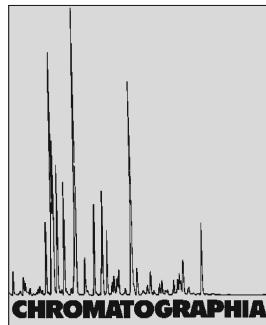


LC-UV Assay of Tolperisone HCl from Sustained Release Matrix Tablets



2010, 71, S109–S113

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Received: 21 October 2009 / Revised: 15 January 2010 / Accepted: 12 February 2010

Online publication: 23 March 2010

Abstract

Tolperisone HCl is a central muscle relaxant, which was incorporated in a matrix system formulated with poly(ethylene oxide)-PEO, in order to achieve adequate gastric residence time. This tablet presents considerable analytical difficulties in the quantitative determination of the drug, because the PEO matrix causes significant increase of viscosity in the samples. Our purpose was to develop a reproducible sample preparation method, which is adapted from parameters of the in vitro dissolution test and validate an LC-UV analytical method, which allows good recovery of the drug (99.97%). The developed analytical method was suitable for quantitative analysis of tolperisone HCl in matrix tablets.

Keywords

Column liquid chromatography
UV detection
Sustained release matrix tablet
Poly(ethylene oxide)
Tolperisone HCl

Introduction

Tolperisone HCl has been used for more than 40 years in therapy, it is a central

muscle relaxant used for the treatment of extrapiramidal movement disorders [1]. It has a local anaesthetic effect, like lidocaine [2], as it blocks the voltage gated sodium channels [3, 4]. The drug has no serious side effects, though anaphylactic reactions can occur [5], so tolperisone HCl is still one of the best

centrally acting muscle relaxants in therapy [6]. Tolperisone HCl is extremely water soluble, it is more stable in acidic medium ($\text{pH} < 4.5$), because it is disposed to decomposition in aqueous solution, which is faster at higher pH [7–9]. Only few works are engaged in the analytical determination of tolperisone HCl [10–13]. These methods focus on the active ingredient content in conventional dosage forms, in which the drug release is not modified by a special formulation, no excipient is used to present difficulties for the analytical assay.

This paper deals with the description of an analytical method validation process that was developed for the quantitative determination of tolperisone HCl from a matrix system. The main difficulty faced was the fact that the previously reported analytical methods were inadequate for the determination of the drug, because of the complex excipient system of the floating tablet. Tolperisone HCl was formulated in a gastroretentive dosage form, wherein the drug was coated with a melt wax layer, then these coated particles were incorporated into a matrix dosage form formulated with poly(ethylene oxide) (PEO) acting as the swelling polymer [14]. This complex excipient system was responsible for the sustained release of tolperisone and this

Presented at: 8th Balaton Symposium on High-Performance Separation Methods, Siófok, Hungary, September 2–4, 2009.

dosage form was designed in order to keep the drug in the stomach, where its stability is not affected by the higher pH of the distal gastrointestinal regions. During this time, the swelling of the polymer (PEO) forms a hydrophilic gel matrix [15, 16], which presents considerable analytical difficulties, because the polymer matrix causes significant increase of viscosity [17–21].

Therefore, the aim of the work was the validation of an LC-UV method that can be adequately used for the quantitative determination of the average and individual tolperisone content of the sustained release tablets. In order to demonstrate specificity and selectivity in the presence of excipients and possible degradation products, the behaviour of the matrix and the placebo tablets under forced stress conditions was also investigated. Since a validated method for such a complex composition helps the recovery of any active ingredient from these types of matrix systems, it might as well be applicable to similar dosage forms.

Experimental

Materials

Tolperisone hydrochloride was obtained from Gedeon Richter (Budapest, Hungary). Gradient solvents used for chromatography were purchased from Sigma-Aldrich (Budapest, Hungary). A hydrophobic wax, glyceryl palmitostearate (Precirol ATO 5, Gattefossé, France) and a hydrophilic polymer, poly(ethylene-oxide) (PEO WSR 303, Colorcon, USA) were used for the preparation of tolperisone HCl sustained release matrix tablets.

In Vitro Dissolution Studies

In vitro dissolution tests were performed using 900 mL of pH = 1.2 HCl as the dissolution medium in a USP paddle apparatus at 50 rpm and at 37 ± 0.5 °C in a Hanson SR8-Plus dissolution tester (Hanson Research, USA). Samples (5 mL) were taken regularly for 8 h. Every sample was filtered (0.2 µm

regenerated cellulose membrane filter), and diluted with pH = 1.2 HCl (20 fold). Every time the dissolution medium was replaced with fresh pH = 1.2 HCl. Tolperisone HCl content was determined at 260 nm using a UNICAM UV-Vis UV2 Spectrophotometer (UNICAM, UK).

Preparation of Calibration, Stock and Quality Control Solutions for the Developed and Validated Analytical Method

0.0150 g tolperisone HCl was dissolved in 100 mL 0.1 N HCl for the preparation of a 15 µg mL⁻¹ solution. 1 mL of the stock solution was diluted to 10 mL with 1:1 = acetonitrile:water mixture. Calibration solutions in the concentration range of 7.5–22.5 µg mL⁻¹ were prepared by dilution of the stock solution.

Preparation of Sample Solution

Twenty tablets were weighed and the average weight was calculated for the calibration. Ten tablets were crushed to a fine powder and homogenized. 700 mL 0.1 N HCl was poured into a 1,000 mL volumetric flask, then 635 mg of the powder was dispersed evenly on the surface of the liquid. The flask was placed for 15 min at 40 °C using a water bath. After this, the sample was mixed by a heating magnetic stirrer at 40 °C, 300 rpm for 30 min. The solution was then cooled to room temperature and sufficient 0.1 N HCl was added to make up to volume. After homogenization, this solution was filtered through a 0.2 µm regenerated cellulose membrane filter (Agilent Technologies, Germany). The further dilution was made with the sample solvent (1:1 = acetonitrile:water) to give a final concentration of 15 µg mL⁻¹.

Chromatographic Separation

Chromatographic separation analysis was performed with an Agilent Technologies (Palo Alto, CA, USA) LC 1100

liquid chromatograph with HP Chemstation software, version A10.02 (Agilent, Germany). The system consisted of a binary pump, an on-line degasser, an autosampler, a column heater, and a diode-array detector (DAD). Separation was achieved on a 4.6 mm × 250 mm, 5 µm particle, YMC-Pack ODS-AQ C-18 column (YMC-Europe, Germany) with acetonitrile-phosphate buffer solution (pH = 5) as the mobile phase at a flow rate of 1.0 mL min⁻¹. A gradient program was developed by mixing eluent A (phosphate buffer solution/ACN 970:50 (v/v)) and eluent B (phosphate buffer solution/ACN 220:800 (v/v)) as follows: 0 min 30% B, 0–3 min 30–44% B, 3–9 min 44–100% B, 9–12 min 100% B, 12–13 min 100–30% B, 13–20 min 30% B. The column temperature was 25 °C and the injection volume was 20 µL. Chromatograms were recorded at 260 nm by use of DAD.

Stress Conditions

Hydrolytic degradation was studied by heating the samples at 80 °C in 0.1 N hydrochloric acid and 0.1 N sodium hydroxide for 4 h. Oxidative degradation was studied by treating the samples with 3% hydrogen peroxide at room temperature for 4 h. Photodegradation was induced by exposing the samples in a photostability chamber to 200 Wh/m² of near UV for 24 h at 25 ± 1 °C. The samples were stored at 75 °C for 24 h, and at 50 °C 75% moisture for 1 week.

Results and Discussion

Development of the Sample Preparation Method

The formulated sustained release matrix tablet comprises a swellable polymer (PEO WSR 303), which has an extremely high molecular weight (M_{PEO} : 7,000,000) and viscosity. In the early stages of the analytical measurements it was rapidly seen that the typical sample preparation method (fluid–fluid extraction combined with sonication) could not be adequately

used for the active ingredient determination, because PEO swells and binds the drug, leading to a marked loss of tolperisone (recovery: 93.16%). In order to achieve better recovery, different solvents (organic, inorganic and their mixtures) and various techniques (mixing and shaking) were examined. Unfortunately, these studies did not result in an effective method. However, it was seen in the 8 h dissolution studies (Fig. 1.) that the total amount of tolperisone HCl was released and the high viscosity of the swollen polymer did not disturb the detection of the drug. Based on these phenomena, it was concluded that a thorough examination of the parameters of the dissolution test could lead to the understanding of what may have caused the low recovery. Primarily, the volume of the dissolution medium is 900 mL, which is large enough for the polymer to evenly distribute and to dilute, therefore the increase of viscosity was not as pronounced as in the case of the analytical method. Secondly, the relatively high temperature (37 °C) helped the softening of the wax. Due to the dilution of the polymer and the softening of the wax the binding of the drug was less likely to occur. Furthermore, the mixing action of the paddle (50 rpm) also proved to be advantageous, because the viscosity of the PEO solution was lower than when sonicated. The presence of these circumstances during the dissolution studies led to 100% dissolution of the drug. Following the understanding of the described conditions, they were adapted for the analytical sample preparation.

Validation Procedure of the LC-UV Analytical Method

The analytical method, which was suitable for the quantitative determination of the active ingredient in a sustained release matrix tablet, was validated in accordance with the corresponding ICH guideline [22]. According to the guideline the following requirements have to be met in order to obtain a validated method: system suitability, specificity and selectivity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness and solution stability. The results obtained are detailed below.

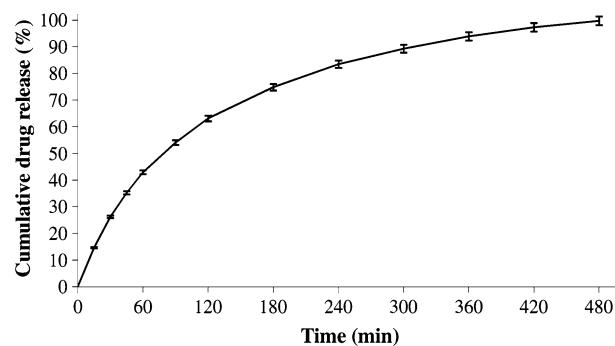


Fig. 1. Dissolution curve of the tolperisone HCl from sustained release matrix tablet (Mean \pm SD, $n = 6$)

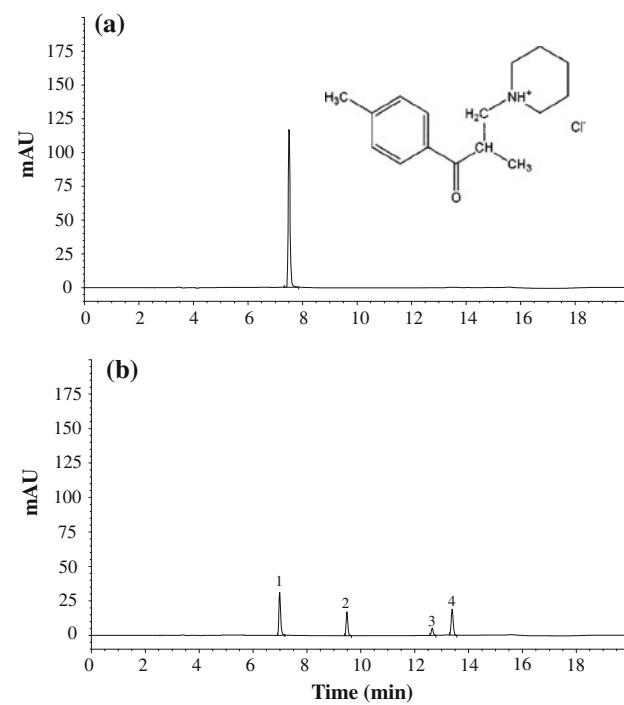


Fig. 2. a Specificity of the sample solution (sample preparation: 0.1 N HCl and acetonitrile:water = 1:1). b Specificity of the sample solution under pH stress condition (sample preparation: 0.1 N NaOH and acetonitrile:water = 1:1). 1 = tolperisone; 2 = unknown degradation product; 3 = p-methyl-propiophenone; 4 = 2-methyl-1-(4-methylphenyl) prop-2-en-1-one

System Suitability Data

System suitability parameters were measured using five replicate injections of a standard solution containing $15 \mu\text{g mL}^{-1}$ of tolperisone HCl to verify the system performance. The relative standard deviations (RSD) calculated for the retention time, symmetry, capacity factor, peak area and theoretical plates were 0.20, 0.61, 0.38, 0.13, and 0.53%, respectively. The results show that the parameters tested were within the acceptable range ($\text{RSD} < 2.0\%$), therefore it was

concluded that the method suitability was justified.

Specificity and Selectivity

Specificity was performed to exclude the possibility of interference with other compounds (e.g. excipients, degradation products) in the region of elution of tolperisone. The specificity and selectivity of the method were tested under normal and stress conditions, such as follows: pH, O_2 , heat, heat and moisture,

Table 1. Robustness of the standard solution ($n = 6$) and of the sample solution ($n = 6$). The effect of eluent composition, column temperature and changing the chromatographic column were analyzed

Parameters	Standard solution					Sample solution	
	t_R (min)	k ($k = 1\text{--}10$)	T ($0.8 \leq T \geq 2.0$)	N ($N \geq 2000$)	RSD (%)	Recovery (% \pm SD)	RSD (%)
Acetonitrile content	Normal	7.5	1.92	0.92	57,637	0.59	99.63 \pm 0.59
	-1%	7.3	1.98	0.91	48,226	0.65	100.59 \pm 0.65
	+1%	7.2	1.88	0.92	45,809	0.67	100.39 \pm 0.64
Column temperature	25 °C	7.2	1.76	1.01	57,175	0.53	99.69 \pm 0.61
	25 - 5 °C	7.45	1.98	0.91	44,581	0.59	100.88 \pm 0.48
	25 + 5 °C	6.35	1.54	0.89	28,985	0.48	100.86 \pm 0.54
Column change	YMC I	7.5	2.0	0.91	66,373	0.70	99.63 \pm 0.59
	YMC II	7.6	2.04	0.89	62,372	0.40	100.59 \pm 0.65
	Restek	8.03	2.21	0.81	55,741	0.30	100.39 \pm 0.64

YMC YMC-Pack ODS-AQ C-18 column, 4.6 mm \times 250 mm, 5 μm (YMC-Europe, Germany)
Restek Restek Ultra Aqueous C18 column 4.6 mm \times 250 mm, 5 μm (Restek, Germany)

Table 2. Intra-day precision for tolperisone HCl in the standard and sample solution ($n = 6$); inter-day precision for tolperisone HCl in the standard solution (dilution of the stock solution) ($n = 3$)

Time (48 h)	Intra-day		
	Standard solution	Sample solution	Storage sample solution (75 °C, 24 h)
Mean area (\pm SD)	525.11 \pm 0.61	521.23 \pm 0.54	515.80 \pm 0.78
RSD (%)	0.12	0.10	0.15
Inter-day			
Time (week)	Stock solution (1. parallel)	Stock solution (2. parallel)	Stock solution (3. parallel)
0.	526.30	531.20	534.90
1.	526.15	531.00	534.60
2.	525.90	531.10	533.95
3.	524.70	529.70	533.10
4.	525.30	529.10	532.90
Mean area (\pm SD)	525.67 \pm 0.66	530.42 \pm 0.71	533.89 \pm 0.88
RSD (%)	0.13	0.13	0.17

light loading. The results of the tests proved that the components other than the drug did not produce a detectable signal at the retention place of tolperisone. Peaks of the degradation products (2-methyl-1-(4-methylphenyl) prop-2-en-1-one, *p*-methyl-propiophenone) were separated from each other, from the peak of the tolperisone and its impurities with an R_s value of ≥ 1.5 . Figure 2a, b show chromatograms of the solution prepared from the tablet and of the pH stressed solution, respectively.

Linearity

Linearity was evaluated by regression analysis in the calibration range of 7.5–22.5 $\mu\text{g mL}^{-1}$. The equation of the straight line was $y = 33.653x - 4.6981$

and the correlation coefficient was $R^2 = 0.9996$ ($n = 5$).

Accuracy

Accuracy was measured after dissolving a placebo and then spiking the solution with known concentrations of tolperisone in the concentration range of 7.5–22.5 $\mu\text{g mL}^{-1}$. The equation of the straight line was $y = 0.9903x$ and the correlation coefficient was $R^2 = 0.9999$ ($n = 5$).

Precision

In order to prove precision, repeatability and intermediate precision were analyzed. Repeatability was examined by measuring the mean recovery of tolperi-

sone from the matrix tablets. The result was 99.54% and the relative standard deviation (RSD) was 0.72% ($n = 6$). Intermediate precision was proved by checking the mean recovery values of six tablets, measured by two analysts. The measurements of the two analysts were 99.99 and 100.08%, the RSD values were 0.15 and 0.12% ($n = 6$ –6), respectively. The precision of content uniformity was also analyzed (3×10 tablets), the results were 99.96, 99.95, and 99.91%, the RSD was <2.0%.

Limit of Detection and Limit of Quantification

The limit of detection of the method was calculated from the standard deviation (σ) of the responses for triplicate blank injections and the slope (S) of the cali-

bration plot, using the formula $LOD = 3.3 \sigma S^{-1}$. The LOD was $2.475 \mu\text{g mL}^{-1}$. In this work the calibration range was narrowed to enhance the accuracy of the analysis. For this reason the limit of quantification (LOQ) was $7.5 \mu\text{g mL}^{-1}$. Although the values of both LOD and LOQ are fairly high compared to the usual range of several ng, they were accepted because due to the high drug content of the tablets the active ingredient content of the samples was always greater than the LOQ.

Robustness and Solution Stability

The robustness of the method was investigated by deliberately changing certain factors such as follows: the effect of eluent composition, column temperature and changing the chromatographic column. The changes in the results obtained were monitored by the RSD values ($<2\%$, Table 1) indicating that none of the changes had a significant effect on the analysis. To demonstrate their stability, standard solutions were stored at room temperature for 48 h and analyzed periodically. Three parallels of stock solution were stored at 8°C for 4 weeks. Within this time the standard solutions were prepared freshly by dilution of the stock solutions on the day of the tests. The stability of these standard solutions was measured once a week. The results confirmed that the retention time and peak area of tolperisone were unchanged (RSD $< 2\%$) (Table 2). The standard solution can be considered stable for 48 h, and the stock solutions and the standard solutions were stable for 28 days. Every measured result corresponds to the validation requirements of the drug content assay.

Conclusions

The objective of our work was to develop a robust and reproducible sample preparation and an analytical LC-UV method which is suitable to determine the content uniformity of a complex matrix tablet formulation. The developed sample preparation method is adapted from parameters of the in vitro dissolution test. The developed gradient elution analytical LC-UV method enables the determination of the tolperisone HCl in sustained release matrix tablets. The recovery of the active ingredient was found to be 99.97%, so it was suitable for quantitative analysis. The method was also validated, and results obtained meet the requirements of validation.

Acknowledgments

The authors wish to thank Katalin Ganzler for the advices and the Gedeon Richter Plc. for providing tolperisone HCl.

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