	0.86	63.68
25.25	0.691	50.18
26.25	0.687	49.86
27.25	0.68	50.1
28.25	0.68	49.3
47.92	0.62	44.51
48.92	0.62	44.51
49.91	0.62	44.51
50.92	0.62	44.51

Table3.11-b: Stability of rutin under 0.1N HCl

Time (hours)	Abs of of 0.1N HCl of 0.05 mg/ml at 360nm	% Assay
Zero time	1.17	88.45
One	1.18	89.25
2.2	1.17	88.45
3.2	1.15	86.86
25.2	1.16	87.65
26.2	1.16	87.65
27.2	1.16	87.65
28.2	1.16	87.65
34.75	1.17	88.45
35.75	1.17	88.45
36.75	1.160	87.65
37.75	1.16	87.65

Table 3.11-c: stability of rutin under 0.3%H2O2

Time (hours)	Abs of 0.05 mg/ml in 0.3% H_2O_2 at 360nm	% assay
At Zero time	1.37	104.43%
One	1.34	102.04%
2.08	1.27	96.44%
3.17	1.22	92.45%
18.42	1.09	82.06%
19.42	1.06	79.66%
20.42	1.07	80.46%
21.42	1.07	80.46%

Table 3. 11-d: stability of rutin under 254 nm UV light

Time(hours)	Concentration(mg/ml)	absorption	%assay
0.92	0.04	1.03	96.58
1.92	0.04	1.03	96.58

The results show that rutin tablets are only stable in the UV light, and slightly degraded in the H_2O_2 as the percentage assay has dropped from 104% to about 80%. However, the results demonstrate that instant degradation has occurred to the tablet after the addition of the alkaline and acidic solution to it, the assay was about 60-80%.

3.5 Weight variation:

Weight variation was also performed according to USP. The results of the weight variation of 20 tablets are shown in **Table 3.12.** According to the US Pharmacopeia the test will pass only if not more than two of the individual weights deviates from the average weight by ± 7.5% and none deviates by more than twice that percentage. Our results show that the variation for any of tested tablets was not more than 2.60 from the mean weight.

Table 3.12: Weight variation test

Tablet No	Weight of tablet (mg)	% Weight variation	Tablet No	Weight of tablet (mg)	% Weight variation
1	442.1	1.22	**11**	444.4	0.70
2	444.4	0.70	**12**	446.2	0.30
3	446.2	0.30	**13**	448.4	0.19
4	446.1	0.32	**14**	445.9	0.37
5	447.7	0.03	**15**	445.4	0.37
6	446.9	0.15	**16**	459.2	2.60
7	446.8	0.17	**17**	448.3	0.17
8	448.5	0.21	**18**	450	0.55
9	455	1.66	**19**	444	0.79
10	451.5	0.88	**20**	444	0.79
Average weight= 447.55 mg					

3.6 Content uniformity test:

The uniformity content test was performed according to the USP. The result of the content uniformity of 10 tablets were chosen randomly and the assay test was performed as in **Table 3.13** According to the pharmacopeia the test will pass if the relative standard deviation (RSD) is ≤ 15 and no value is outside 85-115%. The test fails if one or more values are outside75-125%.

Our result show that RSD value of the assayed tablets was 1.05% and no tablet % assay was out the limit 85-115%. The results clearly demonstrate that our formulated tablets comply with the content uniformity test.

Table 3.13: Content uniformity results

No	Weight (g)	% Assay
1	0.450	99.7
2	0.460	100.0
3	0.450	97.7
4	0.440	95.0
5	0.450	96.7
6	0.440	93.9
7	0.440	93.5
8	0.450	97.3
9	0.450	97.1
10	0.450	97.7
Mean value	**0.450**	**96.8**
SD	**0.0063**	
RSD	**1.05%**	

3.7 Dissolution Profile:

Drug dissolution testing is a routine test that gives information about the drug release to assess the quality of the dosage form. It is also an essential test in the drug development to predict an in vivo drug release profile.

The USFDA recently included the similarity and dissimilarity factors f2, f1 respectively in its various guidance documents and stated different criteria for dissolution profile comparison. The dissolution profile comparison is done prior to in vivo study, to compare an in vitro dissolution profiles using f1 and f2 factors as a surrogate data. The similarity factor was computed from the average mean of the dissolution data. Similarity factor (f2) of 50-100 indicates similarity of two tested products. However, a difference factor (f1) of f 0-15 ensures minor difference between two products [52].

At first the dissolution profile for the three tablet formulations developed in our research lab was performed. These formulations were designed to have different release profiles. The three locally formulated tablets were tested for dissolution in the selected dissolution medium phosphate buffer pH 6.8. The results clearly demonstrate that formula 1 has the best dissolution among three formulations (**Table 3.14 & Figure 3.4).** Thus in our study we selected forrmula1 for our tablet for further study , including the shelf, accelerated and stress stability study. The results show a moderate dissolution (26.7%) after 45 minutes, but it reaches a plateau after approximately 20 minutes. The low dissolution of the tablet was expected due to the low solubility of the rutin in aqueous media. The results also clearly demonstrate that formula 1 has the best dissolution profile among the other three formulations. Moreover, formula1 was superior to the locally and internationally marketed Rutin® 500mg tablets of Solgar (the results are shown in later figures).

Table 3.14**: Dissolution profile of three local formulations at the dissolution medium pH6.8 using apparatus 2 at 50rpm.**

Time(min)	%dissolved of formula1	%dissolved of formula2	%dissolved of formula3
0	0±.00	0.0±0.00	**0**.0±0.00
5	11.3±0.55	9.5±2.02	7.0±0.77
10	16.5±0.68	14.0±1.58	11.6±0.74
15	20.7±0.14	18.3±1.80	15.2±1.80
20	22.7±0.36	20.7±1.35	17.9±1.22
25	24.3±0.27	22.0±1.36	19.8±1.46
30	25.5±0.61	23.0±1.26	20.8±1.16
35	26±0.62	23.9±1.01	21.9±0.85
40	26.6±0.49	24.4±0.10	22.7±0.63
45	26.7±0.50	24.8±0.09	23.4±0.53

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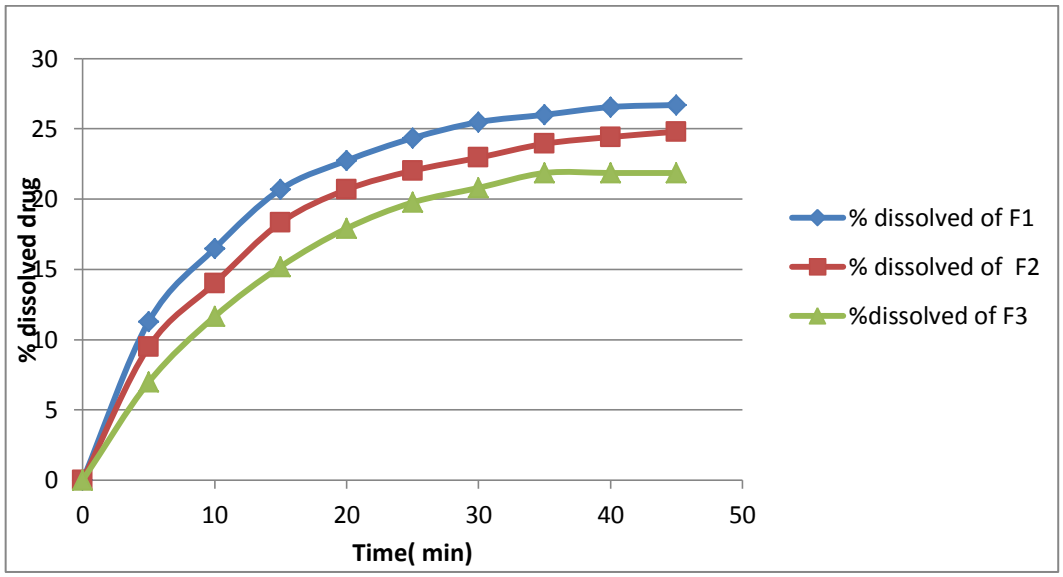

Figure3.4: Dissolution profile of three local formulation at the dissolution media pH6.8

In order to test the discriminatory dissolution profile of the other dissolution media; formula1 was tested in a three different dissolution media namely; phosphate buffer 6.8, phosphate buffer 4.5 and 0.1N HCl. The similarity and dissimilarity factors f2 and f1 were then calculated and the summary results are shown in **Table 3.15.** The results show similarity profile between the three medium.

Table 3.15: Summary of similarity and dissimilarity factor of formula1 in three different media.

	Formula1		
	Dissolution medium		
	0.1N HCl	**pH 4.5 phosphate buffer**	**pH 6.8 phosphate buffer**
f2	66	65	55
f1	52	48	57

Furthermore, the dissolution test for the formulated rutin tablet (formula 1) and for marketed Rutin® tablet of Solgar were tested using phosphate buffer of pH 6.8. The results in (**Table3.16 & Figure 3.5**) show slightly higher dissolution for the formula 1 tablet compared to Rutin® tablets

marketed by Solgar. The similarity factor (f2) and dissimilarity factor (f1) of their dissolution profile was calculated. The result of the dissolution for formula1 and the Rutin® tablets of Solgar revealed a similarity factor (f2) of 55. Moreover, we statistically tested if there is any statistical difference between the two dissolution profiles using SPSS-16 statistical package program and the results revealed that there was no statistical difference (P>0.05). However, the high dissimilarity (f1) value (57) is still confusing; but can be explained by big difference seen in the beginning of the dissolution profile before it reaches the plateau. The overall results show that the dissolution of our formulated tablets is slightly better marketed Rutin® tablets of Solgar tablet.

Table 3.16: Dissolution of formula 1 compared to Rutin® tablets of Solgar at pH 6.8

Time (min)	% Dissolved of Rutin® Solgar at phosphate buffer PH 6.8	% Dissolved of formula1 at phosphate buffer PH 6.8
0	0±0.00	0±.00
5	6.8±0.96	11.3±0.55
10	9.9±1.23	16.5±0.68
15	12.5±1.15	20.7±0.14
20	14.3±0.71	22.7±0.36
25	15.3±0.57	24.3±0.27
30	16.1±0.41	25.5±0.61
35	19.0±0.84	26±0.62
45	18.2±0.88	26.7±0.50
f2=	55	
f1=	57	

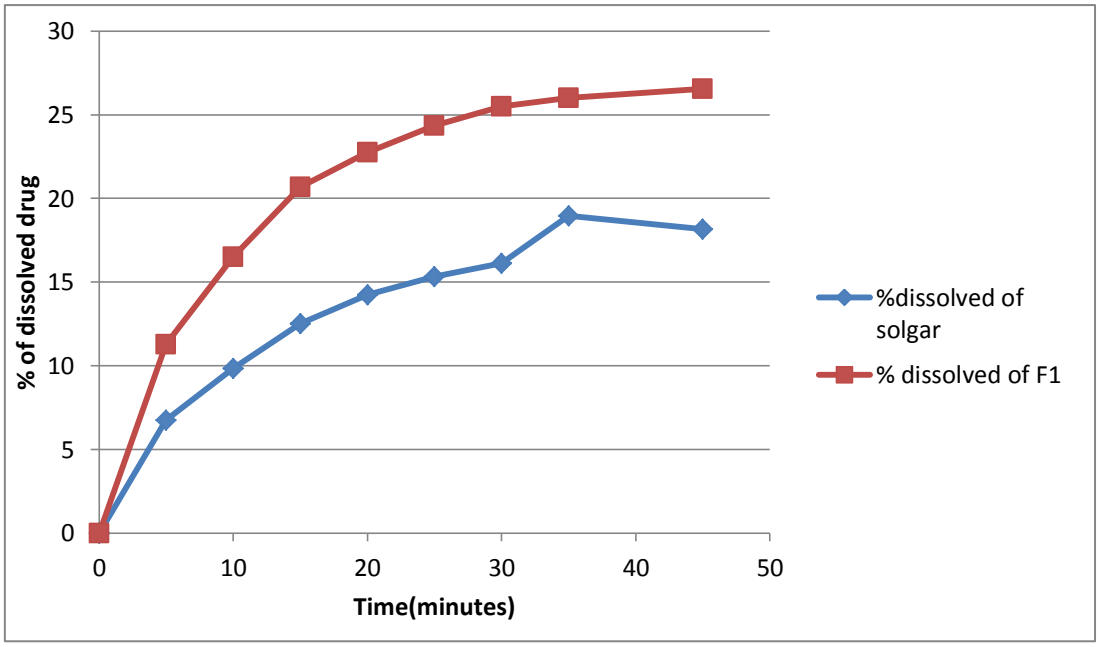

Figure3.5: Dissolution profile for formula1 and Rutin® of Solgar at pH 6.8

Additionally, the dissolution profile was checked in 0.1N HCl dissolution media and the results are shown in (**Figure3.6 & Table3.17**). The result again demonstrate high similarity (f2) value of (66) and the SPSS statistical testing show no significant difference (P>0.05).

Table 3.17: Dissolution of formula1 and Rutin® of Solgar at 0.1N HCl

Time(min)	%dissolved of Rutin® of Solgar at 0.1 N HCl	% dissolved of formula1 at 0.1 N HCl
0	**0.00**±0.00	**0**.00±0.00
5	4.60±1.16	9.65±0.41
10	7.47±0.48	11.61±0.27
15	8.73±0.21	13.24±0.35
20	9.56±0.26	14.23±0.30
25	10.13±0.24	15.00±0.43
30	10.52±0.19	15.60±0.28
35	10.91±0.16	15.90±0.20
40	11.33±0.24	16.23±0.17
45	11.53±0.28	16.63±0.24
f2=	**66**	
f1=	**52**	

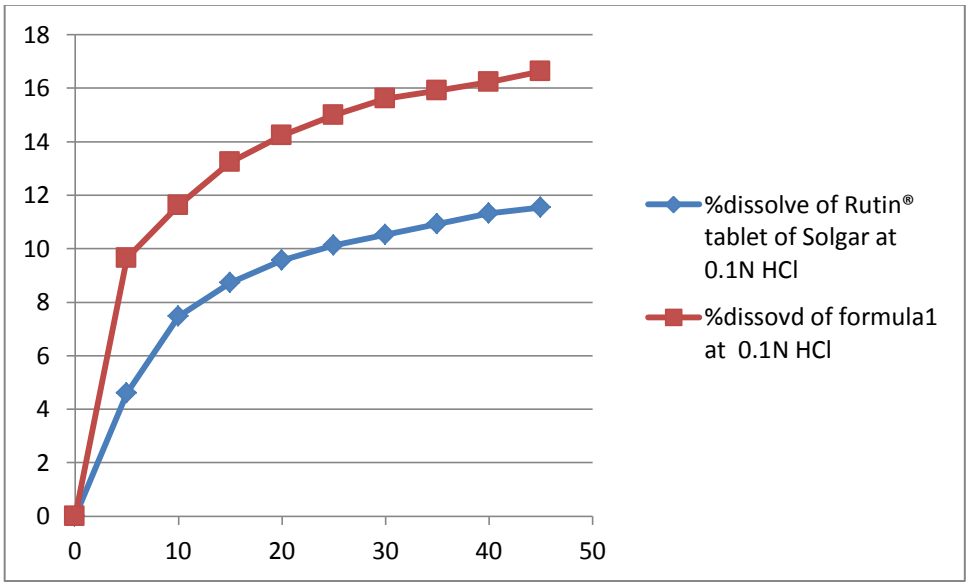

Figure 3.6: Dissolution profile of formula1 and Rutin® of Solgar at 0.1NHCl

Again the dissolution profile for the formulated tablets (formula1) and the Rutin® of Solgar were also tested at phosphate buffer pH 4.5. The result also was similar to previous results of the other dissolution media showing

a slightly higher dissolution for our locally formulated tablets (**Figure 3.7 and Table 3.18)**.

Table3.18: Dissolution of formula1 and Rutin® 500 Solgar at pH 4.5 Phosphate buffer

Time(min)	%dissolved of Rutin® of Solgar at 4.5 PH phosphate buffer	% dissolved of formula1 at 4.5 PH phosphate buffer
0	0±0.00	0±0.00
5	6.30±0.27	8.30±0.78
10	8.33±0.25	12.07±0.36
15	9.18±0.31	13.95±0.66
20	10.24±0.31	15.41±0.67
25	10.84±0.28	16.27±0.70
30	11.21±0.27	16.97±0.64
35	11.54±0.39	17.3±0.60
f1=	48	
f2=	65	

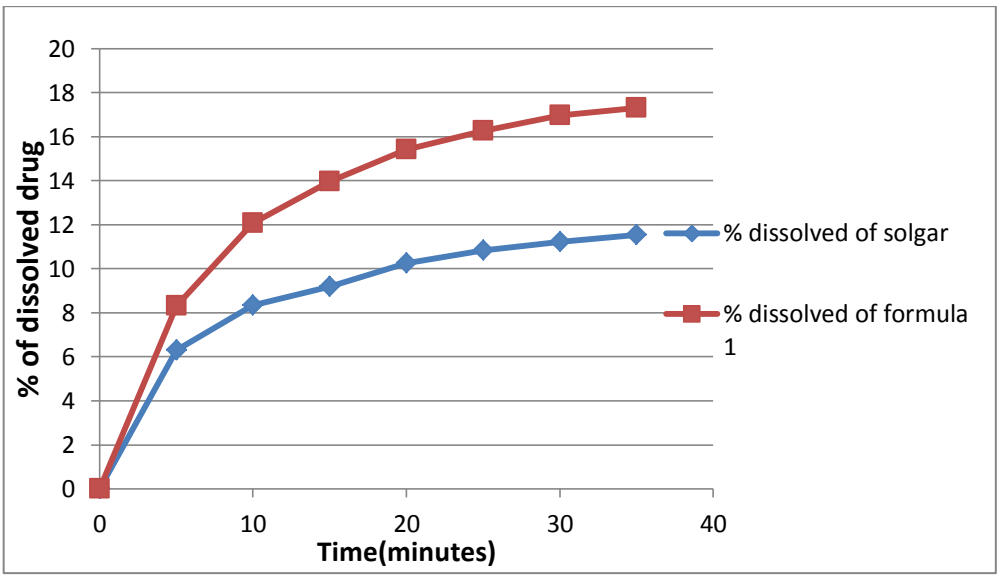

Figure 3.7: Dissolution profile for formula1 and Rutin® 500 Solgar at 4.5PH phosphate buffer

3.8 Determination of the tablet specification of the locally formulated tablets:

Some of the physical parameters of our locally formulated including the disintegration, hardness, thickness and diameter was determined as a tablet specification.

3.8.1 Disintegration test

The disintegration of the tablet was performed using USP specified disintegration apparatus. The tablets were placed in the specified baskets and observed for complete disintegration. The formulated tablets were seen to disintegrate totally after 4 minutes.

3.8.2 Hardness, thickness and diameter of the formulated tablets:

The tablets were tested for its hardness, thickness and diameter simultaneously using Erwecka multi check instrument. The average value of the tested parameters will be considered as our tablet specification. **Table 3.19** show the detailed data of the tested parameters.

Table 3.19: Hardness, diameter and thickness of the formulated tablet.

No	Weight (mg)	Thickness (mm)	Hardness (N)	Diameter (mm)
1	444.3	2.30	260	13.08
2	450.9	2.34	249	13.02
3	443.6	2.29	243	13.01
4	446.9	2.35	234	12.99
5	445.9	2.32	228	13.01
6	440.7	2.30	227	13.08
7	447.0	2.29	251	13.03
8	447.5	2.32	282	13.09
9	446.0	2.29	270	13.00
10	440.7	2.30	284	12.99
Average	445.4	2.31	253	13.03
Min	440.7	2.29	227	12.99
Max	450.9	2.35	284	13.09

3.9 Quality control of Rutin® 500mg Solgar tablet:

To examine if our validated method can be used as an assay quality control method to test various rutin formulation available in the local and international market; we performed an assay testing on Rutin® tablet of Solgar and the results are summarized in **Table 3.20.**

Table3.20 Percentage assay of Rutin® tablets of Solgar.

Day	Absorbance Sample No.			Average	% Recovery Assay (%)
	1	2	3		
1	1.1	1.09	1	1.06	99.58
2	1.02	1.02	1.1	1.05	98.28
3	1.1	1.02	1.07	1.06	99.91
Mean					99.26
SD					0.86
%RSD					0.87

Chapter Four

4. Conclusion and Future work

GMP and analytical method validation are very important not only for human manufactured drugs but also for food supplements and herbals as they are consumed by human. Herbals and food supplements take little care and regulations internationally and locally.

In this study; we developed tablet formulation of rutin 250mg in our research labs, and we developed a validated method for analysis and quantification of rutin in our formulated tablets. The method is also applicable to the international and other rutin containing tablets available in the market.

The solubility of rutin was examined in different solvents and then the maximum absorption of rutin was determined and found to have a λ_{max} of 360nm.

All excipients used in tablet formulation showed no interference with the λ_{max} absorption of rutin in the tablet. The method showed good linearity, accuracy, precision and specificity. The dissolution profile of the formulated tablet and Rutin® of Solgar was studied in three dissolution media of 0.1N HCl, Phosphate buffer PH 4.5 and 6.8. The dissolution results clearly demonstrate a slightly higher dissolution profile for the formulated tablet compared to Rutin® of Solgar. This is probably due to differences in formulation composition and method of preparation of formulated rutin compared to that of marketed rutin.

Stability indicating study and accelerated studies were performed on our formulated tablet in different stress conditions including: acidic, basic,

hydrogen peroxide, and UV light. The results showed the solution was stable in UV light and a slight degradation was observed in 0.3% H_2O_2. Meanwhile, instant degradation happened in acidic and alkaline conditions but the degradative compounds probably have no interference with the selected λ_{max}. The shelf and accelerated stability were also performed on the formulated tablet and showed that the formulated tablets are stable for at least 150 days, if stored at room temperature or even at 40°C.

Some physical parameter tests were performed on the formulated tablets; these include the content uniformity and weight variation, results were within the acceptance criteria of USP. Additionally, the disintegration, hardness, thickness and diameter of the formulated tablets were determined and included in the tablet specification certificate.

Finally, this study highly recommends a simple ,validated analytical method for herbal and food supplements manufacturers in Palestine for use in the registration and quality control of their products.

The future work is summarized as following:

1. The dissolution method development and validation of Rutin need to be studied in more details
2. The formulatrd tablet need more modification to enhance the solubility of rutin tablets, for example by adding some surfactants
3. The rutin active ingredient can be chemically modified in order to enhance its solubility
4. To do more stability study of formulated tablets in its final packaging.

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جامعة النجاح الوطنية

كلية الدراسات العليا

الإستحداث والتثبت من طرق تحليل وذوبان مادة الروتين المطورة كأقراص صيدلانية في مختبراتنا البحثية

إعداد

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إشراف

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قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الصيدلانية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس- فلسطين.

2015

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الملخص

يمكن تصنيف مادة الروتين كفلافونات، كما ويصنف ايضا من ضمن المواد الملونة والفيتامينات. يتوافر بكثرة في معظم الاطعمة والفواكه والخضراوات والمشروبات ذات الاصل النباتي. لقد جذبت هذه الماده نظر الباحثين وذلك بسبب ماتملكه من اثار طبية مما جعلها مستخدمة كعلاج في مختلف الامراض. يتوافر الروتين في مختلف الاشكال الدوائية التي تعطى عن طريق الفم، اما على شكل كبسولات أو اقراص واما لوحدها او مع الاشتراك مع غيرها من المواد الفعالة. تتوافر مادة الروتين في عدة مستحضرات صيدلانية على نطاق واسع في مختلف الاسواق الدولية وكذلك في السوق الفلسطيني.

لقد قمنا في هذه الدراسة بصناعة اقراص صيدلانية من مادة الروتين وبعيار 250ملغم وتم تطوير طريقة تحليل سهلة وبسيطة للتحقق من كمية مادة الروتين الموجودة لدينا في الاقراص التي تم تصنيعها في مختبراتنا البحثية وكذلك مقارنتها بتلك الموجودة في الاسوق الدولية والمصنعة من قبل شركة سولجار والمسماة بروتين 500ملغم. وقد تم التحقق من صحة الطريقة وفقا للمبادئ التوجيهية الدولية لل ICH وكذلك حسب ال USP.

تم فحص ملف سرعة الذوبان للاقراص التي تم تصنيعها في مختبراتنا البحثية وكذلك تم دراسة حياة الرف و الدراسات الثباتية المتسارعة لنفس الاقراص المصنعة في مختبراتنا البحثية.

واظهرت النتائج ان الطريقة المطورة هي طريقة صالحة وجيدة من ناحية Linearity وكذلك Accuracy والدقة.وكانت طريقة التحقق حساسة، حيث كانت اقل كمية يمكن تحديدها(LOD) واقل كمية يمكن قياسها كميا (LOQ) هي 4.34*10^−3 و 0.013ملغم\مل على التوالي.

اظهرت النتائج ان الاقراص التي تم تصنيعها في مختبراتنا البحثية كانت مستقرة في ظروف الدراسات الثباتية المتسارعة وكذلك على درجة حرارة الغرفة لمدة 150 يوم فقط مع انخفاض طفيف وتحمل في مقايسة٪ مع عدم وجود تأثير ضار على الخصائص الفيزيائية للأقراص. كما واظهرت النتائج ايضا ان ملف سرعة الذوبان للاقراص التي تم تصنيعها في مختبراتنا كانت افضل واسرع قليلا في محلولPhosphate buffer مقارنة بتلك المصنعة من قبل شركة سولجار.

وتشجع هذه الدراسة الشركات والمصانع المصنعة للمنتجات العشبية والمكملات الغذائية وبخاصة تلك الموجودة في فلسطين من اجل تطوير وتحسين المنتجات العشبية وتطبيق الاساليب التحليلية المحققة وذلك للتأكدمن صحة وجودة منتجاتها.

وفي الختام، نجحنا في تطوير طريقة تحليل محققة وذلك للتأكد من صحة وجود وكمية الروتين الموجودة في الاقراص المصنعة محليا وكذلك تركيبات الروتين المتاحة والموجودة في الأسواق المحلية والدولية. وأظهرت الدراسة بأن الاقراص التي تم تصنيعها في مختبراتنا البحثية كانت لديها سرعة ذائبية افضل من تلك التي لدى شركة سولجار وكانت الاقراص المصنعة في مختبراتنا البحثية مستقرة في الحالات العادية، وكذلك تحت ظروف الإجهاد.