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FORMULATION OF COATED TABLETS OF DRY HAWTHORN EXTRACT AND THE DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR EVALUATING IT'S *IN VITRO* DISSOLUTION

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ABSTRACT

Extracts of hawthorn (*Crateagus sp*.) have been indicated in the treatment of cardiovascular diseases, however, tablets produced from dry extract due to a multiple of factors such as inconsistent botanical ingredients, moisture content and hygroscopicity turn to be hard with prolong disintegration. Dissolution test is increasingly being employed for the assessment of solid herbal medicinal dosage form due to its ability to predict the bioavailability of active therapeutic agent from herbal products. The aim of this study was to formulate coated tablets containing dry extracts of hawthorn leafs and flowers and to develop and validate a reverse phase high performance liquid chromatography (RP-HPLC) method for assessing *in vitro* dissolution profile of hawthorn tablet. Comparative extraction procedure studies showed that percolating the plant material with ethanol and extracting with ethyl acetate produce extract with the highest amount of total flavonoids calculated either as rutin or hyperoside and the highest hyperoside content. Assessment of two disintegrating agents; Sodium starch glycolate (SSG) and cross-linked polyvinyl pyrrolidone (PVPP) showed that PVPP was a better agent for this formulation. Disintegration time also improved when distilled water was used as granulation fluid compared to alcoholic solutions. The disintegration time of coated tablets (CT), CT_1 and CT_2 were 8.83±0.41min and 9.33±0.52min respectively. The RP-HPLC method was validated as per ICH guidelines. The validation studies demonstrated that the proposed method is simple, selective, accurate and reliable and can be used for routine dissolution analyses of hawthorn tablets. The dissolution profile of the formulated tablets showed Q-values of 89.952% and 86.3765% for CT_1 and CT_2 respectively.

INTRODUCTION: Tablets of Hawthorn (*Crataegus pinnatifida Bge (Rosaceae)),* extract is indicated in chest constrictions, stabbing pericardial pain, angina pectoris and palpitation [1]. The use of hawthorn in cardiovascular diseases has been shown to be common in most cultures [2].

It has also been shown to have anti diabetic, antioxidant, anti-inflammatory and a mild to moderate sedative effect [3-7]. These biological activities have been attributed to the phenolic component such as quercetin, isoquercitrin, chlorogenic acid, vitexin-4$^{//}$-O-glucoside, vitexin-2$^{//}$-O-rhamnoside, vitexin, rutin and hyperoside [8-10].

The rate of disintegration can be the rate-limiting step in the dissolution process and it's particularly important for the dissolution of drugs from coated dosage forms [11]. Slow or incomplete disintegrations reduce the bioavailability of active substances[12]. Dry herbal extract often increase tablet hardness and prolong disintegration time largely due to its hygroscopic nature and thus present a major problem in tablet formulation [13-16].

For quality control purposes, dissolution tests are frequently conducted for synthetic drugs but scarcely used for herbal products [17]and this has been stated to be problematic [18-21]. Certain conditions and manufacturing processes can limit drug release and affect its pharmacological activity [22]. Absorption of drugs is controlled by their dissolution and variation in dissolution can lead to variation in their therapeutic or toxic effects [23], thus the need for evaluating the dissolution profile of herbal products.

To maximize the opportunity and overcome challenges associated with herbal medicines, new technologies to modernize their preparations are being evaluated[24] and employing these new technologies and processes can ensure the efficacy and safety of herbal products [25]. Dissolution of herbal products may add to the innovative processes in the pharmaceutical industry [26].

The goal of the present study was to develop coated hawthorn tablet with suitable disintegrating time and to develop and validate a RP-HPLC method for assessing its dissolution.

MATERIALS AND METHODS:

Materials: Microcrystalline cellulose (MCC) PH-101 was obtained from Shandong Liaocheng A Hua Pharmaceutical (China), Sodium Starch Glycolate (SSG) from Sheridan & Sons (Germany), Cross-linked Polyvinyl Pyrrolidone (PVPP) from ISP Technologies, INC, (USA), Rutin 98% HPLC grade and Hyperoside 98% HPLC grade from Biotech (China), Tetrahydrofuran HPLC grade was obtained from Concord Technology (China) and Tianjin Guangfu Fine Chemical Research Institute (China). Dried leafs and flowers of hawthorn (*Crateagus pinnatifida*) was obtained from the local market.

All chemicals and reagents was of analytical grade.

Methods:

1. **Identification of Plant Material:** The dried plant material was identified by thin layer chromatography [1] (TLC). Rutin and hyperoside was used as reference samples. The reference samples was dissolved in ethanol to prepare a 0.1mg/ml solution of each. 2g of powdered plant material was decocted under reflux for 1.5 h with 50mls of dilute ethanol TS (52.9mls of 95% ethanol diluted to 100ml with distilled water) in a round bottom flask. It was filtered and the ethanol completely removed *in vacuo*. 10mls of distilled water was added and washed two times with 20ml of petroleum ether (30-60^{o}C).

   The aqueous portion was then extracted twice with 20ml of ethyl acetate and ethyl acetate extract evaporated to dryness. The residue was then dissolved in 2ml of ethanol and used as the test solution. Using a polyamide plate and a mixture of ethanol, acetone and water in the ratio 7:5:6 as the mobile phase, 2ul of the samples was applied and developed. After development, the plate was dried in air and sprayed with aluminum chloride in ethanol TS. It was then dried under hot air and examined under UV light at 365nm.

2. **Extraction of Hawthorn leaves and flowers:** Hawthorn leaves and flowers was extracted with distilled water, 45% ethanol and 70% ethanol to obtain three different solvent liquid extract E_1, E_2, E_3 respectively. 150g of hawthorn leafs and flowers was decocted under reflux for 1.5h twice (1: 8 drug to solvent ratio). The extracts were then combine and concentrate in a rotary evaporator *in vacuo* at 40^{o}C for the ethanol extracts and 50^{o}C for the aqueous extract. The concentrates were placed on a water bath to completely evaporate solvent. It was further dried in a vacuum oven at 50^{o}C for 5h to obtain dried extracts DE_1, DE_2, and DE_3 of E_1, E_2, E_3 respectively. A fourth dried extract, DE_4, was prepared by percolation. 500g of dried powder of hawthorn leafs and flowers were moisten with ethanol and allowed to stand for 4h, after which it was packed into a percolator. It was macerated for 48h with ethanol and then percolated (drug to solvent ratio, 1:6). The ethanol was removed under vacuum.

The extract was concentrated to a density of 1.01 and diluted with an equal volume of water. It was then washed with 0.167 volume of petroleum ether (60-90^{o}C). The petroleum ether layer was discarded and the aqueous layer extracted with 0.7 volume of ethyl acetate [1]. The ethyl acetate extract was concentrate at 40^{o}C *in vacuo* in a rotary evaporator and placed on a water bath to completely remove the solvent. It was further dried in a vacuum oven at for 5h at 50^{o}C. A second batch of raw material was obtained and extracted by the CH.P method described above and coded DE_5. The dried extracts were grinded using a mortar and pestle into fine powders and sieved through No. 80 sieve.

3. **Moisture content:** The percentage moisture contents of the dry powdered extracts were determined using Sartorius MA100 infrared moisture content analyzer.

4. **Assay for Total Flavonoids as Rutin [1]:** Rutin was dissolved in ethanol to obtain reference solution containing 0.2mg/ml. To 1, 2, 3, 4, 5, and 6ml of reference solution in a 25ml volumetric flask, 1ml of 5% solution of Sodium nitrite was added. After 6min, 1ml of 10% solution of Aluminum nitrate was added and allowed to stand for another 6min after which 10ml of Sodium hydroxide TS was added and then made up to the 25ml mark with distilled water. The absorbance was measured after 15min at 500nm using a corresponding reagent solution as blank. Absorbance was plotted against concentration to obtain the standard curve.

   0.15g of DE_1, DE_2, DE_3, DE_4 and DE_5 was added to 25ml of dilute ethanol TS in separate stopped conical flasks, ultrasonicated for 5min and allowed to stand for 3h after which it was filtered. 2ml of filtrates were then transferred into 25ml volumetric flasks and distilled water was added to volume to serve as test solutions. 2ml of test solutions were transferred into separate 25ml volumetric flask and 4ml of distilled water added to each. 1ml of 5% solution of Sodium nitrite was added and after 6min 1ml of 10% solution of Aluminum nitrite added. After another 6min, 10ml of Sodium hydroxide TS was added and distilled water added to make the volume.

   Distilled water was added to 2ml of each test solution in a 25ml volumetric flask to serve as the corresponding blank. The absorbance was measure in triplicate at 500nm.

   The percentage content of total flavonoids in each extract was then calculated.

5. **Assay for Total Flavonoids as Hyperoside [27]**: Stock solutions were prepared by dissolving 0.10g of extracts in 100ml of ethanol. To assay an extract, 5ml of its stock solution was evaporated *in vacuo* to dryness in a round-bottom flask. The residue was dissolved in 8ml of a mixture of 10 volumes of methanol and 100 volumes of glacial acetic acid and transfer into 25ml volumetric flask. The round-bottom flask was rinsed with 3ml of the same mixture and transferred into the 25ml volumetric flask. 10ml of a solution containing 25g/L of boric acid and 20g/L of oxalic acid in anhydrous formic acid was added and made to volume with anhydrous acetic acid.

   The absorbance was measured after 30min at 410nm using a compensation liquid as blank. The percentage content of total flavonoids calculated as hyperoside was determined from the expression:

$$\frac{A \times 1.235}{m}$$

Taking specific absorbance to be 405nm.

A = absorbance at 410nm; m = mass of the extract examined in grams.

6. **Percentage content of Hyperoside in extracts by RP-HPLC:** The external standard method of reverse phase high performance liquid chromatography (RP-HPLC) was use for the assay using COMETRO 6000 HPLC system under the following conditions: column, Diamonsil C_{18} (200mm X 4.6mm, 5µm); UV detector set at 363nm; column temperature, 25^{o}C; mobile phase, methanol-acetonitirile-tetrahydro-furan- 5% solution of glacial acetic acid (1:1:19.4:78.6); flow rate, 1ml/min. The reference solution was 25µg/ml of hyperoside in dilute ethanol TS. To prepare the test solution, 0.15g of extract was added to 50ml of dilute ethanol TS and ultrasonicated for 30min. It was then filtered using a 0.45µm micro porous membrane.

7. **Formulation of Tablets:** Tablets were compressed to contain 64mg of dried extracts, (the minimum dose prescribe by the CH.P) by the wet granulation method using the formulation table below (**Table 1**). To prepare granules, the extract and MCC were mixed by passing it through No. 80 sieve for 20 times. The granulation fluid was added, mixed well and the wet mass passed through No. 18 sieve. The wet granules were dried at 40^{o}C for 2 h in an oven. Agglomerates were eliminated by forcing the dried granules through No. 20 sieve. The disintegrating agent was added, mixed well and the lubricant added. The formulation was then compressed using a single punch tablet machine with a spherical punch of diameter 10mm.

TABLE 1: FORMULATION OF TABLETS

Code	Extract (%)	MCC (%)	Extract/MCC ratio	SSG (%)	PVPP (%)	MS (%)	Granulation Fluid	Compression Force (N)	Total Weight (mg)
	DE_2								
T_1	16	64	1: 4	19	-	1	95% ethanol	70N	400
T_2	16	64	1:4	19	-	1	95% ethanol	100N	400
T_3	21.33	64	1:3.0005	13.67	-	1	70% ethanol	100N	300
T_4	21.33	69.67	1: 3.2663	8	-	1	70% ethanol	100N	300
T_5	21.33	73.67	1: 3.4538	4	-	1	70% ethanol	100N	300
T_6	21.33	64	1: 3.0005	-	13.67	1	70% ethanol	100N	300
T_7	21.33	69.67	1: 3.2663	-	8	1	70% ethanol	100N	300
T_8	21.33	64	1: 3.0005	13.67	-	1	45% ethanol	100N	300
T_9	21.33	64	1: 3.0005	13.67	-	1	Distilled water	100N	300
T_{10}	21.33	64	1: 3.0005	-	13.67	1	45% ethanol	100N	300
T_{11}	21.33	64	1: 3.0005	-	13.67	1	Distilled water	100N	300
T_{12}	21.33	67.67	1: 3.1725	-	10	1	Distilled water	100N	300
T_{13}	21.33	70.67	1: 3.3132	-	7	1	Distilled water	100N	300
T_{14}	21.33	73.67	1: 3.4538	-	4	1	Distilled water	100N	300
T_{15}	21.33	75.67	1:3.5476	-	2	1	Distilled water	100N	300
T_{16}	32	54	1: 1.6875	-	13	1	Distilled water	100N	200
T_{17}	32	57	1: 1.7813	-	10	1	Distilled water	100N	200
T_{18}	32	60	1: 1.8750	-	7	1	Distilled water	100N	200
T_{19}	32	63	1: 1.9688	-	4	1	Distilled water	100N	200
T_{20}	25.6	69.40	1: 2.7109	-	4	1	Distilled water	100N	250
	DE_3								
T_{21}	21.33	67.67	1: 3.1725	-	10	1	Distilled water	100N	300
T_{22}	21.33	70.67	1: 3.3132	-	7	1	Distilled water	100N	300
T_{23}	21.33	73.67	1: 3.4538	-	4	1	Distilled water	100N	300
T_{24}	21.33	75.67	1:3.5476	-	2	1	Distilled water	100N	300
	DE_4								
T_{25}	21.33	67.67	1: 3.1725	-	10	1	Distilled water	100N	300
T_{26}	21.33	70.67	1: 3.3132	-	7	1	Distilled water	100N	300
T_{27}	21.33	73.67	1: 3.4538	-	4	1	Distilled water	100N	300
T_{28}	21.33	75.67	1:3.5476	-	2	1	Distilled water	100N	300
	DE_5								
T_{29}	21.33	64	1: 3.0005	-	13.67	1	Distilled water	100N	300
T_{30}	21.33	67.67	1: 3.1725	-	10	1	Distilled water	100N	300
T_{31}	21.33	70.67	1: 3.3132	-	7	1	Distilled water	100N	300
T_{32}	21.33	73.67	1: 3.4538	-	4	1	Distilled water	100N	300
T_{33}	21.33	75.67	1:3.5476	-	2	1	Distilled water	100N	300

8. **Friability:** Friability of the tablets was measured as the percentage of weight loss of 20 tablets tumbled in a friabilator. After 100 rotations in 4 minutes, the tablets were dusted and the percentage weight loss calculated.
9. **Tablet Coating:** Tablets produced by T_{29} and T_{30} were coated with OPADRY® II white (85F18422) in 50% ethanol, 8%w/w to obtain coated tablets CT_1 and CT_2 respectively using a conventional coating pan.

10. **Disintegration Test:** Disintegration of both coated and uncoated tablets was carried out using ZB-1C intelligent disintegration apparatus (Tianjin University Precision Instrument Factory). Six tablets from each formulation was tested using 900ml of distilled water as medium at 37 ± 1^{o}C with the timer set at 30min.

11. **Development and Validation of RP-HPLC Method for Dissolution Assay:**

a. **Instrumentation:** Agilent 1200 HPLC system under the following conditions: column, Diamonsil C_{18} (200mm X 4.6mm, 5µm); UV detector set at 363nm; column temperature, 25^{o}C; mobile phase, methanol-acetonitirile-tetrahydrofuran-5% solution of glacial acetic acid (1:1:19.4:78.6); flow rate, 1ml/min.

b. **Preparations of Standard Stock Solution:** 2.5mg of hyperoside was accurately weighed into a 25ml volumetric flask and made to volume with dilute ethanol TS to obtain a solution of 100µg/ml concentration. 1ml of this was taken into a 10ml volumetric flask and diluted to the mark with dilute ethanol TS to obtain a concentration of 10µg/ml.

c. **Preparation of Working Standard Solution:** 0.2, 0.4, 0.6, 0.8, 1 and 1.2ml of the 10µg/ml standard solutions was taken into 10ml volumetric flasks and diluted to the mark with dilute ethanol TS to prepare 0.2, 0.4, 0.6, 0.8, 1, and 1.2µg/ml concentrations respectively.

d. **Selectivity:** To demonstrate selectivity, 1µg/ml working solution was injected and the chromatogram recorded.

e. **Calibration Curve/Linearity:** Each of the working standard solutions was injected in triplicates and their peak areas recorded. The mean peak areas were then plotted against the concentrations.

f. **Precision:** The methods precision was evaluated by repeatability and intermediate precision studies. To demonstrate repeatability, the 0.8µg/ml working standard solution was injected 8 times, during the same day under the same experimental conditions. The peak areas were recorded and the %RSD calculated.

Intermediate precision was evaluated by intraday and inter day analysis. For intraday analysis, 0.6, 0.8 and 1µg/ml working standard solutions were injected six times each. Their peak areas were recorded and their %RSD calculated. The inter day analysis was for five consecutive days by six determinations with the 0.6, 0.8 and the 1µg/ml working standard solutions. Their peak areas were recorded and %RSD calculated.

g. **Accuracy:** To ensure that the proposed method is reliable and accurate, recovery studies were conducted by analyzing samples in triplicate at concentrations equivalent to 50%, 100% and 150% of the theoretical concentration of hyperoside in tablet. The percentage recovery and %RSD for each level was then calculated.

h. **Limits of Detection (LOD) and Limit of Quantification (LOQ):** Serial dilutions of the working standard solutions were injected and the LOD/LOQ determined by the Signal-to-Noise ratio.

i. **Robustness:** Deliberate changes were made to the chromatographic conditions and analysis carried out using the 1µg/ml working solution. The conditions varied were flow rate, column temperature and Tetrahydrofuran from a different manufacturer. The retention times and the peak area were recorded and the %RSD of the peak areas determined.

j. **Saturation Solubility Studies:** Equilibrium solubility studies as per the amount of hyperoside in saturated solutions of hawthorn extract was conducted. Water, Hydrochloric acid solution, pH 1.2, Phosphate buffer solutions, pH 4.5 and 6.8, and 0.5, 1, 1.5, and 2% Sodium dodecyl sulphate (SDS) solution were selected as mediums.

Excess hawthorn extract was placed in 10-ml glass tubes each containing 5ml of one of the above mentioned solutions. The glass tubes were sealed and agitated at 37^{o}C for 48h using rotation of 150rpm in a thermostated shaking water bath. After equilibrating, the solutions were filter through 0.45µm micro porous filters, diluted appropriately and their hyperoside content determined in triplicate by HPLC.

k. **Dissolution Studies:** Dissolution studies using six tablets were conducted with USP Dissolution test apparatus II (EWERKA DT 820, Germany) in 750ml of 2% SDS solution as medium, using the paddle method at 50 rpm at 37^{o}C. Aliquots of 10mls were withdrawn at 10, 20, 30, 40, 50 and 60 minutes intervals and filtered. The volume was kept constant by adding 10mls of the dissolution medium. The filtered samples were assayed for hyperoside using the validated HPLC method.

RESULTS AND DISCUSSION: The plant material was successfully identified as that of hawthorn leafs and flowers by the TLC as shown in **figure 1**. The spots marked H represents the hyperoside spots and those marked R denotes the rutin spots with retention factors of 0.73 and 0.55 respectively.

The percentage moisture content of the extracts were within acceptable limits (**Table 2**). The standard curve of the rutin spectrometry showed linearity for the range of concentrations of rutin (9.168-55.008µg/ml) with regression coefficient, R^2, of 0.9998. The line equations was y = 87.461 + 1.127. DE_5 contained the highest amount of total flavonoids either as rutin or hyperoside but contained a lesser amount of hyperoside compared to DE_4 as shown in **Table 3**. It is worth noting that, whiles the total flavonoids calculated as rutin fell below the stipulated value of not less than 80.0 percent in the CH.P for all the extract, the total flavonoids calculated as hyperoside for DE_1, DE_4, DE_5 were above the Eur.P limits of 2.5 percent for aqueous and 6.0 percent for hydro alcoholic extracts. In 2008, the global market for traditional medicines was estimated at US$83 billion annually [28]. To maximize international trade in traditional medicinal products and reduce trade limitations, methods of qualification and quantification need to be harmonized.

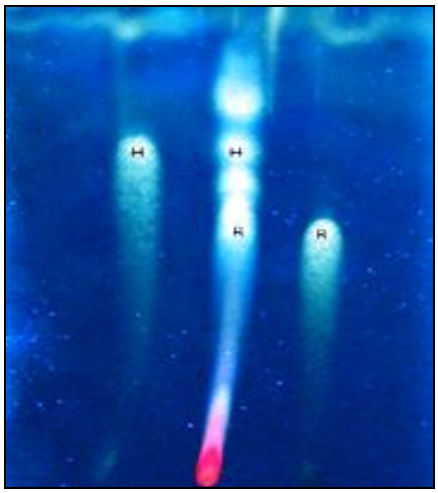

FIGURE 1: PHOTO OF TLC UNDER UV-365NM. H= HYPEROSIDE SPOTS, R= RUTIN SPOTS

TABLE 2: MOISTURE CONTENT

Extract	% Moisture Content
DE_1	4.88
DE_2	4.86
DE_3	2.94
DE_4	2.29
DE_5	1.4

TABLE 3: TOTAL FLAVONOIDS AND HYPEROSIDE CONTENT IN EXTRACTS

Extract	% Content of Total Flavonoids calculated as Rutin	% Content of Total Flavonoids calculated as Hyperoside	% Content of Hyperoside
DE_1	25.3937	4.7278	0.2143
DE_2	41.8482	5.8081	0.3358
DE_3	44.7197	5.8696	0.4010
DE_4	46.6403	7.6872	0.9160
DE_5	58.1100	13.8000	0.8292

Two disintegrating agents; SSG and PVPP, with varying amounts were evaluated to access their effect on tablet disintegration. Binders were not used in this formulation as the extract exhibited a strong binding effect. Plant extracts have been show to act as strong binders [29]. This is illustrated by the fact that tablets with lower weight containing the same amount of the extract either had a longer disintegrating time within the 30min experimental time or failed the test even though they contained the same percentage of disintegrating agents and filler. For example, T_{14}, T_{19} and T_{20} contained 4 percent of PVPP. Whiles T_{19} failed to disintegrate within 30min, T_{20} disintegrated in 20.67min and T_{14} had a disintegrating time 5.67min. Also compare T_{12} and $T_{17,}$ T_{13} and T_{18}. The binding effect of the extract seems to decline with increasing extract to MCC ratio thereby enhancing disintegration.

The complexity of indentifying an appropriate extract-filler-disintegrating agent combination for producing tablets (containing extract with binding ability) with good disintegration time was highlighted in this study. It has been observed that disintegrating agents are not effective in formulations which exhibit long disintegration time due to binding properties of the herbal extract not being reduced by a filler [29].

For 300mg of tablets containing DE_2, DE_3 or DE_4, 7 percent PVPP resulted in disintegration times lower than those containing a higher or lower percentage of the same. However, tablets formulated with DE_5 showed a different behavior; disintegration time improved with increasing percentage of PVPP. Again, tablets formulated with DE_5 showed significant differences in disintegration times compared with those of DE_4 though the extraction method and composition of formulations were the same. Though environmental conditions can bring about slight changes, this wide difference could be attributed to the fact that the physico-chemical properties of the extracts as well as their interaction with other components in the formulation are not exactly the same. It is therefore imperative to access the disintegration times of tablets when different extracts are used, especially when raw materials are not of the same batch.

SSG showed a better disintegrating ability compared to PVPP at the same percentage in tablets of equal weight when 70% ethanol was used as granulation fluid; comparing the disintegration time of T_3 and T_6 or T_4 and T_7. However, PVPP showed a superior effect on tablet disintegration better than SSG when 45% ethanol or distilled water was use as granulations fluid; see disintegration times for T_8 and T_{10}; and T_9 and T_{11}. In general terms, disintegration time improves when lower percentage of ethanol was use regardless of the disintegrating agent. Distilled water was the best granulation fluid found in this study as it improved the disintegration of the tablets. It may therefore be suggested that alcohol increases the binding ability of the extracts.

The disintegration times for the coated tablets were 8.83±0.41 and 9.33±0.52 for CT_1 and CT_2 respectively. Their percentage weight gains after coating were 7% and 9% respectively.

The disintegration times for the various formulations are shown in **table 4**.

All the tablets showed acceptable friability when pressed at 100N.

TABLE 4: DISINTEGRATION TIME AND FRIABILITY

Code	Disintegration time (min)	% Friability
T_1	4.00 ±1.41	12.95
T_2	5.00 ±1.10	0.05
T_3	11.50 ±2.95	0.33
T_4	13.83 ±1.47	0.07
T_5	17.17 ±2.64	0.07
T_6	11.67 ±2.88	0.39
T_7	15.33 ±2.16	0.07
T_8	11.17 ±1.84	0.13
T_9	10.00 ±1.67	0.07
T_{10}	5.67 ±1.86	0.07
T_{11}	5.00 ±0.63	0.13
T_{12}	4.33 ±0.52	0.13
T_{13}	3.67 ±0.52	0.01
T_{14}	5.67 ±0.52	0.00
T_{15}	10.00 ±1.27	0.07
T_{16}	21.83 ±2.99	0.00
T_{17}	24.50 ±3.27	0.00
T_{18}	2 Tablets failed to disintegrate within 30min	0.00
T_{19}	No disintegration within 30min	0.09
T_{20}	20.67±1.37	0.08
T_{21}	13.17±2.56	0.00
T_{22}	6.17±0.98	0.00
T_{23}	8.17±0.98	0.00
T_{24}	9.17±0.98	0.00
T_{25}	12.17±1.94	0.00
T_{26}	3.83±0.41	0.00
T_{27}	4.67±0.52	0.00
T_{28}	5.17±0.75	0.00
T_{29}	6.67±0.82	0.00
T_{30}	7.33±1.36	0.00
T_{31}	13.17±2.02	0.00
T_{32}	16.00±2.53	0.00
T_{33}	23.33±3.77	0.00
CT_1	8.83±0.41	-
CT_2	9.33±0.52	-

Method Development and Validation: Method validation was done in accordance with the International Conference on Harmonization (ICH) guidelines [30]. The graph was linear across the chosen range of concentrations with regression coefficient R^2 of 0.9999. The regression line had a slope of 15.143, a y-intercept of -0.1222 and a residual sum of squares of 0.009418. The line equation was y = 15.143x - 0.1222.

Selectivity was demonstrated by injecting a standard hyperoside solution and recording the chromatogram, **Figure 1.**

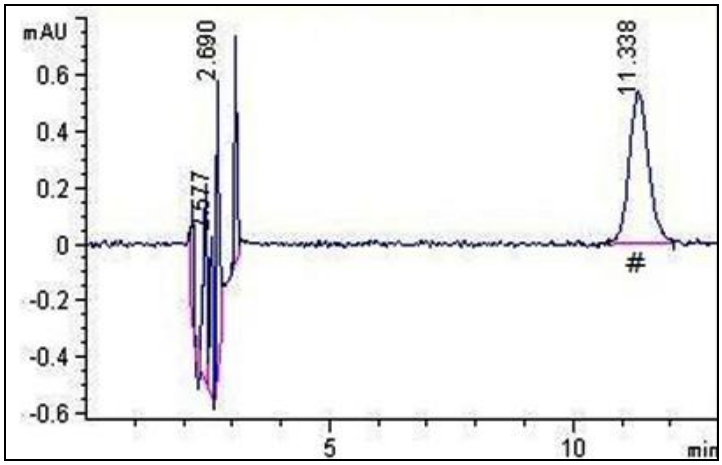

FIGURE 1: STANDARD CHROMATOGRAM OF HYPEROSIDE; #- STANDARD PEAK OF HYPEROSIDE

Precision was demonstrated by repeatability and intermediate precision. The %RSD for repeatability was 0.621963, that for intraday and inter day analysis were 0.611981, 0.682309, 1.066869 and 0.277034, 0.590566, 0.966917 respectively for the chosen concentrations of 0.6, 0.8 and 1µg/ml. The very low %RSDs indicates that the proposed method is precise.

The percentage recoveries at the level of 50%, 100% and 150% were 100.40, 103.04 and 100.95 with %RSD between 0 and 1.01. Thus, the method is accurate and reliable.

The LOD and LOQ were found to be 0.06µg/ml and 0.1µg/ml respectively.

Changes in the flow rate, column temperature and THF manufacturer did not result in significant changes in the retention times and peak areas. The %RSDs were 0.383197, 0.666667 and 0.383197 which are within acceptable range and thus method can be said to be robust;

Medium Selection: Results from the saturation equilibrium solubility studies showed that the highest amount of hyperoside was releas in the 2% SDS solution. 2% SDS solution was therefore selected as medium for the dissolution studies.

TABLE 5: RESULT OF SOLUBILITY STUDIES

Medium	Solubility (mg/ml)
Water	0.6224
Hydrochloric acid solution, pH 1.2	0.5503
Phosphate buffer, pH 4.5	0.5422
Phosphate buffer, pH 6.8	0.6585
0.5% SDS	0.9088
1% SDS	1.1275
1.5% SDS	1.1944
2% SDS	1.2948

Dissolution studies: Figure 2 shows a typical chromatogram of dissolution samples. The developed RP-HPLC method was able to detect and quantify the released of hyperoside.

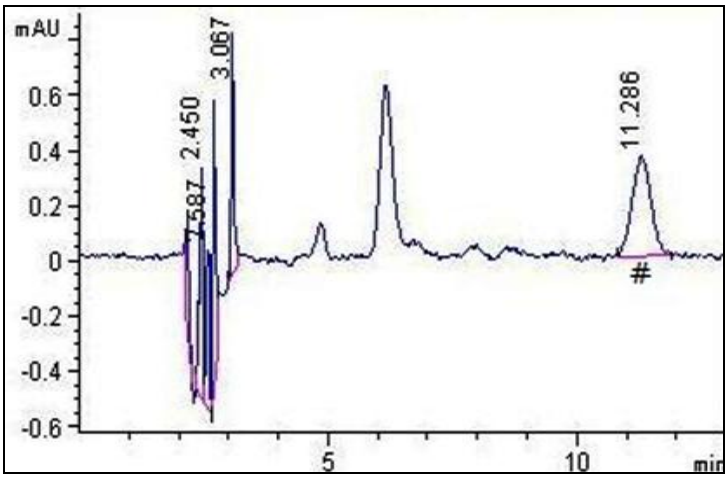

FIGURE 2: A TYPICAL CHROMATOGRAM OF DISSOLUTION SAMPLE: #- HYPEROSIDE PEAK

The results of the dissolution test are presented as percentage of hyperoside released at the preset times, **Figure 3.**

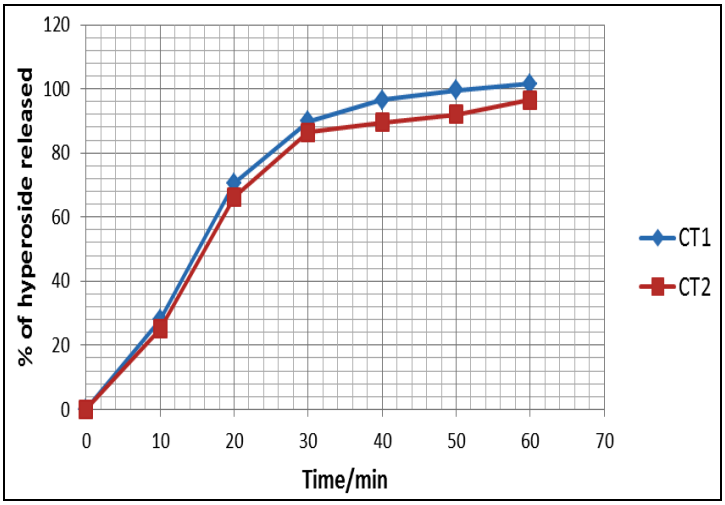

FIGURE 3: DISSOLUTION PROFILE OF COATED TABLETS CT_1 AND CT_2

It was observed that, the amount of hyperoside released from CT_1 was higher at all the set time than that released from CT_2. The amount released was 4% higher for CT_1 in 30 minutes and 5% higher after the run time; 60 minutes.

Although there is no pharmacopoeia specification for dissolution of this formulation, the Q-value was set at least 75% after 30 minutes. The Q-value for CT_1 was found to be 89.952% and that for CT_2 was 86.3765%, therefore both tablets meet the set specification.

CONCLUSION: Coated hawthorn extract tablets with acceptable disintegration time and dissolution has been produced. HPLC method for assessing the dissolution of herbal tablets has been recommended as against spectrophotometry since identifying the matrix used by manufacturers could prove impossible [26]. A RP-HPLC method which is simple, selective, accurate and reliable has been developed for assessing the *in vitro* dissolution of hawthorn extract tablet. This method can be applied for the routine quality control analyses of hawthorn tablet.

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REFERENCES:

1. Pharmacopoeia of the People's Republic of China. China Medical Science and Technology Publishing House, Beijing, English Edition, Volume I, 2010; 69-70, 662-663.
2. Herbal Medicines Association, B., British Herbal Pharmacopoeia. Bournemouth: British Herbal Medicines Association; 1984.
3. Wang T, An Y, Zhao C, Han L, Boakye-Yiadom M, Wang W, Zhang Y: Regulation Effects of Crataegus pinnatifida Leaf on Glucose and Lipids Metabolism. J. Agric. Food Chem., 2011; dx.doi.org/10.1021/jf1049062
4. Brchm, M. *et al*.: Comparative antitumor activity of doxorubicin plus two radical scavenging plant extract preparations *(Ginkgo biloba, Crataegus oxyacantha)* and of doxorubicin alone, Conbrib Oncol., 1995;48, 48-52
5. Dr Duke's phytochemical and ethnomedicinal data base (online). Available at URL: http://www.ars-grin.gov/duke/index.html
6. Della Loggia, R. *et al*.: Depressive effect of *Crataegus oxyacantha L*. on central nervous system in mice. Sci. Pharm., 1983; 51, 319-24
7. Rewerski, V., Piechocki, T., Rylski, M., Lewak, S: Some pharmacological properties of crataegeus oxyacantha compounds and isolated oligomeric procyanidins. ArzneimForsch Drug Res. 1971; 21, 886-88
8. Veveris, M.; Koch, E.; Chatterjee, S. S: Crataegus special extract WS 1442 improves cardiac function and reduces infarct size in a rat model of prolonged coronary ischemia and reperfusion. Life Sci., 2004; 74, 1945-1955.
9. Asgary, S., Naderi, G. H., Sadeghi, M., Kelishadi, R., Amiri, M: Antihypertensive effect of Iranian *Crataegus curvisepala Lind*: a randomized, double-blind study. Drugs Exp. Clin. Res., 2004; 30, 221¨-225.
10. Tadic VM, Dobric S, Markovic GM, Dordevic SM, Arsic IA, Menkovic NR, Stevic T: Anti-inflammatory, gastroprotective, free-radical-scavenging, and antimicrobial activities of hawthorn berries ethanol extract. J. Agric. Food Chem., 2008; 56: 7700-7709.
11. Siepmann J, Siepmann F, Florence AT, , 'Factors Influencing Oral Drug Absorption and Drug Availability' in AT Florences and J Siepmann (eds), Modern Pharmaceutics, Informa Healthcare USA, Inc., New York, Edition 5, Volume 1, 2009:134.
12. Von Eggelkraut-Gottanka SG, Abed SA, Muller W, Schmidt PC: Roller compaction and tabletting of St. John's wort plant dry extract using a gap width and force controlled roller compactor. I. Granulation and tabletting of eight different extract batches., Pharm. Dev. Technol., 2002;7 (4) 433-45
13. Soares LAL, Ortega GG, Petrovick PR, Schmidt PC: Optimization of tablets containing a high dose of spray-dried plant extract: A technical note. AASP PharmSciTech., 2005;E: 367-371
14. Eder M, Mehnert W: The importance of concomitant compounds in plant extracts. Pharmazie.1998; 53: 285-93.
15. Onunkwo GC, Egeonu HC, Adikwu MU, Ojile JE, Olowosulu AK: Some physical properties of tabletted seed of Garcinia kola (HECKEL), Chem. Pharm. Bull., 2004; 52: 649-53
16. Rocksloh H., Rapp FR, Abu Abed S, Muller W, Reher M, Gauglitz G, Schmidt PC: Optimization of crushing strength and disintegration time of a high-dose plant extract tablet by neural networks, Drug Dev. Ind. Pharm., 1999; 25: 1015-25
17. Taglioli V, Bilia AR, Ghiara C, Massi G, Mercati V, Vincieri FF: Evaluation of the dissolution behaviour of some commercial herbal drugs and their preparations. Pharmazie, 2001; 56: 868-870.
18. Bempong DK, Houghton PJ: Dissolution and absorption of caffeine from guarana. J Pharm Pharmacol, 1992; *44*: 769771.
19. Kressmann S, Biber A, Wonnemann M, Schug B, Blume HH, Muller WEJ: Influence of pharmaceutical quality on the bioavailability of active components from *Ginkgo biloba* preparations. Pharm. Pharmacol., 2002; *54*: 1507-1514.
20. Westerhoff K, Kaunzinger A, Wurgilics M, Dressman J, Shubert MZJ:. Biorelevant dissolution testing of St John's wort products. Pharm. Pharmacol., 2002; *54*: 1615-1621
21. Kratz JM, Terrazas CB, Motta MJ, Reginatto FH, Simões CMO: Determinação da composição quimica e dos perfis de dissolução *in vitro* de medicamentos a base de *Ginkgo biloba* disponiveis no mercado brasileiro. Lat Am J Pharm., 2008; 27: 674-680.
22. Ansel HC, Popovich NG, Allen Jr. LV: Formas farmaceuticas e sistemas de liberação de farmacos, Baltimore, Editorial Premier, 2000; 568.
23. Ngo SNT: When do differences in dissolution profiles predict clinical problems? J Clin Pharm Therapeut., 2007; 32:111-112.
24. Vasconcelos EAF, Medeiros MGF, Raffi FN, Moura TFAL: Influincia da temperatura de secagem e da concentração de Aerosil ®200 nas caracteristicas dos extratos secos por aspersão da *Schinus terebinthifolius Raddi* (Anacardiaceae). Rev Bras Farmacogn., 2005; 15: 243-249.

25. Alves FNR: Desafio para a inovação em fitomedicamentos no contexto da industria farmaceutica nacional. Rev Fitos., 2005; 1: 18-29.
26. Costa ART, Alves SF, da Conceicao EC, Garrote CFD, Paula JR, Bara MTF: Dissolution test of herbal medicines containing *Passiflora* sp., Braz. J. Pharmacogn., 2010; 25.
27. European Pharmacopoeia. Council of Europe, Strasbourg, France, Editions 6, Volume I, 2008: 2036-2037
28. Traditional Medicine: Global Situation, Issue and Challenges. Geneva, World Health Organization, 2011 (WHO/EMP/MIE/2011.2.3). Available at: http://apps.who.int/medicinedocs/documents/s18063en/s18063en.pdf
29. Kucinskaite A, Sawicki W, Breidis V, Sznitowska M: Fast disintegrating tablets containing *Rhodiola rosea L.* Acte Poloniae Pharmaceutica- Drug Research, 2007; 64: 63-67.
30. International Conference on Harmonization, 1995, 'Q2A: Text on Validation of Analytical Procedures', Federal Register 60(40), 11260-11262.

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