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RESEARCH ARTICLE

Validation of High Performance Liquid Chromatography Methods for Determination of Meloxicam and Tenoxicam from Transdermal Therapeutic Systems

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Objective: The aim of this study was to develop and validate two HPLC methods for the quantification of meloxicam and tenoxicam from transdermal therapeutic systems. **Methods**: Based on 1.0% hydroxypropyl methylcellulose 15000, transdermal patches containing meloxicam or tenoxicam were prepared by solvent evaporation technique. Analytical performances of the HPLC methods for the quantification of meloxicam and tenoxicam from such systems were assessed in terms of specificity, linearity, detection limit, quantification limit, recovery and precision. **Results and discussion**: The linearity of the method was assessed through a calibration curve in the 1.0 - 75.0 μg·mL-¹ concentration range, with a regression coefficient higher than 0.999. The detection limit and the quantification limit were found to be 0.46 μg·mL-¹ and 1.39 μg·mL-¹, for meloxicam; and 0.88 μg·mL-¹, respectively 2.64 μg·mL-¹ for tenoxicam. According to the European Pharmacopeia 5.0 the mean recovery was found to be between 75% and 125%. As performance criteria for precision was used the RSD% which were lower than 2.0% for both methods. **Conclusions**: The proposed liquid chromatography methods provide selective, linear and precise results for the quantification of meloxicam and tenoxicam from transdermal therapeutic systems. The presence of a single peak in the chromatograms of the analyzed transdermal patches with meloxicam or tenoxicam, certify the successful determination of the active pharmaceutical ingredient in the prepared patches.

Keywords: meloxicam, tenoxicam, transdermal therapeutic system, high performance liquid chromatography

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) represents one of the oldest classes of therapeutic agents. Despite this, they still are of a great interest in treatment of rheumatic diseases. Conventional pharmaceutical forms may reduce patient compliance by the required multiple administrations. Therefore, the interest in incorporating these drugs into transdermal therapeutic systems (TTSs) has increased in the recent years. Administration of NSAIDs through the transdermal route confers the advantages of maintaining a constant blood concentration of the active ingredient and to reduce the well known gastric side effects [1-3]. Meloxicam (MX), chemically known as 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1, 2-benzothiazine-3-carboxamide-1, 1-dioxide and tenoxicam (TX), chemically known as 4-hydroxy-2-methyl-N-2-pyridinyl-2H-thieno (2,3-e)-1,2-thiazine-3-carboxamide-1,1-dioxide, are two substances that belonging to NSAIDs class, which selectively inhibits the enzyme cyclooxygenase-2, being frequently indicated in the treatment of inflammatory diseases [4-6]. Development of transdermal patches involves multiple quality studies for their evaluation [7-11]. Among these types of tests, determining the drug content is one of the most important. The UV spectrophotometrically assay is one of the most common methods used to quantify meloxicam and tenoxicam from TTSs [12-17], mainly because of the low cost. Despite this, a high performance liquid chromatography (HPLC) with ultraviolet detection is more accurate, reproductible and has the advantage of small volume samples.

For this reason, the purpose of this study was to develop two HPLC methods for the quantification of meloxicam and tenoxicam from transdermal therapeutic systems. A validated method can give a real and interpretable information about the analyzed samples, which can generate trusted results.

Methods

Preparation of TTS

Transdermal patches containing 1.3264 mg·cm-² MX (Techno Drugs & Intermediates Ltd. Mumbai, India) or 1.3264 mg·cm-² TX (Nantong Chemding Chephar Co. Ltd. Jiangsu, China) as active pharmaceutical ingredients, were prepared according to a method that has been described in another study [18]. Were acquired TTSs by solvent evaporation technique, with 1.0% hydroxypropyl methylcellulose 15000 (Shin-Etsu Chemical Co., Ltd. Tokyo, Japan). Other substances that were used: propylene

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glycol (Scharlau Chemie, Barcelona, Spain), Tween 20 (Sigma Aldrich Co., France); ultrapure water (Millipore Direct-QS. water distiller), absolute ethanol (Chemical Company, Romania). A placebo patch was prepared as described above without adding the active pharmaceutical ingredient (API).

Validation study

Equipment: HPLC Agilent Technologies 1100 Series (USA) with quaternary pump, degasser, automatic injector, column thermostat, ultraviolet detector and software (Chemstation software); Waters Symmetry C8 column, 4.6x150 mm, 5 μm; analytical balance AB54S (Mettler Toledo, Schweiz); ultrasonic bath T700H (Elma Transsonic).

Chromatographic conditions for MX: mobile phase - phosphate buffer (KH2PO4 20mM, pH 3.0): acetonitrile (60:40 v/v); 1.0 ml/min flow; detection at 362 nm; column temperature was set at 35^{0}C; volume injected 5 μl.

Chromatographic conditions for TX: mobile phase - phosphate buffer (KH2PO4 20mM, pH 3.0): acetonitrile (65:35 v/v); 1.0 ml/min flow; detection at 360 nm; column temperature was set at 35^{0}C; volume injected 5 μl.

HPLC solvents: acetonitrile (Merk, Germany) and methanol (Merk, Germany) with HPLC analytical grade; ultrapure water; the mobile phases were prepared with a mixture of a buffer solution KH2PO4 (Merk, Germany) 20 mM with pH 3 adjusted with phosphoric acid (Merk, Germany).

Analytical performances of the HPLC methods for MX and TX were assessed in terms of specificity, linearity, detection limit, quantification limit, recovery and precision. The calculation of the validation parameters was performed using Microsoft Office Excel 2010 (Microsoft Corporation, USA).

– Method specificity was evaluated by comparing the results acquired for a standard solution of API with a placebo sample. As performance criteria for specificity was used the absence of any interference from excipients in the retention time of the analyte.
– The linearity of the method was assessed through a calibration curve in the 1.0 - 75.0 μg·mL-^{1} concentration range. An initial stock solution of 10 mg·mL-^{1} API was prepared in a volumetric flask with phosphate buffer pH 7.4. This solution was diluted in five different standard solutions with concentrations between 1.0 to 75.0 μg·mL-^{1}. As performance criteria for linearity were used: a linear correlation obtained by plotting the area ratio of API as a function of API concentration and a regression coefficient higher than 0.999 acquired with a least square linear regression analysis. All analyses were performed in triplicate.
– Limit of detection (DL) and limit of quantification (QL) were determined according to the ICH guidelines [19]. Four methods are approached for determining the DL and QL: based on visual evaluation; based on signal-to-noise; based on the standard deviation of the response and the slope; based on the recommended data. In our study the method based on the standard deviation of the response and the slope was approached.

$$DL = (3.3*\sigma)/S \qquad QL = 10*\sigma/S$$

where: σ - was calculated based on the calibration curve (as standard deviation was used the standard deviation of y-intercepts of regression lines); S - slope of the calibration curve.
– Recovery of API was determined in triplicate and was done by comparing the results obtained from API assay from TTS with the results acquired from the standard drug solution with the same concentration. For API assay, TTS samples of 0.7539 cm² were dissolved in a phosphate buffer solution pH 7.4 into a 25 mL volumetric flask, obtaining a final concentration of 40 μg·mL-^{1} of the API. The concentrations of API presented in the samples were determined from the standard curve.
– The precision of the method was analyzed by calculating two parameters: repeatability (intra day precision) and repeatability over different days (intermediate precision). As performance criteria for precision was used the relative standard deviation RSD% which must be lower than 2.0%. The repeatability was done by analyzing in replicate (3 times), three levels of concentration: 50.0 μg·mL-^{1}, 25.0 μg·mL-^{1}, 5 μg·mL-^{1} for MX, and 75.0 μg·mL-^{1}, 50.0 μg·mL-^{1}, 25.0 μg·mL-^{1} for TX, during the same day and under the same experimental conditions. The intermediate precision was done by analyzing sample solutions prepared at the same concentration level, in 3 different days and under the same experimental conditions.

Results and discussion

During methods development and for optimization of chromatographic conditions for quantification of MX and TX from transdermal patches, many compositions of the mobile phase, wavelengths and flow rates were tested. Under the conditions described before, MX showed a characteristic peak at 3.08 min, and the characteristic peak of TX appeared at 3.33 min. Compared with the data reported in the literature [20,21], the retention times of MX and TX have been improved.

– *Specificity*. The results acquired with the placebo patches showed that none of the excipients eluted in the same retention time as the API.
– *Linearity*. Taking into account the peak area response at 362 nm for MX and 360 nm for TX the linearity was observed over the concentration range of 1.0 to 75.0 μg·mL-^{1}. For each drug the correlation coefficient (R^2) was found to be high (0.9999). The analytical curve (Fig.1) data for both API are presented in Table I: slope, intercept and R^2. Statistical data analyze proves that is a linear relationship between the variables (Table I). The chromatograms corresponding to the linearity analyze for MX and TX are shown in Fig.1.
– *Limit of detection*. Limit of quantification. For MX the DL and QL were found to be 0.46 μg·mL-^{1} and 1.39

Table I. Linearity parameters for meloxicam and tenoxicam

Concentration level (µg·mL-1)	Meloxicam				Tenoxicam			
	$A_{Series\ 1}$	$A_{Series\ 2}$	$A_{Series\ 3}$	$A_{Average}$	$A_{Series\ 1}$	$A_{Series\ 2}$	$A_{Series\ 3}$	$A_{Average}$
1	13.2	13.0	13.0	13.1	14.6	14.5	14.1	14.4
5	70.5	70.9	70.8	70.7	68.8	69.7	71.1	69.9
25	348.1	347.5	345.5	347.0	349.8	338.7	340.3	342.9
50	697.2	695.5	696.7	696.5	690.9	693.2	691.3	691.8
75	1049.2	1044.7	1046.5	1046.8	1042.6	1040.3	1041.9	1041.6
Statistical parameters								
Mean equation	Y=13.954x-0.5495				Y=13.875x-0.7939			
Slope	**13.9540**				**13.8750**			
Intercept	**- 0.5495**				**- 0.7939**			
R^2	**0.9999**				**0.9999**			
$^{*}t_{calculated}$	**- 0.92**				**- 0.63**			
$^{*}t_{tabulated}$=2.16	If $t_{calculated} < t_{tabulated}$ (Ordonate at origin does not differ significantly of 0)							
$^{**}C_{calculated}$	**0.657**				**0.67**			
$^{**}C_{tabulated}$=0.68	If $C_{calculated} < C_{tabulated}$ (Determination groups variants are homogeneous)							
$^{***}F_{calculated}$	**3.27**				**2.20**			
$^{***}F_{tabulated}$=3.71	If $F_{calculated} < F_{tabulated}$ (Equation is valid)							

*Student's t test; **Cochran test; ***Fischer test

µg·mL-1 respectively; for TX the DL value was 0.88 µg·mL-1 and the QL was 2.64 µg·mL-1. Similar results were acquired in other studies. For example, in 2009 Mrunalini C. Damle et al obtained for MX a DL value of 219 ng·mL-1 and for QL 722 ng·mL-1 respectively [22]. For quantitative of TX in tablets Singh AK et al [21] obtained as DL a value of 0.35 µg·mL-1 and a QL value of 1.20 µg·mL-1.

– *Recovery*. Using the proposed HPLC method the mean recovery of MX was found to be 97.41±2.02% and for TX it was 87.17±7.43 %. The low recovery of TX may be caused by a experimental error such as a non-homogeneous dispersation of the API during preparation.

A typical chromatograms for API assay are presented in Fig.2.

– *Precision*. The method precision was evaluated in terms

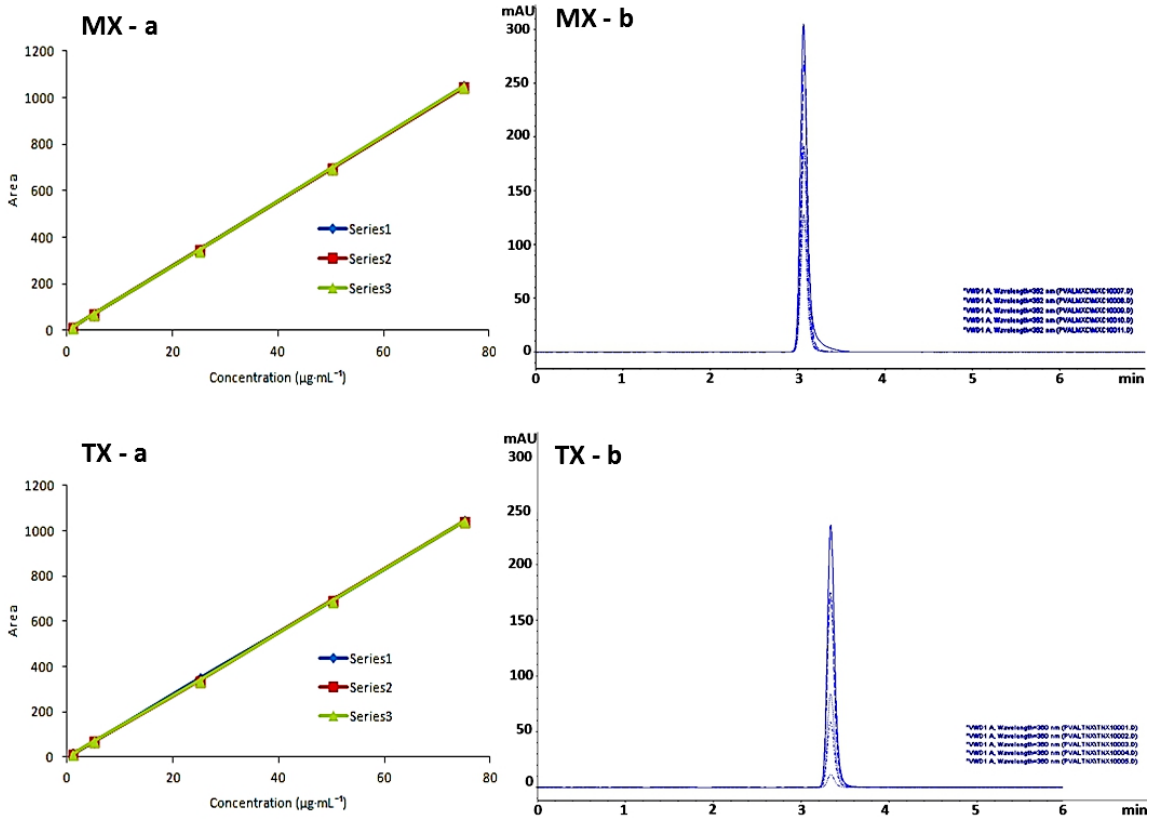

Fig.1. Linearity profiles (a) and chromatograms (b) corresponding to standard solutions of calibration curbes for MX and TX in the 1.0 - 75.0 µg·mL-1 concentration range

Table II. Precision parameters for meloxicam and tenoxicam

Concentration level (µg·mL^{-1})	Repeatability			Intermediate precision		
	Meloxicam					
	50	25	5	50	25	5
	Area - A -					
$A_{Series\ 1}$	701.1	350.5	70.9	697.2	351.1	70.5
$A_{Series\ 2}$	700.5	351.0	70.8	698.3	351.3	70.8
$A_{Series\ 3}$	700.6	351.3	70.9	700.5	350.2	70.9
$A_{Average}$±SD	**700.7±0.3**	**350.9±0.4**	**70.9±0.1**	698.6±1.6	350.8±0.5	70.7±0.2
RSD%	**0.037**	**0.094**	**0.066**	**0.196**	**0.136**	**0.240**
	Retention time (min) - T_R -					
$T_{R\ Series\ 1}$	3.070	3.076	3.077	3.064	3.067	3.069
$T_{R\ Series\ 2}$	3.073	3.076	3.076	3.067	3.069	3.071
$T_{R\ Series\ 3}$	3.070	3.077	3.078	3.069	3.070	3.073
$T_{R\ Average}$±SD	**3.071 ±0.002**	**3.076 ±0.001**	**3.077 ±0.001**	**3.067 ±0.002**	**3.069 ±0.001**	**3.071 ±0.002**
RSD%	**0.046**	**0.015**	**0.026**	**0.067**	**0.040**	**0.053**
Concentration level (µg·mL^{-1})	Tenoxicam					
	75	50	25	75	50	25
	Area - A -					
$A_{Series\ 1}$	1041.9	691.3	253.9	1042.6	693.2	253.9
$A_{Series\ 2}$	1041.2	690.8	253.8	1040.3	691.3	254.3
$A_{Series\ 3}$	1040.9	690.9	254.1	1043.2	690.9	254.7
$A_{Average}$±SD	**1041.3±0.5**	**691.0±0.2**	**253.9±0.1**	**1042.0±1.5**	**691.8±1.2**	**254.3±0.4**
RSD%	**0.040**	**0.031**	**0.049**	**0.119**	**0.145**	**0.128**
	Retention time (min) - T_R -					
$T_{R\ Series\ 1}$	3.337	3.335	3.336	3.336	3.335	3.335
$T_{R\ Series\ 2}$	3.335	3.335	3.336	3.337	3.336	3.337
$T_{R\ Series\ 3}$	3.336	3.337	3.335	3.339	3.337	3.338
$T_{R\ Average}$±SD	**3.336 ±0.001**	**3.336 ±0.001**	**3.336 ±0.001**	**3.337 ±0.002**	**3.336 ±0.001**	**3.337 ±0.002**
RSD%	**0.024**	**0.028**	**0.014**	**0.037**	**0.024**	**0.037**

of inter- and intra-day repeatability and was expresses as RSD %. As shown in Table II, RSD% was found to be below the set 2.0% for all control samples. RSD% ranged from 0.037% to 0.240% and 0.031% to 0.145% for all three levels of MX concentrations and TX, respectively.

Conclusions

In this study two efficient and simple HPLC methods were developed and validated for the analysis of MX and TX in TTS. Based on the current study and on the statistical data, the proposed liquid chromatography methods provide selective, linear and precise results for the quantification of MX and TX from TTS. An advantage of the methods used is the short duration of the API assay (TR for MX: 3.08 min; TR for TX: 3.33 min). The presence of a single peak in the chromatograms of the analyzed TTS with MX and of the TTS with TX, certify the successful determination of the API in the prepared TTS. From this point of view this method can be used successfully to determine MX and TX in TTS.

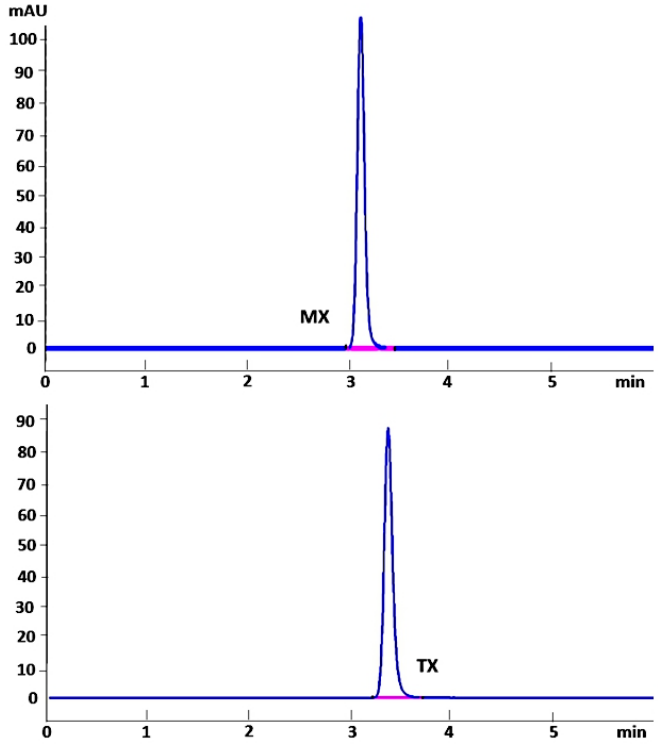

Fig.2. Typical chromatograms for API assay

Acknowledgments

The determinations have been performed in the Drug Testing Laboratory of University of Medicine and Pharmacy of Tîrgu Mureş (https://erris.gov.ro/Drug-Testing-Laboratory), using for data interpretation a special spreadsheet created in Excel (software Microsoft Excel).

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Conflict of interest

None to declare

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