

ข้อที่ควรพิจารณาสำหรับเกณฑ์ที่ใช้ในการควบคุมคุณภาพของผลิตภัณฑ์ฟ้าทะลายโจร

The Consideration of Quality Control Criteria for *Andrographis paniculata* Products

สุภาวดี ดาวดี (Supawadee Daodee)^{1*}

จินดา หวังบุญสกุล (Jinda Wangboonskul)²

กนกวรรณ จารุกัจฉา (Kanokwan Jarukamjorn)¹

บังอร ศรีพานิชกุลชัย (Bung-orn Sripanidkulchai)²

บทคัดย่อ

แอนโดรกราโฟไลด์เป็นสารสำคัญที่พบในฟ้าทะลายโจร อย่างไรก็ตามยังมีข้อจำกัดในการควบคุมคุณภาพ วิธีการวิเคราะห์โดย HPLC ที่ได้ผ่านการทดสอบความถูกต้องได้ถูกนำมาใช้ในการวิเคราะห์หาปริมาณแอนโดรกราโฟไลด์ในผลิตภัณฑ์ฟ้าทะลายโจร เกณฑ์ที่ใช้ในการควบคุมคุณภาพได้เขียนแบบวิธีใน USP XXII และ BP2002 ซึ่งใช้ในการควบคุมคุณภาพของยาแผนปัจจุบันทั่วไป โดยจะมีการหาปริมาณแอนโดรกราโฟไลด์ ความเท่าเทียมกันของยาแต่ละหน่วย การทดสอบการละลาย ผลิตภัณฑ์ฟ้าทะลายโจรที่จำหน่ายในประเทศไทยจำนวน 8 ชนิดได้ถูกนำมาใช้ในการศึกษาครั้งนี้ ปริมาณแอนโดรกราโฟไลด์ของผลิตภัณฑ์เหล่านี้จะมีค่าระหว่าง 0.93% ถึง 3.01% ของน้ำหนักแห้ง (%RSD 1.20 ถึง 5.40) ความเท่าเทียมกันของยาแต่ละหน่วยจะถูกคำนวณในรูปของมิลลิกรัมแอนโดรกราโฟไลด์ต่อหนึ่งหน่วย ซึ่งมีค่าระหว่าง 2.96 ถึง 11.35 มิลลิกรัม (%RSD 3.49 ถึง 10.34) ปริมาณแอนโดรกราโฟไลด์แต่ละหน่วยที่ละลายใน 100 นาที จะมีค่าอยู่ในช่วง 3.19-11.19 มิลลิกรัม สำหรับตัวทำละลาย 0.1 N กรดไฮโดรคลอริก ส่วนตัวทำละลายที่เป็นสารละลายอะซิเตทบัฟเฟอร์ pH 4.5 อยู่ในช่วง 2.89-10.01 มิลลิกรัม และช่วง 1.51-10.60 มิลลิกรัมในสารละลายบัฟเฟอร์ pH 6.8 ผลจากการทดสอบความคงตัวชี้ให้เห็นว่าปริมาณแอนโดรกราโฟไลด์ จะลดลงไปเรื่อยๆ ประมาณ 8-17% ในเวลา 3 เดือน เมื่อเทียบกับปริมาณเริ่มต้นในสภาวะปกติ และอัตราการสลายตัวจะมีค่าเพิ่มขึ้นในสภาวะเร่ง

Abstract

Andrographolide is the main active constituent in *Andrographis paniculata*. However, the quality control of *A. paniculata* products is limited. A validated analytical method using HPLC was used for determination of andrographolide in *A. paniculata* products. The quality testing methods, adopted from the pharmacopoeias, USPXXVII and BP2002, were assay of andrographolide content, the uniformity of dosage unit and dissolution testing. Eight *A. paniculata* commercial products marketed in Thailand were used in this study. The content of andrographolide in dried powder form in the products ranged from 0.93 % to 3.01 % (%RSD 1.20 to 5.40). The uniformity of dosage unit was calculated in terms of mg andrographolide content per dosage unit which was between 2.96 and 11.35 mg (%RSD 3.49 to 10.34). The amount of andrographolide dissolved within 100 minutes per unit were 3.19 - 11.19, 2.89-10.01 and 1.51-10.60 mg in 0.1 N hydrochloric acid solution, acetate buffer pH 4.5 and phosphate buffer solution pH 6.8, respectively. The results from stability testing indicated that the andrographolide content gradually decreased approximately 8-17% of the initial value in 3 months at ambient conditions and the degradation rate was much higher in accelerated condition.

คำสำคัญ: แอนโดรกราโฟไลด์ ฟ้าทะลายโจร การควบคุมคุณภาพ

Keywords: andrographolide, *Andrographis paniculata*, quality control

¹ผู้ช่วยศาสตราจารย์ คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น

²รองศาสตราจารย์ คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น

*corresponding author, e-mail: csupawad@kku.ac.th

Introduction

Andrographis paniculata (Burm.f.) Nees, also known commonly as King of Bitters, is a plant in the family Acanthaceae, and has been used for centuries to treat gastrointestinal tract and upper respiratory infections, fever, herpes, sore throat and a variety of other chronic and infectious diseases. The most medicinally active phytochemical in this plant is andrographolide (Sharma et al., 1992). It has a very bitter taste. The appearance of andrographolide is colorless crystals and it is called one of the diterpene lactone compounds. Andrographolide is believed to enhance functionalities of the immune system. It inhibits the *in vitro* proliferation of different tumor cell lines and results suggest that andrographolide is an interesting pharmacophore with anticancer and immunomodulation activity (Sriram et al., 2003). Andrographolide showed anticancer activity on diverse cancer cells representing different types of human cancers (Kumar et al., 2004). A significant anti-inflammatory effect has been demonstrated for both the pure diterpene andrographolide and for the extract of this plant (Madav et al., 1996). In the same study, an antipyretic effect was also observed. Moreover, andrographolide showed an anti-allergic action which might be related to the total anti-inflammatory effect (Gupta et al., 1998).

The analysis of andrographolide has been previously reported by using various techniques such as TLC-HPTLC (Saxena et al., 2000), chemiluminescence (Wang et al., 1994), gravimetry (Srivastava et al., 1959; Sengupta et al., 1949), colorimetry (Maiti et al., 1959), micellar electrokinetic chromatographic method (Cheung et al., 2001), HPLC (Jain et al., 2000; Panossian et al.,

2000; Kumaran et al., 2003; Srivastava et al., 2004), high speed counter-current chromatography (Du et al., 2003) and gas chromatography-mass spectrometry (Panossian et al., 2000). In this study, the developed HPLC method was validated and used in the determination of andrographolide in *A. paniculata* products.

Products of *A. paniculata* dried powder in pharmaceutical dosage forms (capsule/tablet) have been widely marketed as an alternative medicine in Thailand. These products are produced from many companies and the *A. paniculata* dried powder is obtained from many sources. The major compound, andrographolide, has been accepted as the main active compound in this herb as shown by its pharmacological activities, as mentioned above. From the knowledge of biodiversity, variation of level of the components in plant obtained from different cultivated areas is common. This leads to the uncertain efficacy of most herbal medicines. Stability of the active component is another crucial part of the quality testing program because the instability of the product modifies the essential requisites such as quality, efficacy and safety. The stability of the compound is affected by changing of environmental factors such as temperature and humidity. This study considered appropriate methods for quality control of *A. paniculata* products. The applied method in this study included the assay of amount of andrographolide, uniformity of dosage unit, dissolution and stability testing of the products which followed the guidelines used for modern medicine in the pharmacopoeias.

Material and methods

The pure compound used as a standard of andrographolide was obtained from the isolation of

A. paniculata dried powder which had been characterized and identified by TLC, HPLC, NMR and MS. All solvents were of AR and HPLC grade and were purchased from Merck (Darmstadt, Germany). Eight commercial *A. paniculata* products from 6 manufacturers were purchased from the drug stores located in the inner city of Khon Kaen province, Thailand. Seven of them contained ground dried *A. paniculata*; six were in capsule dosage form (samples A, B, C, E, F and G) and sample H was in tablet form. Only sample D was the dried extract powder of *A. paniculata* filled in capsules. A Hewlett Packard LC-1100 gradient liquid chromatography instrument, equipped with autosampler system and a photodiode array detector was used in the determination of andrographolide under the following operating conditions; acetonitrile-10 mM phosphate buffer, pH 2.0 (50:50, %v/v) as mobile phase, flow rate 1.0 ml/min. The analysis was performed on a Hypersil ODS column (250 x 4.0 mm, 5 μ m) and andrographolide was detected at 230 nm. The optimized HPLC method was validated for selectivity, linearity, accuracy, precision, detection limit and limit of quantitation. The internal standard used in this analysis was naproxen sodium. This HPLC method was accurate, reliable and can be used in the determination of andrographolide since precision of retention time, within-day and between-day precision were lower than 2%RSD. The assay was linear over the range from 4.2 to 33.6 g/ml ($r^2 > 0.999$). The limit of detection and quantitation were 0.022 and 0.055 g/ml, respectively (Daodee et al., 2002).

Assay amount of andrographolide in A. paniculata products

A sample solution of *A. paniculata* product was prepared by accurately weighing approximately 300

mg dried powder from 20 dosage units (ground to make uniform), then dissolved in 25 ml of methanol and sonicated for 60 minutes. A small portion of sample solution was filtered through 0.45 μ m Acrodisc filter. Fifty μ l of the resulting solution and 20 μ l of internal standard solution (naproxen sodium 0.2 g/ml) were further transferred to an autosampler vial containing 930 μ l of mobile phase solution then mixed. Sample solutions were analyzed directly after preparation by injecting 20 μ l into the HPLC system under the described chromatographic conditions. The amount of andrographolide was calculated by comparing the peak area ratio of andrographolide and internal standard to the calibration curve of standard andrographolide solution.

Uniformity of dosage unit

Weight variation and content uniformity method (United States Pharmacopoeia XXVII and British Pharmacopoeia 2002) were adopted to represent the uniformity of dosage unit of *A. paniculata* products in this study. For the weight variation, thirty units of each *A. paniculata* product were selected, ten units were used and the net content weight of each individual unit was determined. Mean and percent relative standard deviations of each product were calculated.

For content uniformity, thirty units of each *A. paniculata* product were selected for the study. The andrographolide in each individual unit was dissolved in 25 ml methanol. Fifty μ l of the filtered solution and 20 μ l of internal standard solution (naproxen sodium 0.2 g/ml) were diluted and adjusted to volume (1000 μ l) by mixture of mobile phase before injecting 20 μ l into the HPLC system described above. The contained amount was calculated from the calibration curve using linear regression.

Calibration curve preparation

The calibration curves for assay amount of andrographolide in *A. paniculata* products and the content uniformity were prepared by using five concentrations of standard andrographolide, prepared in mobile phase, ranging from 4 to 32 g/ml. Twenty μ l of each standard was injected into the HPLC system. Peak areas were measured. Calibration equations were obtained by linear regression of peak area ratio (andrographolide/internal standard) on concentration.

Dissolution testing

To determine the compliance of the dissolution, Apparatus II, as described in United States Pharmacopoeia XXVII, was used in this study. For each product, one unit was placed in each of the six vessels which were partially immersed in a suitable water bath. The water bath permitted holding the temperature inside the vessels at 37 ± 0.5 °C during the test and keeping the bath fluid in constant, smooth motion. The paddle formed from a blade and a shaft was used as the stirring element and set to rotate smoothly at 100 rpm. Three mediums, 0.1 N hydrochloric acid solution, acetate buffer pH 4.5 and phosphate buffer pH 6.8 were used to perform the dissolution profiles of each *A. paniculata* products. The sample solution of each unit was withdrawn within the time interval specified at 10, 20, 40, 60, 80 and 100 min. The collected solutions were filtered through Acrodisc (0.45μ m). Six hundred μ l of the resulting solution, 20 μ l of internal standard solution and 380 μ l of acetonitrile were pipette into a vial, mixed and 20 μ l was then injected into the HPLC system. The amount of andrographolide dissolved for each time interval from each unit was calculated from the calibration curve using linear regression. The standard

andrographolide solution used for preparing the calibration curve in the dissolution test ranged from 0.5–15 g/ml and was prepared in the same solvent proportion as in the sample solution.

Stability testing for A. paniculata products

Three *A. paniculata* products in capsule dosage form were kept in white plastic boxes and stored under two different conditions for a period of 3 months. The uncontrolled condition was performed at ambient temperature and humidity and the accelerated condition was performed in a stability chamber at 45 ± 0.5 °C with 75 ± 5 %RH. The frequency of sampling to determine the content of andrographolide for each product was set at 0, 1, 2 and 3 months for both conditions. The method under the topic of assay amount of andrographolide in *A. paniculata* products was used.

Results and discussion

The amount of andrographolide in A. paniculata product

The content of andrographolide in eight *A. paniculata* products was analyzed using the method described above and calculated. The percent of milligrams andrographolide in the dried powder ranged from 0.93 to 3.01% (%RSD 1.20 to 5.40).

The uniformity of dosage unit

The results of the weight variation and the content uniformity of eight *A. paniculata* products are shown in Figures 1 and 2, respectively. The mean net weight of *A. paniculata* powder per unit of the products ranged from 285.2 \pm 15.2 to 377.0 \pm 20.8 mg (%RSD 2.76 to 5.53). The content uniformity was expressed in terms of the average amount of andrographolide contained per unit which ranged from 2.96 \pm 0.19 to 11.35 \pm 0.87 mg (%RSD 3.49 to

10.34). A high variation among products was found which may be caused by the different sources of raw materials used and the particle size of *A. paniculata* powder in the formulation. However, further study should be done to support this hypothesis.

Dissolution testing

The results of dissolution test from eight *A. paniculata* products in three mediums, 0.1 N hydrochloric acid, acetate buffer (pH 4.5) and phosphate buffer (pH 6.8), are shown in Table 1 and their profiles are shown in Figure 3. The amounts of andrographolide dissolved in 100 minutes from these products were 3.19 to 11.19 mg, 2.89 to 10.01 mg and 3.21 to 10.60, respectively. No interference from the opaqueness and the pigments from the capsules were detected at the selected wavelength of 230 nm. The variation of amount dissolved of andrographolide from each product was as high as 3.5 fold. However, this was not unexpected due to the diversity of the different sources of raw materials obtained.

Stability testing for A. paniculata products

Andrographolide, the major constituent of *A. paniculata*, was used to represent the stability of the products in this study. The andrographolide remaining after 3 months' storage in ambient conditions was 82.67 to 92.31% which gradually decreased approximately by 8–17% of the initial value. In accelerated conditions of 3 months' storage, andrographolide remaining was 50.55 to 84.61 % which decreased approximately by 15–49 % of the initial value. The results indicated that the degradation rate of andrographolide in accelerated conditions was much higher than in ambient conditions. However, high variation of degradation rate among the products was found. This may be due to the different formulation and the manufacturing processes. Moreover the

different raw materials' sources, particle size and humidity remaining in the dried *A. paniculata* powder may be the main issues about the instability of these products.

Conclusion

The rationale of the uncertainty of effectiveness of *A. paniculata* products may be shown in this study by the results of high variation of the level and instability of andrographolide itself. This leads to difficulty setting quality control by adopting the criteria and methods following the guidelines from the pharmacopoeias. High variation of andrographolide level leads to unreliable label claims for the products. Shelf life of the product is too short for the commercial scale. A better formulation process such as film-coating or better packing material could improve its stability. The European Agency for the Evaluation of Medicinal Products (EMA, 2001) has recently accepted a limit of 10% of the initial assay content for the constituents with therapeutic activity. Control of the main active constituent level of the raw material before formulation has to be performed for consistency of products. The expiration date should be set to assure the effectiveness of the product. In our opinion, criteria for the quality control of this product should be set but the limitation range should be set wider than the criteria set for modern medicine in pharmacopoeias.

However, the best way of using herbal medicine is to isolate a single pure compound of the active constituent and follow the long process of drug development. This may include the modification of the chemical structure to give the right physicochemical properties, testing for the pharmacological activities and toxicity before starting the drug formulation and

quality control of the products. The final step is the clinical trial.

Reliable, accurate and sensitive methods to determine the amount of the active compound in the products are needed for the drug development processes. The HPLC method developed in this study provides a good determination of andrographolide for the purpose of quality control as shown by the validation values. The advantage of this method is the short analysis time since a large number of samples can be analyzed in one day.

Acknowledgements

The authors are indebted to the Center of Research and Development of Herbal Health Products and the Faculty of Pharmaceutical Sciences, Khon Kaen University for financial support and facilities.

References

- Cheung, H.Y., Cheung, C.S. and Kong, C.K. 2001. Determination of bioactive diterpenoids from *Andrographis paniculata* by micellar electrokinetic chromatography. **J. Chromatogr. A.** 930(1-2): 171-176.
- Daodee, S., Wangboonskul, J., Jarukamjorn, K. and Sripanidkulchai, B. 2004. An HPLC method developed for applying in quality control of *Andrographis paniculata* products. In: **Angural AASP Conference**, Beijing, Republic of China (Poster presentation).
- Du, Q., Jerz, G. and Winterhalter, P. 2003. Separation of andrographolide and neoandrographolide from the leaves of *Andrographis paniculata* using high-speed counter-current chromatography. **J. Chromatogr. A.** 984(1): 147-151.
- Gupta, P.P., Tandon, J.S. and Patnaik, G.K. 1998. Antiallergic activity of andrographolides isolated from *Andrographis paniculata* (Burm.F) Wall. **Pharm. Bio.** 36(1): 72-74.
- Jain, D.C., Gupta, M.M., Saxena, S. and Kumar, S. 2000. LC analysis of hepatoprotective diterpenoids from *Andrographis paniculata*. **J. Pharm. Biomed. Anal.** 22(4): 705-709.
- Kumar, R.A., Sridevi, K., Kumar, N.V., Nanduri, S. and Rajagopal, S. 2004. Anticancer and immunostimulatory compounds from *Andrographis paniculata*. **J. Ethnopharm.** 92: 291-295.
- Kumaran, K.S., Thirugnanasambantham, P., Viswanathan, S. and Murthy, M.S. 2003. An HPLC method for the estimation of andrographolide in rabbit serum. **Ind. J. Pharm.** 35: 109-112.
- Madav, S., Tandan, S.K., Lal, J. and Tripathi, H.C. 1996. Anti-inflammatory activity of andrographolide. **Fitotherapy** 67: 452-458.
- Maiti, P.C., Kanji, S.K. and Chatterjee, R. 1959. Studies in Kalmegh extracts. **Ind. J. Pharm.** 21(6): 169-171.
- Panossian, A., Ovhanisyan, A. and Mamikonyan, G. 2000. Pharmacokinetic and Oral bioavailability of andrographolide from *Andrographis paniculata* fixed combination Kan Jang in rats and human. **Phytomedicine** 7(5): 351-364.
- Saxena, S., Jain, D.C., Gupta, M.M., Bhakuni, R.S., Mishra, H.O. and Sharma, R.P. 2000. High-performance thin-layer chromatographic analysis of hepatoprotective diterpenoids from *Andrographis paniculata*. **Phytochem. Anal.** 11(1): 34-36.

- Sengupta, S.P., Banerjee, S. and Chakravarty, D. 1949. Studies in the specification of Indian medicinal plants. **Ind. J. Pharm.** 11: 77-78.
- Sharma, A., Lal, K. and Handa, S.S. 1992. Standardization of Indian crude drug Kalmegh by high pressure liquid chromatographic determination of andrographolide. **Phytochem. Anal.** 3: 129.
- Sriram, R., Kumar, R.A., Deevi, D.S., Satyanarayana, C. and Rajagopalan, R. 2003. Andrographolide, a potential cancer therapeutic agent isolated from *Andrographis paniculata*. **J. Exp. Ther. Onc.** 3(3): 147.
- Srivastava, S.K., Bose, P.C., Ray, G.K. and Mukherjee, B. 1959. Estimation of andrographolide in Kalmegh Source. **Ind. J. Pharm.** 21: 229-230.
- Srivastava, A., Misra, H., Verma, R.K. and Gupta, M.M. 2004. Chemical fingerprint of *Andrographis paniculata* using HPLC, HPTLC and densitometry. **Phytochem. Anal.** 15(5): 280-285.
- Wang, B.Q., Pang, Z.G. and Wang, C.Y. 1994. Indirect chemiluminescence to quantify andrographolide. **Huaxue Tongbao.** 7: 50.

Table 1. The amount of andrographolide dissolved within 100 minutes of eight *A. paniculata* products in three mediums; 0.1 N hydrochloric acid, acetate buffer, pH 4.5 and phosphate buffer, pH 6.8.

Product	Dosage form	Amount of andrographolide dissolved in 100 minutes (mg)		
		0.1 N HCl	Acetate buffer (pH 4.5)	Phosphate buffer (pH 6.8)
A	capsule	11.19	10.01	10.60
B	capsule	6.80	6.24	5.85
C	capsule	5.94	3.73	3.21
D	capsule	3.19	1.65	1.51
E	capsule	3.78	2.89	3.36
F	capsule	6.31	5.61	5.35
G	capsule	7.42	5.70	4.89
H	tablet	9.11	8.40	8.36

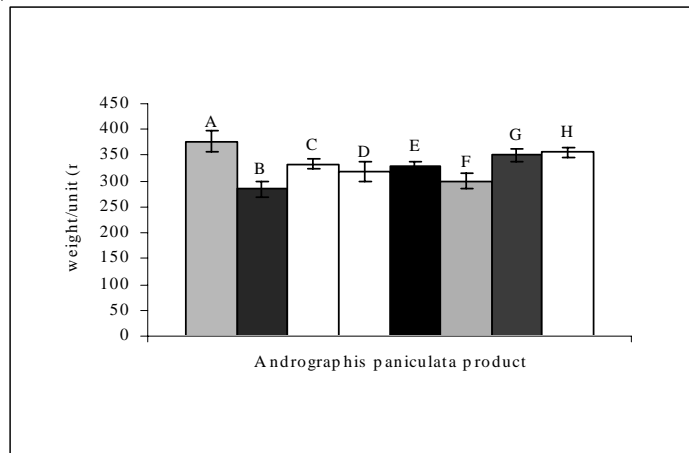


Figure 1. Result of weight variation for each *A. paniculata* product (A-H). The average weight of the products is from 285.2 ± 15.2 to 377.0 ± 20.8 mg.

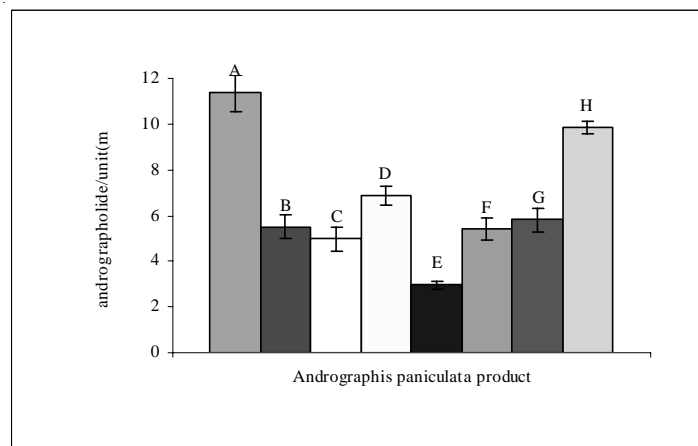


Figure 2. Result of content uniformity for each *A. paniculata* product (A-H) expressed in terms of the mean amount of andrographolide per unit.

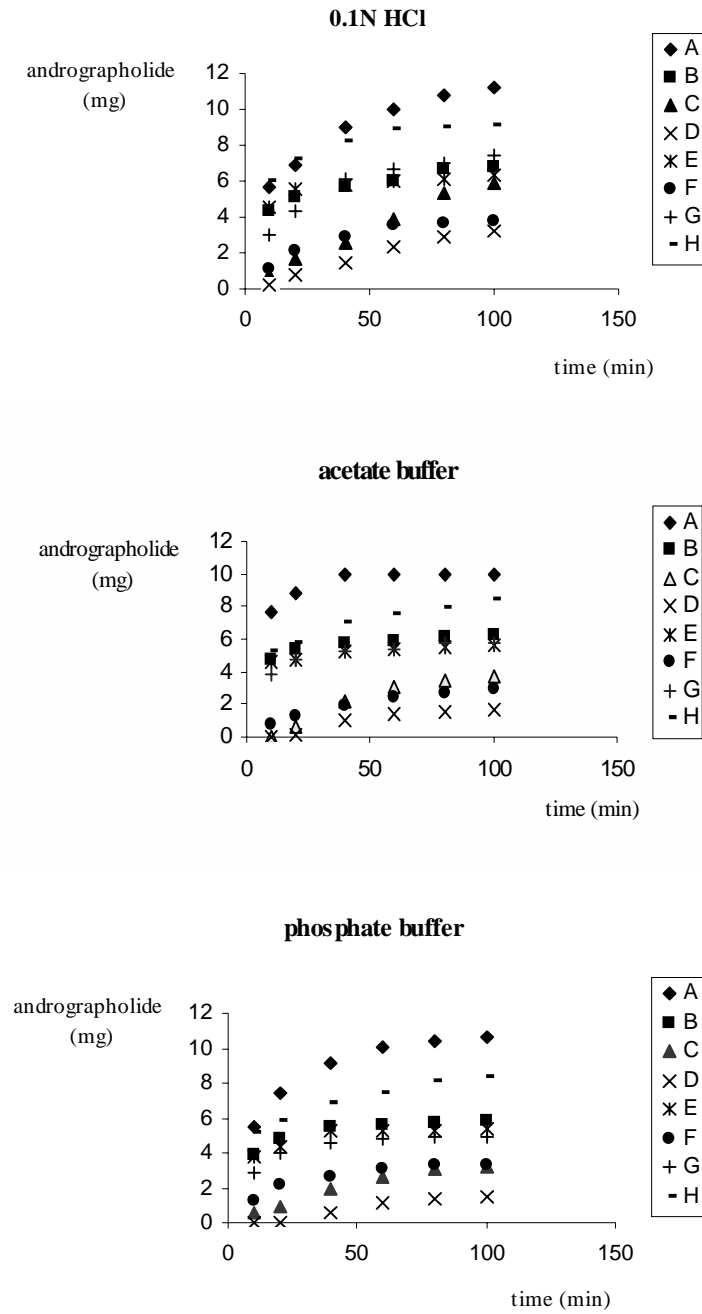


Figure 3. Dissolution profile of *A. paniculata* products (A-H) in three mediums; 0.1 N hydrochloric acid, acetate buffer pH 4.5 and phosphate buffer pH 6.8.