DEVELOPMENT AND VALIDATION OF DISSOLUTION TEST METHOD FOR ANDROGRAPHOLIDE FROM FILM COATED POLYHERBAL TABLET FORMULATION

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ABSTRACT

The objective of present study was to develop and validate a dissolution test for polyherbal hepatoprotective tablets containing andrographolide with bio-relevant media. Different dissolution conditions such as basket (type I)/paddle (type II) agitation, rotation speed, dissolution medium and volume were evaluated. The dissolution samples were analyzed and validated using a high-performance liquid chromatographic method (HPLC). The separation of the analyte from sample matrix was achieved using an Inertsil RP-18 column with UV detection at 226 nm. Thorough validation of the assay based on pre-defined criteria included specificity, accuracy, precision, linearity and robustness. In addition, filter suitability, standard and sample solution stability was also demonstrated. The best dissolution profile was obtained using type II (paddle) at 75 rpm, 900 ml of medium with pH 4.5 where over 80% of andrographolide was dissolved after 120 min. The method showed specificity, accuracy, precision, linearity and robustness within the acceptable range. Both the HPLC and dissolution method were validated and could be proposed as a pharmacopeial standard to assess the performance of polyherbal formulations containing andrographolide.

Keywords: Dissolution, Polyherbal formulation, Andrographolide, *Andrographis paniculata.*

INTRODUCTION

Dissolution is the process by which a solid substance (Active/marker constituent) dissolves in a specified volume of aqueous medium. Dissolution rate is the amount of substance that goes into solution under standardized conditions of liquid/solid interface, temperature, and solvent composition. In the sector of pharmaceutical production and quality control, the dissolution test can be used to detect quality deviations in parameters such as content uniformity and ensure reproducibility between production batches [1].

Herbal products are medicines whose active principles are exclusively derived from vegetable drugs. They are characterized by knowledge of the effectiveness and risks of their use, as well as by the reproducibility and constancy of their quality [2, 3]. In contrast to synthetic medicinal products, polyherbal formulations contain number of herbal medicinal plant extracts, and each of these extracts further contain multiple chemical constituents in varied amounts, some or all of them may be active. This makes the process of developing a dissolution test significantly more complicated than the process for a pharmaceutical product, which typically contains one or two well-characterized active ingredients. Compendial standards for nutraceuticals and natural products are in their infancy, and there are currently only four herbal monographs published in the USP that specify dissolution parameters. Till date very little data is published on the application of dissolution in the development and testing of polyherbal medicinal products. European, British and Japanese pharmacopeias have general chapters for dissolution harmonized with the USP, but these pharmacopeias do not have monographs with specifications for poly herbal formulations [4].

Commercially available polyherbal formulations vary widely in quality and in the concentration of their active constituents and this variation could help to explain the variable efficacy of herbal medicines and dietary supplements in clinical trials. Wide variation in the active constituents of the herbal preparations undermines the practice of herbal medicines itself [5]. Therefore, production of quality botanical medicines has become a challenge to regulatory authorities, scientific organization and manufacturers [2, 6, 7].

In the present study an attempt has been made to develop a dissolution method for a polyherbal formulation containing four standardized herbal extracts namely *A. paniculata* (10% andrographolide), *Boerhaavia diffusa* (0.25% Boeravinone B and E), *Phyllanthus amarus* (0.5% of Phyllanthin and Hypophyllanthin) and *Picrorrhiza kurroa* (4% Kutkoside and Picroside-I). Among all the above mentioned extracts andrographolide is selected based on its

relevant pharmacological activity [8], its concentration in the final product, its aqueous solubility and the commercial availability. The aqueous solubility of the selected marker compound is evaluated to set the sink condition. The biomarker compound has limited aqueous solubility; therefore a change in the volume of media, use of co-solvents like anionic or nonionic surfactants or by alteration of media pH has also been undertaken to improve solubility.

In addition, adequate analytical procedures from literature are applied for the determination of the amount of andrographolide dissolved during dissolution testing. The method intended for this purpose was not sufficiently sensitive, selective and robust in presence of excipients and other extracts present in formulation [9-14]. Therefore, a new HPLC method was established, to quantify andrographolide in above mentioned polyherbal formulation and to investigate its release characteristics from selected product available on the Indian market.

MATERIALS AND METHODS

Materials

Concentrated hydrochloric acid, orthophosphoric acid, acetic acid reagent grade, and acetonitrile, methanol HPLC grade were obtained from Merck (Mumbai, India). Sodium acetate, potassium dihydrogen phosphate, sodium hydroxide, sodium lauryl sulphate (reagent grade) were obtained from Fisher Scientific (Mumbai, India). Standard andrographolide (Purity 98%) was obtained from Sigma Aldrich (Bangalore, India). Water for dissolution and chromatography was purified by using Milli-Q purification unit (Millipore, Milford, MA, USA). Disposable latex-free 10ml plastic syringes were supplied by Hindustan Syringes and Medical Devices Ltd. (Faridabad, India), syringe filters, 0.45 μm MDI Nylon-66 Membrane Disc filters were obtained from Advanced Microdevices Pvt. Ltd. (Ambala, India) and Whatman No.1 filter papers from Whatman International Ltd. (Maidstone, UK).

Instrumentation

HPLC analysis was performed on a Waters (Millipore, Billerica, MA) system equipped with gradient pump 600F, 717 autosampler, 600E system controller and 2996 photodiode array detector. All system operations as well as data acquisition and integration were controlled with *Empower 2* software. The analysis was carried out on an Inertsil ODS-4 (250 × 4.6 mm, i.d.; 5 μm, GL Sciences Inc, Tokyo, Japan) column, using a gradient elution. Different mobile phases were tested in order to find the best conditions for separating andrographolide from the other constituents of the

finished product. The optimal composition of the mobile phase was solvent A, orthophosphoric acid (0.1% v/v) in Milli-Q water (pH 2.2); solvent B acetonitrile. For the first 22 minutes the composition of the mobile phase was kept constant at 25% B, and then changed to 95% B in 2 minutes. The mobile phase was kept constant at 95% B for next 6 minutes and the column was reequilibrated for 5 minutes until the next sample was injected. All solvents were degassed with sonication on an ultrasonic water bath before they were used for analysis. The flow rate of 1 ml/min resulted in a back pressure of approximately 1800 psi. UV detection was carried out at 226 nm, the absorption maximum of andrographolide, and the injection volume was 20 µl.

Solubility studies

The solubility studies were carried out for andrographolide in order to find an aqueous dissolution medium that ensures sink conditions during dissolution testing. Dissolution media containing 0.1%, 0.5%, 1% w/v of sodium lauryl sulphate, 0.1M and 1N HCl, acetate buffer, pH 4.5 and phosphate buffer, pH 6.8 were prepared in accordance with USP.

Accurately weighed amount of *A. paniculata* extract (standardized for 10% andrographolide) equivalent to 10 mg andrographolide was placed into volumetric flask with glass stopper, and 100 ml of different dissolution media were added. The flasks were capped and placed into a shaking water thermostatic bath at 37±0.5 ◦C. Samples were taken after 24 h, filtered through 0.45µm nylon syringe filters and analyzed by UV at 226 nm. The solubility in each medium was determined in triplicate.

Dissolution testing

Dissolution testing was performed using a qualified Electrolab TDT-08L (Mumbai, India) dissolution tester operated as USP Apparatus 2 (paddle) at 50rpm or 75rpm and at 37±0.5 ºC. Nine hundred milliliters of 0.1M HCl, acetate buffer, pH 4.5 and phosphate buffer, pH 6.8 were used as the dissolution media. Each dissolution test was performed with 6 tablets. Sample aliquots were collected at 15, 30, 45, 60, and 120 min, filtered through 0.45µm nylon filters and analyzed by the above mentioned HPLC procedure.

RESULTS AND DISCUSSION

Validation of HPLC method

The HPLC method used for analysis was validated according to current ICH guidelines [15]. In addition, filter suitability, solution stability and robustness were evaluated. Samples were drawn at 15, 30, 45, 60 and 120 min for the validation work. At the time of validation the sample was prepared by spiking a solution of drug into a dry excipient blend to minimize the manual errors.

Solubility studies

Andrographolide was found to be poorly soluble in water, 0.1M HCl, 0.1M acetate buffer and 0.1M phosphate buffer. The highest solubility of andrographolide was in 1%w/v sodium lauryl sulphate which facilitates the drug release process at the solid/liquid interface and micelle solubalization in the bulk. Although, from the solubility studies it was found that sodium lauryl sulphate significantly improved the solubility but 0.1M HCl, 0.1M acetate buffer and 0.1M phosphate buffer being physiologically relevant media, were chosen to optimize the dissolution test[16] Table (1).

Specificity

Tablets of the *A. paniculata* placebo (*Phyllanthus amarus* extract; *Picrorhiza kurroa* extract; *Boerhaavia diffusa* extract; microcrystalline cellulose RQ 101, RanQ Remedies Pvt. Ltd. India; methylparaben and propylparaben, Alta Lab Ltd. India; crospovidone XL, Boai NKY Pharmaceutical Ltd. China; croscarmellose sodium, Aditya Chemicals, India; colloidal silicon dioxide, Evonik Degussa Corp, USA, and magnesium stearate, Amithi Drugs, India) was used as the blank sample to rule out possible interference from the other ingredients and excipients during HPLC method development. One tablet was placed in the dissolution vessel containing 900 ml of the dissolution medium (phosphate buffer pH 6.8) at 37±0.5 ºC and stirred for 1 h at 75rpm using USP Apparatus II. An aliquot was collected, filtered through a 0.45µm syringe filter and analyzed by HPLC, no interference from the excipients or other phytoconstituents of the polyherbal formulation with the peak of interest was observed (Fig. 1).

Accuracy and Precision

The accuracy of the method was demonstrated by the recovery of spiked sample solutions where dissolution medium containing placebo formulation was spiked with andrographolide at about 50%, 100%, and 120% of the labeled amount. The mean recovery percent ranged from 98% to 102% Table (2). The injection precision of the method was evaluated by performing six replicate injections of a sample at the working concentration (4.4µg andrographolide/ml). The sample was a blend of drug and excipients. The peak area % RSD was 0.22% which was considered acceptable. The % RSD of the percent assay for andrographolide was calculated for six separate preparations at the working concentration (4.4µg andrographolide/ml). The % R.S.D. values were 0.7%, which was considered acceptable.

Intermediate precision was performed by two analysts, each testing two sample preparations on three separate days and using two different instruments and analytical columns. Fresh sample and standard solutions were independently prepared on each day of analysis. The intermediate precision results are shown in Table (3). These results were considered acceptable.

Linearity

Calibration curves were prepared at 6 different concentrations ranging from 0.4 to 5.5 µg/ml to cover the range from approximately 10% of the lower labeled dose strength (4 mg *A. paniculata* extract/tablet equivalent to 0.4 mg andrographolide in 900 ml) and up to 125% of the highest labeled amount (50mg *A. paniculata* extract /tablet equivalent to 5mg andrographolide in 900 ml). Individual samples were prepared at each concentration level from one stock solution.

A linear relationship was observed between peak areas of andrographolide and its nominal concentration with correlation coefficients of 0.9975 with a slope of 52626 and a *y*-intercept of 9648. The *y*-intercept was within 10% of the response at the 100% level. These results were considered acceptable.

Standard and sample solution stability

The stability of working standard and sample solutions was determined. The stock standards were prepared in methanol and subsequently diluted with dissolution media for the preparation of working standards. The solutions were stored under normal laboratory conditions (capped in flask at ambient temperature, unprotected from light). Recovery was determined using an external standard prepared on the day of analysis and the results were compared with the initial time-point. Stock standards were found to be stable up to 24 hours. Results are shown in Table (4).

Robustness

The experimental results of the ruggedness study are summarized in Table (5). Critical chemical and instrumental chromatographic parameters such as the gradient composition and flow rate of the mobile phase, pH and column temperature were deliberately varied in the range of ±3.0% compared to their optimal values. Recovery values obtained using samples at the 100% level confirmed the robustness of the HPLC method, since the obtained values were within the acceptance limits (95.0–105.0%).

Filter suitability

Filter suitability was evaluated using the dissolution medium for solution containing *A. paniculata* extract and excipients at 10% of the working concentration, solution containing *A. paniculata* extract without excipients at 10% of the working concentration, solution containing *A. paniculata* extract and excipients at 100% of the working concentration and solution containing *A. paniculata* extract without excipients at 100% of the working concentration. The above

solutions were prepared in a dissolution vessel and stirred at 50 rpm at 37 °C for 120 min. Samples were filtered and fractions collected at 1, 2, 3, 5, 7 and 10 ml. The syringe filters (0.45 μm membrane disc filters) showed recoveries between 98.2 and 101% after discarding the first 5ml of filtrate.

Optimization of dissolution test

The dissolution test was optimized in terms of dissolution medium, paddle (type II)/basket (type I) agitation and rotation speed. Initial dissolution experiments were carried out under the following experimental conditions: 500 ml water as the dissolution medium, medium temperature of 37.0±0.5 °C, rotation speed of basket 50 rpm. To construct the dissolution profile, sampling was performed at 5, 10, 20, 30, 60 and 120 min. A typical dissolution profile obtained under these experimental conditions is shown in (Fig. 2).

Study of dissolution media

Pure water is not considered as an "ideal" dissolution medium, mainly due to its extremely low capacity against potential pH variations during the dissolution tests. However, andrographolide being a poorly water-soluble compound, 0.1% sodium lauryl sulphate in water was used to improve dissolution. Additionally, three alternative media 0.1M HCl to simulate gastric fluid pH conditions, acetate buffer (pH 4.5) to simulate duodenal pH conditions and phosphate buffer (pH 6.8) to simulate intestinal pH conditions were also employed.

Under the experimental conditions described above, all the dissolution media have shown similar release profile but the % cumulative release of andrographolide was still less than 80%. To improve the dissolution rate of andrographolide phosphate buffer (being the least soluble media) was used as dissolution media for the further experiments. After optimization the method was applied to all the dissolution media described above to simulate different physiological conditions (Fig. 2D).

Basket (type I) versus paddle (type II) agitation

Using phosphate buffer as the dissolution medium, experiments were carried out to compare the performance of basket versus

paddle agitation, rotating at 50 rpm in both cases. The experimental results are presented in (Fig. 2A). The result shows that the dissolution rate was faster when paddle was used. Paddle (type II) agitation was therefore selected for further studies.

Effect of dissolution media volume

The comparison of 500 and 900 ml media was carried out to get the better release profile. Increasing the media volume significantly improved the dissolution rate in a given span of time. Therefore 900 ml was selected as the final media volume for the further dissolution testing (Fig. 2B).

Effect of the paddle's rotation speed

The effect of the rotation speed of the paddle on the dissolution profile of tablets containing andrographolide was examined at 50, 75 and 100 rpm. Increasing the rotation speed resulted in faster dissolution kinetics in the timeframe of 15-45 min, although the profiles of 75 and 100 rpm coincided thereafter but were found to be better than 50 rpm (Fig. 2C). Therefore, the rotation speed of 75 rpm was selected for further experiments.

Robustness of the dissolution test

The ruggedness of the dissolution test was validated by deliberately introducing small variations, in the range of ±3% compared to the optimal values, to critical parameters including the pH of the dissolution medium (6.7–6.9), temperature (36.0–38.0ºC) and basket rotation speed (72–78 rpm). The obtained dissolution profiles (12 tablets in all cases) were compared to those obtained under the optimal conditions by calculating similarity factors ($f2$) derived by the following equation [17]:

$$f2 = 50 \times \log \{[1 + (1/n) \sum_{t=1}^{n}(Rt - Tt)^2]^{-0.5} \times 100\}$$

where n is the number of time points, Rt the dissolution value of the reference (pre-change) batch at time t and Tt is the dissolution value of the test (post-change) batch at time t. Generally, $f2$ values greater than 50 (50–100) indicate similarity or equivalence of the compared dissolution profiles. The experimental results are presented in Table 6. The values of the similarity factor ranged between 78 and 85 in all cases, confirming the ruggedness of the dissolution test.

Table 1: Apparent solubility studies

Medium	Concentration μg/ml (n=3)	SE
Water	4.4	0.35
0.1M HCl	9.7	1.10
1M HCl	7.0	0.88
0.1%w/v SLS	44.7	5.14
0.5%w/v SLS	82.1	1.88
1.0%w/v SLS	109.0	4.18
pH 4.5	7.7	0.56
pH 6.8	2.6	0.75

The apparent solubility of andrographolide in different dissolution media at 37ºC

Table 2: Accuracy of the HPLC assay method

Amount of Standard Added (μg/ml)	Average Amount Found (μg/ml)	Average Recovery (%)	Range of Recovery (%)	% RSD
2.22	2.24	100.9	99.3 - 102.2	1.43
4.44	4.43	100.5	98.5 - 101.3	1.51
5.33	5.22	97.92	97.7 - 98.2	0.22

Table 3: Intermediate precision results

Test day	Analyst	Instrument	Column	Number of replicates	Drug content (%, w/w)
1	A	A	A	2	12.3, 11.9
2	A	B	A	2	7.4, 8.9
3	A	A	B	2	13.0, 13.3
4	B	B	A	2	10.3, 11.0
5	B	A	B	2	6.8, 7.6
6	B	B	B	2	8.3, 9.5
Mean, % RSD					10.04, 0.22

Table 4: Standard and sample solution stability study

Sample	Concentration µg/ml	% of Initial at 5h	% of Initial at 15h	% of Initial at 24h
Tablet formulation				
Acetate buffer	4.4	98.7	98.5	98.9
Phosphate buffer	4.4	97.5	97.7	97.2
0.1M HCl	4.4	99.1	98.9	98.7
Working Standard				
Acetate buffer	4.4	98.3	98.7	98.1
Phosphate buffer	4.4	98.7	98.1	98.6
0.1M HCl	4.4	98.1	98.5	97.9

Table 5: Robustness of the HPLC method

Chromatographic condition	% Recovery ± S.D.
Optimal condition	99.76±0.7
Variation of the mobile phase flow rate	
0.9 ml/min	98.63±0.7
1.1 ml/min	101.34±0.4
Variation of the gradient time	
+2 min	98.84±0.9
-2 min	97.69±0.5
Variation in the mobile phase pH	
pH 2.1	97.93±1.1
pH 2.3	98.34±0.9
Variation in column temperature	
29ºC	97.69±1.2
31ºC	98.46±1.0

Table 6: Robustness of the dissolution method

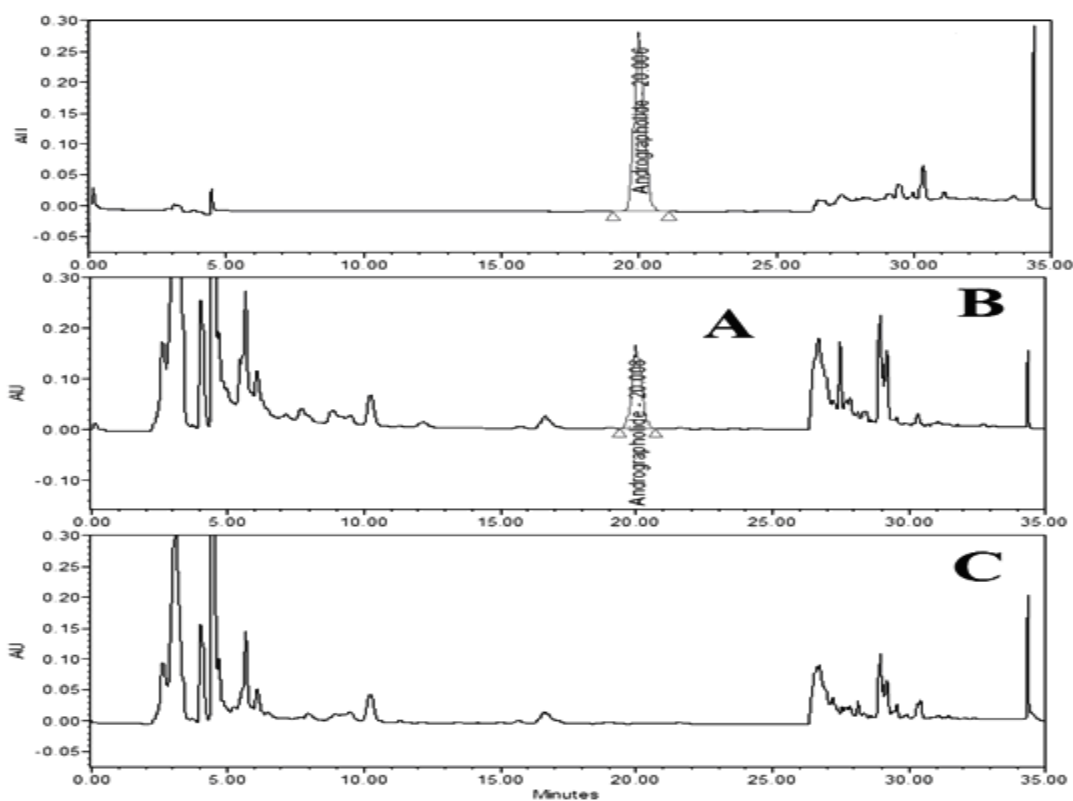| Dissolution parameters	Similarity factor ($f2$)
Optimal condition	100
Variation of the paddle rotation speed	
72 rpm	81
78 rpm	85
Variation in the dissolution medium pH	
pH 6.7	78
pH 6.9	82
Variation in column temperature	
36ºC	80
38ºC	84

Fig. 1: Typical chromatograms of: (A) standard andrographolide; (B) polyherbal formulation; (C) *A. paniculata* placebo in dissolution medium.
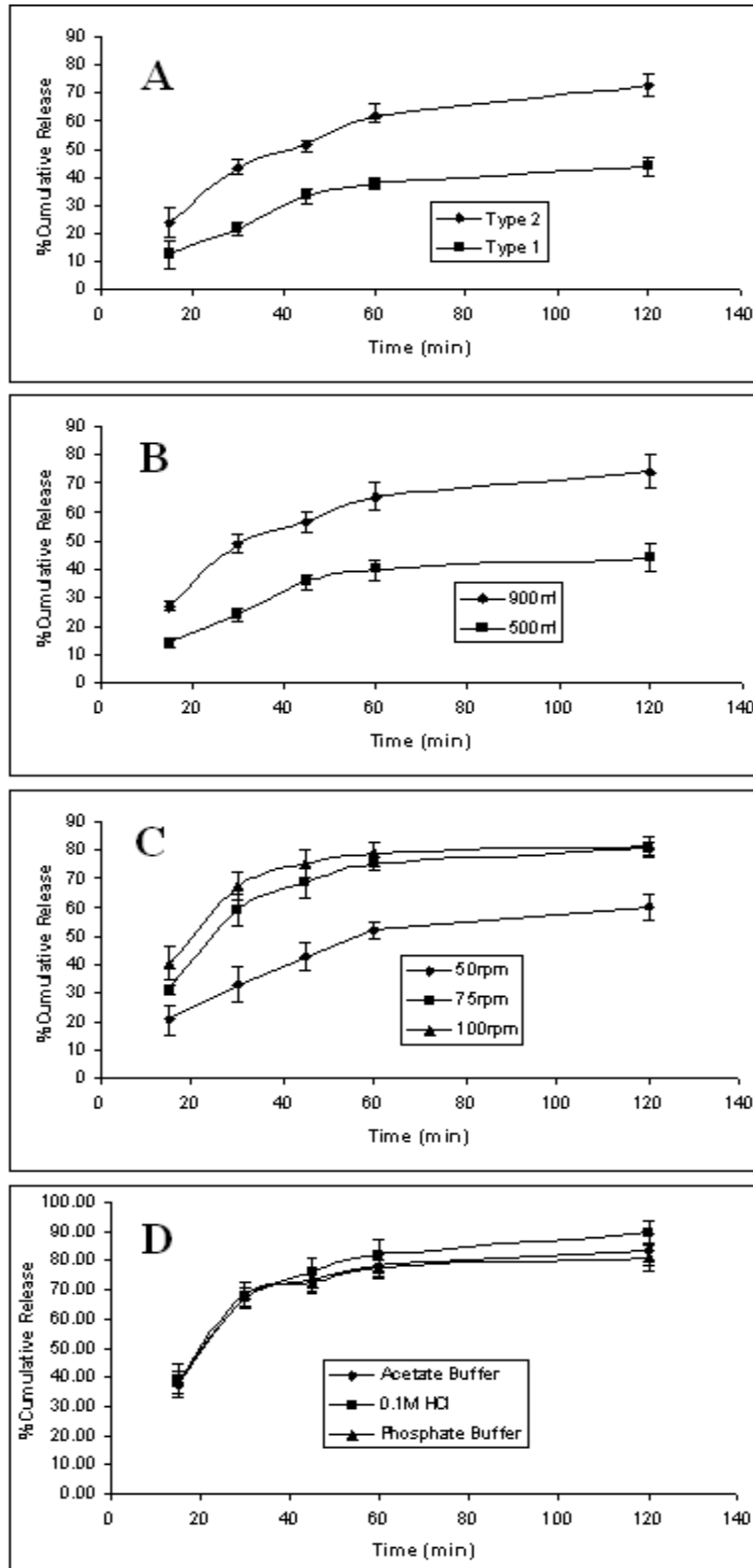
Fig. 2: Optimization of the dissolution test: (A) basket vs. paddle at 50 rpm, (B) effect of dissolution medium volume, (C) effect of paddle rotation speed and (D) dissolution of andrographolide at the optimized condition.

CONCLUSION

A dissolution test for a polyherbal tablet formulation containing the standardized extract of *A. paniculata* has been optimized and validated. All necessary analyses were carried out by a HPLC method that has been developed and validated for this purpose. To the best of our knowledge it is the first attempt towards the development of dissolution study for the active constituent (andrographolide) from polyherbal tablet formulation.

The developed method is quite discriminative to detect any manufacturing changes. Thorough validation demonstrated the

accuracy, precision, selectivity and robustness of the analytical method. The developed method is successfully used by the Quality control department for the routine testing of the dissolution profile of the commercial batches of the product.

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