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Simultaneous Determination of Tinidazole and Lidocaine in Pharmaceutical Preparations by HPLC with Photodiode Array Detection

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

A selective, simple and new reversed phase high performance liquid chromatographic method (HPLC) was developed and validated for simultaneous determination of tinidazole (TIN) and lidocaine (LID) in the ovule dosage form in this study. Developed method was performed with gradient elution by getting C_{18} (250x4.6mm,5µm) reversed phase HPLC column, mobile phase containing 10mM potassium phosphate buffer (pH3.0), acetonitrile at 1.0 mL/min. Temperatures for column and autosampler were adjusted at 25Cº. The chromatographic separation was carried out at 220 nm wavelength. Retention times were found as 6.5 min for LID and 8.2 min. for TIN. The purity of each substance was evaluated getting a photodiode array detector. The linearity ranges were 75.0-195.0 µg/mL for TIN and 25.0-65.0 µg/mL for LID. The limit of dedection (LOD) and the limit of quantitation (LOQ) results were 0.05625 µg/mL and 0.225 µg/mL for TIN, 0.01875 µg/mL and 0.075 µg/mL for LID, respectively. The simple, sensitive and reproducible method was applied for simultaneous determination of TIN and LID in pharmaceutical preparations successfully.

Keywords: HPLC; lidocaine; ovule; pharmaceutical preparations; tinidazole.

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ABBREVIATIONS

HPLC : High performance liquid chromatography
TIN : Tinidazole
LID : Lidocaine
LOD : Limit of detection
LOQ : Limit of quantitation

1. INTRODUCTION

The chemical name of TIN is 1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitro-1*H*-imidazole and its closed formula is $C_8H_{13}N_3O_4S$. TIN, a white or light yellow crystalline powder with a molecular weight of 247.3 g/mol, is practically insoluble in water, soluble in acetone and methylene chloride, sparingly soluble in methanol. Its melting point is in the range of 125ºC- 128ºC [1]. The chemical formula of TIN is illustrated in Fig. 1.

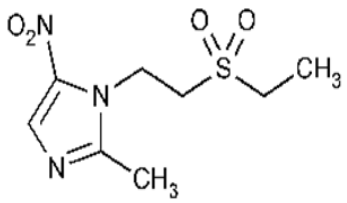

Fig. 1. Chemical formula of TIN

The effect of TIN against anaerobic bacteria and protozoa is through penetration of the drug into the microorganism cell and damage to DNA strands or inhibition of synthesis. TIN acts against protozoa as well as obligate anaerobic bacteria [2,3]. Its antiprotozoal activity includes Trichomonas vaginalis, Entamoeba histolytica and Giardia lamblia. Tinidazole acts on Gardnerella vaginalis and most of the anaerobic bacteria (Bacteroides fragilis, Bacteroides melaninogenicus, Bacteroides spp., Clostridium spp., Eubacterium spp., Peptostreptococcus spp. and Veillonella spp.) with bacterial vaginosis treatment. The mechanism of action of TIN is still not clearly explained. It mediates the reduction of the nitro group and the low oxidation-reduction potential produced by the ferredoxin system and anaerobic bacteria only. Therefore, although TIN can penetrate the cell membranes of both types of microorganisms, anaerobes can take up more TIN than aerobes. The reduction produces increased diffusion, which increases the uptake of reactive intermediates and TIN [3].

The chemical name of LID hydrochloride is 2-(diethylamino)-N-(2,6-dimethylphenyl) acetamide hydrochloride monohydrate and its closed formula is $C_{14}H_{23}ClN_2O,H_2O$. Its molecular weight is 288.8 g/mol and LID HCl is a white or almost white crystalline powder, very soluble in water, freely soluble in 96% ethanol. Its melting point is between 74°C-79°C [4]. The chemical formula of LID hydrochloride is illustrated in Fig. 2.

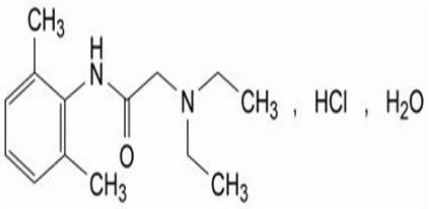

Fig. 2. Chemical formula of LID hydrochloride

LID stabilizes and blocks nerve conduction to the neuron membrane and exerts a local anesthetic effect. It stops phase-4 diastolic depolarization and reduces automaticity in Purkinje fibers and keeps the effective refractory period and action potential duration short in Purkinje fibers and ventriculus muscle (Vaughan-Williams class IB antiarrhythmic). LID increases the diastolic impulse threshold of Purkinje fibers and the fibrillation threshold of the ventricles. In normal heart tissues, the His-Purkinje system, ventricular muscle and A.V. - no change in node conduction. Conduction is reduced or increased in ischemic tissues [5,6]. When evaluated from its minimum effective concentration, it exhibits approximately three times greater local anesthetic effect than procaine. The important electrophysiological effect of LID on automaticity of the heart is that it decreases the rate of spontaneous diastolic depolarization. It is thought that this situation is related to lowering the base sodium current and increasing the potassium current. This effect has not been clarified in sinoatrial node cells. It does not change the resting membrane potential and the voltage threshold needed in the formation of the action potential, and reduces the excitability. Although it was stated that it did not affect the rapid depolarization in phase 0, it was determined that these in vitro experiments were performed with the use of low K^+ concentration and in the experiments performed by keeping the K^+ concentration at a normal level, LID applied within the limits suitable for the therapeutic concentration decreased the ejection rate of the action potential. It does not change the action potential time in the atrium myocardium and shortens it considerably in the ventricular

myocardium with Purkinje fibers. By shortening the refractory period in the last two places to a lesser extent, the result of this effect is partially corrected in pathological conditions, the asynchrony that occurs in depolarization-repolarization conditions in both mentioned places. It shortens the Q-T distance on ECG [7].

Commercially available triple combination of tioconazole, TIN and LID; while it has therapeutic properties on candida vaginitis, bacterial vaginosis and trichomoniasis, the dual combination of TIN and LID is predicted to have therapeutic properties on bacterial vaginosis and trichomoniasis. If the patient does not have candida vaginitis, the use of the product in the dual combination will eliminate the extra active substance intake and a specific treatment will be applied only for bacterial vaginosis and trichomoniasis.

When the analyzes of the predicted combination of TIN and LID were examined, it was determined that there was no simultaneous assay method of these two active substances in the literature. There are some methods for both active substances separately or simultaneous with other active substances; Various methods have been encountered such as thin layer chromatography [8], micelles liquid chromatography [9], high performance liquid chromatography [10], gas chromatography-mass spectrometry [11] and liquid chromatography [12].

In this study, it was aimed to develop, validate and apply to pharmaceutical preparations a new HPLC method, which will be the first in the literature, to be used in simultaneous determination of TIN and LID. The developed method can be used in routine analyzes in the pharmaceutical industry.

2. MATERIALS AND METHODS

2.1 Reagents and Solutions

The TIN and LID HCl reference standards used in the study were obtained from EP. The chemicals used are methanol (HPLC grade, J.T.Baker), acetonitrile (HPLC grade, J.T.Baker), potassium dihydrogen phosphate (Merck), potassium hydroxide (Merck), orthophosphoric acid (85%) (Merck).

2.1.1 Mobile phase solution

1.36 g of potassium dihydrogen phosphate was weighed and dissolved with 1000 mL of water, and the pH of the solution was adjusted to 3.0 with orthophosphoric acid. The obtained solution was filtered through 0.45 µm filters and made ready for analysis as mobile phase A. It was filtered through 0.45 µm filters with mobile phase B acetonitrile sieve.

2.1.2 TIN stock solution

300.0 mg of TIN was weighed exactly, dissolved with methanol in a 100 mL volumetric flask and made up to volume (equivalent to 3000 µg/mL TIN).

2.1.3 LID stock solution

123.26 mg LID HCl (equivalent to 100 mg LID) was weighed exactly, dissolved with methanol in a 100mL volumetric flask and made up to volume (equivalent to 1000 µg/mL LID).

2.1.4 Standard solutions

5 mL of TIN stock solution and 5 mL of LID stock solution were taken and transferred to a 100 mL volumetric flask. Complete to volume with solvent.

2.1.5 Sample solution

The mean ovule weight of 10 ovules containing 300 mg of TIN and 100 mg of LID was weighed precisely. It was grated with a stainless steel grater and made homogeneous. The grated ovule samples were kept in the refrigerator for 15 minutes for convenience before weighing. One ovule weight sample (2500 mg ovule weight) was weighed into 100mL volumetric flask with 6 different weighings. 50 mL of methanol was added to each flask and kept in a hot water bath set at 60ºC for 5 minutes until the base in the sample melted. The volumetric flasks were taken from the hot water bath and brought to room conditions, and their volume was filled with methanol. Each flask was kept in an ice bath for 20 min to collapse the base in the sample solution. After the precipitation was observed, the sample solutions in each flask were filtered through the blue band filter. 5 mL of the filtrates were transferred to 100 mL volumetric flasks. They were completed to volumes with solvent. Each solution was filtered through a 0.45 µm PVDF filter.

2.1.6 HPLC conditions

This study was carried out on Shimadzu LC-20A PDA device with Shimadzu LC-20AT pump unit,

Shimadzu SIL-20AC autosampler, Shimadzu SPD M-20A detector and Shimadzu CTO-10ASvp column furnace units. The separation occurred in a C18 (Waters Symmetry 5 µm, 250 x 4.6 mm) reversed phase HPLC column, at a flow rate of 1.0 mL/min, at a wavelength of 220 nm, at a column temperature of 25°C and an injection volume of 20 µL.

2.2 Method Validation

In the assay validation study in accordance with the ICH guidelines, the parameters of selectivity, repeatability (precision), linearity, working range, accuracy, detection and quantitation limits, robustness, and solution stability were studied [13].

3. RESULTS AND DISCUSSION

In this study, a specific HPLC method was developed for the simultaneous determination of TIN and LID as active substances in the ovule pharmaceutical preparation. The highest absorbance wavelengths were determined as 316 nm for TIN and 205 nm for LID, respectively.

Primarily optimum chromatographic conditions were determined where the separation was the best and the tailing factor was the most suitable. Although the symmetry factor meets the system suitability parameter requirement for the Waters Symmetry and Phenomenex LUNA C18 columns in the method development studies, since the values in the Phenomenex LUNA column were found to be higher than the Waters Symmetry column, C18 is a 4.6 mm diameter, 250 mm long, 5 µm particle diameter column. The study was carried out with the (Waters Symmetry) column. 10mM potassium phosphate buffer (pH: 3.0), acetonitrile gradient elution mobile phase was chosen. The 25°C column temperature and the 25°C sample split (autosampler) temperature were obtained at a wavelength of 220 nm in the PDA detector using a flow rate of 1.0 mL/min. Retention times were determined as 8.2 minutes for TIN and 6.5 minutes for LID. The relevant chromatogram is illustrated in Fig. 3.

In method validation, in the selectivity parameter, the active substances did not interfere with placebo, solvent and methanol, and the active substance peaks were pure; detected by the relevant spectra (Fig. 4 and Fig. 5).

In the developed method, the linear ranges were found to be 75.0-195.0 µg/mL for TIN and 25.0-65.0 µg/mL for LID. The linear regression equation for TIN was found as y=15439912.97925x+746,14411 (r^2=0.999) and for LID; y=34376112,94340x+16690.05250 (r^2=0.999). In the reproducibility study in the precision parameter, the relative standard deviations were 0.305% for TIN and 0.244% for LID. The relative standard deviations in the intermediate precision study were 1.438% for TIN and 0.389% for LID. In the intermediate precision study, the % differences between the results of different analysis were 0.090% for TIN and 0.140% for LID. In the accuracy study, recovery values were calculated as 98.74% - 99.67% for TIN and 99.83%-100.33% for LID. LOD and LOQ calculated from the signal/noise values were 0.05625 µg/mL and 0.225 µg/mL for TIN and 0.01875 µg/mL and 0.075 µg/mL for LID, respectively.

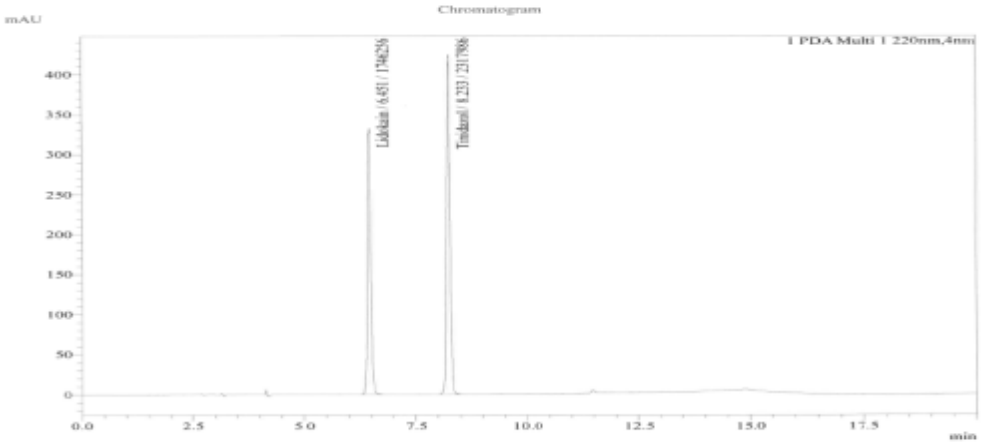

Fig. 3. Chromatogram of TIN and LID obtained with a C18 (250 mm x 4.6mm, 5µ) Waters Symmetry HPLC column

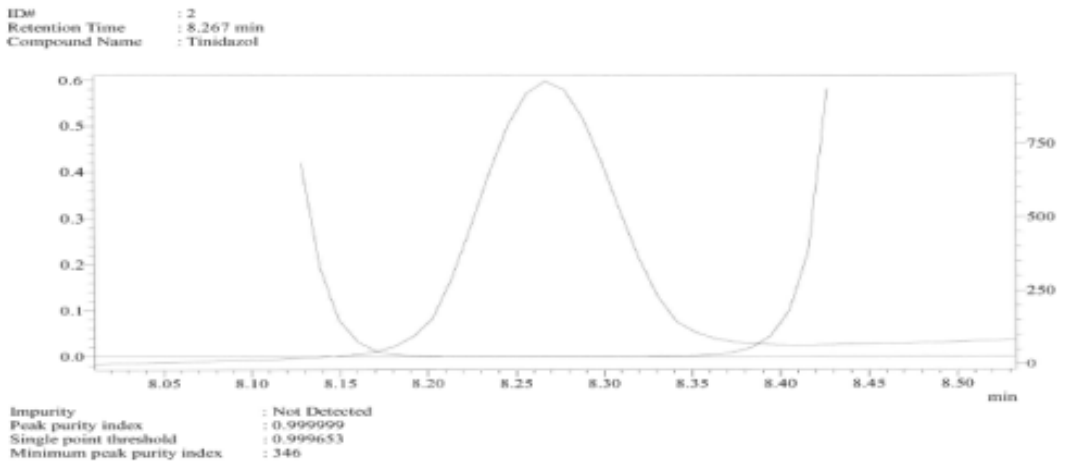

Fig. 4. Peak purity spectrum of TIN.

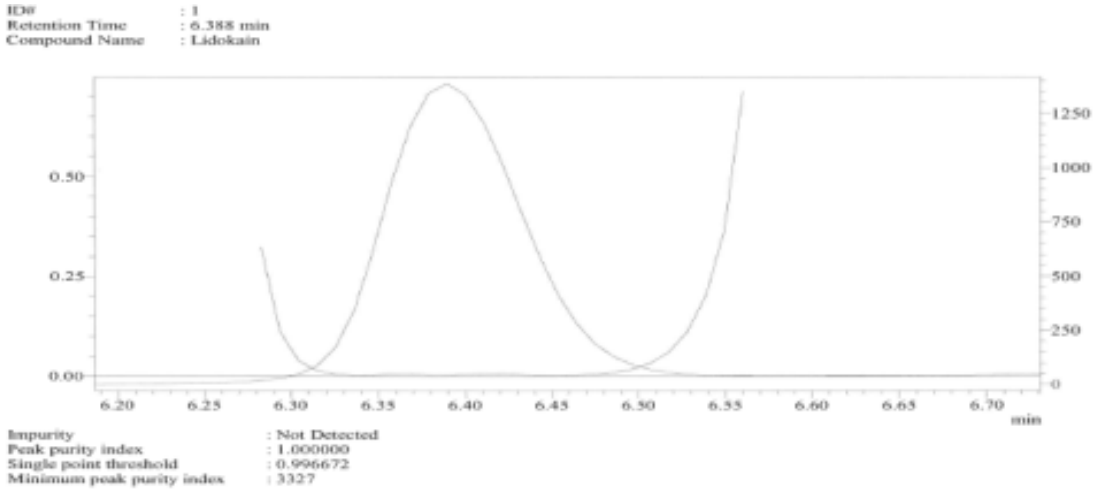

Fig. 5. Peak purity spectrum of LID

Changes in mobile phase buffer pH, column temperature, flow rate, mobile phase buffer concentration, use of different brands of columns in the robustness parameter did not cause a significant change in system compatibility parameters. The filter effect was studied in standard and sample solutions, and the PVDF filter, in which the amount of filtrate did not change significantly, was found suitable for the method. In studies for solution stability, it has been shown that standard and sample solutions remain stable at +25ºC and +4ºC for 48 hours.

In the literature research, no study was found regarding the simultaneous determination of TIN and LID in pharmaceutical preparations. It was determined that HPLC [14,15] and HPLC/MS/MS [16,17] methods were generally used in studies conducted separately for both active ingredients. HPLC/MS/MS device is not available in every laboratory due to its high cost. HPLC method [14,18-21] is preferred because of its advantages such as easy accessibility and more economical use of chemicals and materials.

4. CONCLUSION

In this study, a specific, accurate, sensitive, stable and original HPLC method for the simultaneous determination of TIN and LID in pharmaceutical preparations was developed and validated for the first time in the literature. The developed method can be used safely in quality control analyzes in pharmaceutical industry.

CONSENT

It is not applicable.

ETHICAL APPROVAL

We conducted our research after obtaining proper IEC approval.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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