Palestinian Medical and Pharmaceutical Journal

Volume 2 | Issue 1 Article 6

3-2-2017

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Recommended Citation

Abu alhasan, Murad; Qutob, Tasneem; Obedat, Sana; Yahyia, Dania; and G Watson, David (2017) "Dissolution Method Development and Validation of Rutin Tablet," *Palestinian Medical and Pharmaceutical Journal*: Vol. 2 : Iss. 1 , Article 6.
Available at: https://doi.org/10.59049/2790-0231.1042

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Abstract

Rutin is a flavonoid glycoside that has shown wide range of pharmacological applications with various beneficial medical effects. Rutin is poorly soluble in water and could limit its absorption from the gastrointestinal tract. The aim of this project was to develop and validate a simple UV analytical method for the evaluation of Rutin release from tablet dosage form. The method was developed by testing solubility of Rutin in different concentrations of sodium lauryl sulfate. The dissolution method was then validated in accordance with international guidelines. The results showed that the best dissolution was achieved in phosphate buffer pH 6.8 containing 3% SLS. The percent released was almost 100% after 55 minutes. The developed method was found to be linear, precise and accurate in the range (0.04-0.1mg/ml). The analytical method was also found to be selective for Rutin. In conclusion we successfully developed a dissolution method that is easy and feasible. The validated analytical dissolution method could be used by quality control labs and could be adopted by the official international pharmacopeias.

Keywords

Rutin, Dissolution, Validation, Tablets

Palestinian Medical and Pharmaceutical Journal (PMPJ). 2017; 2(1): 55-62

Dissolution Method Development and Validation of Rutin Tablet

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Received: (21/11/2016), Accepted: (2/3/2017)

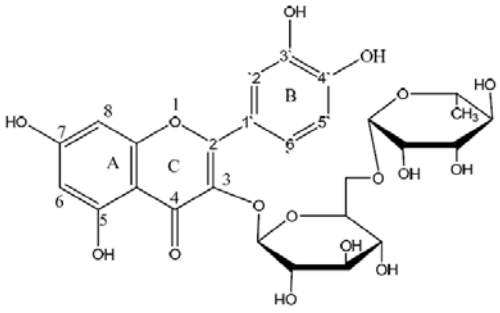
ABSTRACT

Rutin is a flavonoid glycoside that has shown wide range of pharmacological applications with various beneficial medical effects. Rutin is poorly soluble in water and could limit its absorption from the gastrointestinal tract. The aim of this project was to develop and validate a simple UV analytical method for the evaluation of Rutin release from tablet dosage form. The method was developed by testing solubility of Rutin in different concentrations of sodium lauryl sulfate. The dissolution method was then validated in accordance with international guidelines. The results showed that the best dissolution was achieved in phosphate buffer pH 6.8 containing 3% SLS. The percent released was almost 100% after 55 minutes. The developed method was found to be linear, precise and accurate in the range (0.04-0.1mg/ml). The analytical method was also found to be selective for Rutin. In conclusion we successfully developed a dissolution method that is easy and feasible. The validated analytical dissolution method could be used by quality control labs and could be adopted by the official international pharmacopeias.

Keywords: Rutin, Dissolution, Validation, Tablets.

INTRODUCTION

Rutin is a yellow crystalline rhamnoglucoside of the flavonoid quercetin, it is very slightly soluble in water (1); each 12.5 mg of Rutin is soluble in 100 ml of water (2-4). The chemical formula of Rutin is $C_{27}H_{30}O_{16}.3H_2O$ and its chemical structure is shown in Figure 1.



$3.H_2O$

Figure (1): Chemical structure of Rutin.

Rutin is abundantly found and distributed in plants such as in buckwheat and various fruits (5, 6). Rutin is considered a non-toxic molecule and has advantage over other flavonoids as it behaves as pro-oxidant agent and catalyzes oxygen production([6). The main disadvantage of the molecule is its poor solubility in aqueous media, explaining its poor oral or topical bioavailability and being a drawback to its conversion in adequate dosage forms(7).

Rutin proved to have many pharmacological properties such as antitumor, myocardial protective, antihypertensive effects, and has been used in the treatment of peripheral vascular diseases because of its vascular-protective properties (8, 9).

Dissolution testing is used mainly in the development stage of drug products to guide development of new formulations, to assist in proper selection of excipients and to optimize the manufacturing process for optimization of the therapeutic effectiveness, stability assessment, and in quality control purposes in order to ensure uniformity between production lots (10). Dissolution of the active pharmaceutical ingredient (API) is affected by several factors such as the medium in which the drug is being dissolved, the temperature of the medium, the dissolution apparatus and the affinity for the solid particles for the dissolution medium. In the development of a dissolution procedure these components must be properly chosen and developed to provide

a method that is accurate, precise and reproducible in the laboratory (11-13).

Developing dissolution test methods for poorly water-soluble drug products has been an important task to formulation scientists and is of particular importance, where the absorption is dissolution-rate limited (BCS class II drugs). At the same time, development of a dissolution method for this group of drugs is very challenging; difficulties are usually encountered in selecting a dissolution medium of acceptable volume and composition as well as a good discrimination power. General strategies to enhance their dissolution patterns rely upon either optimizing the dissolution medium pH, or adding solubilizers such as surfactants (14, 15). The types of surfactants that were used in the dosage form to increase the dissolution include anionic, cationic and nonionic forms. Moreover, surfactants influence tablet disintegration rates, producing a finer dispersion of disintegrated particles with a correspondingly larger surface area for drug dissolution (14, 16).

The objective of this study is to develop a simple and feasible dissolution method to quantify dissolved Rutin of commercially available Rutin tablet. The solubility of Rutin will be enhanced using anionic surfactant. The percentage of the surfactant and dissolution medium will be optimized to have the best Rutin solubility. The method will then be validated to demonstrate that the test procedure is suitable for its intended purpose. Validation of a dissolution method will take into account the validation parameters including: specificity, accuracy, linearity, precision and robustness. The validation process must follow the ICH guidelines (13, 17, 18).

MATERIALS AND METHODS

Materials, Chemicals and Reagents

Rutin trihydrate powder (99%) was purchased form (Sigma Aldrich-Germany), sodium lauryl sulfate (SLS) surfactant was purchased form (Sigma Aldrich- USA). Potassium dihydrogen phosphate was purchased form (Alfa Aesar- Germany). The other entire reagents used were of analytical grade and were purchased form reliable resources these reagents include: Sodium Dihydrogen Phosphate, Sodium hydroxide, Sodium Acetate, Glacial Acetic Acid and HCl 37% solution. Freshly deionised water was used throughout the study. Product Solgar tablets containing 500mg Rutin per tablet was purchased from local community pharmacy. In-house prepared Product Rutin Tablets, containing 500mg of Rutin were prepared in our research laboratories, the following excipients were used in the tablet formulation: Colloidal Silicon Dioxide, Croscarmellose Sodium and Microcrystalline Cellulose. All these tablet excipients were given as a gift from Jerusalem Pharmaceuticals Company-Palestine.

Instrumentation

The following instruments were used in this research project: UV/visible Spectrophotometer (JENWAY-Model7315) using 10mm quartz cells was used for all absorbance measurements. Six-station Paddle Dissolution Tester (HSIANGTAI, DT-6) in accordance with USP general methods, pH meter (JENWAY -3510), hotplate stirrer (LabTech, ES35A), analytical balance (Nevada Weighing, Radwag- AS 220.R2).

Solutions preparation

All solutions used throughout the research project; phosphate buffer (pH 6.8), 0.1N HCl and acetate buffer pH 4.5. were prepared according to directions in USP monograph (19).

Dissolution development procedure

Determination of λ max absorption

At first the λ max absorption was determined using UV-Vis spectrophotometer by scanning Rutin in the range of 200nm-800nm.

Determination of saturation solubility and sink conditions

In the early stages of dissolution method development, it is of important to characterize the medium to be chosen properly in order to evaluate the performance of the dosage form. Solubility data were used as a basis for the selection of a dissolution medium for Rutin. The saturation solubility of Rutin was determined at 37° in different media and expressed as mg/mL. The term sink conditions

(20) is defined as the volume of medium at least three times greater than that required to form a saturated solution of drug substance. Sink conditions were determined in the following media: 0.1N HCl, pH 4.5 acetate buffer, pH 6.8 phosphate buffer, pH 6.8 phosphate buffer containing 3.0% of Sodium Lauryl Sulphate (SLS) and water containing different concentrations of SLS (i.e., 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 4.0%, and 5.0%). Experiments were conducted in triplicate. Excess amount of Rutin was added in each medium in a 10-ml screw-capped vial. The vials were shaken continuously in a shaker for 24 h at 37°. The solutions were kept aside at the same temperature for 4 h until equilibrium was achieved. The equilibrated samples were immediately filtered through 0.45 µm polyamide filter and analyzed spectrophotometrically at 360 nm after appropriate dilutions with medium.

Dissolution release profile comparison of formulated Rutin

The dissolution tests were carried out on both a commercial Rutin 500mg tablet from Solgar, and in-house prepared Rutin 500mg Tablets by employing USP Apparatus II at 37±0,5°. Each dissolution test was performed in triplicate. In each dissolution testing a paddle speed of 100 rpm, different dissolution media (water, phosphate buffer pH 6.8 and phosphate buffer pH 6.8 containing 3.0% SLS) with 900ml of were used.

Sampling aliquots of 10 ml were withdrawn from each vessel and the same volume of the dissolution medium was replaced to maintain a constant total volume of 900 ml. The times schedule of sampling were every 5 minutes for the first hour then every 10 minutes for the next half hour and the last sample was collected after 30 minutes. After the end of each test time, sample aliquots were filtered through 0.45µm polyamide membrane filter, diluted with respective dissolution medium, when necessary and analyzed spectrophotometrically.

Method Validation

In this present research work, the developed UV spectrophotometric dissolution method was validated as per ICH guidelines(17). Different validation parameters were examined in this dissolution development method these validation parameters include: specificity, linearity, range, accuracy, and robustness.

The specificity parameters of the dissolution method were performed by examining the absorbance of the placebo solution which consisted of a solution of phosphate buffer pH 6.8 with 3% SLS and all the excipients of the formulated Rutin. The placebo concentrations were determined based on the Handbook of Pharmaceutical Excipients and calculated for an average weight of Rutin tablet content (21). The excipients per Rutin tablet were as follows: aerosol (2mg), AcDiSol® (4mg), MCC(72mg) and magnesium stearate (2mg).

Linearity was tested across a range of 5 concentrations and was evaluated from the linearity plot by examining the square of the correlation coefficient (R^2).The linearity of our developed method was examined by preparing a series of Rutin standard solutions (0.04,0.05, 0.06, 0.08, and 0.1 mg/ml).The absorbance of the tested solutions was plotted against their concentration. The regression line equation and the R^2 value were examined to check the linearity of the method.

The accuracy was performed by measuring the recovery of known amounts of Rutin in the dissolution vessels. Three concentrations (20%, 100% and 120) of the theoretical Rutin concentration were spiked and the measurements were done in triplicate. The average recovery percentage was calculated.

Precision of the method was determined by measuring the repeatability, intraday precision and inter-day precision. The analytical solution of three concentration levels (20%, 100% and 120%) was measured six times on three different days. The precision of the method was determined by measuring the repeatability. (intra-day precision) in the same day and the intermediate precision (inter-day precision) in two different days, all are expressed as RSD (%). The precision of the data between days was demonstrated by ANOVA statistical testing(22).

The robustness of the analytical procedure is the measure of its capacity to remain

unaffected by small deliberate variations in parameters internal to the procedure. We intentionally varied three parameters: the pH of the buffer, the absorbance, and analyst. The phosphate buffer pH 6.8 was intentionally varied by ±0.2 pH units. Rutin solution in phosphate buffer 6.8 containing 3% SLS was measured at three different wavelengths: 358nm, 360nm, and 362nm. Lastly the absorbance of Rutin solution was measured by two different analysts. The robustness of the method was examined by calculating the %RSD and applying an ANOVA statistical test.

RESULTS

Dissolution method development

The UV-Vis spectra of the Rutin solution revealed two absorption maxima, one was in the UV region (255nm) and the other was in the visible region (360nm) (Figure 2).

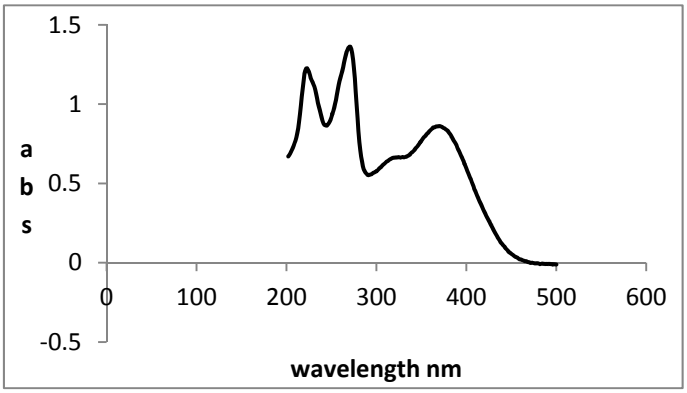


Figure (2): Absorption spectrum of Rutin 2% aq. Solution in the range of 200-500 nm.

The solubility results of Rutin in different proposed dissolution media are summarized in Table 1. The data demonstrated that the sink condition is provided by using Phosphate buffer containing 3% SLS in which the concentration of the dissolved Rutin was 1.690mg/ml.

Table (1): Solubility results of Rutin in different proposed dissolution media.

Medium	Solubility of Rutin (mg/ml)	Sink condition (solubility >1.667mg/ml)*
Water	0.16	No
Water containing 0.5% SLS	0.336	No
Water containing 1.0% SLS	0.047	No
Water containing 1.5% SLS	0.636	No
Water containing 2.0% SLS	0.770	No
Water containing 2.5% SLS	0.936	No
Water containing 3.0% SLS	1.635	Almost
Water containing 4.0% SLS	1.539	Almost
Water containing 5.0% SLS	1.619	Almost
0.1N HCl containing 3.0% SLS	0.720	No
Acetate Buffer pH 4.5 containing 3.0% SLS	1.080	No
Phosphate Buffer pH 6.8 containing 3.0% SLS	1.200	No
Phosphate Buffer pH 6.8 containing 3.0% SLS	1.690	Yes

* Calculated from 500mg/900 ml x 3 = 1.667mg/ml.

Dissolution release profiles comparison

The dissolution profile results showed a significant difference in the dissolution profile of Rutin 500mg (in house prepared) tablet in the three dissolution media namely: Distilled water, Phosphate buffer pH 6.8, and 3% SLS in phosphate buffer pH 6.8 media.

The results showed that the best dissolution was achieved in phosphate buffer pH 6.8 containing 3% SLS because the sink condition was provided. The percent released was almost 100% after 55 minutes, while in water alone and in the buffer alone did not exceed 28.06, and 37.82 % respectively after two hours (Figure 3).

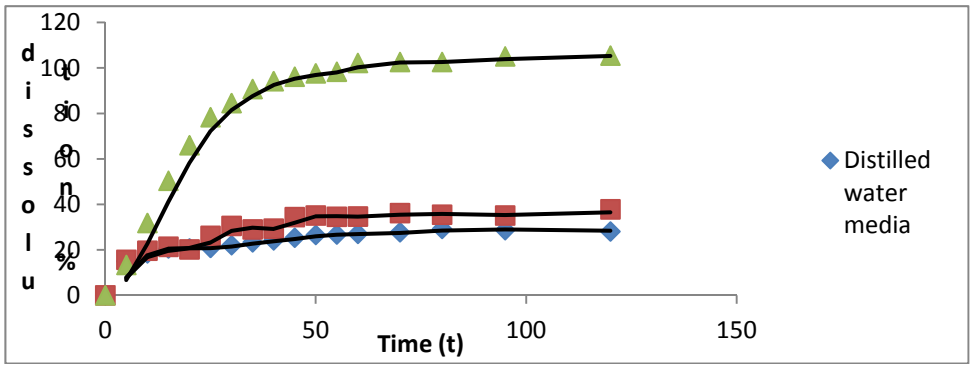


Figure (3): Dissolution profile of Rutin tablets in different dissolution media.

The dissolution comparison between in-house formulated Rutin tablets 500mg and Rutin® 500 mg tablet-Solgar showed a slightly higher dissolution rate for the marketed Rutin tablet of Solgar. The dissolution profiles of the two products are shown in Figure 4.

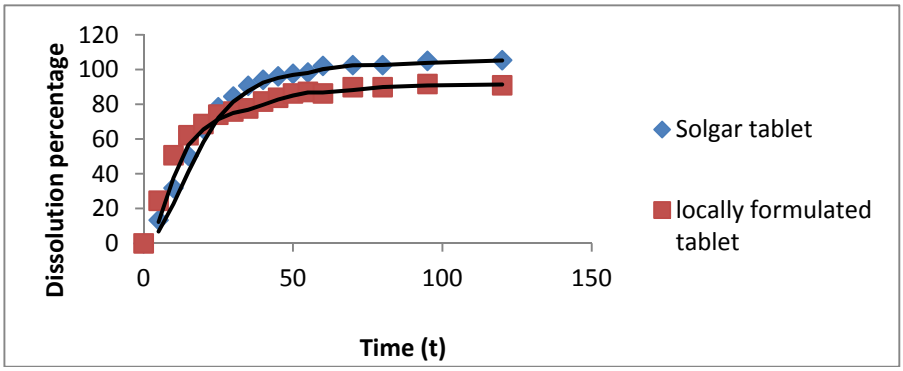


Figure (4): Comparison between in-house formulated tablet and Solgar Rutin ® tablet.

Validation

The selectivity of the method was examined by testing the absorbance of the tablet excipients and the Rutin solution separately. The results show that the developed method was selective. The absorption spectrum of dissolution medium shows no absorbance peak at 360 nm. These results clearly show that at this wavelength there are no interferences from the excipients. The overlay of absorption scans of the tablet excipients and Rutin are shown in Figure 5.

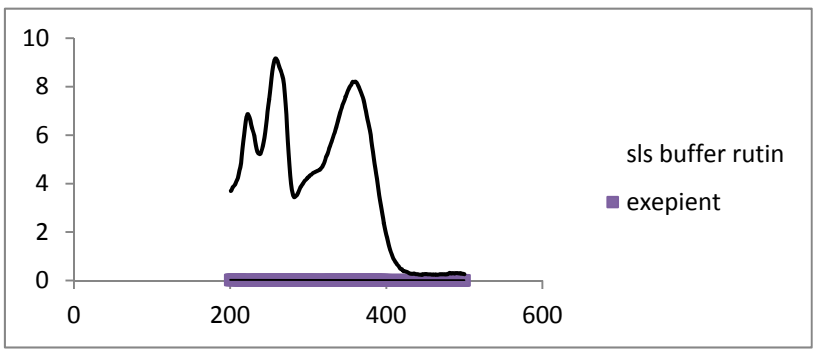


Figure (5): Overlay absorption spectrum of dissolution medium of excipients and Rutin.

Linearity and range results showed that the calibration curve equation was (y = 21.844x + 0.0457) and square of the correlation coefficient (R^2) was = 0.9969 (Figure 6). The results clearly demonstrate that the method is linear in the tested dissolution range of (0.04-0.1mg/ml).

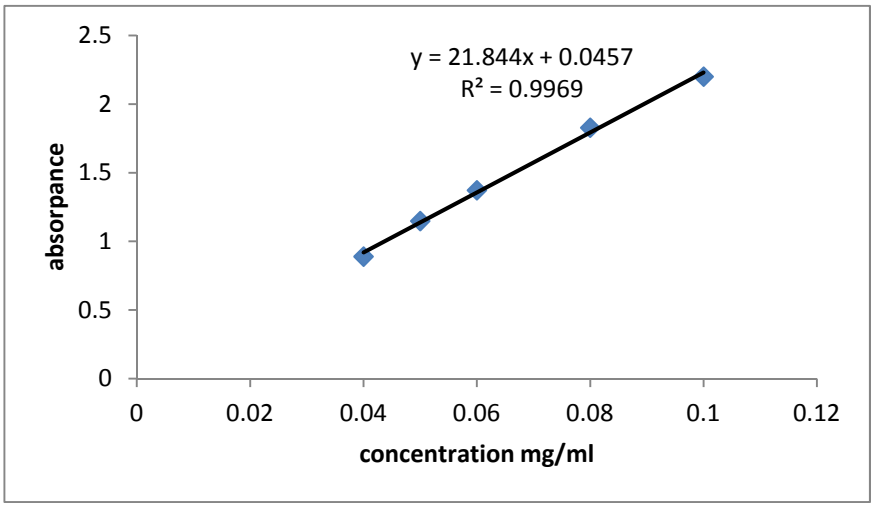


Figure (6): Linearity curve of Rutin standards in phosphate buffer pH 6.8 containing 3% SLS at 360 nm.

The results showed good recovery percentage (97.8 -0.98). This result clearly demonstrates the accuracy of the developed method. The calculated concentration and the percentage recovery are shown in Table 2.

Table (2): Percentage recovery of Rutin at three concentration levels.

Concentration %	% recovery
20%	98%
100%	97.8%
120%	97%

The precision results showed %RSD in the range of 0.5-3.11 among the samples prepared on the same day under the same conditions. The detailed results of % RSD for each recovery level is shown in Table 3. The table also shows the results of the ANOVA test which shows no significant difference between the readings on different days. The ANOVA test showed no significant difference between the results of the three tests done on the first , fifth and sixth day. However, the reading of 100% was an exceptional case; this could be due to low number of tested samples.

Table (3): Inter-day and intra-day precision assay of Rutin tablet.

Percentage assay level	% RSD			P-value
	1st day	5th day	6th day	
20%	0.902967	0.232028	1.16206	0.571
100%	0.578643	2.4264	3.110845	0.04
120	2.043047	0.96432	1.812164	0.571

Our developed method proved to be robust; the results showed that intentional variability of the measuring wave length, the media pH and the analyst did not considerably affect the consistency of results (Table 4). The results show that there is no variability among the results; none of the %RDS exceeded 5 and none of the P values were > 0.05.

Table(4): Robustness results at different variable parameters.

Parameters	%RSD	P value
Different Analysts	3.08	0.51
Variation in pH ±2	5	0.07
Variation in the wavelength 360nm±2	4.7	0.06524

DISCUSSION

In this research project a UV-spectrophotometric dissolution method was developed to analyze very slightly soluble Rutin in tablet dosage form. Developing dissolution test methods for poorly water-soluble drug products has been an important task to formulation scientists and very challenging. The choice of apparatus is based on the dosage form performance in vitro test system. The apparatus used in dissolution of solid dosage form (immediate release, modified release) products are either type 1 or type 2. In our case we used apparatus type 2 because of its availability in our laboratory.

The method development involved determination of the maximum absorption. Two λ max were observed one in UV region and the other was in the visible region. The λ max absorption of the visible region was selected for the analysis of Rutin. This wavelength was selected to avoid any

possible interference from the excipients which might absorb in the UV region.

The choice of a medium, like any other experimental conditions for dissolution testing, should be linked to appropriate physiological characteristics which are similar to those of the gastrointestinal tract(23). The most common dissolution medium is dilute hydrochloric acid, however other media commonly used include buffers at physiological pH and stimulated gastric or intestinal fluid(13).In this case, we examined the dissolution of Rutin in three media (hydrochloric acid, sodium acetate buffer, and phosphate buffer) in order to see which one was suitable for dissolving it. The solubility results of the three media demonstrate that phosphate buffer was the best media for dissolving Rutin and the acidic media (0.1N HCl) was the least suitable.

Sodium Lauryl Sulfate (anionic surfactant) has been proven as a reagent of choice for dissolution studies because it is inexpensive and it possesses good solubilizing capacity at relatively low concentrations. Several authors reported that SLS could enhance dissolution of poorly soluble drugs above its critical micelle concentration (14, 24).

In this present research work, the developed UV spectrophotometric dissolution method was validated as per ICH guidelines. Different validation parameters were examined in this dissolution development method these validation parameters include: specificity, linearity, range, accuracy, and robustness. These results clearly show that method is accurate and linear in the range of 0.04-0.1mg/ml.The absorbance measurement at wavelength (360) has no interferences from the excipients which make the method selective for Rutin. Our developed method proved to be robust; the intentional variability of the measuring wave length, the media pH and the analyst did not considerably affect the consistency of result. This proves that a small variation of the method conditions do not affect the result.

The developed method might be adapted as an official method in an international pharmacopeia and can be adapted by quality control labs to analyze Rutin tablets in the local and international market. Our developed dissolution method will enable herbal medicine factories to apply this dissolution method in the quality control of their similar herbal products.

CONCLUSION

In conclusion we successfully developed a simple and feasible UV- visible spectrophotometric analytical method to quantify the dissolution of Rutin tablets. The method used SLS surfactant in a phosphate buffer pH 6.8 as a dissolution medium to release and solubilize Rutin to about 100%. The method was further validated in accordance with the international guidelines. The method was found to be linear, specific, accurate and robust. This developed method could be used for quality control labs and could be adopted by the official international pharmacopeias.

CONFLICT OF INTERESTS

The authors report no conflicts of interest in this manuscript.

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