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STABILITY INDICATING ANALYTICAL METHOD VALIDATION FOR SIMULTANEOUS ESTIMATION OF FENTICONAZOLE NITRATE, TINIDAZOLE AND LIDOCAINE BY REVERSED PHASE-HPLC

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Keywords:

Tinidazole, Fenticonazole Nitrate, Lidocaine Base, Analytical Method Validation, Degradation

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ABSTRACT: The current study aims to develop a reversed-phase HPLC method for quantifying the active substances of Fenticonazole Nitrate (FENTI), Tinidazole (TINI) and Lidocaine base (LIDO) simultaneously and to validate the proposed method for the intended analytical application in the bulk and pharmaceutical dosage form by ICH guidelines. In this work, the behaviour of f FENTI, TINI and LIDO was studied under various degradative conditions, and a stability-indicating LC method was developed to separate the degradation products and quantify the drug in their presence. The mobile phase Phosphate buffering agent: Methanol: Acetonitrile (60:30:10; v: v: v), Hypersil Phenyl type silica column, with flow rate 1.0ml, detection wavelength 210nm, column temperature at 40ºC and Methanolis used as diluents. System Suitability, Specificity, Precision, Linearity, Accuracy, and Robustness parameters were all validated. The system suitability parameters were investigated by injecting the standard six times, and the results were well below the acceptance threshold. Linearity parameter was investigated at levels ranging from 80% to 120%, with a r^2 value of 0.999 discovered. The formulation was tested using the above method and 102 % Fenticonazole Nitrate, 102% Tinidazole and 101% Lidocaine were found. So, this method can be used for routine analysis to estimate Fenticonazole Nitrate, Tinidazole, and Lidocaine in Pharmaceutical dosage form.

INTRODUCTION: The chemical name of TINI is 1- [2-(Ethyl sulfonyl) ethyl]-2-methyl-5-nitro-1H-imidazole, and its closed formula are $C_8H_{13}N_3O_4S$. It is an almost white or pale yellow crystalline powder with a molecular weight of 247.3 g/mol. It has a solubility in acetone and methylene chloride, is sparingly soluble in methanol, and is practically insoluble in water. The melting point range is between 125ºC to 128ºC [1]. The chemical formula of TINI is figured in **Fig. 1**. Tinidazole is an antiparasitic drug in the nitroimidazole class with strong antiprotozoal activity [2].

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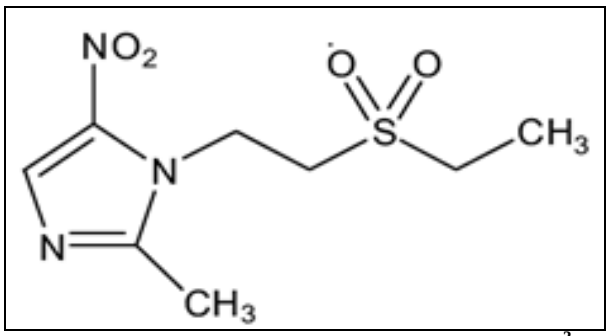

FIG. 1: CHEMICAL STRUCTURE OF TINI [3]

The chemical name of FENTI is 1-[(2RS)-2-(2,4-Dichlorophenyl) – 2 - [[4-(phenylsulfonyl) benzyl] oxy] ethyl]-1H-imidazole nitrate and its closed formula is $C_{24}H_{21}C_{l2}N_3O_4S$. It is almost white or almost white crystalline powder with a molecular weight of 518.4g/mol. It has a solubility in dimethylformamide and in methanol, is sparingly soluble in anhydrous ethanol, and practically insoluble in water. The melting point range is between 134ºC to 137ºC [1]. The chemical formula of FENTI has figured in **Fig. 2**. Fenticonazole Nitrate This molecule has a broad range of activity against dermatophytes and yeasts [4].

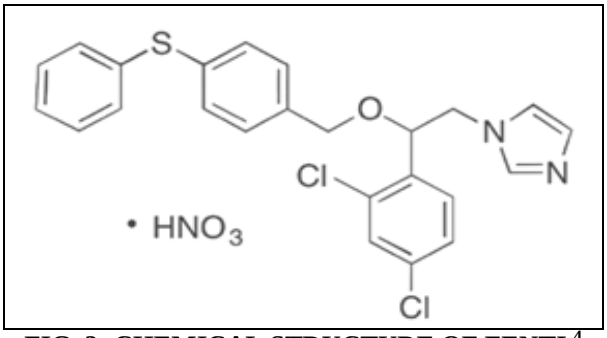

FIG. 2: CHEMICAL STRUCTURE OF FENTI [4]

The chemical name of LIDO is 2-(Diethylamino)-N-(2,6-dimethylphenyl) acetamide, and its closed formula is $C_{14}H_{22}N_2O$. It is white or almost white, crystalline powder with a molecular weight of 234.3 g/mol. It has a solubility in very soluble in ethanol (96 percent) and in methylene chloride and is practically insoluble in water. The melting point range is between 66ºC to 70ºC [1]. The chemical formula of LIDO is figured in **Fig. 3**. LIDO is a local anaesthetic used for infiltration anaesthesia, regional nerve blocks, surface anaesthesia, and the treatment of ventricular arrhythmias [5].

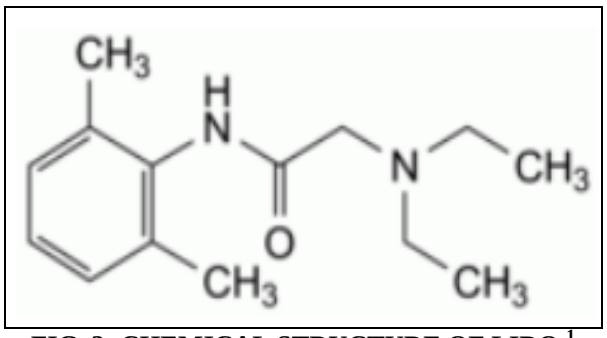

FIG. 3: CHEMICAL STRUCTURE OF LIDO [1]

HPLC, is a modern technique for standardizing both single and compound formulations; it is a much more accurate and valid method. The separation technique of HPLC is based on a stationary phase and a liquid mobile phase [6]. Depending on the size of the stationary phase used, separations are accomplished through partitioning, adsorption, or ion exchange [7]. Reversed-phase HPLC (RP-HPLC) has aqueous, moderately polar stationary, and mobile phases.

Literature Review: No study was found on the simultaneous determination of FENTI, TINI, and LIDO in pharmaceutical preparations. R. B. Ahmed *et al.* (2019) have developed and validated an HPLC method for determining tinidazole pharmaceutical dosage forms, ensuring satisfactory precision and accuracy and determining lower concentrations of the drug in its solid combined dosage form. They claim that the method is simple, accurate, economical, and rapid. It can be used for routine laboratory analysis and is suitable for quality control of raw materials and formulations. It is also stated that their method is more environmentally friendly than the pharmacopeia method [8]. Determination conditions of the assay method are at isocratic on a reverse phase C18 column with amobile phase is a mixture of methanol: pH 6.8 Buffer (50:50, v: v) and TINI was determined at the wavelength of 317 nm with a retention time 5.8 min. T. A. Silva *et al.* (2019) have developed a dependable and robust analytical method for FENTI active substance; it can be used for stability studies and quality control of cream formulations containing that active substance [9].

Objective: To perform an analytical RP-HPLC method development with a specific, precise, accurate, linear, simple, rapid, validated, and cost-effective for estimating FENTI, TINI, and LIDO quantities in a combined dosage form.

METHODS AND MATERIALS:

Reagents and Solutions: The FENTI, TINI, and LIDO reference standards used in the study were obtained from EP. The chemicals used are Methanol (HPLC Grade, J.T. Baker), Asetonitril (HPLC Grade, J.T. Baker), Potassium Dihydrogen Phosphate (Merck), Potassium Hydroxide (Merck).

Mobile Phase Solution: For Mobile Phase, Dissolve 2.72 g Potassium Dihydrogen Phosphate in 800 ml distilled water. Adjust the pH 5.0 with 1 M Potassium Hydroxide and dilute to 1000 ml with

water. Filter from 0.45 µm membrane filter. For Mobil Phase A ,directly mix pH 5.0 Phosphate Buffer: Methanol: Acetonitrile with the ratio (600:300:100; v: v:v). For Mobile Phase B, Acetonitrile is directly used.

LIDO Stock Solution: Weigh 4 mg of LIDO Reference standard accurately into a 20 mL volumetric flask. Add 10 mL of methanol and dissolve by keeping in an ultrasonic bath for approximately 5 minutes. (Equivalent to 0.2mg/ml LIDO).

FENTI Stock Solution: Weigh 12 mg of FENTI Reference Standard accurately into a 10 mL volumetric flask. Add 5 mL of methanol and dissolve by keeping in an ultrasonic bath for approximately 5 minutes. (Equivalent to 1.2 mg/ml FENTI).

TINI Stock Solution: Weigh accurately 40 mg of TIN Reference Standard into a 20 mL volumetric flask. Add 10 mL of methanol and dissolve by keeping in an ultrasonic bath for approximately 5 minutes. (Equivalent to 0.2mg/ml TINI).

Mix Standard Solution: 1ml of each stock solutions were taken and transferred to a 10ml volumetric flask. Complete to volume with methanol. (Equivalent to 0.02mg/ml LIDO, 0.12 mg/ml FENTI, 0.02 mg/ml TINI).

Sample Solution: The average ovule weight of five ovules containing 600mg FENTI, 1000mg TINI, and 100mg LIDO was precisely weighed. Ovules are homogeneously grated with a stainless-steel grater. Sample with 1440mg weight (Equivalent to 240 mg of FENTI, 400 mg of TINI and 40 mg of LIDO) was weighed into a 200 mL volumetric flask. Add about 100 mL of methanol and place the flask in the water bath (63 ± 2°C) until the mixture is completely melted. Allow to cool the solution to room temperature. Dilute to the volume with methanol and mix. Keep the flask in an ice-bath again to precipitate the hard fat. Filter through slow flow filter paper (blue band). Discard the first 5 mL of the filtrate. Dilute 5 mL of this solution to 50 mL with the methanol and mix. Filter through a 0.45 µm Nylon filter.

Chromatographic Condition: Shimadzu LC-20A PDA device with Shimadzu LC-20AT pump unit, Shimadzu SIL-20AC autosampler, Shimadzu SPD M-20A detector, and Shimadzu CTO10ASvp column furnace units were used in this study. The separation took place in a Phenyltype reversed-phase HPLC column (BDS Hypersil Phenyl, 5µ 250 x 4.6 mm) at a flow rate of 1.0 mL/min, a wavelength of 210 nm, a column temperature of 25ºC and an injection volume of 10 µL.

Method Validation: The parameters of Specificity, Repeatability (precision), Linearity, Range, Accuracy, and Robustness were studied in the assay validation study by the ICH guidelines.

Forced Degradation and Stability-Indicating Study: Forced degradation studies under different conditions were carried out on ovule samples according to the following conditions:

Photo-degradation*:* The study is performed with a photostability cabinet.(Brand of cabinet: Binder) Samples are taken into the cabinet, and the prepared quinine standard is divided into two parts; one part is wrapped with aluminium foil, and the other is left exposed to light, and they are taken beside the samples. Photostability cabinet parameters are adjusted, and samples are exposed to minimum 1.2 million lux radiation. Quinine standards placed in the cabinet are used to confirm that required radiation is applied to the cabinet under control at the end of the study. The quinine standard wrapped with aluminium foil is used as a reference in the measurements, and the absorbance difference between it and the decayed quinine standard is calculated. The quinine standard and the quinine standard wrapped with aluminum foil are measured at a wavelength of 400 nm at certain periods. The samples are taken out of the cabinet on the day the absorbance difference between the two solutions is over 0.50.

Acidic and Basic Conditions: 5 ovule samples are taken, grinded, and mixed. Approximately 5500 mg of grinded sample (equivalent to 600 mg of FENTI, 2000 mg of TINI, and 100 mg of LIDO) is weighed into a 200 mL volumetric flask. Add 8 ml of 0.1N HCl solution and keep it in an oven set at 80°C for 2,8 and 24 hours. After 2,8 and 24 hours add 8 ml of 0.1 N NaOH solution and neutralize. Add about 100 mL of methanol and place the flask in the water bath (63 ± 2°C) until the mixture is

completely melted. Allow to cool the solution to room temperature. Dilute to the volume with methanol and mix. Keep the flask in an ice-bath again to precipitate the hard fat. Filter through slow flow filter paper (blue band). Discard the first 5 mL of the filtrate. Dilute 2 mL of this solution to 50 mL with the methanol and mix. Filter through a 0.45 µm nylon filter

Oxidation with H_2O_2: 5 ovule samples are taken, grinded, and mixed. Approximately 5500 mg of grinded sample (equivalent to 600 mg of FENTI, 2000 mg of TINI, and 100 mg of LIDO) is weighed into a 200 mL volumetric flask. Add 8 ml of 3 % H2O2 solution for 1, 2, 4, 8, and 24 hours. After 1, 2, 4, 8 and 24 hours add about 100 mL of methanol and place the flask in the water bath (63 ± 2°C) until the mixture is completely melted. Allow to cool the solution to room temperature. Dilute to the volume with methanol and mix. Keep the flask in an ice bath again to precipitate the hard fat. Filter through slow-flow filter paper (blue band). Discard the first 5 mL of the filtrate. Dilute 2 mL of this solution to 50 mL with the methanol and mix. Filter through a 0.45 µm filter

Thermal Degradation: 5 ovule samples are taken, grinded, and mixed. Approximately 5500 mg of grinded sample (equivalent to 600 mg of FENTI, 2000 mg of TINI, and 100 mg of LIDO) is weighed into a 200 mL volumetric flask. Keep it in a photostability cabinet set at 50% RH humidity and 60°C for 24, 48 and 72 hours. After 24, 48, and 72 hours add about 100 mL of methanol and place the flask in the water bath (63 ± 2°C) until the mixture is completely melted. Allow to cool the solution to room temperature. Dilute to the volume with methanol and mix. Keep the flask in an ice-bath again to precipitate the hard fat. Filter through slow flow filter paper (blue band). Discard the first 5 mL of the filtrate. Dilute 2 mL of this solution to 50 mL with the methanol and mix. Filter through a 0.45 µm filter.

Realistic limits based on the approach exposure to energy in slight excess of accelerated storage (40°C for 6 months) have been set in the study. Under certain stress conditions, the study was terminated because the drug can be regarded as "stable" to the particular stress condition. Increasing stress conditions to force degradation can lead to pathways not representative of "real world" degradation. Such degradation will cause unnecessary method development for separating components that will never be observed under realistic conditions.

RESULTS AND DISCUSSIONS: A specific HPLC method for the simultaneous determination of FENTI, TINI, and LIDO as active substances in the ovule pharmaceutical preparation was developed in this study. In the specificity parameter, the retention times for TINI were 3.8 minutes, 5.5 minutes for LIDO, and 15.8 minutes for TINI. The relevant chromatogram is shown in **Fig. 4.** The system suitability results are given in **Table 1.**

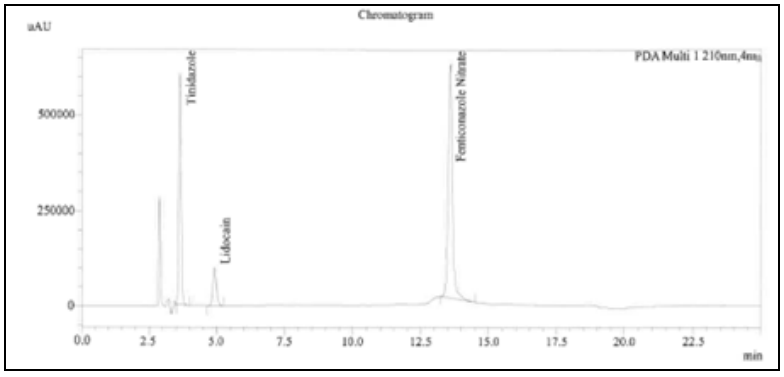

FIG. 4: TINI, LIDO AND FENTI CHROMATOGRAM WAS OBTAINED USING A PHENYL (250 MM X 4.6MM, 5µM) BDS HYPERSIL HPLC COLUMN

TABLE 1: SYSTEM SUITABILITY RESULTS

Peak Name	Retention Time	Area	Tailing Factor	Theoretical Plate Number
FENTI	13.57	6755151	1.3	181432
LIDO	4.93	854246	1.2	36862
TINI	3.63	3966461	1.2	29571

The active substances were not interfered with placebo, solvent, or methanol in the selectivity parameter, and the active substance peaks were pure; detected by the chromatographic system **Table 2**. The filter effect was investigated in standard and sample solutions, and the PVDF filter was found to be suitable for the method because the amount of filtrate did not change significantly. Standard and sample solutions are stable at +25ºC and +4°C for 48 hours in solution stability studies. In the specificity parameter, the carryover effect was investigated, and as a result of sequential sample and solvent injections, no carryover peak was observed.

TABLE 2: PEAK PURITY OF STANDARD AND SAMPLE SOLUTION

Peak Name	Impurity	Minimum Purity Index
Standard Solution (TINI)	ND	202
Sample Solution (TINI)	ND	283
Standard Solution (LIDO)	ND	2721
Sample Solution (LIDO)	ND	4325
Standard Solution (FENTI)	ND	234
Sample Solution (FENTI)	ND	328

In the linearity parameter, the linear ranges were found to be 99.0-148.0 µg/mL for FENTI, 162.0-242.0 µg/mL for TINI and 324.0- 486.0 µg/mL for LIDO. The linear regression equation for FENTI was found (r^2=0.999); for TINI was found (r2=0.999); for LIDO was found (r^2=0.999). In the repeatability study in the precision parameter, the relative standard deviations were 0.84% for FENTI and 0.90% for TINI, and 0.76% for LIDO.

The relative standard deviations in the intermediate precision parameter were 0.61% for FENTI and 0.59% for TINI, and 1.33 % for LIDO. In the accuracy parameter, for FENTI, recovery values were calculated as 100% at 80%, 101% at 100%, and 101% at 120%.

For TINI, recovery values were calculated as 100% at 80%, 101% at 100%, and 101% at 120%. For LIDO recovery values were calculated as 101% at 80% level, 100 % at 100% level and 101 % at 120% level. In the robustness parameter, changes in mobile phase buffer pH, column temperature, flow rate did not result in a significant change in system compatibility parameters. Our study performed forced degradation experiments on ovule samples to generate potentially relevant degradants and test their chromatographic behaviour using the developed method. Hydrolytic (using strongly acidic and basic media) and oxidative degradation studies were carried out either at room temperature or with heating assistance.

In acidic medium, the Ovule sample was exposed to 0.1 N HCl solution and 80ºC for 24 hours, TINI was degraded by 0.4%, LIDO by 1.7%, and FENTI 1.5%. The yields were found 98.0%, 102.4% and 98.7%, respectively. In basic medium, Ovule sample was exposed to 0.1 N NaOH solution and 80ºC for 24 hours, TINI was degraded by 0.4%, LIDO by 0.8% and FENTI 1.3%.

The yields were found 98%, 99% and 98%, respectively. In oxidative medium, Ovule sample was exposed 3% H_2O_2 solution for 24 hours, TINI was degraded by 0.4%, LIDO by 2.4% and FENTI 22.6%. The yields were found 99 %, 101 % and 100%, respectively.

In thermal exposure, Ovule sample was exposed to heat and humidity for 48 hours, TINI was degraded by 0.2%, LIDO by 0.5% and FENTI 0.5%. The yields were found 97.3% for TINI, 98.7% for LIDO and 98% for FENTI, respectively. In photo exposure, Ovule sample was exposed to light in the photostability cabinet for 144 hours, TINI was degraded by 0.2%, LIDO by 1.0% and FENTI 3.1%. The yields were found 98%, 100% and 99%, respectively **Table 1.**

As a result of the stress test study, it was determined that combined pharmaceutical dosage did not show significant degradation in acidic, basic conditions, exposure to light, temperature, and humidity. However, a significant degradation occurred in the study with hydrogen peroxide and in the study with temperature.

The results obtained in the mass balance and yield study carried out under this condition have shown that the impurities formed because of the degradation can be detected. The results obtained in the mass balance and yield study carried out under this condition have shown that the impurities formed as a result of the degradation can be detected.

TABLE 3: MASS BALANCE RESULTS AND YIELD FOR COMBINED PHARMACEUTICAL DOSAGE OVULE SAMPLE

	Condition of degradation	Time (hours)	Assay (%)	Total Impurity (%)	Total Mass Amount (%)	Yield (%)
TINI	Acidic	24	100	0.4	101	98
LIDO		24	101	1.7	102	102
FENTI		24	99	1.5	100	99
TINI	Basic	24	100	0.4	100	98
LIDO		24	99	0.8	99	99
FENTI		24	99	1.3	100	98
TINI	Oxidative	24	101	0.4	101	99
LIDO		24	98	2.4	101	101
FENTI		24	80	22.6	101	100
TINI	Photolytic	144	101	0.2	101	98
LIDO		144	99	0.9	100	100
FENTI		144	98	3.1	101	99
TINI	Thermal	72	100	0.2	100	97
LIDO		24	89	6.01	95	95
FENTI		72	88	6.74	95	94

CONCLUSION: In this study, a specific, precise, responsive, stable, and unique, reliable stability indicating HPLC method for simultaneous determination of FENTI, TINI and LIDO in pharmaceutical preparations was developed and validated study. The developed method can be used safely in a combined pharmaceutical dosage form quality control analysis. The method's reliability is ensured by performing various validation parameters and successful application to the submission batch of the product.

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