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Simultaneous determination of Lansoprazole and Metronidazole in Pharmaceutical Dosage Form by Reversed Phase- High Performance liquid Chromatography

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ABSTRACT

A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for the simultaneous estimation of Lansoprazole and Metronidazole in Pharmaceutical dosage forms. The method was carried out on a C18 (25 cm×4.6 mm i.d, 5 µm particle size) column with a mobile phase consisting of Acetonitrile ,water and Triethanol amine(pH 7.3 by addition of O-phosphoric acid) (40:60:1 v/v) at a flow rate of 1 ml/min. Detection was carried out at 290nm with UV detector. The retention time of Metronidazole and Lansoprazole was 3.14 and 10.13 min, respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantitation .The calibration curves were linear with R^2 between 0.99to 1.0 over a concentration range of 5-25 µg/ml for Metronidazole and 2-10 µg/ml for Lansoprazole . The RSD for intraday and interday precision was <1.0%.The proposed method can be used for the estimation of these drugs in combined dosage forms. Degradation studies showed that solutions of both the drug were stable for 5 hr at room temperature but both the drug showed degradation when subjected to thermal and photodegradation.

KEYWORDS: Lansoprazole, Metronidazole, RP-HPLC.

INTRODUCTION

Lansoprazole[1,2], chemically , is (*RS*)-2-([3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methylsulfinyl)-1*H*-benzo[*d*]imidazole. It is used for Treatment of ulcers of the stomach and duodenum, and NSAID-induced ulcers and Adjunctive treatment of Helicobacter *pylori* infection, alongside antibiotics. Metronidazole[3,4] is chemically 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol. Metronidazole is primarily used to treat: bacterial vaginosis, pelvic inflammatory disease , pseudo membranous colitis, aspiration pneumonia. It is also often used to eradicate Helicobacter pylori along with other drugs and to prevent infection in people recovering from surgery.

UV spectroscopy , HPLC,HPTLC and Spectrofluorimetric methods have been described in the literature for the determination of Lansoprazole[5-13] and Metronidazole[14-16] individually and in combination with other drugs like Pantoprazole,Spiramycin and Omeprazole etc . However, there is no RP-HPLC method reported for the simultaneous quantitative estimation of these drugs in combined dosage forms. Fixed dose combination containing Lansoprazole and Metronidazole is prepared in house.The aim of this work was to develop an RP-HPLC[17] method with ultraviolet detection for the simultaneous determination of Lansoprazole and Metronidazole in pharmaceutical dosage forms. Further degradation studies were performed[18]. The present RP-HPLC method was validated according to International Conference of Harmonization (ICH) guidelines[19-21].

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Experimental

Reagents , standards and samples

Acetonitrile HPLC grade and Triethanol amine AR grade were procured from Thomas Baker pvt.Ltd., Mumbai. Orthophosphoric acid AR grade was procured from S D fine chemicals Ltd, Mumbai. Water HPLC grade was obtained from a Milli-Q RO water purification system.

Gift sample of Lansoprazole and Metronidazole were procured from Meyer Organics Pvt Ltd., Navi Mumbai. at 98.2 and 99.5 % purity respectively.

Mobile phase was prepared by addition of acetonitrile and water(40:60, v/v),1 mL triethanol amine, adjusted P^H 7.3 with orthophosphoric acid. It was filtered through a 0.2 µ membrane filter and degassed.

Standard stock solution (100 µg/mL) and all dilutions (2 µg/mL to 10 µg/mL)of Lansoprazole and (5 µg/mL to 25 µg/mL) of Metronidazole were prepared in the mobile phase comprising acetonitrile and water (40:60, v/v),1 mL triethanol amine, adjusted to P^H 7.3 with orthophosphoric acid separately.

Formulation blend of 20 tablets , each containing Lansoprazole 20 mg and Metronidazole 250 mg with polymers like HPMC K 100 M and Eudragit S 100 , diluent like microcrystalline cellulose and PVP K 30 as a binder was prepared in house.

HPLC Instrument

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-20AD pump, SPD-20A/ 20AV UV detector, Rheodyne sample injector port with 20 µl loop volume and Spinchrome software. A Phenomenex C18 column (25 cm × 4.6 mm i.d., 5 µ) was used for the separation of combination of drug. The mobile phase was delivered at a flow rate of 1.0 ml/min with detection at 290 nm. The injection volume was 20 µL; Analysis was performed at an ambient temperature

Solution preparation

(a) Preparation of standard stock solution:- A standard stock solutions having a concentration of 100 µg/ml were prepared by dissolving the pure drugs Lansoprazole and Metronidazole separately in the mobile phase.

(b) Preparation of solution for the calibration curve :-Dilutions of 2,4,6,8,10µg/mL and 5,10,15,20 and 25 µg/mL of Lansoprazole and Metronidazole respectively were prepared by addition of aliquots of 0.2,0.4,0.6,0.8 & 1 ml (for Lansoprazole) and 0.5, 1.0,1.5, 2.0 and 2.5 mL (for Metronidazole) of standard stock solution of drug separately in 10 mL calibrated volumetric flask. The volumes were adjusted to the mark with the mobile phase.

(c) Procedure for sample preparation for tablet blend analysis:-Tablet blend of 20 tablets was prepared according to the formula(Table 1) with geometric mixing. Blend was sifted finally through 80 #. From the tablet blend powder equivalent to 20 mg of Lansoprazole and 250 mg of Metronidazole was weighed accurately and transferred in to 100 ml volumetric flask containing HPLC grade methanol for dissolving both the drugs. Volume was further adjusted with HPLC grade methanol. Mixture was then sonicated for 30 min for the complete dissolution of the drugs and was further filtered through 0.45 µm Whatman filter paper in order to remove the excipients. A 1 ml volume of filtrate was diluted to 100 ml with the mobile phase. Thus, the test concentration was containing 2 µg/mL of Lansoprazole and 25 µg/mL of Metronidazole.

Table 1. Formula for the of blend of tablet.

Ingredient	Quantity for 1 tablet(mg)	Qty for 20tablets.(mg)
Lansoprazole	20	400
Metronidazole	250	5000
HPMC K 100 M	70	1400
MCC	25	500
PVP K 30	5	100
EUDRAGIT S 100	30	600
WT. of tablet	400	

(d) Dilutions for precision studies :- Precision of the method was determined intraday and inter-day by three replicate readings at three concentration levels(4, 6 and 8 µg/mL) of Lansoprazole and (10, 15 and 20 µg/mL) of Metronidazole.

(e) Dilutions for Recovery studies:-Accuracy of the method was studied by addition of standard drug solution to pre-analyzed sample at three different levels: 80, 100 then 120% of the test concentration, which was 2 µg/mL for Lansoprazole and 25 µg/mL for Metronidazole.

(f) Dilutions for thermal degradation study:- Sample of 20 mg of Metronidazole and Lansoprazole was subjected to 60° C for 6 hrs. and standard solutions of 10 µg/mL of both the drugs were prepared with mobile phase.

(g) Dilutions for photo-degradation study:-Standard solutions of 10 µg/mL of Metronidazole and Lansoprazole prepared in mobile phase were subjected to UV light chamber (365 nm)for 3 hr.

Validation

(a) Robustness studies :- Robustness of the method was determined by carrying small changes, deliberate changes in the flow rate and mobile phase composition. The flow rate of 1 ml/min was changed by ±0.1 ml/min and mobile phase composition 40+60, v/v was changed by 3% of both the components.

(b) LOD and LOQ determination :- The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3).LOD can be calculated using the formula

$$LOD = 3.3\sigma / S$$

And The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). LOQ calculated with the formula $LOQ = 10\sigma / S$

Where σ = SD of the response and S=slope of the calibration curve.

(c) System suitability testing :- To verify the resolution and

reproducibility of the system for the analysis to be performed, system suitability studies are used. Parameters like theoretical plates, tailing factor, resolution and reproducibility (RSD for retention time and area of six replicated) were determined and compared against the specifications set for the method. The results of the system suitability and system precision are presented in Table 2.

Table 2 Results of system suitability testing(10 ug/ml solution of combination)

Drug	Retention time(min)	Area	No of theoretical plates	Resolution	Asymmetry
Metronidazole	3.14	151.981	5485	6.22	1.571
Lansoprazole	10.137	262.424	4312	17.802	0.903

(d) Solution stability studies :- In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 hr at room temperature. The results showed that for both solutions, the retention time and peak area of Lansoprazole and Metronidazole remained almost unchanged (% RSD less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 hr, which was sufficient to complete the whole analytical process.

RESULT AND DISCUSSION

Method development and Optimization

The pharmacopieal method for Lansoprazole makes use of buffer and acetonitrlile(P^H 7.3) in the mobile phase. As both drugs remain unionized at neutral P^H, P^H 7.3 was selected for the present method. Therefore, Triethanol amine buffer with P^H adjusted to 7.3 was selected as a mobile phase component. O-Phosphoric acid was used to adjust P^H of mobile phase buffer component as it helps to maintain good peak shape. When Triethanolamine buffer was combined with Acetonitrile ,it provided good resolution and peak shape. Thus, Acetonitrile – Triethanolamine buffer with P^H adjusted to 7.3 was chosen as mobile phase. By trying various proportions of Acetonitrile and Triethanolamine buffer ,the final proportion selected was 40:60 v/v. Table 3 lists the optimal chromatographic conditions.

UV absorption spectra of both drugs in the range of 200-400nm showed the optimum absorbance at 290nm (Figure 1).Therefore, it was selected as the detection wavelength.

Table 3 Conditions used for chromatographic analysis

Parameter	Conditions
Mobile phase	Acetonitrile ,water and Triethanol amine(pH 7.3 by addition of O-phosphoric acid) (40:60:1v/v)
Flow rate	1ml/min
Detection wavelength	290nm
Sample injector	20µl loop
Column	Phenomenex C18 (25 cm×4.6 mm i.d, 5 µm particle size)

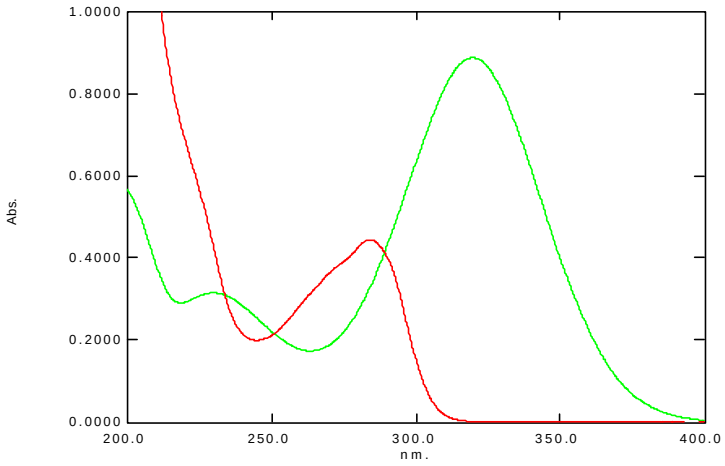

Figure 1: UV spectra of lansoprazole and metronidazole

A flowrate of 1ml/min provided a runtime of about 11 min and was used as the flowrate.C18 is a common stationary phase suitable for many pharmaceuticals , and it was also found to be suitable in this case.

With these selected method parameters, system suitability testing indicated good resolution (Figure 2) and reproducibility for the analysis to be performed. The results of system suitability testing are shown in Table 2.

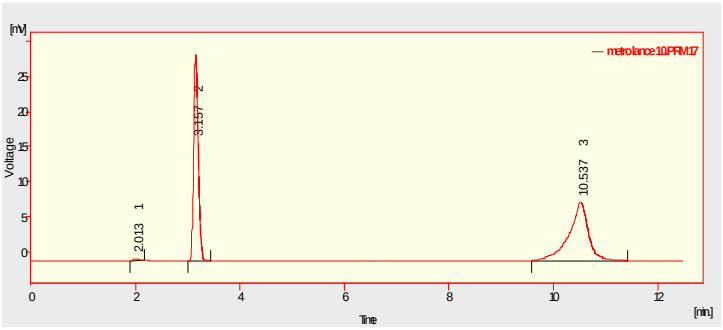

Figure 2: Chromatograph for system suitability testing (10 ug/ml solution of combination)

The method was validated for various parameters according to ICH guidelines. The results of method validation are shown in Table 4.

Table 4. Results of validation studies

Parameter	Results	
	Lansoprazole	Metronidazole
Linearity	0.994	0.991
RSD%	<2	<2
Mean recovery%	100.92	100.65
LOD,µg/ml	0.3	0.67
LOQ, µg/ml	0.9	2.01
Range,µg/ml	2-10	5-25

Method validation

The linearity of the method was determined at five concentration levels ranging from 2 to 10 µg/ml for Lansoprazole and 5 to 25 µg/ml for Metronidazole .The calibration curve was constructed by plotting area against concentration of drugs. The slope and intercept value for calibration curve was y=35.35x-2.837 (R^2 =0.994) for Lansoprazole and y=22.65x + 34.29 (R^2 =0.991) for Metronidazole (Figure 3 and 4 respectively). The results show that an excellent correlation exists between area and concentration of drugs within the concentration range.

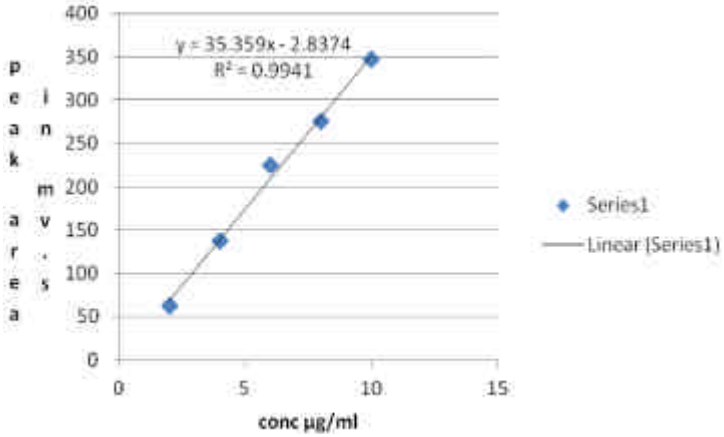

Figure 3. calibration curve for Lansoprazole

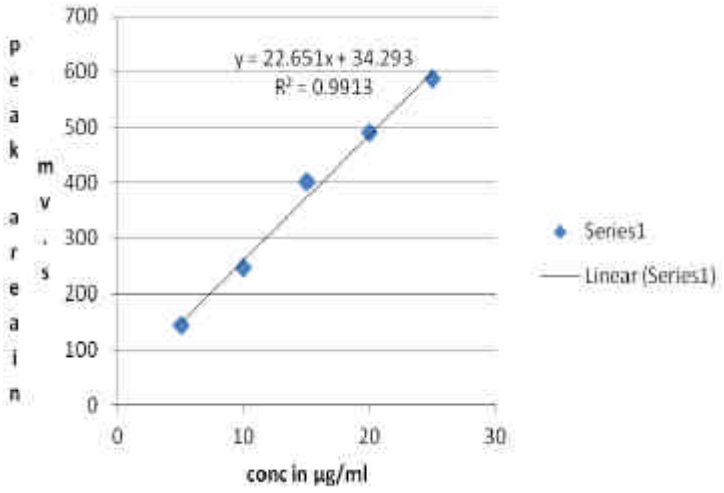

Figure 4: Calibration curve for Metronidazole

According to ICH guidelines for assay procedures of active substance or finished product, the range should be 80-120% of the test concentration (15).Therefore , a range of 2-10 µg/ml was selected for Lansoprazole and 5-25 µg/ml for Metronidazole. The results of precision study are given in Table 5.

Precision was evaluated as repeatability according to ICH guidelines. It was determined at three concentration levels with three replicates at each level. For all three concentration levels, RSD obtained was less than 1.0% for both Lansoprazole and Metronidazole. The results of the precision study are given in Table 5.

Table 5. Results of method Precision study

Drug	Conc,µg/ml	Mean area	SD	RSD,%
Lansoprazole	4	137.58	0.3469	0.228
	6	224.44	0.933	0.514
	8	275.27	0.335	0.153
Metronidazole	10	246.1	0.356	0.145
	15	401.2	0.293	0.072
	20	490.69	0.357	0.073

The recovery studies were carried out at 80,100 and 120 % of the test concentration. The results ranged from 99.76 to 101.51% for Lansoprazole and 99.56% to 101.84% for Metronidazole. Results of recovery studies are shown in Table 6 .

Table 6. Results of the recovery study

Added, % of test conc.	Amount of drug after standard addition,µg/ml		Mean area		Amt found		Recovery%	
	LNS	MTZ	LNS	MTZ	LNS	MTZ	LNS	MTZ
80	3.6	45	125.67	1074.16	3.65	44.83	101.51	99.56
100	4	50	139.65	1182.86	4.06	50.27	101.50	100.56
120	4.4	55	150.98	1349.39	4.38	56.01	99.76	101.84

Robustness studies were carried out after deliberate alterations of flow rate and mobile phase compositions. It was observed that the small changes in these operational parameters did not lead to changes of retention time in peak of interest. Results of robustness studies are shown in Table 7.

Table 7. Results of the Robustness study

	Flow rate (1ml/min)		RSD,% P^H(7.3)		Ratio of mobile phase(60+40,v/v)	
Drug	+0.1ml/min	-0.1ml/min	+0.1	-0.1	+3%	-3%
Metronidazole	0.66	0.54	0.4	0.5	0.67	0.74
Lansoprazole	0.54	0.65	0.78	0.89	0.54	0.87

The LOD and the LOQ was found to be 0.3 and 0.9 for Lansoprazole and 0.67 and 2.01 for Metronidazole. Results of stability study showed that solutions of Lansoprazole and metronidazole were stable over the period of 5 hr at room temperature. Thermal degradation and

photodegradation studies of Lansoprazole showed 22.3% and 14.49% degradation (Figure 5 and 6 respectively). Thermal degradation and photodegradation studies of Metronidazole showed 27.41% and 57.68 % degradation (Figure 7 and 8 respevtively) .

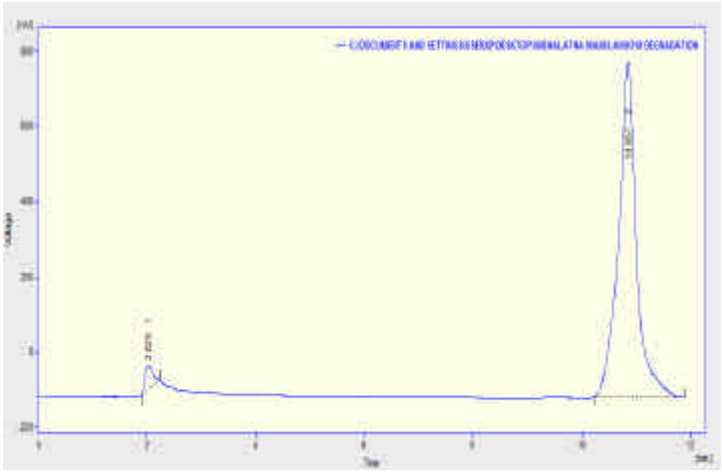

Figure 5: Lansoprazole thermal degradation

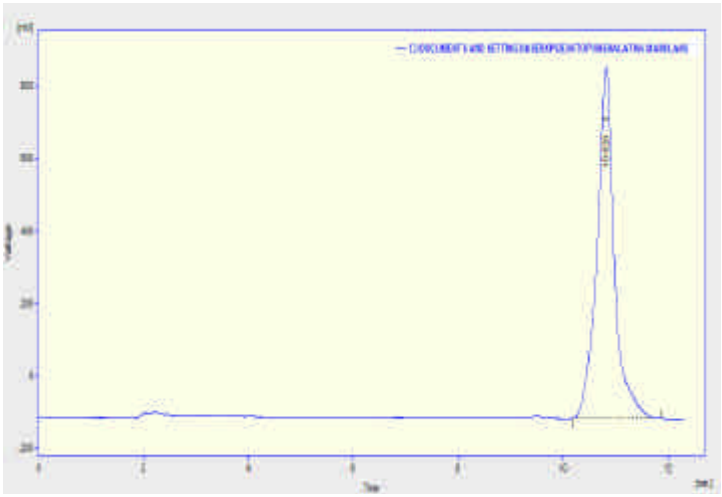

Figure 6: Lansoprazole photo degradation chromatograph

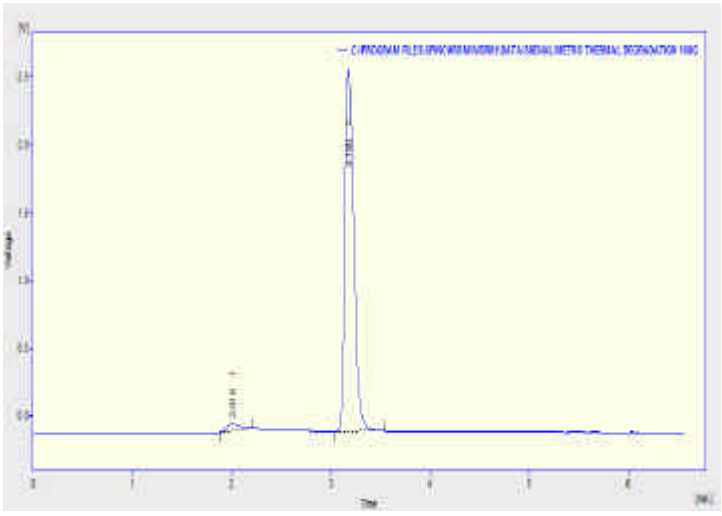

Figure 7: Metronidazole Thermal degradation.

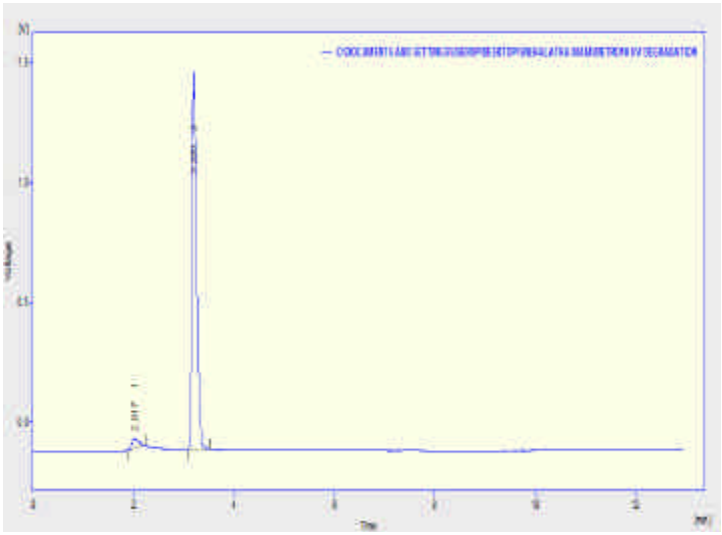

Figure 8: Metronidazole photodegradation.

Table 8. Results of the tablet blend assay study

Label claim, µg/ml		Amount found, µg/ml		Percent of label claim	
MTZ	LNS	MTZ	LNS	MTZ	LNS
25	2	24.96	1.98	99.84	99
25	2	24.89	1.95	99.56	97.5
25	2	25.02	2.01	100.08	100.5
25	2	24.68	1.96	98.72	98
25	2	25.00	2.02	100	101

The proposed method was evaluated for the assay of tablet blend containing Lansoprazole and Metronidazole prepared in-house. Five replicate determinations were carried out on the tablet blend. Mean content was 99.2% for Lansoprazole and 99.64% for Metronidazole. Results of tablet blend analysis are shown in Table 8.

Conclusions

The developed RP-HPLC method for the simultaneous determination of Lansoprazole and Metronidazole is simple, precise, accurate, reproducible and highly sensitive. The developed method was validated based on ICH guidelines. Hence, this method can be routinely used for the simultaneous determination of Lansoprazole and Metronidazole in pure and pharmaceutical formulations.

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