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Development of high performance liquid chromatography assay method of tramadol hydrochloride injection

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Abstract Currently, ultraviolet-visible spectrophotometry and titration methods are used for assay tests of tramadol hydrochloride injection and raw material in the Korean Pharmacopoeia XI (KP XI). Titration has also been used in the British Pharmacopoeia (BP 2013) for the assay test of tramadol hydrochloride, and the HPLC assay for tramadol hydrochloride raw material has been used in the United States Pharmacopeia (USP 39). In this study, we developed an alternative HPLC assay method for tramadol hydrochloride injection that is up to date and specific, and employs the same method as tramadol hydrochloride capsules. Validation of the HPLC method was conducted to determine linearity, precision, accuracy, system suitability, and robustness. The linearity of the calibration curves in the desired concentration range was good ($r^2 > 0.9999$). RSDs of intra-day precision obtained were 0.05-0.08 % and inter-day precision obtained were 0.08-0.19 %. Accuracy was obtained with recoveries in the range of 98.16 % and 100.90 %. As a result of the system's suitability, the RSD of both retention time and the peak area obtained were 0.07 %. The values of the plate number and tailing factor of tramadol hydrochloride obtained were 7076 and 1.16, respectively. Because of the intermediate precision and robustness of the developed assay, it is expected to become a valuable tool for revising the Korean Pharmacopoeia (KP XI).

Key words: HPLC, Korean Pharmacopoeia (KP XI), Tramadol hydrochloride injection, Assay, Validation.

1. Introduction

Since analytical science and technology have been continuously developed, the assay tests for drugs also have been upgraded regularly in pharmacopoeias in many nations. The Korean Pharmacopoeia (KP)[1] also has been revised 10 times to ensure safety and efficacy of pharmaceutical products through appropriate test methods in accordance with international harmonization. However, some test methods are not up to date so that it is crucial to establish new methods to have KP function properly.

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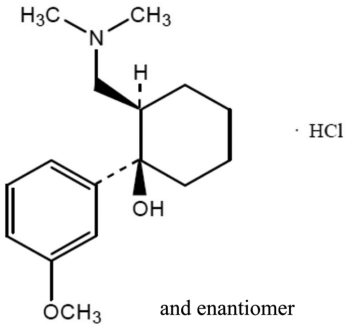

Fig. 1. Chemical structure of tramadol hydrochloride.

Tramadol (2-(dimethylaminomethyl)-1-(3-methoxyphenyl)cyclohexanol hydrochloride) (*Fig.* 1) is a synthetic codeine analogue. Tramadol has central analgesic properties with effects similar to opioids, such as morphine and codeine, acting on specific opioid receptors. Used as a narcotic analgesic for severe pain, it can be addictive and weakly inhibits norepinephrine and serotonin reuptake.[2] Therefore, an appropriate analytical method is necessary for the assay tests of tramadol hydrochloride in commercial formulations. High performance liquid chromatography (HPLC) is utilized for the assay test of tramadol hydrochloride in raw material and capsules according to United States Pharmacopeia (USP 39)[3] and British Pharmacopoeia (BP 2013).[4] However, in KP XI, HPLC is only suggested to quantitate tramadol hydrochloride in capsules while the assay test for tramadol hydrochloride raw material and injection respectively are still titration and ultra violet – visible spectrophotometry which are less specific than HPLC.

For the above reasons, the objective of the present work is to develop a reliable, simple, affordable HPLC method for quantitation of tramadol hydrochloride in injection. Validation was conducted following the International Conference on Harmonization (ICH) and Korean Food and Drug Administration (KFDA) Validation Protocols.[5-7]

2. Experimental

2.1. Chemicals and reagents

Tramadol hydrochloride standard was purchased from Sigma-Aldrich (St. Louis, USA). Tramadol hydrochloride injection was supplied by Daihan Pharm Co. Ltd (Ansan, Korea). Trifluoroacetic acid, HPLC grade acetonitrile and methanol were obtained from Daejung Chemicals and Metals Co. (Siheung, Korea). Purified water was prepared in our laboratory. All other chemicals were of analytical reagent grade.

2.2. Instrumental conditions

Experiments were conducted on Shimadzu HPLC system consisted of following components: DGU – 20A5R Degasser, two 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 Auto-sampler, G1316 Colcom (Column Oven) and G1314AVWD Detector (Agilent Technology, Santa Clara, USA) was used.

For the HPLC condition, a Phenomenex Luna C18 (2) column (250 × 4.6 mm I.D., 5 μm) was used for the analysis of tramadol hydrochloride. Mobile phase included mixture of trifluoroacetic acid in water (0.2:100) and acetonitrile (705:295, v/v). Flow rate was 1.0 mL/min. Inject volume was 10 μL. UV detection was at 270 nm.

2.3. Sample Preparation

Standard solution: 50 mg of tramadol hydrochloride standard was dissolved in 100 mL water to obtain a 500 μg/mL stock solution. This solution was diluted to make a 100 μg/mL standard solution.

Sample solution: A volume of the injection containing the equivalent of 50 mg of tramadol hydrochloride was transferred to a 100 mL volumetric flask. Water was added to the flask to make 100 mL solution. This solution was diluted so that a 100 μg/mL solution was obtained. A portion of this solution was passed through a 0.45 μm membrane filter 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 tramadol hydrochloride stock solution and diluting with water to obtain final concentrations of 20; 60; 100; 150; 200 and 250 μg/mL and used for evaluation of the linearity, accuracy, precision. Linearity was estimated by coefficient of determination (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 (80; 100 and 120 μg/mL). Accuracy was expressed as recovery rates evaluated by standard addition method: three concentrations (80; 100 and 120 μg/mL) were spiked into 100 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 tramadol hydrochloride in injections. The study was conducted on 6 samples prepared from injections as mentioned above. The amount of tramadol hydrochloride in sample was calculated by following expression:

Tramadol hydrochloride ($C_{16}H_{25}NO_2$·HCl) (mg)
$= m \times (A_T / A_S)$

Where

m (mg) is the amount of tramadol hydrochloride 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 Luna C18 (2) column (250 × 4.6 mm I.D., 5 μm) was used for the analysis of tramadol hydrochloride. Mobile phase included mixture of trifluoroacetic acid in water (0.2:100) and acetonitrile (705:295, v/v). Flow rate was 1.0 mL/min. Inject volume was 10 μL.

UV spectrum of 100 μg/mL tramadol hydrochloride solution in water was shown in *Fig*. 2. 270 nm was chosen as detection wavelength because of sufficient detectability and smooth of baseline. Typical chromatogram was shown in *Fig.* 3(b).

3.2. Linearity

Calibration curves showed good linearity in the concentration range 20 ~ 250 μg/mL (*Table* 1). The

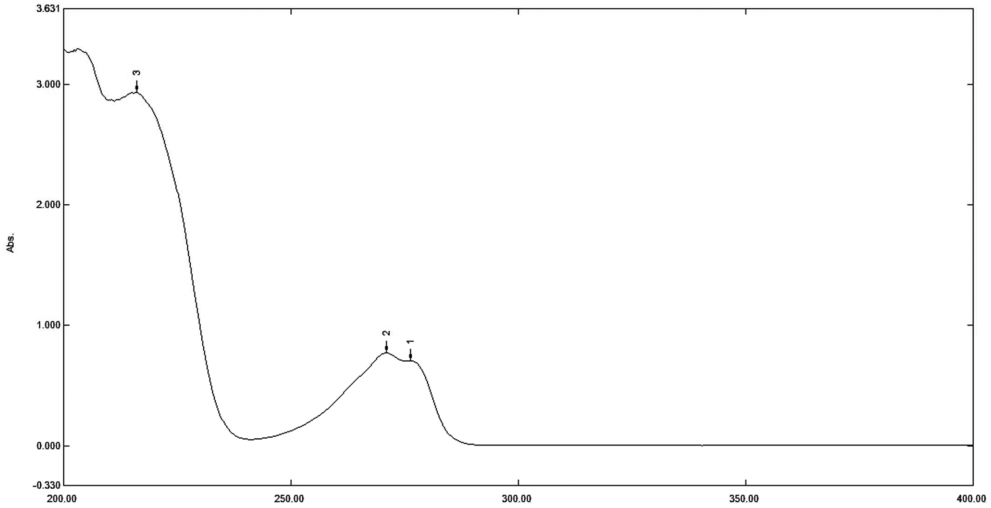

Fig. 2. UV spectrum of 100 μg/mL tramadol hydrochloride solution in water. (1) 276 nm (2) 270 nm (3) 215 nm.

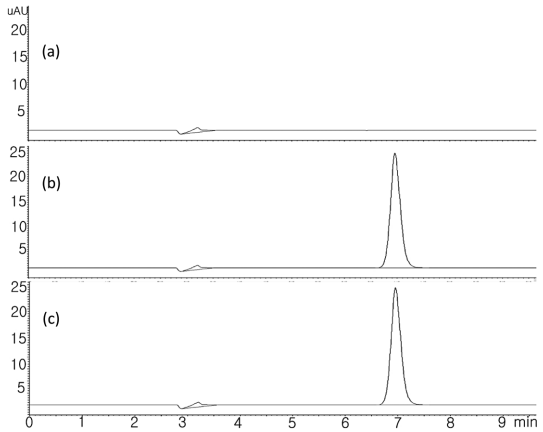

Fig. 3. Typical chromatograms of (a) blank mobile phase sample, (b) 100 μg/mL Tramadol HCl standard solution, (c) sample solution prepared from injection. Condition: Luna C18 (2) column (250 × 4.6 mm I.D., 5 μm), mobile phase: ((Trifluoroacetic acid : Water = 0.2:100) : Acetonitrile = 705:295), flow rate: 1.0 mL/min, inject volume: 10 μL, detection at 270 nm.

Table 1. Results of linearity validation

Parameter	
Regression equation	y = 3209.2x + 2445.3
Range (μg/mL)	20 – 250
Correlation of determination (r^2)	0.9999
Number of data points	6
Slope ± SD	3209.2 ± 5.9
Intercept ± SD	2445.3 ± 611.1

SD: Standard deviation

equation of the calibration line obtained is: y = 3209.2x + 2445.3. The coefficient of determination 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: 80; 100 and 120 μg/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 0.05 ~ 0.08 % and the inter-day precision was 0.08 ~ 0.19 %.

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 (%)
80	0.05	101.6	0.12	100.7
100	0.08	100.9	0.08	101.1
120	0.08	101.3	0.19	101.5

Table 3. Recovery tests for tramadol hydrochloride injections (n = 3)

Added conc. (μg/mL)	Recovery	
	Mean (%)	RSD (%)
80	100.90	0.63
100	99.06	0.53
120	98.16	0.46

3.4. Accuracy (Recoveries)

Results of recovery studies by standard addition method were ranged from 98.16 % to 100.90 % (*Table* 3). This also suggested that there was no interference from excipients in determining content of tramadol hydrochloride in injections.

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 100 μg/mL solution analyses to evaluate system suitability of method (*Table* 4). RSD % of retention time and peak areas were less than 0.1 %. The average number of theoretical plates was 7076 and average tailing factor was 1.16.

Robustness of the method was checked by making small deliberate changes in the content of trifluoroacetic acid (0.2 ± 0.1 %) and flow rate (1.0 ± 0.1 mL/min).

Table 4. System suitability data (n = 6)

Retention time (RSD %)	Peak area (RSD %)	Average number of theoretical	Average tailing factor
0.07	0.07	7076	1.16

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 0.2 %, number of theoretical plates were more than 5000 and symmetric factor was not less than 0.90 and not more than 1.3.

Intermediate precision was studied by using Agilent HPLC system. Results showed that there was an increase in retention time - about 0.5 minutes later compared to Shimadzu system. Asymmetric factor was 0.85 and the number of theoretical plates was about 5741. RSD % of peak area was 0.45 %.

3.6. Application

This analytical method was applied to quantitate the content of tramadol hydrochloride in injections. The results of assay test in 6 samples of commercial injections were recorded in *Table* 5. The average content of tramadol hydrochloride in the formulation was 99.6 %, RSD % of samples was 0.12 %. 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 tramadol hydrochloride in injections. The method was validated and found to be sensitive, accurate and precise.

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References

1. Ministry of Food and Drug Safety, The Korean Pharmacopoeia, 11th Edition, Republic of Korea (2014)
2. National Cancer Institute, https://ncit.nci.nih.gov/ncitbrowser/ConceptReport.jsp?dictionary=NCI_Thesaurus&ns=NCI_Thesaurus&code=
3. The United States Pharmacopeial Convention, *The United States Pharmacopeia*, USP 39 NF **34**, United States (2016).
4. Medicines & Healthcare Products Regulatory Agency, *British Pharmacopoeia*, **7th Edition**, United Kingdom (2013).
5. ICH Guideline. Q2(R1): validation of Analytical Procedures: Text and Methodology Q2(R1) in ICH Harmonised Tripartite Guideline (2005).
6. Ministry of Food and Drug Safety No. 2009-173 (2009. 12. 15), Republic of Korea.
7. Ministry of Food and Drug Safety, No. C0-2012-2-005 (2012. 09. 19), Republic of Korea.