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ESTIMATION OF METRONIDAZOLE, FURAZOLIDONE AND LOPERAMIDE IN VETERINARY FORMULATION BY RP-HPLC

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ABSTRACT

A RP-HPLC method for the simultaneous estimation of Metronidazole (MTZ), Furazolidone (FZD) and Loperamide (LOP) was developed in the present study. The chromatographic elution was carried out on Phenomenex C_{18} column (150×4.6mm) with 5µm particle size using Acetonitrile: Double distilled water in the ratio of 10:90% v/v as mobile phase and the detection was carried out at 230nm. The Retention times were found to be 5.35, 6.84 and 16.59 for MTZ, LOP and FZD respectively. The developed method was validated according to ICH guidelines. The method was found to be linear over a concentration range of 160-240 µg/ml for MTZ and LOP and 60-140 µg/ml for FZD respectively. Accuracy of the method was assessed by recovery studies and the percentage recovery was found to be within 99.34 – 100.90% w/w. The proposed method was simple, accurate, precise and sensitive. Hence the method can be released for routine quality control analysis of these drugs in Pharmaceutical and Veterinary formulations.

KEY WORDS: HPLC, Metronidazole, Furazolidone, Loperamide, Veterinary formulation.

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INTRODUCTION

Metronidazole is a nitroimidazole derivative which is a bactericidal, amoebicidal and trichomoncidal. It was reduced by low-redox-potential electron transfer proteins (e.g. nitroreductases such as ferredoxin) to unidentified polar product(s) which lack the nitro group. The reduction product(s) appears to be responsible for the cytotoxic and antimicrobial effects of the drug which include disruption of DNA and inhibition of nucleic acid synthesis. [1-5]

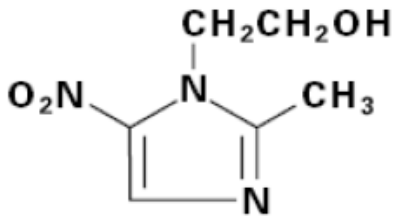

Figure 1
Metronidazole.

Furazolidone is one of the synthetic antimicrobial nitro furans. It is a stable, yellow, crystalline compound 3-(5-nitrofurfurylideneamino)-2-oxazolidinone. It has a broad antibacterial spectrum covering the majority of gastrointestinal tract pathogens including E. coli, staphylococci, Salmonella, Shigella, Proteus, Aerobacter aerogenes, Vibrio cholerae and Giardia lamblia. Its bactericidal activity is based upon its interference with several bacterial enzyme systems; this antimicrobial action minimizes the development of resistant organisms. It neither significantly alters the normal bowel flora nor results- in fungal overgrowth. The brown colour found in the urine with adequate dosage is of no clinical significance.[1-4,6]

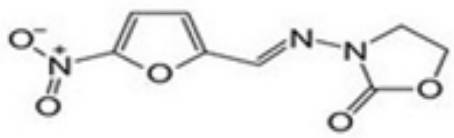

Figure 2
Furazolidone.

Loperamide is an ant diarrheal agent. Loperamide is chemically related to haloperidol, acting directly on the intestinal wall and inhibits peristalsis. It acts more rapidly and is longer acting than diphenoxylate and codeine. It was also found effective in reducing the amount of discharge from ileostomies. It acts by lowering the intestinal motility and affects water and electrolyte movement through the bowel. Inhibits peristaltic activity by a direct effect on circular and longitudinal muscles of the intestinal wall.[1-4,7]

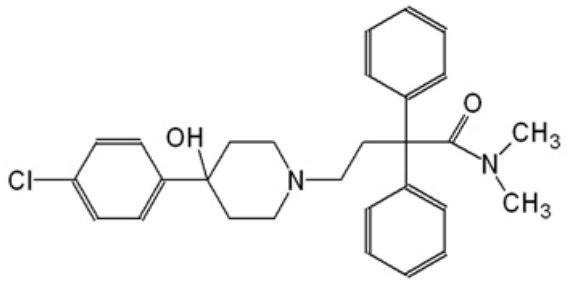

Figure 3
Loperamide.

On literature survey, a number of methods have been developed for estimation of MTZ, FZD and LOP individually and in combinaton with other drugs. Metronidazole was estimated using UV Spectroscopy[8] in combination with Tinidazole[9] and by Liquid Chromatography[10] in combination with Spiramycin[11]. Furazolidone was estimated by HPLC and HPTLC in combination with Tinidazole[12,13]. Similarly Loperamide was estimated by HPLC individually in various formulations[14,15]. The combination of MTZ and FZD was estimated by UV, HPLC and HPLC methods[16-20]. No HPLC methods was found for the MTZ, FZD and LOP in combination. The present work is an attempt to develop an analytical method for estimation of three drugs in formulation and to validate the method according to ICH guidelines.

MATERIALS AND METHODS

Raw materials

The raw materials (Metronidazole (98.90%), Furazolidone (98.75%), and Loperamide (99%) were obtained from Pond Chy Pharmaceuticals, Pondicherry as gift samples and used as reference materials throughout the experiment without any prior treatment. The reagents used were of analytical grade and are purchased from RANKEM and SRL chemicals Pvt Ltd.

Preparation of solvent mixture

The solvent mixture was prepared by mixing equal volumes of Acetonitrile and double distilled water and sonicated for 20 mins, which was used for further analysis.

Preparation of Standard Stock Solutions

Metronidazole (MTZ)

250mg of Metronidazole was accurately weighed and dissolved in 100ml of solvent mixture, sonicated to aid complete dissolution; further the volume was made up to 250ml with the same to get a final concentration of 1mg/ml (1000µg/ml).

Furazolidone (FZD)

250mg of Furazolidone was accurately weighed and dissolved in 100ml of solvent mixture, sonicated to aid complete dissolution; further the volume was made up to 250ml with the same to get a final concentration of 1mg/ml (1000µg/ml).

Loperamide (LOP)

250mg of Loperamide was accurately weighed and dissolved in 100ml of solvent mixture, sonicated to aid complete dissolution; further the volume was made up to 250ml with the same to get a final concentration of 1mg/ml (1000µg/ml).

Preparation of sample solution

Twenty tablets (Triogyl – MTZ-200mg, FZD – 100mg and LOP – 2mg) were weighed and the average weight was determined and powdered. From the powdered mixture a weight equivalent to the label claim of Loperamide was accurately weighed and dissolved in 50ml solvent mixture.

The solution was sonicated for 20min and the volume was made up to 100ml with the same. The solution was filtered and the filtrate was used for further analysis.

Method Development

Optimisation of Chromatographic conditions

- Column : Phenomenex C_{18} column 150×4.6mm, 5µm particle size
- Mobile phase : Acetonitrile : Double distilled water (10:90% v/v)
- Software : Win chrome
- Run Time : 20 minutes
- Flow rate : 1ml/min
- Injection volume : 20µl
- Detection : 230 nm

Under the optimized chromatographic conditions, 20µl of the standard mixture was injected and the standards were eluted and scanned at 230nm using UV Detector, where all the three drugs showed a good resolution and the R_t values were tabulated in Table 1.

Method Validation *[21,22]*

Linearity

Preparation of solutions for Linearity

i. Linearity 1

[80%- (MTZ) 160µg,(FZD)60µg,(LOP)160µg]

1.6ml of Metronidazole, 0.6ml of Furazolidone and 1.6ml of Loperamide standard stock solutions were pipetted out in to a 10ml standard flask and final volume was made with solvent mixture.

ii. Linearity 2

[90%- (MTZ) 180µg, (FZD)80µg, (LOP)180µg]

1.8ml of Metronidazole, 0.8ml of Furazolidone and 1.8ml of Loperamide standard stock solutions were pipetted out in to a 10ml standard flask and final volume was made with solvent mixture.

iii. Linearity 3

[100%-(MTZ)200µg,(FZD)100µg, LOP-200µg]

2.0ml of Metronidazole, 1.0ml of Furazolidone and 2.0ml of Loperamide standard stock solutions were pipetted out in to a 10ml standard flask and final volume was made with solvent mixture.

vi. Linearity 4

[110%- (MTZ)220µg,(FZD)120µg, LOP-220µg]

2.2ml of Metronidazole, 1.2ml of Furazolidone and 2.2ml of Loperamide standard stock solutions were pipetted out in to a 10ml standard flask and final volume was made with Solvent mixture.

v. Linearity 5

[120%- (MTZ)240µg,(FZD)140µg, LOP-240µg]

2.4ml of Metronidazole, 1.4ml of Furazolidone and 2.4ml of Loperamide standard stock solutions were pipetted out in to a 10ml standard flask and final volume was made with Solvent mixture.

20µl of each of the linearity solutions were injected and the detection was carried at 230nm and the chromatograms were recorded. The results were tabulated in Table 2 and 3 and the chromatograms and calibration graphs were shown in Figure 4, 5, 6 and 7.

Method Precision

20µl of the sample solution equivalent to 100% concentration of linearity solution was injected six times. The chromatograms were recorded at 230 nm and presented in Figure 8. The % RSD values were calculated and found to be within the limits according to ICH guidelines. The results were tabulated in Table 4.

System Precision

20µl of the standard linearity solution equivalent to 100% concentration was injected six times and the chromatograms were recorded at 230 nm. The chromatograms showing system precision was shown in Figure 9 and the results were tabulated in Table 5. The % RSD values were calculated and found to be within the limits according to ICH guidelines.

Accuracy

Accuracy of the method was assessed by recovery studies. Standard addition method was employed.

Preparation of solutions for Accuracy

i. Solution 1

To 1ml of sample solution, 1.6ml of standard Loperamide solution, 1.6ml of standard Metronidazole solution and 0.6ml of standard Furazolidone solution were added and the volume was made up to 10ml with solvent mixture.

ii. Solution 2

To 1ml of sample solution, 2.0ml of standard Loperamide solution, 2.0ml of standard Metronidazole solution and 1.0ml of standard Furazolidone solution were added and the volume was made up to 10ml with solvent mixture.

iii. Solution 3

To 1ml of sample solution, 2.4ml of standard Loperamide solution, 2.4ml of standard Metronidazole solution and 1.4ml of standard Furazolidone solution were added and the volume was made up to 10ml with solvent mixture.

20µl of the solution was injected six times and scanned at 230nm. The % recovery was calculated and the chromatograms were shown in Figure 10 and the results were tabulated in Table 6.

RESULTS

Table 1
Table showing Retention time

Drug	R_t Value
Metronidazole	5.35
Loperamide	6.84
Furazolidone	16.59

Linearity

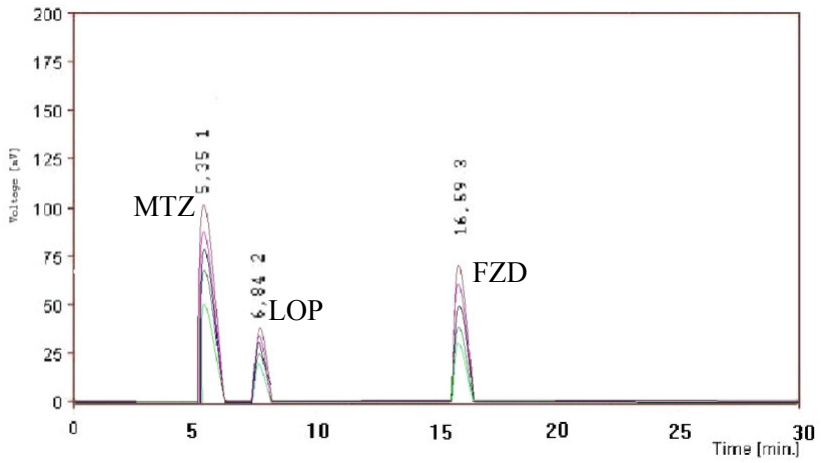

Figure 4
The chromatogram showing linearity of MTZ, FZD and LOP at 230nm.

Table 2
Linearity data

S.No.	Drugs	Concentration (µg/ml)	Peak area
1	MTZ	160	3596
		180	4002
		200	4498
		220	5029
		240	5495
2	LOP	160	548
		180	639
		200	757
		220	869
		240	982
3	FZD	60	1857
		80	2249
		100	2757
		120	3204
		140	3658

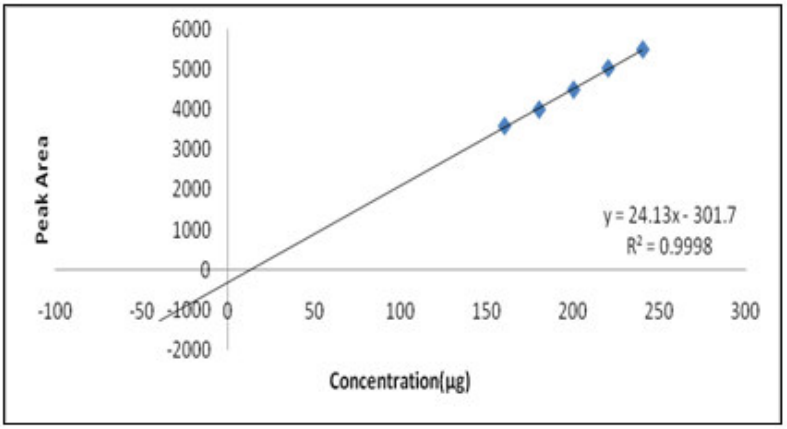

Figure 5
Calibration graph of Metronidazole

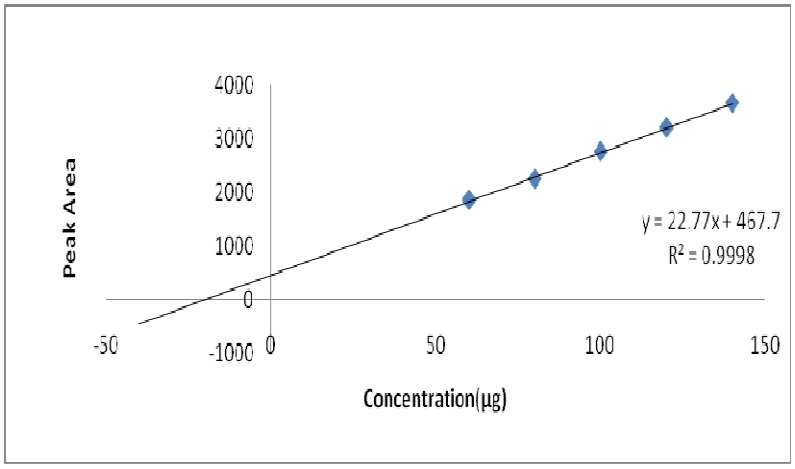

Figure 6
Calibration Graph of Furazolidone

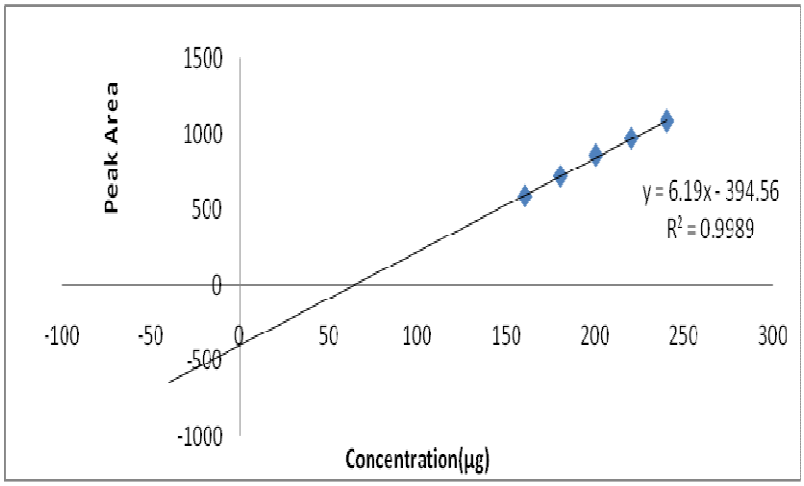

Figure 7
Calibration Graph of Loperamide

Table 3
Linearity parameters of Metronidazole, Furazolidone and Loperamide

Drug	Linearity range (µg//ml)	R^2	Slope	Intercept	LOD (µg/ml)	LOQ (µg/ml)
MTZ	160-240	0.9998	24.13	301.7	11.92	36.12
LOP	160-240	0.9989	6.19	394.56	44.35	134.40
FZD	60-140	0.9998	22.77	467.7	12.63	38.28

Precision

Method Precision

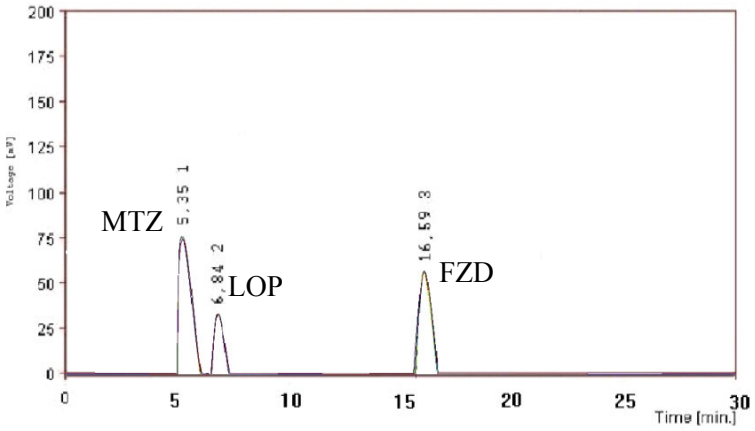

Figure 8
Chromatogram showing the precision of the method.

Table 4
Method Precision

Drug	Concentration (µg/ml)	Amount estimated (µg/ml)	Drug content (% w/w)	SD	%RSD
MTZ	200	199.62	99.81	32.66	0.65
LOP	200	196.06	98.03	11.79	1.74
FZD	100	100.79	100.79	29.11	1.46

Table 5
System Precision showing Mean S.D, % RSD.

Drug	Concentration (µg/ml)	Peak area	Average	SD	% RSD
MTZ	200	4193 4265 4356 4298 4202 4279	4265	55.83	1.30
LOP	200	819 839 834 811 827 842	829	12.58	1.51
FZD	100	1996 1975 2022 1987 2058. 2002	2007	32.57	1.62

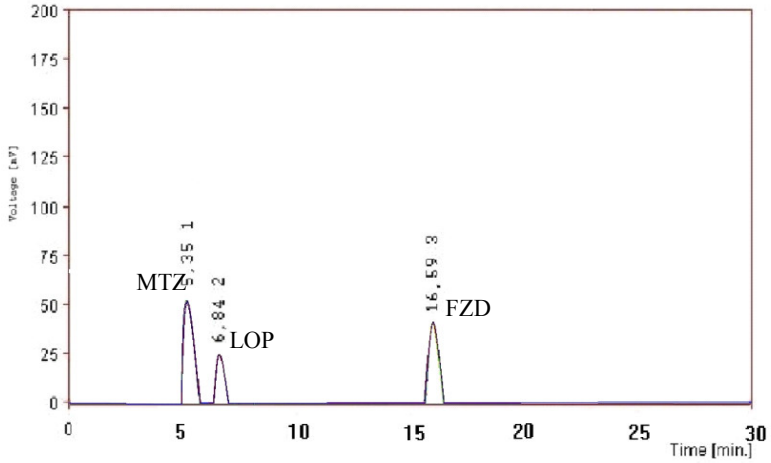

Figure 9
Chromatogram showing the system precision.

Accuracy

Table 6
Results showing accuracy of the method

Drug	Recovery Level (%)	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	SD	%RSD
MTZ	80	200	160	360.87	100.43	0.47	0.23
	100	200	200	401.81	100.90		
	120	200	240	439.91	99.95		
LOP	80	200	160	360.61	100.40	0.02	1.24
	100	200	200	400.82	100.43		
	120	200	240	440.37	100.45		
FZD	80	100	60	160.64	100.64	0.94	0.94
	100	100	100	199.34	99.34		
	120	100	140	241.18	101.18		

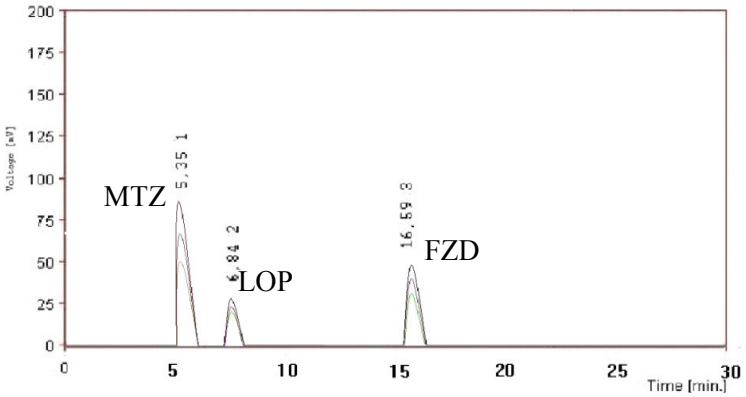

Figure 10
Chromatogram showing Accuracy results.

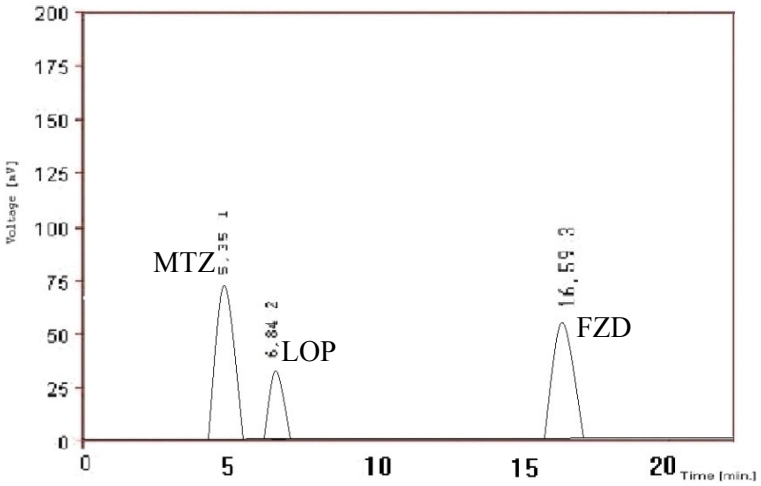

Figure 11
Sample chromatogram of the drugs in Veterinary formulation

Table 7
System Suitability Parameters

Parameters	MTZ	LOP	FZD	Limit
Retention time (mins)	5.35	6.84	16.59	----
Capacity Factor	4.35	5.84	15.89	----
Tailing factor	1.54	1.84	1.03	NMT 2
No. of Theoretical plates	2530	2316	2728	NLT 2000
Resolution	2.70	----		
	----	6.44		

Table 8
Summary of validation parameters

Parameters	Results			Acceptance criteria
	MTZ	LOP	FZD	
R_t value	5.35	6.84	16.59	--
Linearity- Range (µg/ml)	160-240	160-240	60-140	--
Correlation coefficient	0.9998	0.9998	0.9998	0.999 -1.0
Slope	24.13	6.19	22.77	--
Intercept	301.70	394.56	467.70	--
System Precision (% RSD)	1.30	1.51	1.62	< 2
Method Precision (% RSD)	0.65	1.74	1.46	< 2
Percentage recovery (%w/w)	99.95-100.90	100.40-100.45	99.34-100.64	97-103

DISCUSSION

The optimized chromatographic conditions selected for the present study showed good resolution between the drug peaks with less retention time and good response for all the three drugs at 230nm. The Retention times were found to be 5.35, 6.84 and 16.59 for MTZ, LOP and FZD respectively. The method was found to be linear over a concentration range of 160-240 µg/ml for MTZ and LOP and 60-140 µg/ml for FZD respectively. The estimated % RSD for system and method precision were found to be less than 2 as per ICH guidelines. Accuracy of the method was assessed by recovery studies and the percentage recovery was found to be within 99.34 – 100.90% w/w.

CONCLUSION

The newly developed HPLC method was validated by evaluating various validation parameters as per ICH guidelines. The results obtained were found to be within the prescribed limits. The proposed method was simple, accurate, precise and sensitive. Hence the method can be released for routine quality control analysis of these drugs in Pharmaceutical and Veterinary formulations.

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