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Determination of triflusal in raw material and capsules by HPLC

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Abstract Currently, the Korean Pharmacopoeia (KP XI) recommends HPLC and potentiometric titration (which is less specific than HPLC) for the determination of triflusal content in capsules and raw materials, respectively. Additionally, the British Pharmacopoeia (BP 2017) and European Pharmacopoeia (EP 8.0), which include a monograph for triflusal in raw materials only, describe a titration method for the assay. The latest version of the United States Pharmacopoeia (USP 39) and Japanese Pharmacopoeia (JP 17) still have not published monographs for triflusal and its preparations. To improve the specificity and efficacy of the assay, we present an HPLC method to determine triflusal content in both raw materials and capsules. The proposed method was validated in accordance with the requirements of the International Conference on Harmonization. A good linear relationship was achieved for triflusal in the range of 200-1250 μg/mL with a coefficient of determination of approximately 0.9996. The relative standard deviations (RSDs) of inter- and intraday precision were 0.73-1.12 % and 0.34-0.51 %, respectively. The recovery percentage of triflusal was in the range of 98.80–101.31 %. Because its system suitability, intermediate precision, and robustness were satisfactory, this method could be suitable for determining triflusal content in raw materials and capsules.

Key words: HPLC, Triflusal, Assay, Validation, Capsule

1. Introduction

Triflusal (*Fig.* 1) is chemically described as 2-hydroxy-4-trifluoromethyl benzoic acid. It is a drug of the salicylate family and chemically related to acetylsalicylic acid.[1,2] Triflusal is a thromboxane synthesis inhibitor as well as an antiplatelet drug.[3] In many studies, triflusal appears to be equally or more effective and safe than acetylsalicylic acid plus dipyridamole and clopidogrel alone.[1]

Currently, most of the commercial products that contain triflusal are prepared as capsules. The Korean Pharmacopoeia (KP XI)[4] recommends HPLC as the assay method for triflusal in capsules and potentio-

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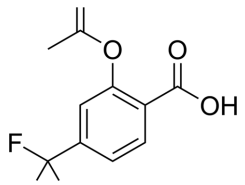

Fig. 1. Chemical structure of triflusal.

metric titration for triflusal in raw material, which is not as specific as HPLC. However, in KP XI, the HPLC method for the determination of triflusal in capsules still has some limitations. In particular, the capacity factor was relatively small (1.30) and the number of theoretical plates was less than 2000 (about 1800).

On the other hand, British Pharmacopoeia (BP 2017)[3] and European Pharmacopoeia (EP 9.0)[5] have only a monograph for triflusal raw material which apply titration method for the assay test. The latest version of the United State Pharmacopoeia (USP 39)[6] and Japanese Pharmacopoeia (JP 17)[7] still have not published monographs for triflusal and its preparations.

Since the establishment of the first edition in 1958, KP has been revised 10 times to ensure safety and efficacy of pharmaceutical products through appropriate test methods. Thus, replacement of non-specific or conventional methods with more appropriate and updated methods as well as assurance of laboratory and environmental safety are considered in every revision of the KP.

For the above reasons, the objective of the present study was to develop an alternative HPLC method, which is reliable, simple, and affordable for quantitating triflusal in raw material and capsules. Validation was conducted following the International Conference on Harmonization (ICH)[8] and Korean Food and Drug Administration Validation Protocols.[9,10]

2. Experimental

2.1. Chemicals and reagents

Triflusal material and capsules were supplied by Shinpoong Pharmaceutical (Ansan, Korea). HPLC-grade acetonitrile and methanol were obtained from Daejung Chemicals and Metals Co. (Siheung, Korea). Potassium phosphate monobasic was purchased from Duksan Pure Chemicals Co. (Ansan, Korea). Purified water was prepared in the laboratory. All other chemicals were of analytical reagent grade.

2.2. Instrumental conditions

Experiments were conducted on a Shimadzu HPLC system consisting of the following components: DGU – 20A5R Degasser, 2 LC – 20 AD pumps, SIL – 20A autosampler, SPD-20A UV – Vis Detector, CBM – 20A communication bus module (Shimadzu Corporation, Kyoto, Japan) and CO-965 Column Oven (Jasco Corporation, Tokyo, Japan).

In intermediate precision validation, Agilent 1100 HPLC system included G1379A Degasser, G1312 Binary Pump, G1313 autosampler, G1316 Colcom (Column Oven), and G1314AVWD Detector (Agilent Technology, Santa Clara, USA).

For the HPLC analysis of triflusal, a Phenomenex NH_2 column (250 × 4.6 mm I.D., 5 μm) was used. The mobile phase was composed of acetonitrile and 0.05 mol/L phosphate buffer previously adjusted to pH 4.5 with potassium hydroxide (1:1, v/v). The flow rate was 1.0 mL/min. Injection volume was 20 μL. UV detection was at 250 nm.

2.3. Sample preparation

Standard solution: 100 mg of triflusal was dissolved in 20 mL mobile phase to obtain a 5000 μg/mL stock solution. This solution was diluted with mobile phase to prepare a 500 μg/mL standard solution.

Sample solution: The contents of 20 capsules were obtained as completely as possible, and the average weight per capsule was determined. After the combined contents were mixed, a quantity of the powder containing the equivalent of 100 mg of triflusal was transferred to a 20 mL volumetric flask. Mobile phase was added to about 50 % of the capacity of the flask. For completely dissolving triflusal, the content of the flask was sonicated for 2 min, cooled to room temperature, and diluted with mobile phase to 20 mL. The resultant solution was diluted to 500 μg/mL. After filtering this solution, it was used as the sample solution.

2.4. Method validation

The method was validated according to the ICH Q2 (R1) guideline with regard to limits of detection (LOD) and quantification (LOQ), linearity, precision, accuracy, and robustness. Calibration curves were prepared by taking an appropriate volume of triflusal stock solution and diluting with mobile phase to obtain final concentrations of 200, 350, 500, 750, 1000, and 1250 μg/mL and used for the evaluation of linearity, accuracy, and precision. Linearity was estimated by the coefficient of determination (r^2) of the regression lines from 6 repeated analyses of the desired concentration range. LOD and LOQ were based on signal-to-noise ratio, 3:1 and 10:1, respectively from six repeated analyses. Precision (relative standard deviation, RSD %) of the method was assessed by 6 analyses on a single day (intra-day) and on 3 different days (inter-day) of standard solutions at concentrations corresponding to 80, 100, and 120 % of the analysis concentration (400, 500, and 600 μg/mL). Accuracy was expressed as recovery rates evaluated by standard addition method: three concentrations (400, 500, 600 μg/mL) were spiked into 500 μg/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 triflusal in capsules. The study was conducted on 6 samples prepared from capsules as mentioned above. The amount of triflusal in the sample was calculated by the following equation:

$$\text{Triflusal } (C_{10}H_7F_3O_4)\ (\text{mg}) = m\ \frac{A_T}{A_S} \times 1000$$

Where

m (g) is the amount of triflusal weighed,

A_S (μAU*s) is area of standard,

A_T (μAU*s) is area of sample.

3. Results and Discussion

3.1. Chromatography

A Phenomenex NH_2 column (250 × 4.6 mm I.D., 5 μm) was used for the analysis of triflusal. Different compositions of the mobile phase and flow rate were investigated to determine the optimal chromatographic conditions. Recently, in KP XI, mixture of acetonitrile and 0.05 mol/L phosphate buffer pH 4.5 (3:1, v/v) was used as the mobile phase for the determination of triflusal in capsules. The flow rate was 1.2 mL/min. However, in this condition, the capacity factor (k') was relatively small (1.30), and the number of theoretical plates (N) was less than 2000 (about 1800). As shown in *Fig.* 2, when the ratio of acetonitrile in the mobile phase and the flow rate were decreased, k' and N increased. At the ratio of acetonitrile: 0.05 mol/L phosphate buffer pH 4.5 = 1:1 (v/v), the capacity factor of triflusal was 2.56, and the number of theoretical plates increased to about 6600.

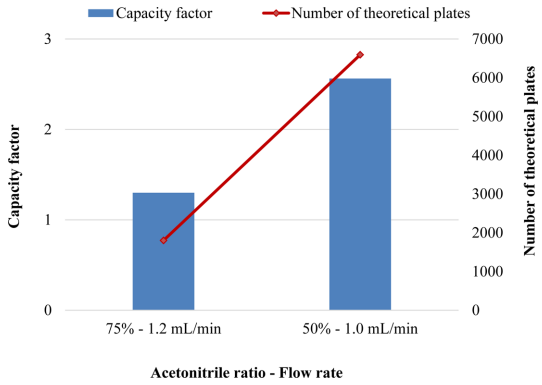

Fig. 2. Investigation of HPLC condition.

Therefore, the optimized HPLC condition used for the analysis of triflusal included: a Phenomenex NH_2 column (250 × 4.6 mm I.D., 5 μm), acetonitrile and 0.05 mol/L phosphate buffer previously adjusted to pH 4.5 with potassium hydroxide (1:1, v/v) as mobile phase, flow rate at 1.0 mL/min, injection volume of 20 μL, and UV detection at 250 nm. A typical chromatogram is shown in *Fig.* 3(b). In comparison with conventional method, the proposed method presented a significant improvement in terms of peak shape (tailing factor 1.13), retention factor (k' rose from 1.30 to 2.56) and column efficiency (N increased from 2000 to more than 6000).

3.2. Linearity

Calibration curves showed good linearity in the

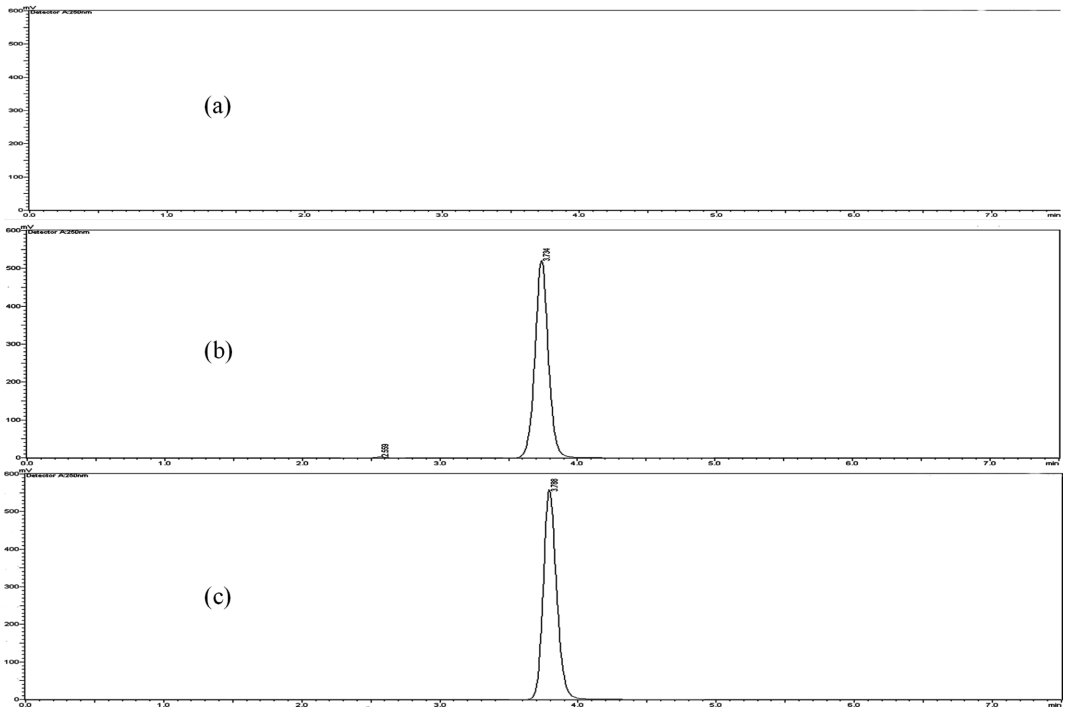

Fig. 3. Typical chromatograms of (a) blank (mobile phase), (b) standard solution, (c) sample solution. Condition: NH2 column (250 × 4.6 mm I.D., 5 μm), acetonitrile and 0.05 mol/L phosphate buffer pH 4.5 (1:1, v/v), flow rate: 1.0 mL/min, inject volume: 20 μL, detection at 250 nm.

Table 1. Results of linearity of proposed and conventional methods

Parameter	Proposed method	Conventional method
Regression equation	y = 7068.3x + 197926.5	y = 6118.8x + 254491
Range (μg/mL)	200 – 1250	200 – 1250
Coefficient of determination (r^2)	0.9996	0.9982
Number of data points	6	6
Slope ± SD	7068.3 ± 115.9	6118.8 ± 86.7
Intercept ± SD	197926.5 ± 8695.5	254491 ± 9725.3
Limit of detection (μg/mL)	0.125	0.5
Limit of quantification (μg/mL)	0.5	1.0

SD: Standard deviation

concentration range of 200-1250 μg/mL (*Table* 1). The equation of the calibration line obtained was: y = 7068.3x + 197926.5. The coefficient of determination was 0.9996. This linearity range was also tested with conventional method. As a result, conventional method showed an inferior linearity with the coefficient of determination about 0.9982.

The LOD and LOQ concentrations of optimized method were estimated to be 0.125 and 0.5 μg/mL, which was half as much as those of conventional method (*Table* 1). These results suggested that the proposed HPLC method is more sensitive than the conventional method.

3.3. Precision

The precision of the method was assessed by determining the intra-day assay RSD % of the analysis (n = 6) of the standard solution at 3 concentrations (400,

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

Conc. (μg/mL)	Intra-day (n=6)		Inter-day (n=12)	
	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)
400	0.73	100.70	0.37	100.68
500	1.12	100.89	0.51	101.02
600	0.82	101.07	0.34	100.79

Table 3. Recovery tests for triflusal in raw material and capsules (n=3)

Added conc. (%)	Raw material		Capsules	
	Mean of recovery (%)	RSD (%)	Mean of recovery (%)	RSD (%)
80	101.31	0.79	98.80	0.12
100	100.64	1.12	100.12	0.13
120	100.12	0.55	99.52	0.10

Table 4. System suitability data (n=6)

Retention time (RSD%)	Peak area (RSD%)	Theoretical plates number	Tailing factor
0.46	0.60	6597.63	1.13

500, and 600 μg/mL). The solution at each concentration was analyzed in triplicate on 3 consecutive days. Results obtained are shown in *Table* 2. The intra-day precision for each concentration was 0.73-1.12 % and the inter-day precision was 0.34-0.51 %.

3.4. Accuracy (Recovery)

Recoveries by the standard addition method ranged from 100.12 % to 101.31 % for raw material and from 98.80 % to 100.12 % for capsules (*Table* 3). This also suggested that there was no interference from excipients in determining the content of triflusal in capsules.

3.5. System suitability, robustness, and intermediate precision

RSD % of retention time and peak areas, the number of theoretical plates, and tailing factor were measured after analyzing the 500 μg/mL solution 6 times to evaluate system suitability of the method (*Table* 4). RSD % of retention time and peak areas was 0.46 % and 0.60 %, respectively. The number of theoretical plates was about 6600, and the tailing factor was 1.13.

Robustness of the method was checked by making small deliberate changes in the pH of phosphate buffer (4.5 ± 0.2) and flow rate (1 ± 0.1 mL/min). In both cases, except changes in retention time, the results of the method were not affected: RSD % of retention time and peak areas (n = 6) was not more than 0.17 % and 0.13 %, respectively, the number of theoretical plates was more than 6000, and the tailing factor was not less than 1.19 and not more than 1.29.

Intermediate precision was studied by using an Agilent 1100 HPLC system. The number of theoretical plates using this system was higher by 4000 than that when using the Shimadzu HPLC system. Symmetry factor was 0.99 and retention time was about 3.67 min. RSD % of retention time and peak areas (n = 6) were 0.35 % and 1.11 %, respectively.

Table 5. Contents of triflusal capsules (n=6)

Sample	Claimed value	Assay	
		Content (%)	RSD (%)
Capsule A	300 mg	100.94	0.54

3.6. Application

This analytical method was applied to quantitate the content of triflusal in capsules. The results of the assay of 6 samples of commercial capsules are shown in *Table* 5. The average content of triflusal in the formulation was 100.94 %, and RSD % of samples was 0.54 %. A typical chromatogram of the sample is shown in *Fig.* 3(c).

4. Conclusions

This paper describes a simple HPLC method for the determination of triflusal in raw material and capsules. The method was validated and found to be sensitive, accurate, and precise. In comparison with the conventional method, the proposed method was turned out to be superior in terms of sensitivity, peak shape and column efficiency.

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