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Determination of the quantity of tolperisone hydrochloride in tablets by high performance liquid chromatography

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Abstract: In attempt to contribute in official monographs of Korean Pharmacopoeia, an HPLC method was developed and fully validated for the determination of tolperisone hydrochloride in tablets which have never been published in other forgein Pharmacopoeia. Analysis was carried out in an ODS column (250 × 4.6 mm I.D., 5 μm) with common solvents include acetonitrile and ammonium hydrophosphate buffer as mobile phase. The assay was validated according to International Conference on Harmonization (ICH) guidelines. The method has good linearity in the range of 5 – 200 μg/mL tolperisone. Intra-day precision varied between 0.04 and 0.10 %. Relative standard deviations of inter-day precision ranged between 0.43 and 1.24 % for peak area. The percentage recovery of the tolperisone ranged between 99.8 and 101.2 % in material. Recoveries in tablets were ranged between 98.7 and 100.8 %, thus confirmed the suitability of method for estimation of tolperisone hydrochloride in tablet dosage form.

Key words: tolperisone, HPLC, assay, validation, tablet dosage form

1. Introduction

Tolperisone which chemically described as 2-Methyl-1-(4-methylphenyl)-3-piperidin-1-ylpropan-1-one hydrochloride (*Fig.* 1) is a centrally acting muscle relaxant that is prescribed for symptomatic treatment of spasticity and muscle spasm.[1] In spite of worldwide marketing, only Japanese and Korean Pharmacopoeia[2-3] have published tolperisone hydrochloride monographs of material which use titration method for assay test. Korean Pharmacopoeia 11 (KP XI) also contains monographs of tolperisone hydrochloride tablets using UV spectrophotometric method and tolperisone hydrochloride injection using HPLC method.

Nowadays, High Performance Liquid Chromatography (HPLC) methods were extensively applied in drugs quality control due to its sensitivity, precision, repeatability and automation in comparison with

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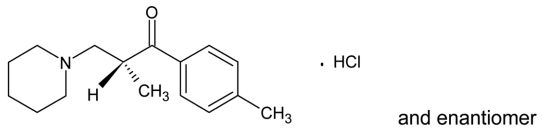

Fig. 1. Structure of tolperisone hydrochloride.

other conventional methods such as spectrophotometry or titration. Countries around the world have been spending many efforts to upgrade their National Pharmacopoeia using HPLC as recommended method.

Many authors have reported HPLC method for determination of tolperisone hydrochloride in many aspects including simultaneously quantitation with other drugs such as lidocain,[4-5] eperisone,[6] paracetamol,[7] tizanidine,[8] determination of related impurities which required complicated gradient profile,[9] determination tolperisone in plasma using mass spectrophotometric detector,[8,10,11] enantiomeric separation by new generation chiral column.[12] In estimation of tolperisone in bulk material and capsules,[13] Satyanarayana. P. V. V *et al.* developed an isocratic reversed phase – HPLC method using an ODS column and mixture of methanol and acetonitrile (90:10 v/v) as mobile phase which is highly toxic and volatile.

For the above reasons, the objective of the present work is to develop a reliable, simple, affordable and less hazardous HPLC method for quantitation of tolperisone hydrochloride in tablets and substitute current UV method in KP XI. Validation was conducted following the International Conference on Harmonization (ICH).[14]

2. Experimental

2.1. Chemicals and reagents

Tolperisone hydrochloride material and tablets were kindly supported by Shinpoong Pharmaceutical (Ansan, Korea). HPLC grade acetonitrile and methanol were obtained from Daejung Chemicals and Metals Co. (Siheung, Korea). Ammonium phosphate dibasic 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

Shimadzu UV 1800 Spectrophotometer was used to determine maximum absorption wavelength of tolperisone hydrochloride solution.

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 (250 × 4.6 mm I.D., 5 μm) was thermo-stated at 25 ºC for the analysis of tolperisone. Different ratios of acetonitrile and 0.02 M ammonium hydro-phosphate pH 5.0 were investigated as mobile phase. Flow rate was 1.0 mL/min. Inject volume was 10 μL. UV detection was at maximum absorption wavelength.

2.3. Sample preparation

Standard solution: 20 mg of tolperisone hydrochloride was dissolved in 20 mL mobile phase to obtain a stock solution of 1000 μg/mL of tolperisone hydrochloride. Standard solution was prepared by taking appropriate volume of tolperisone stock solution and diluting with mobile phase to obtain final concentration of 100 μg/mL of tolperisone hydrochloride

Sample solution: 20 tablets were weighed and powdered. A quantity of the powder containing the equivalent of 100 mg of tolperisone hydrochloride 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 tolperisone, the content of the flask was sonicated for 15 min, cooled to room temperature and diluted with mobile phase to volume. Resultant solution was quantitatively diluted so that a concentration of 100 μg/mL of tolperisone hydrochloride 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, LOD and LOQ, precision, accuracy and robustness.

Calibration curves were prepared by taking appropriate volume of tolperisone stock solution and diluting with mobile phase to obtain final concentrations of 5; 10; 20; 50; 100; 150; 200 μg/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. Detection and quantitation limits were established by signal – to – noise ratio. Precision (relative standard deviation, RSD %) of the method were assessed by five 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 standard solution (recovery of material) and 100 μg/mL sample solution (recovery of tablets). The experiments were performed in triplicate.

2.5. Application of the method

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

$$\% \text{ tolperisone} = \frac{M_w \times A_S}{M_0 \times A_T} \times 100\%$$

Where

M_w (g) is the amount of tablet powder equivalent to 100 mg tolperisone,

M_0 (g) is the amount of tablet powder weighted in experiment,

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. Variety ratios of acetonitrile and 0.02 M ammonium hydrophosphate pH 5.0 were investigated as mobile phase. As shown in *Fig.* 2, when the ratio of acetonitrile in mobile phase increased, tolperisone eluted significantly faster and peak shape was more symmetrical. However, eluting too fast resulted in limited retention and theoretical plates. Thus, the composition of acetonitrile and 0.02 M ammonium hydrophosphate pH 5.0 (40:60 v/v) was chosen. The effects of pH and column temperature slightly affected peak shape and retention time. At pH 5 and 25 °C, the best peak shape was observed. Maximum absorption wavelength was detected at 259 nm (*Fig.* 3). Therefore, optimal condition include acetonitrile : 0.02 M ammonium hydrophosphate pH 5.0 (40 : 60

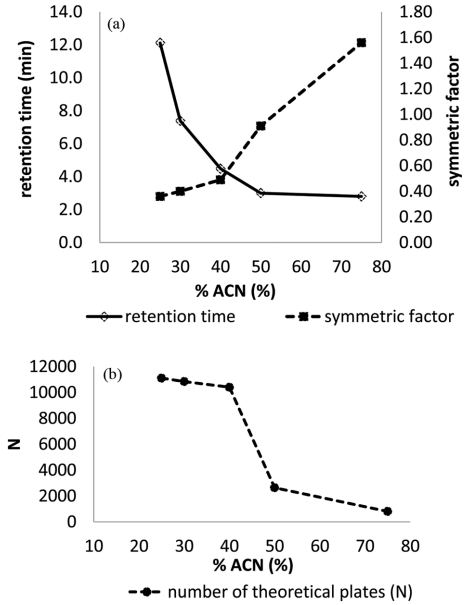

Fig. 2. Effect of content of acetonitrile in mobile phase on retention time, symmetric factor (a) and number of theoretical plates (b) of tolperisone. Condition: Phenomenex Luna C18(2) column (250 × 4.6 mm I.D., 5 μm), mobile phase: Acetonitrile : 0.02 M ammonium hydrophosphate buffer (pH 5), 25 °C. Detection: UV 259 nm, flow rate 1.0 mL/min.

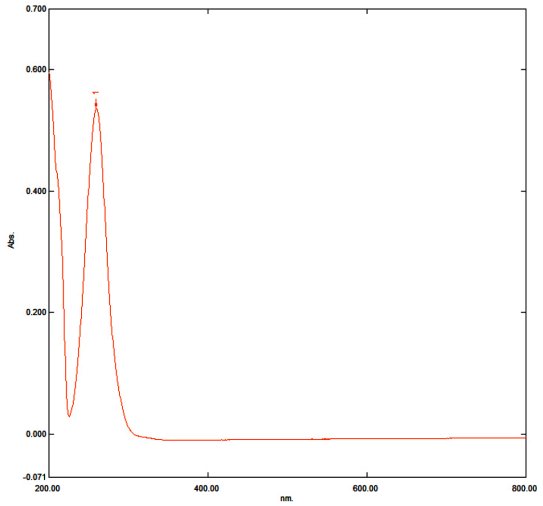

Fig. 3. UV spectrum of tolperisone hydrochloride standard solution.

v/v) as mobile phase, Phenomenex Luna C18(2) column (250 × 4.6 mm I.D., 5 μm) thermostated at 25 °C, flow rate at 1 mL/min, injection volume 10 mL and detection at 259 nm. Typical chromatograms were shown in *Fig.* 4(a) and 4(b).

Table 1. Results of linearity validation. Condition: Luna C18(2) column (250 × 4.6 mm I.D., 5 μm) thermostated at 25 °C, flow rate at 1 mL/min, injection volume 10 μl and detection at 259 nm

Parameter	Tolperisone
Regression equation	y = 31.218 x – 10.247
Range (μg/mL)	5 – 200
Correlation coefficient (R^2)	0.9999
Number of data points	7
Slope ± SD	31.218 ± 0.256
Intercept ± SD	10.247 ± 2.047
LOD/LOQ (μg/mL)	0.02/0.05

3.2. Linearity and limit of detection (LOD), quantitation (LOQ)

Calibration curves showed good linearity in the concentration range 5 ~ 200 μg/mL (*Table* 1). The equation of the calibration line obtained is: y =

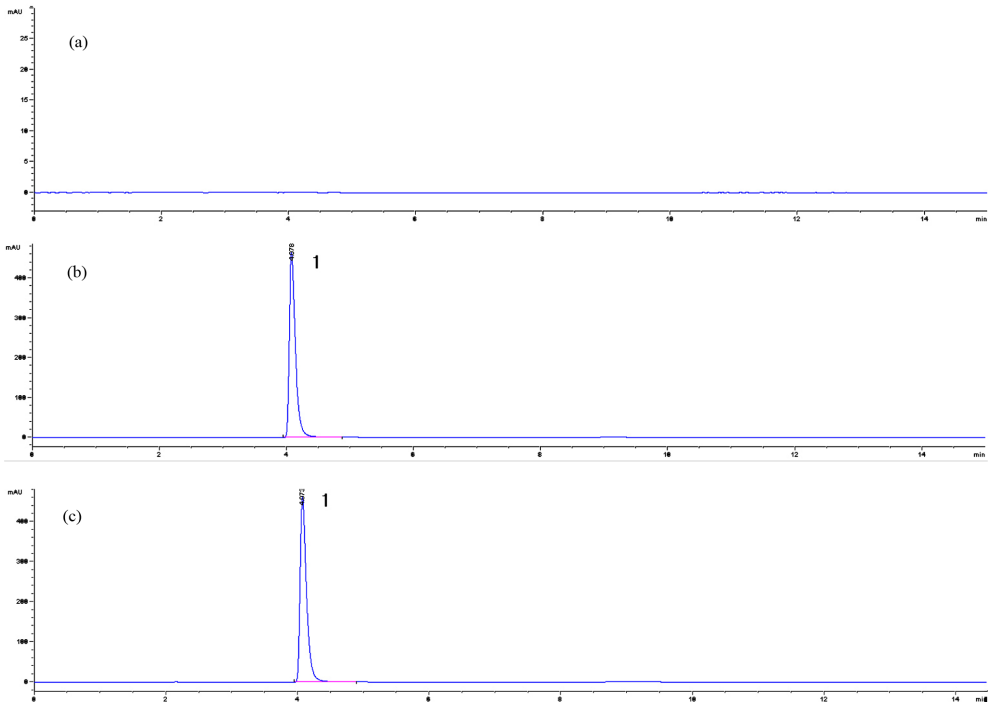

Fig. 4. Typical chromatogram of (A) blank mobile phase sample, (B) 100 μg/mL tolperisone hydrochloride standard solution, (C) sample solution prepared from tablets. Condition: Phenomenex Luna C18(2) column (250 × 4.6 mm I.D., 5 μm), mobile phase: Acetonitrile : 0.02 M ammonium hydrophosphate buffer (pH 5) (40:60, v/v), 25 °C. Detection: UV 259 nm, flow rate 1.0 mL/min. Peak 1. Tolperisone

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

Conc. (μg/mL)	Precision (RSD %)	
	Intraday (n=5)	Interday (n=11)
80	0.10	0.43
100	0.04	1.03
120	0.09	1.24

Table 3. Recovery for tolperisone tablets (n = 3)

Added conc. (μg/mL)	Measured conc. (μg/mL)	Recovery	
		Mean (%)	RSD (%)
80.00	78.98	98.7	0.15
100.00	98.96	99.0	0.26
120.00	120.92	100.8	0.11

31.218 x – 10.247. The correlation coefficient was 0.9999. The LOD and LOQ concentration were estimated to be 0.02 and 0.05 μg/mL, when signal – to – noise ratios of 3 and 10 were used as criteria.

3.3. Precision

The precision of the method was assessed by determining the intra-day assay relative standard deviation (RSD %) of the analysis (n = 5) 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 0.04 ~ 0.10 % and the inter-day precision was 0.43 ~ 1.24 %.

3.4. Accuracy (Recoveries)

Results of recovery studies by standard addition method were ranged from 99.8 % to 101.2 % for material. For tablets, recoveries were from 98.7 % to 100.8 % (*Table* 3). This also suggested that there was no interference from excipients in determining content of tolperisone in tablets.

Table 4. System suitability data (n = 6) using Luna C18(2) column (250 × 4.6 mm I.D., 5 μm) thermostated at 25 °C, flow rate at 1 mL/min, injection volume 10 μL and detection at 259 nm

Retention Time (RSD%)	Peak Area (RSD%)	Plate number	Symmetric factor
0.15	0.43	10401	0.50

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 0.15 % and 0.43 %, respectively. The number of theoretical plates was 10401 and symmetric factor was 0.50.

Robustness of the method was checked by making small deliberate changes in the content of acetonitrile in mobile phase (40 ± 2%) 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 1.03 %, number of theoretical plates were more than 10000 and symmetric factor was not less than 0.49.

Intermediate precision was studied by using Shimadzu HPLC system. Results showed that there was an increase in retention time - about 0.8 minutes late compared to Agilent 1100 system. Tailing factor (10 %) was 1.70 and the number of theoretical plates was about 7894. RSD% of peak area was 0.21 %.

3.6. Application

This analytical method was applied to quantitate the content of tolperisone in tablets. The results of assay test in 6 samples of commercial tablets were recorded in *Table* 5. The average content of tolperisone hydrochloride in the formulation was 99.3 %, RSD% of samples was 0.28 %. A typical chromatogram of sample is shown in figured 4C. Although, there was a major impurity peak eluted at 9.08 min which did not occur in the chromatogram of

Table 5. Contents of tolperisone in tablets (n = 6)

Sample	Claimed value	Assay	
		Content (%)	RSD (%)
Tablet A	100 mg	99.30	0.28

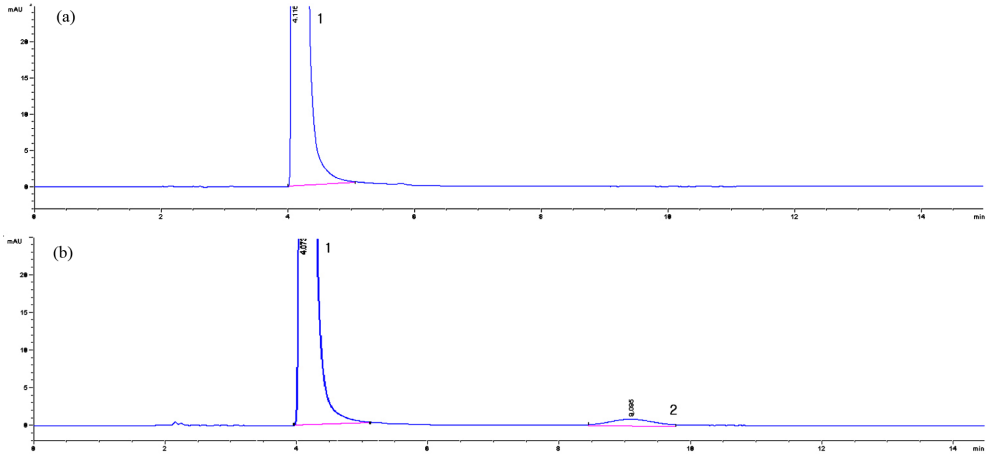

Fig. 5. Typical chromatogram shows well resolved impurity with tolperisone in pharmaceutical formulations. (A) 100 μg/mL tolperisone hydrochloride standard solution, (B) sample solution prepared from tablets. Peak 1: toperisone, peak 2: impurity.

standard (*Fig.* 5), the resolution between tolperisone and impurity peaks was more than 5, thus, calculation of the main peak area was not interfered. On the other hand, analysis time had to be adjusted to 15 minutes.

4. Conclusions

The above proposed study describes a simple HPLC method for the determination of tolperisone in tablets. To compromise between retention time, symmetric factor and number of theoretical plates, 40 % of acetonitrile in mobile phase was chosen as an optimal ratio. In this study, only one compound (Tolperisone hydrochloride) was analyzed and the chromatograms showed that there were no interferences from excipients and impurities. And validation result also showed there was no problem for quantitation even though the symmetric value was 0.60. The method was validated and found to be sensitive, accurate and precise. Within reasonable running time of 15 minutes, the method was convenient and effective for the assay test of tolperisone hydrochloride tablets.

Acknowledgements

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References

1. Martindale, The Extra Pharmacopoeia 30th edition, A. G. Lipman, The Pharmaceutical Press, London, 1211 (1993).
2. The Japanese Pharmacopoeia, 17th Edition, The Ministry of Health, Labour and Welfare, Japan (2016).
3. The Korean Pharmacopoeia, 11th Edition, Ministry of Food and Drug Safety, Republic of Korea (2014).
4. N. Youngvises, B. Liawruangrath, and S. Liawruangrath, *J. Pharm. Biomed. Anal*., **31**, 629-638 (2003).
5. S. Liawruangrath, B. Liawruangrath, and P. Pibool, *J. Pharm. Biomed. Anal*., **26**, 865-872 (2001).
6. N. R. Rao and S. S. Raju, *Chirality*, **25**(10), 622-627 (2013).
7. I. C. Nimila, P. Balan, N. Chiranjeevi, and V. V. V. M. Kumar, *Int. J. Pharm. Pharm. Sci.*, **4**(5), 84-85 (2012).
8. N. Miura, T. Saito, T. Taira, T. Yamagiwa, S. Morita, and S. Inokuchi, *J. AOAC Int.*, **97**(6), 1546-1551 (2014).
9. T. V. R. Raju, R. K. Seshadri, S. Arutla, T. S. S. J. Mohan, I. M. Rao, and S. R. Nittala, *Sci. Pharm.*, **81**, 123-138 (2013).
10. J. W. Bea, Y. S. Park, U. D. Sohn, C. S. Myung, B. K. Ryu, C. G. Jang, and S. Y. Lee, *Arch. Pharm. Res.*, **29**(4), 399-342 (2006).
11. C. I. Choi, J. I. Park, H. I. Lee, Y. J. Lee, C. G. Jang, J.

W. Bea, and S. Y. Lee, *J. Chromatogr. B*, **911**, 59-63 (2012).
12. Y. Owada, M. Takahashi, S. Iwasa, H. Ichiba, K. Sadamoto, and T. Fukushima, *Biomed. Chromatogr.*, **28**, 102-105 (2014).
13. M. Murali and P. V. V. Satyanarayana, *Der Pharma. Chemica*, **3**(5), 13-19 (2011).
14. ICH Guideline. Q2(R1): validation of Analytical Procedures: Text and Methodology Q2(R1) in ICH Harmonised Tripartite Guideline (2005).