Original Article

SIMULTANEOUS ESTIMATION OF LOPERAMIDE HYDROCHLORIDE AND TINIDAZOLE IN BULK AND FORMULATIONS BY REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: A new, rapid, selective, precise, accurate and economical, isocratic, reverse phase high-performance liquid chromatography method has been developed for simultaneous estimation of loperamide hydrochloride and tinidazole in bulk and in tablet formulations.

Methods: The separation was achieved by using Lithosphere RP C-18, (250 x 4.6 mm, 5 μm) end capped column with a mobile phase containing sodium-1-octane sulfonate buffer: methanol: acetonitrile (40:30:30%v/v/v) pH adjusted to 4.0 (using dilute orthophosphoric acid). The flow rate was 1.0 ml/m and column effluent was monitored at 224 nm. The method was validated as per international conference on chemical harmonization (ICH) guidelines.

Results: Tinidazole and loperamide hydrochloride were eluted at about 3.1 and 5.4 min respectively, indicating the shorter analysis time. The proposed method was found to be accurate, precise and reproducible. The linearity was established in the concentration range of 10-50 μg/ml. Limit of detection (LOD) and Limit of quantification (LOQ) was found to be 0.001 μg/ml and 0.003 μg/ml for loperamide hydrochloride and 0.01 μg/ml and 0.03 μg/ml for tinidazole.

Conclusion: This method can be used for routine analysis of formulations containing any of the above drugs or combinations without any alteration in the chromatographic conditions.

Keywords: Reverse Phase-High Performance Liquid Chromatography (RP-HPLC), Loperamide Hydrochloride, Tinidazole, Simultaneous estimation

INTRODUCTION

Loperamide hydrochloride is one of the long-acting synthetic anti-diarrheal; it's an amide derivative. Chemically, it is 4-(p-chlorophenyl)-4hydroxy-N, N-dimethyl-α-α-diphenyl-1-piperidine butyramide monohydrochloride (See fig. No: 1) [1]. Tinidazole chemically it is, 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro imidazole (fig. 2) which is a nitroimidazole anti-trichomonal agent effective against *Trichomonasvaginalis, Entamoebahistolytica,* and *Giardia lamblia* infections (See fig. 2) [2].
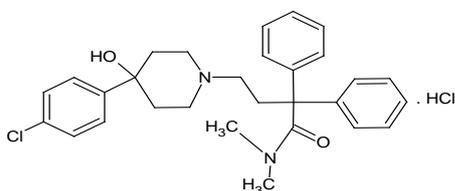
Fig. 1: Structure of loperamide hydrochloride
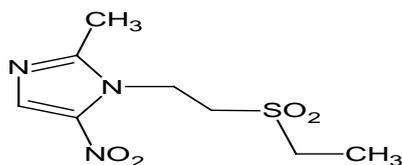
Fig. 2: Structure of Tinidazole

These drugs are being used either alone or in combination for the treatment of diarrhoea and dysentery of amoebic, bacterial or mixed origin. Pharmacologically, anti-diarrheal is prescribed along with anti-flatulants or antibacterials.

Literature survey revealed that there are methods available to estimate loperamide hydrochloride by RP-HPLC [3], Ultraviolet spectroscopy [4] and tinidazole by RP-HPLC [5] individually. But no method was developed and validated for the simultaneous estimation of loperamidehyrocholride and tinidazole.

Hence the present study was conducted to develop and validate developed and validate a suitable method for the simultaneous estimation of loperamide hydrochloride and tinidazole in bulk and formulations as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Loperamide Hydrochloride and Tinidazole were procured from Micro labs, Bengaluru and Karnataka Antibiotics Private Limited (KAPL) Bengaluru, respectively along with Certificate of Assurance (COA). HPLC grade acetonitrile and methanol and analytical grade reagents were purchased from Merck, India. HPLC grade water was collected from Milli-Q3 water purifier system. Class A apparatus were used throughout the experiment. Formulations were purchased from local markets of Bengaluru for recovery studies and specificity determination.

Instrumental and chromatographic system [6, 7]

HPLC system: Shimadzu, 20 AT model attached with pump, degasser, autosampler, Ultraviolet detector.

Mobile phase: Sodium-1-octane sulfonate buffer: methanol: acetonitrile (40:30:30 %v/v/v) pH adjusted to 4.0 (adjusted with dilute ortho phosphoric acid).

Chromatographic column: Lichospere RP C-18, (250 x 4.6 mm, 5 μm) end capped, Merck made.

Flow rate: 1.0 ml/m.

Acquisition time: 10 m.

Detection wavelength: 224 nm

Injection volume: 20 μl

Preparation of working standard solutions of Loperamide Hydrochloride and Tinidazole

Standard preparation

Accurately weighed10 mg of loperamide hydrochloride was dissolved in 10 ml of mobile phase (Stock A) and 10 mg of tinidazole was dissolved in 10 ml mobile phase (Stock A). 10 ml of each above stock solutions were diluted to 100 ml to obtain stock B of 100 μg/ml concentration. It is further serially diluted to contain 10,20,30,40, 50 μg/ml as final concentration using mobile phase.

Sample preparation

Twenty tablets that contain Loperamide Hydrochloride and twenty tablets that contain Tinidazole were weighed and crushed to a fine, homogenous powder. A quantity equivalent to 10 mg was weighed and diluted to 10 ml of mobile phase. 10 ml was further diluted to 100 ml with a mobile phase which was stock B of 100 μg/ml. Stock B was serially diluted to contain 10, 20, 30, 40 and 50 μg/ml of final concentration using mobile phase for both the tablets and are further mixed for the specificity studies.

Optimization of chromatographic conditions

The method was made on trial and error basis and the best resolution was obtained at mobile phase concentration of sodium-1-octane sulfonate buffer: methanol: acetonitrile (40:30:30% v/v/v) pH adjusted to 4.0 (with dilute orthophosphoric acid).

The method was validated as per ICH guidelines. The method was validated in terms of linearity, specificity, accuracy, precision, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Linearity and range

20 μl of each of these working standard solutions of loperamide hydrochloride and tinidazole ranging from 1 to 50 μg/ml were injected into a chromatograph at a flow rate of 1 ml/m. Retention time and peak area obtained were recorded and a standard calibration curve was plotted for loperamide hydrochloride and tinidazole, linearity equations were derived. The Correlation coefficient, % curve fitting was also calculated.

Accuracy

20 μl solution of the resulting mixture was repeatedly injected into the chromatograph, the peak area and chromatogram obtained were recorded and the % recovery of standard loperamide hydrochloride and tinidazole were calculated.

Specificity

20 μl of diluent, working standard of loperamide hydrochloride and tinidazole were injected separately to examine that the loperamide hydrochloride and tinidazole peaks are not affected by the mobile phase and diluent and the chromatogram was recorded.

Precision

System precision

Successive six injections of 20 μl working standard mixture solution (six replicates) were injected into an HPLC chromatograph, the peak area and chromatograms obtained were recorded. The % relative standard deviation was calculated for peak areas of replicates.

Method precision

Intra-day precision

Successive six injections of 20 μl of working standard mixture solutions were injected separately at different intervals in the same day, and chromatograms were recorded. The % relative standard deviation was calculated for the concentration of drug in replicates.

Inter-day precision

Successive six injections of 20 μl of working standard mixture solutions were injected separately on different days, and chromatograms were recorded. The % RSD was calculated for the concentration of drug in replicates.

Intermediate precision

Intermediate precision (Ruggedness) expresses the variations within laboratories variations: (different days, different analysts, different equipment, etc.). The Intermediate precision was performed for loperamide hydrochloride and tinidazole by a different analyst on the different instrument using a different lot of column on a different day.

Limit of detection and limit of quantification

For estimation of LOD and LOQ, visualization method was followed. In visualization method, lower dilutions of working standard solution each of loperamide hydrochloride and tinidazole of 20μl was injected into the chromatograph till the drug solution gives response and peak area. The chromatogram and peak area obtained for different concentrations of loperamide hydrochloride and tinidazole were recorded.

System suitability

20 μl of standard solutions of loperamide hydrochloride and tinidazole were injected into chromatograph and chromatograms were recorded. From the data obtained system suitability parameters like theoretical plates, tailing factor and resolution were calculated.

Robustness

For the method developed, the flow rate of 1 ml/m was used. The robustness study was carried out with the small deliberate change to 0.9 and 1.1 ml/m. 20 μl working standard mixture solutions were injected in chromatograph at a flow rate of 0.9 and 1.1 ml/m, the peak area and chromatograms obtained were recorded.

For the method developed, mobile phase comprising of methanol: sodium dihydrogen orthophosphate (70:30v/v) was used. For Robustness study, the ratio of methanol and sodium dihydrogen orthophosphate buffer were slightly altered from the ratio of (70:30v/v) to (68:32) and (72:28).20μl of working standard mixture solutions of loperamide hydrochloride and tinidazole were injected into the chromatograph with altered mobile phase ratios, the peak areas and chromatograms obtained were recorded, and the % assay was calculated.

RESULTS AND DISCUSSION

Loperamide is opiod receptor agonist that reduces intestinal motility and effective to treat diarrhea [8, 9] while tinidazole antiamoebic drug used to treat dysentery [10, 11]. The combination of loperamide and tinidazole is effective to prevent complications of amoebiasis and hence available in the market. Literature review reveals that there is no analytical methods have been reported to the simultaneous estimation of loperamide hydrochloride and tinidazole in bulk and tablet dosage form till date.

Hence, the newer, simpler, rapid and reliable RP-HPLC method has been established for the simultaneous estimation of loperamide hydrochloride and tinidazole either alone and/or in combination in the formulation. The separation was carried out by using Lichospere RP C-18, (250 x 4.6 mm, 5 μm) end capped Merck made column with mobile phase consisting of sodium-1-octane sulfonate buffer: methanol: acetonitrile (40:30:30 %v/v/v) pH adjusted to 4.0 (with dilute orthophosphoric acid) at a flow rate of 1.0 ml/min with detection at 224 nm.

Developed method for simultaneous estimation

The RP-HPLC method was developed and validated for simultaneous estimation of loperamide hydrochloride and tinidazole in the pure and combined formulation. The results obtained for the entire study are presented here.

Column and standardization of the mobile phase

It was found that peaks of loperamide hydrochloride and tinidazole were well resolved with C 8 column and the solvent system consists of sodium-1-octane sulfonate buffer: methanol: acetonitrile (40:30:30% v/v/v) pH adjusted to 4.0 (with dilute orthophosphoric acid) and this mobile phase was selected for further studies. The 10 µg/ml solutions of loperamide hydrochloride and tinidazole showed isobestic point at 224 nm and it was selected as wavelength maxima for estimation of loperamide hydrochloride and tinidazole.

Estimation of retention time

The retention time of loperamide hydrochloride and tinidazole was found to be 3.1 m and 5.4 m respectively when injected as individual components and in combination. The chromatograms for loperamide hydrochloride and tinidazole are given in the fig. 3 and fig. 4.
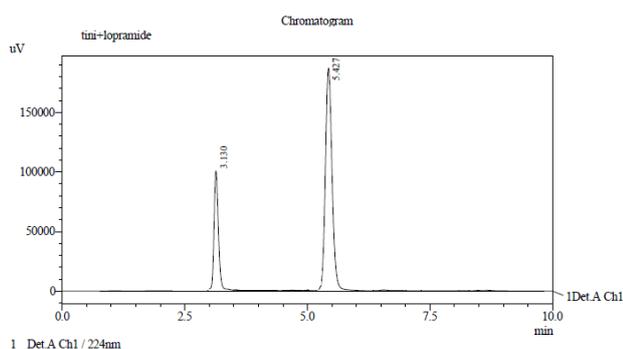

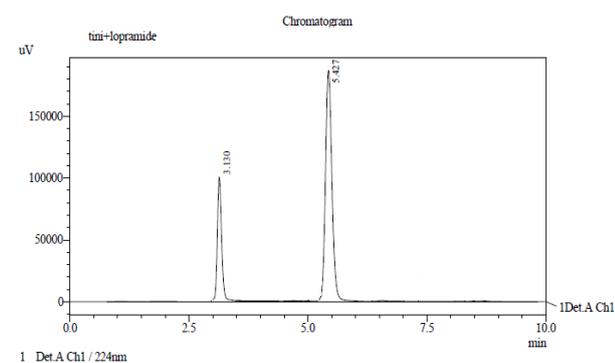**Fig. 3: Chromatogram of Loperamide hydrochloride and Tinidazole**

Fig. 4: Chromatogram of Loperamide hydrochloride and Tinidazole

Validation parameters

Linearity and range

The linearity response for loperamide hydrochloride and tinidazole were observed in the concentration range of 1 to 50µg/ml for both the drugs respectively, with correlation coefficient, percentage curve fittings found to be well within the acceptance criteria limit. The linearity and regression coefficient value is 0.997 and 0.999 for loperamide hydrochloride and tinidazole respectively, and thus the response is linear for the concentration range of 1-50 µg/ml for both (See fig. 5 and fig. 6).

Specificity

As no peaks were found at a retention time of 3.1 m and 5.4m, the proposed method was specific for the detection of loperamide hydrochloride and tinidazole.

Accuracy

The mean percentage recovery for loperamide hydrochloride and tinidazole at three different levels was found to be between 96.20-101.0% and 96.80-101.2% respectively, which was well within the acceptance limit and hence the method was found to be accurate (table 1).
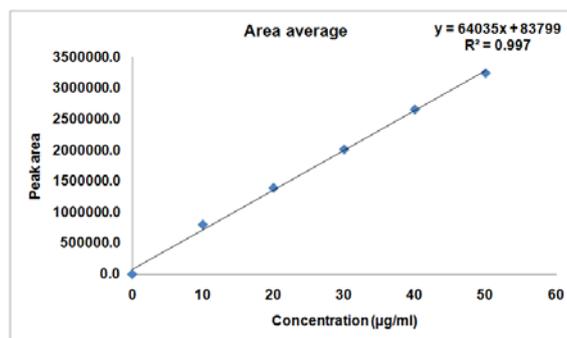

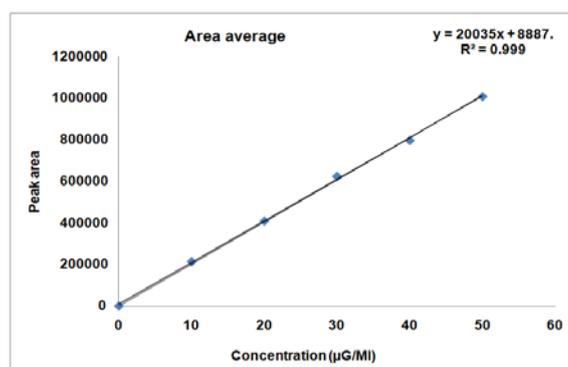**Fig. 5: Linearity of Loperamide hydrochloride**

Fig. 6: Linearity plot for tinidazole

Precision

System precision

The % RSD values of peak area for six replicate injections of loperamide hydrochloride and tinidazole were found to be 1.24 and 0.20 respectively, which are well within the acceptance criteria limit of not more than 2% (table 2).

Method precision

The % RSD values of concentration for method precision of six replicate injections of loperamide hydrochloride and tinidazole were found to be 1.2 and 1.3 respectively. Which are well within the acceptance criteria limit of not more than 2 % (table 2).

Intra-day precision

The % RSD was found to be 1.2 and 1.3 for intraday precision; 1.5 and 1.6 for the inter-day precision of loperamide hydrochloride and tinidazole respectively. As the results were within the acceptance limits, so both methods, as well as the system, provides good precision (table 2).

Inter-day precision

The % RSD was found to be 1.5 and 1.6 for interday precision; 1.66 and 0.36 for the inter-day precision of loperamide hydrochloride and tinidazole respectively. As the results were within the acceptance limits, so both methods, as well as the system, provides good precision (table 2).

Table 1: Results of accuracy of Loperamide hydrochloride and Tinidazole

Level%	Amount of standard loperamide added in µg/ml	Amount of Standard recovered in µg/ml	Recovery of Loperamide hydrochloride in %	Amount of standard Tinidazole added in µg/ml	Amount of Standard recovered in µg/ml	Recovery of Tinidazole in %
75	5	5.02	100.40	5	5.04	100.8
	5	4.81	96.20	5	4.91	98.2
	5	4.92	98.4	5	4.84	96.8
100	10	10.1	101.0	10	9.89	98.9
	10	9.98	99.8	10	10.12	101.2
	10	9.71	97.1	10	10.04	100.4
125	15	14.91	99.4	15	15.03	100.2
	15	15.07	100.4	15	14.91	99.4
	15	14.92	99.46	15	15.07	100.46

*Average of three determinations

Table 2: Results of precision parameters

Precision parameter	% RSD of Loperamide hydrochloride	% RSD of tinidazole	Acceptance criteria
System precision	1.24	0.2	<2.0
Intraday precision	1.2	1.3	<2.0
Interday Precision	1.5	1.6	<2.0
Method precision	1.2	1.3	<2.0

RSD: Relative Standard Deviation

Limit of detection (LOD) and limit of quantitation (LOQ)

It was found from the chromatogram that the concentration of 0.5 µg/ml for loperamide hydrochloride and tinidazole peak or response was observed, but no area was observed.

Hence the LOD for loperamide hydrochloride and tinidazole by visualization was found to be 0.001µg/ml and 0.010 µg/ml and LOQ was found to be 0.003µg/ml and 0.03µg/ml respectively for loperamide hydrochloride and tinidazole (table 3).

System suitability

The system suitability parameters were calculated for loperamide hydrochloride, and tinidazole, Theoretical plates HETP, Tailing factor and Resolution were shown to be well within the acceptance criteria. In the system, suitability found the resolution for all the adjacent peaks more than 2. (table 4).

Table 3: Results of LOD and LOQ

Drug	LOD *(µg/ml)	LOQ * (µg/ml)
Loperamide hydrochloride	0.001	0.003
Tinidazole	0.01	0.03

*n=6, LOD-Limit of detection,LOQ-Limit of Quantification.

Table 4: System suitability parameters

System suitability factor	Loperamide hydrochloride	Tinidazole	Acceptance criteria
Theoretical plates	48,413	35,043	>2000
HETP(mm)	24.30	28.73	-
Tailing Factor	1.1	1.2	<2
Resolution	10.81	0.0	>2

HETP: Height equivalent to a theoretical plate.

Table 5: Results of robustness

Change in	% assay of Loperamide hydrochloride*	%assay of tinidazole*
Flow rate(ml)		
0.9	100.21	99.45
1.0	99.45	100.01
1.1	96.78	98.45
Mobile phase ratio (buffer: ACN: Me-OH		
40:40:20	97.52	98.52
45:30:35	96.86	99.57
40:30:30	99.23	96.85

*n=6, Buffer: ACN: Methanol-Sodium-1-octane sulfonate buffer: acetonitrile: methanol.

Robustness

From the above observation, it was found that % assay of loperamide hydrochloride and tinidazole ranges between 96.78% to 101.21% and 96.85-100.10 % respectively, indicating that the method is robust with respect to a slight change in the ratio of the mobile phase. It is a measure of the capacity of the method to remain unaffected by small but deliberate variation of the operating conditions. This was tested by studying the changes in the pH of mobile phase by 0.5, also by varying amount of buffer by 10% (table. 5).

Recovery

The mean percentage recovery for loperamide hydrochloride and tinidazole at was found to be between 97.8% and 99.50 % respectively (table 6).

Table 6: Results of recovery assay

Amount of Loperamide hydrochloride recovered from formulation in µg/ml	Amount of Tinidazole recovered from formulation in µg/ml	% Assay for Loperamide hydrochloride	% Assay for Tinidazole
9.87	9.95	98.7	99.5

*n=6, The developed method was validated as per ICH guidelines and results were found to be within limits.

CONCLUSION

A simple Rapid and reliable RP-HPLC method has been established for the simultaneous estimation of loperamide and tinidazole either alone and/or in combination in the formulation. The method has several advantages like rapid, simple sample preparation, no need of any special reagents, high sensitivity, etc. It is suitable for analysis of these drugs in the binary formulation in a single isocratic run. This makes the method suitable for routine analysis of the combination product in quality control laboratories.

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CONFLICT OF INTERESTS

Authors hereby declare that there is no conflict of interest with respect this research work

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