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Development of HPLC assay method of fusidate sodium tablets

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Abstract: The Korean Pharmacopoeia (KP XI), British Pharmacopoeia (BP 2013) and Japanese Pharmacopoeia contain monographs for the quality control of raw fusidate sodium and its formulations using high performance liquid chromatography (HPLC). However, the assay method for the determination of fusidate sodium in commercial tablets is titration which is less specific than HPLC. In this study, we present an alternative HPLC method for quantitation of fusidate sodium in tablets. Method validation was performed to determine linearity, precision, accuracy, system suitability, and robustness. The linearity of calibration curves in the desired concentration range was high (r^2 = 0.9999), while the RSDs for intra- and inter-day precision were 0.25-0.37 % and 0.11-0.60 %, respectively. Accuracies ranged from 99.46-100.85 %. Since the system suitability, intermediate-precision and robustness of the assay were satisfactory, this method will be a valuable addition to the Korean Pharmacopoeia (KP XI).

Key words: HPLC, Fusidate Sodium, Assay, Validation, Tablet dosage form

1. Introduction

Fusidate sodium (*Fig.* 1) is a sodium salt form of fusidic acid which is an antibiotic isolated from the fermentation broth of *Fusidium coccineum*. It has similar structure to cephalosporin and acts by inhibiting bacterial protein synthesis.[1] Fusidic acid and its sodium salt show a very high anti-staphylococcal activity[2] and is administered as variety of preparations such as cream, ointment, eye drops or tablets. Available methods for the quantitative determination of fusidic acid are based mainly on microbiological procedures.[2,3] In the 11th revision of the Korean Pharmacopoeia (KP XI), high performance liquid chromatography (HPLC) was applied for assay test of fusidate sodium material, ointment and plaster. However, assay method of sodium fusidate tablet was titration, which is not as specific as HPLC.[4] In British Pharmacopoeia 2013 (BP, 2013), fusidic acid and fusidate sodium material are determined by titration method while fusidic acid cream, eye drops, oral suspension and ointment are quantitated by liquid chromatography.[5]

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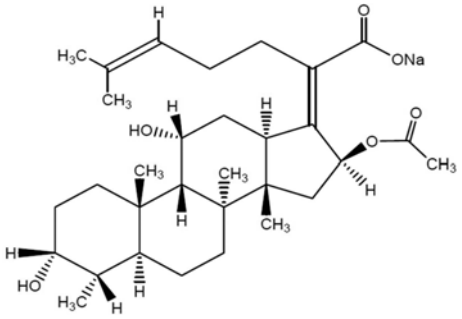

Fig. 1. Chemical structure of fusidate sodium.

The latest version of the United State Pharmacopoeia (USP) still has not published monographs for fusidic acid and its sodium salts.[6] A throughout literature search revealed that no validated method for the determination of fusidate sodium tablets has been reported until now.[7-12]

Since the establishment of the first edition in 1958, the Korean Pharmacopoeia (KP) has been revised 10 times to ensure safety and efficacy of pharmaceutical products through appropriate test methods in accordance with international harmonization. Thus, replacement of non – specific test or conventional methods with updated methods and assurance of laboratory safety and environmental issues are greatly considered in every revision of the KP.

For the above reasons, the objective of the present work is to develop a reliable, simple, affordable HPLC method for quantitation of fusidate sodium in tablets. Validation was conducted following the International Conference on Harmonization (ICH)[13] and Korean Food and Drug Administration (KFDA) Validation Protocols.[14,15]

2. Experimental

2.1. Chemicals and reagents

Fusidate sodium material and tablets were supplied by Dong Wha Pharmaceutical (Seoul, Korea). HPLC grade acetonitrile and methanol were obtained from Daejung Chemicals and Metals Co. (Siheung, Korea). Phosphoric acid was purchased from Samchun Pure Chemicals Co. (Seoul, Korea). Purified water was prepared in the laboratory. All other chemicals were of analytical reagent grade.

2.2. Instrumental conditions

Experiments were conducted on Agilent 1100 HPLC system consisted of following components: G1379A Degasser, G1312 Binary Pump, G1313 Auto-sampler, G1316 Colcom (Column Oven) and G1314AVWD Detector (Agilent Technology, Santa Clara, USA). In intermediate precision validation, Shimadzu HPLC equipment included: DGU – 20A5R Degasser, two LC – 20 AD pumps, SIL – 20A auto-sampler, SPD-20A UV – Vis Detector, CBM – 20A communication bus module (Shimadzu Corporation, Kyoto, Japan) and CO-965 Column Oven (Jasco Corporation, Tokyo, Japan) was used.

For the HPLC condition, a Phenomenex Luna C18 (2) column (150 × 4.6 mm I.D., 5 μm) was used for the analysis of fusidate sodium. Mobile phase was composed of acetonitrile, 0.05 mol/L phosphoric acid and methanol (10:4:1). Flow rate was 1.0 mL/min. Inject volume was 10 μL. UV detection was at 235 nm.

2.3. Sample preparation

Standard solution: 2.0 g of fusidate sodium was dissolved in 100 mL mobile phase to obtain a 20.0 mg/mL stock solution. This solution was diluted with mobile phase to make 1.0 mg/mL standard solution.

Sample solution: 20 tablets were weighed and powdered. A quantity of the powder containing the equivalent of 2.0 g of fusidate sodium was transferred to a 100 mL volumetric flask. Mobile phase was added to about 50 % of the capacity of the flask. For completely dissolve of fusidate sodium, the content of the flask was sonicated for 5 min, cooled to room temperature and diluted with mobile phase to volume. Resultant solution was quantitatively diluted so that a concentration of 1.0 mg/mL of fusidate sodium was obtained. A portion of this solution was passed through a filtrate as the sample solution.

2.4. Validation studies

Method was validated accordingly to ICH Q2 (R1) guideline with regard to linearity, precision, accuracy and robustness.

Calibration curves were prepared by taking appropriate volume of fusidate sodium stock solution and diluting with mobile phase to obtain final concentrations of 0.2; 0.4; 1.0; 1.2; 1.6; 2.0 mg/mL and used for evaluation of the linearity, accuracy, precision. Linearity was estimated by correlation coefficient (r^2) of the regression lines from 6 repeated analyses of the desired concentration range. Precision (relative standard deviation, RSD %) of the method were assessed by six analyses in a day (Intra – day) and in three different days (Inter – day) of standard solutions at concentrations corresponding to 80, 100, 120 % of analysis concentration (0.8; 1.0 and 1.2 mg/mL). Accuracy was expressed as recovery rates evaluated by standard addition method: three concentrations (0.8; 1.0 and 1.2 mg/mL) were spiked into 1.0 mg/mL sample solution. The experiments were performed in triplicate.

2.5. Application of the method

This analytical method was applied to quantitate the content of fusidate sodium in tablets. The study was conducted on 6 samples prepared from tablets as mentioned above. The amount of fusidate sodium in sample was calculated by following expression:

$$\text{Fusidate sodium } (NaC_{31}H_{43}O_6)(mg) = m \frac{A_T}{A_S} \times 1000$$

Where

m (g) is the amount of fusidate sodium weighed

A_S (mAU*s) is area of standard,

A_T (mAU*s) is area of sample.

3. Results and Discussion

3.1. Chromatography

The compositions of the mobile phase were investigated to determine the optimal chromatographic conditions. Recently, mixture of acetonitrile, 0.05

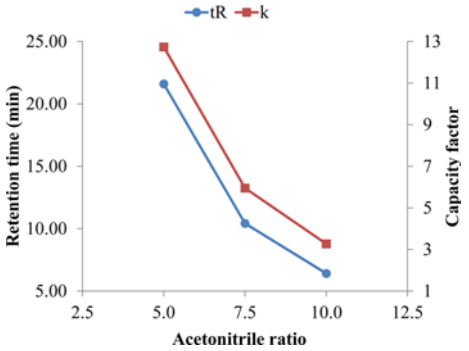

Fig. 2. Investigation of acetonitrile ratio in mobile phase.

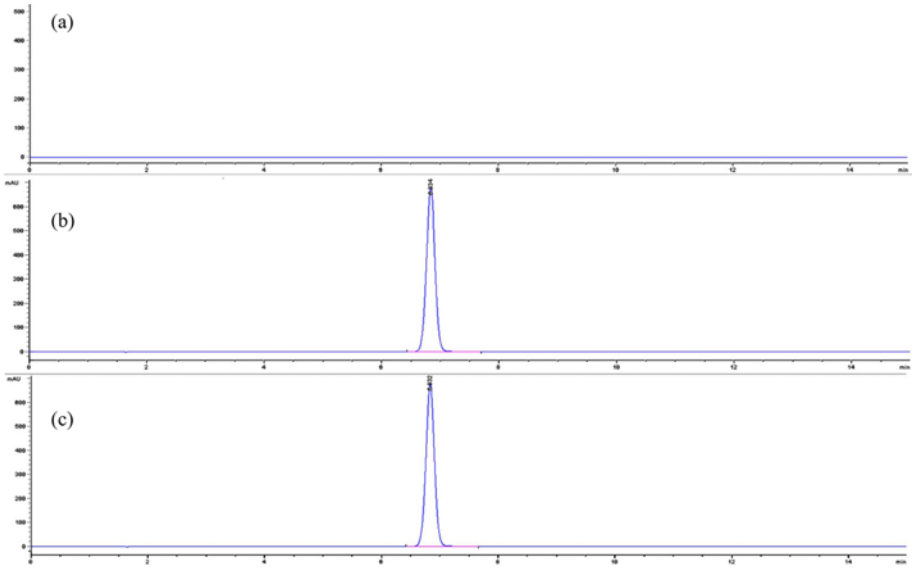

Fig. 3. Typical chromatograms of (a) blank mobile phase sample, (b) 1.0 mg/mL fusidate sodium standard solution, (c) sample solution prepared from tablets. Condition: Luna C18 (2) column (150 × 4.6 mm I.D., 5 μm), mobile phase: acetonitrile : 0.05 mol/L phosphoric acid : methanol (10:4:1), flow rate: 1.0 mL/min, inject volume: 10 μL, detection at 235 nm.

mol/L phosphoric acid and methanol (5 : 4 : 1) was used as mobile phase for the determination of fusidate sodium raw material. However, in this condition, the capacity factor (k) was relatively large: 12.74 and retention time (t_R) of the compound was 21.6 min which required long running time. As shown in *Fig.* 2, when the ratio of acetonitrile in mobile phase was increased, k and t_R were decreased considerably. At the ratio of acetonitrile : 0.05 mol/L phosphoric acid : methanol = 10 : 4 : 1, k value was acceptable (3.27) and retention time of fusidate sodium was 6.83 min which is sufficient enough for routine analysis. Therefore, optimal condition include acetonitrile, 0.05 mol/L phosphoric acid and methanol (10 : 4 : 1) as mobile phase, Phenomenex Luna C18(2) column (150 × 4.6 mm I.D., 5 μm), flow rate at 1 mL/min, injection volume 10 μL and detection at 235 nm. Typical chromatogram was shown in *Fig.* 3(b).

3.2. Linearity

Calibration curves showed good linearity in the concentration range 0.2 ~ 2.0 mg/mL (*Table* 1). The equation of the calibration line obtained is: y = 7.4473 x – 52.11. The correlation coefficient was 0.9999.

3.3. Precision

The precision of the method was assessed by determining the intra-day assay relative standard deviation (RSD %) of the analysis (n = 6) of standard solutions at three concentrations (0.8; 1.0 and 1.2 mg/mL). Three replicates of each concentration were analyzed on each of three consecutive days. Results obtained are shown in *Table* 2. The intra-day precision for each concentration was 0.25 ~ 0.37 % and the inter-day precision was 0.11 ~ 0.60 %.

Table 1. Results of linearity validation. Condition: Luna C18 (2) column (150 × 4.6 mm I.D., 5 μm), mobile phase: acetonitrile : 0.05 mol/L phosphoric acid : methanol (10:4:1), flow rate: 1.0 mL/min, inject volume: 10 μL detection at 235 nm

Parameter	Fusidate sodium
Regression equation	y = 7.4473x – 52.11
Range (mg/mL)	0.2 – 2.0
Correlation coefficient (r^2)	0.9999
Number of data points	6
Slope ± SD	7.4473 ± 0.0159
Intercept ± SD	52.110 ± 7.805

SD: Standard deviation

Table 2. Results of precision (intra/inter-day) validations of the proposed method

Conc. (mg/mL)	Intra-day (n=6)		Inter-day (n=3)	
	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)
0.8	0.25	100.4	0.60	100.5
1.0	0.29	100.6	0.11	100.1
1.2	0.37	99.2	0.30	98.9

Table 3. Recovery tests for fusidate sodium tablets (n = 3)

Added conc. (%)	Recovery	
	Mean (%)	RSD (%)
80	99.46	0.33
100	100.55	0.05
120	100.85	0.72

3.4. Accuracy (Recoveries)

Results of recovery studies by standard addition method were ranged from 99.46 % to 100.85 % (*Table* 3). This also suggested that there was no interference from excipients in determining content of fusidate sodium in tablets.

3.5. System suitability, robustness and intermediate precision

Relative standard deviations of retention time, peak areas and number of theoretical plates, symmetric factor were measured after 6 repeats of 1.0 mg/mL solution analyses to evaluate system suitability of method (*Table* 4). RSD % of retention time and peak areas were 0.03 % and 0.22 %, respectively. The number of theoretical plates was 10727 and symmetric factor was 1.0.

Table 4. System suitability data (n = 6)

Retention time (RSD %)	Peak area (RSD %)	Plate number	Symmetry factor
0.03	0.22	10727	1.00

Table 5. Contents of fusidate sodium tablets (n = 6)

Sample	Claimed value	Assay	
		Content (%)	RSD (%)
Tablet A	250 mg	94.1	0.67

Robustness of the method was checked by making small deliberate changes in the concentration of phosphoric acid (0.05 ± 0.005 mol/L) and flow rate (1 ± 0.5 mL/min). In both case, except changes in retention time, the results of method were not affected: RSD % of peak area (n = 6) was not more than 0.31 %, number of theoretical plates were more than 10000 and symmetric factor was not less than 0.95 and not more than 1.05.

Intermediate precision was studied by using Shimadzu HPLC system. Results showed that there was an increase in retention time - about 0.9 minutes late compared to Agilent 1100 system. Tailing factor (10 %) was 1.1 and the number of theoretical plates was about 9820. RSD % of peak area was 0.20 %.

3.6. Application

This analytical method was applied to quantitate the content of fusidate sodium in tablets. The results of assay test in 6 samples of commercial tablets were recorded in *Table* 5. The average content of fusidate sodium in the formulation was 94.1 %, RSD % of samples was 0.67 %. A typical chromatogram of sample is shown in *Fig.* 3(c).

4. Conclusions

The above proposed study describes a simple HPLC method for the determination of fusidate sodium in tablets. The method was validated and found to be sensitive, accurate and precise. Within reasonable running time of 10 minutes, the method was convenient and effective for the assay test of fusidate sodium tablets.

Acknowledgements

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References

1. Merck Research Laboratories, The Merck Index, 14th Ed., United States (2006).
2. A. H. Hikal, A. Shibl, and S. El-Hoofy, *J. Pharm. Sci.*, **71**(11), 1297-1298 (1982).
3. The Ministry of Health, Labour and Welfare, *The Japanese Pharmacopoeia*, 17th Ed., Japan (2016).
4. Ministry of Food and Drug Safety, *The Korean Pharmacopoeia*, 11th Ed., Republic of Korea (2014).
5. Medicines & Healthcare Products Regulatory Agency, *British Pharmacopoeia*, 7th Ed., United Kingdom (2013).
6. The United States Pharmacopeial Convention, *The United States Pharmacopeia*, USP 39 NF **34**, United States (2016).
7. J. Byrne, T. Velasco-Torrijos, and R. Reinhardt, *J. Pharm. Biomed. Anal.*, **96**, 111-117 (2014).
8. S. Shaikh, M.S. Muneera, O.A Thusleem, M. Tahir, and A. V. Kondaguli, *J. Chromato. Sci.*, **47**, 178-183 (2009).
9. M. Nawaz, M. S. Arayne, N. Sultana, A. Haider, and S. Hisaindee, *Acta Chromatogr.*, **26**, 57-66 (2014).
10. J. Byrne, T. Velasco-Torrijos, and R. Reinhardt, *J. Chromato. Sci.*, **53**, 1498-1503 (2015).
11. H. Sorensen, *J. Chromatography*, **430**, 400-408 (1988).
12. R. Leclercq, R. Bismuth, I. Casin, J. D. Cavallo, J. Croize, A. Felten, F. Goldstein, H. Monteil, C. Quentin-Noury, M. Reverdy, M. Vergnaud, and R. Roiron, *J. Antimicrob. Chemother.*, **45**(1), 27-29 (2000).
13. ICH Guideline. Q2(R1): validation of Analytical Procedures: Text and Methodology Q2(R1) in ICH Harmonised Tripartite Guideline (2005).
14. Ministry of Food and Drug Safety No. 2009-173 (2009. 12. 15), Republic of Korea.
15. Ministry of Food and Drug Safety, No. C0-2012-2-005 (2012. 09. 19), Republic of Korea.