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A NEW HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF INDOMETHACIN FROM TRANSDERMAL THERAPEUTIC SYSTEMS

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ABSTRACT. This study aimed to develop and validate an HPLC method in order to assay the indomethacin from therapeutic transdermal systems (TTS). A TTS with indomethacin and $HPMC_{15k}$ as a film-forming agent was developed. The performance of the method was verified in terms of specificity, linearity, detection limit, quantification limit, and precision. The results proved the developed method specificity no interference being observed in a placebo sample at the retention time corresponding to indomethacin. To evaluate the linearity, concentrations in the range of 1-100 µg/mL were used, proving the existence of a linear relationship between the selected points. The detection limit was 0.93 µg/mL and the quantification limit was 2.80 µg/mL. Indomethacin concentration in the analysed samples indicated a concentration of 95.15±4.15% respecting the official data. The statistical parameter used to evaluate the precision was the relative standard deviation, being used in the case of the evaluated concentrations smaller than 2%. In conclusion, the developed method is specific, reproducible and precise. An advantage of the developed method consists of the short analysis time of 3.53 min, whilst the presence of a single characteristic signal in the TTS samples chromatograms certifies that the indomethacin was quantified successfully from the TTS.

Keywords: *HPLC, TTS, indomethacin*

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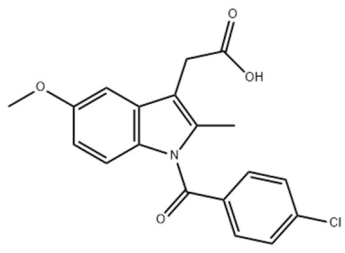
INTRODUCTION

Due to the advantages that the therapeutic transdermal systems (TTS) give, developing this type of pharmaceutical product represents a high-interest alternative to conventional formulations. Developing TTS aims to improve the clinical efficiency of a medicinal molecule and to increase the treatment acceptability and the patient's compliance through the capacity of a system to release a small amount of a drug at a pre-established liberation release. Furthermore, the transdermal patches present advantages when a lack of tolerance regarding oral administration occurs [1-6].

Indomethacin (IND), is indole acetic acid derived chemically named 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl] acetic acid, belonging to the first generation of nonsteroidal anti-inflammatory drugs (NSAIDs), that act through the inhibition of the cyclooxygenase enzymes. It is well known fact that the NSAIDs through their mechanism of action significantly decrease the pain experienced in particular conditions while administrated orally, in addition, gastric side effects might be exhibited. To reduce the NSAIDs side-effects and taking into consideration the high number of patients that require chronically this type of medication, developing TTS gained a high interest, especially related to the ease of administration [7-12].



Molecular formula:
$C_{19}H_{16}ClNO_4$
Molecular weight:
357.8
Physical description:
- pale-yellow to yellow-tan, crystalline powder, odorless
- crystals exhibit polymorphism
Melting point:
158°C
Solubility:
- in water, 0.937 mg/L (at 25°C)
- soluble in ethanol, ether, acetone
Log P:
4.27
Stability:
- sensitive to light
- stable in neutral or slightly acidic media
- it is decomposed by strong alkali

Figure 1. Indomethacin: physical and chemical properties [13]

Considering its applicability, whilst developing TTS it is very important to quantify the active pharmaceutical ingredient from the proposed pharmaceutical formulation. In this regard, the main objective of this study consisted of developing and validating an analytical method for quantifying the IND from the polymeric TTS, through an HPLC method.

RESULTS AND DISCUSSION

To develop and validate an HPLC method to assay IND from the polymeric matrices, the specification found in the literature were considered [14-17]. To optimize the method and the chromatographic conditions, multiple compositions of the mobile phase, wavelength, and flow rate were studied. Thus, respecting the conditions outlined in the Material and Methods chapter, a characteristic signal was obtained at 3.53 minutes.

Method specificity and linearity

Developing and validating an HPLC method to quantify IND from polymeric TTS

Specificity. The comparative evaluation of the results based on the chromatograms registered for the placebo sample and a sample solution proved the lack of interference at the IND retention time.

Linearity. The evaluation of this parameter was obtained in the concentration range of 1-100 µg/mL, the measurements being conducted in triplicate for five levels of concentration. The statistical data included in **Table 1**, proved the linear relations between the variables.

Table 1. Linearity parameters

Concentration level (µg/mL)	Area ± SD	Statistical parameters
		Mean equation: y=4.9722x+0.0241 Slope = 4.9722; Intercept = 0.0241; R^2 = 0.9999
1	5.6±0.36	**Student's t test:** $t_{calculated}$= -0.162; $t_{tabulated}$= 2.160 If $t_{calculated} < t_{tabulated}$, ordonate at origin does not differ significantly of 0
5	25.3±0.73	
25	124.3±0.40	
50	248.3±1.30	**Cochran test:** $C_{calculated}$= 0.498; $C_{tabulated}$= 0.680 If $C_{calculated} < C_{tabulated}$, determination groups variants are homogeneous
75	372.1±0.46	
100	498.1±0.75	**Fischer test:** $F_{calculated}$= 1.171; $F_{tabulated}$= 3.710 If $F_{calculated} < F_{tabulated}$, equation is valid

Detection and quantification limit. By the ICH guide [15], the calculation of the parameters was done considering the following formulas:

*LOD = (3.3*σ)/S,* where: LOD - detection limit; σ - was calculated based on the calibration curve (intercept standard deviation) (y's standard deviation) y, calculated through simple linear regression); S - calibration curve slope.

*LOQ = 10*σ/S,* where: LOQ - quantification limit.

For IND, LOD was 0.93 µg/mL, whilst LOQ was 2.8 µg/mL, the results being similar to the ones found in the literature [14].

Accuracy and precision

Accuracy. Evaluation of this parameter was made through the quantification of IND found in the sample compared to a standard solution with a previously established concentration of 40 µg/mL. The determinations were made in triplicate. The obtained results of the IND average concentration in the analyzed samples indicated a concentration of 95.15±4.15% respecting the Ph. Eur. 10.0 stipulations [19]. A characteristic chromatogram for the IND-TTS samples can be observed in **Figure 2**.

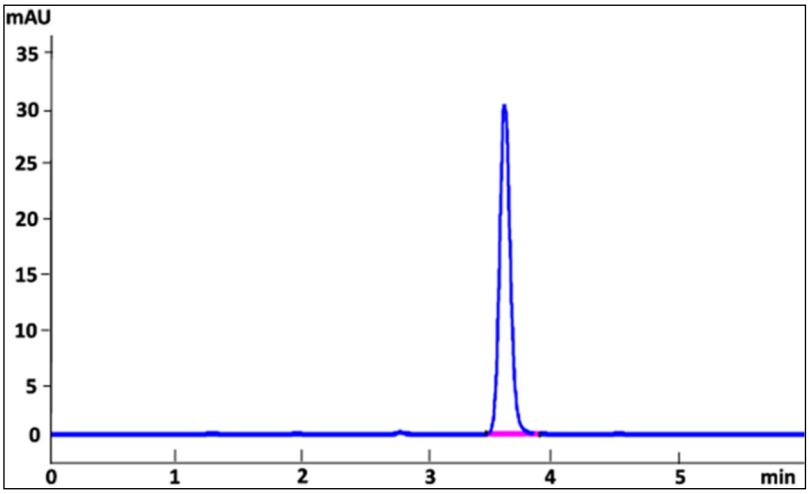


Figure 2. Typical chromatogram for IND assay from a TTS sample

Precision. Two parameters were evaluated (repeatability - intraday precision) and repeatability on different days (interday precision). All determinations were conducted in triplicate.

The precision was verified on three levels of concentrations belonging to the standard solutions: 25, 50, and 100 µg/mL. According to the results found in **Table 2**, RSD% showed values between 0.043-0.601%, corresponding to the pre-established acceptability criteria.

Table 2. Precision intraday and interday

	Precision intraday				**Precision interday**		
Concentration level (µg/mL)	**100**	**50**	**25**		**100**	**50**	**25**
Area±SD	496.9 ±1.4	248.7 ±1.0	124.8 ±0.7		497.2 ±0.9	248.5 ±0.5	124.9 ±0.6
RSD%	0.287	0.419	0.601		0.181	0.221	0.455
			Retention time (min) - T_R -				
$T_{RAverage}$ ±SD	3.530 ±0.007	3.529 ±0.001	3.536 ±0.012		3.530 ±0.003	3.536 ±0.011	3.533 ±0.006
RSD%	0.216	0.043	0.360		0.098	0.327	0.176

Application of the method to in vitro *release study*

The developed method can be successfully applied to study the release profiles of drug and to determine the release rate pattern of the IND from the TTS in dissolution rate studies, without interference.

CONCLUSIONS

The obtained results proved that the method developed to quantify IND is specific, reproducible, and precise. An advantage of this method consists of the short analysis time (t_R=3.53 min). The presence of only one characteristic signal in the chromatograms of the IND-TTS sample certifies the success of the assay for evaluating the drug from the polymeric matrix.

EXPERIMENTAL SECTION

Formulation and development of IND-TTS

Materials: indomethacin (IND) (Sigma Aldrich, Milan, Italy), hydroxypropyl methylcellulose 15k ($HPMC_{15k}$) (Metolose 90SH - 15000 mPa·s, Shin-Etsu Chemical Co., Ltd. Tokyo, Japan), propylene glycol (Scharlau Chemie, Barcelona, Spain), Tween 20 (Sigma Aldrich Co., France), ethanol (Chemical Company, Romania), ultrapure water (Millipore Direct-QS distiller).

Preparation method: solvent casting method (**Table 3**). Steps:

1. IND will be dissolved in a mixture of ethanol and propylene glycol under continuous stirring (500 rpm, Heidolph RYR1 stirrer, Germany) for 30 minutes.
2. The Tween will be dissolved in the available water amount, then the Tween solution will be mixed with the IND solution.
3. The chosen polymer will be dispersed in the formed solution and will be stirred continuously for 1 hour.
4. The formed dispersion will be kept in the water bath to eliminate the air bubbles.
5. The mixture will be cast in Petri dishes (diameter 9.8 cm).
6. Subsequently, the solvent will evaporate and a polymeric film will be formed by keeping the Petri dishes, at 40°C for 24 h.
7. The polymeric films will be packed individually in aluminum foil and will be kept in constant conditions of temperature and humidity constant (20ºC±2ºC, RH≈40%)

Table 3. TTS polymeric matrix type composition

Ingredients (% w/w)	**IND**	**$HPMC_{15k}$**	**Ethanol**	**Propylene glycol**	**Tween**	**Water**
	0.5	1.0	30.0	10.0	1.0	57.5

Similarly, a placebo TTS was prepared, without IND.

Development and validation of the HPLC method

Apparatus: HPLC Agilent Technologies 1100 Series (USA) features a quaternary pump, degasser, automatic injector, thermostated column, UV detector, data acquisition software (ChemStation), AB54S (Mettler Toledo, Sweden), ultrasonic bath T700H (Elma Transsonic).

Solvents for HPLC: acetonitrile (Merck, Germany), methanol (Merck, Germany) with analytical purity, ultrapure water (Millipore Direct-Q S distillation system); phosphate buffer KH2PO4 (Merck, Germany) 20 mM with pH adjusted at 3 using a phosphoric acid solution of 10 % (Merck, Germany)

Calibration and validation protocol

1. Phosphate buffer (KH_2PO_4 20 mM) was prepared by dissolving the KH_2PO_4 in ultrapure water, in a volumetric flask of 1000 mL.
2. Stationary phase: Waters Symmetry Column C8 (4.6x150 mm, 5 µm)
3. Chromatographic conditions:

a. Mobile phase (sonicated 10 min for degassing) composed from: phosphate buffer KH_2PO_4 20 mM, pH=3.0 and acetonitrile 35:65 (v/v%).
b. Flow rate: 1 mL/min.
c. Detection: UV 320 nm.
d. Column temperature: 35°C.
e. Injection volume: 5 µL.

Stock solution - preparation steps:

- 0.0100 g IND were dissolved in 5 mL methanol, subsequently, the solution formed is diluted with phosphate buffer (pH=7.4) until 100 mL using a volumetric flask of 100 mL. Preparation of the stock solution was made daily before starting the chromatographic analysis.
- Preparation of phosphate buffer, pH=7.4: 2.28 g of $NaH_2PO_4 \cdot H_2O$) (Fisher Scientific, UK) and 9.146 g ($Na_2HPO_4 \cdot 2H_2O$) (Merk, Germany) are dissolved in ultrapure water in a 1000 mL volumetric flask.

Preparation of the standard solutions:

Standard solutions were prepared using the stock solution making suitable dilution in order to obtain the following concentrations: 1 µg/mL, 5 µg/mL, 25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL. The stock solution was diluted with phosphate buffer (pH=7.4).

Preparation of IND-TTS:

IND-TTS polymeric matrices with a surface of 0.7539 cm^2 and a theoretical concentration of 1.33 mg/cm^2 of IND were dissolved in phosphate buffer (pH=7.4), in a volumetric flask of 25 mL, obtaining a concentration of 40 µg/mL of the active ingredient.

Preparation of the placebo samples:

The polymeric TTS without IND was evaluated similarly to the ones containing active pharmaceutical ingredient.

Analytical performances evaluation:

The analytical performance of the proposed method was verified in terms of specificity, linearity, detection limit, quantification limit, accuracy, and precision. To calculate the statistical parameters Microsoft Office Excel 2010 (Microsoft Corporation, USA) was used.

Acceptability criteria for HPLC method validation to assay IND from TTS polymeric matrices:

- *Specificity:* the lack of interferences at the retention time corresponding to IND from the standard solution taking into consideration the results regarding the placebo sample.

- *Linearity:* obtaining a linear correlation by representing the active pharmaceutical ingredient signals area as a function of the concentration of IND and a value of the correlation coefficient larger than 0.999.
- *Accuracy:* by the stipulations found in the in-force Ph. Eur. 10 and the 2004 Romanian Pharmacopoeia Supplement [19,20], the acceptability limit ranges between 75-125 %.
- Precision: The calculated RSD% for the signals area and retention times belonging to the standard solutions must be less than 2%.

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