DEVELOPMENT AND OPTIMIZATION OF AN IN VITRO DISSOLUTION METHOD FOR METRONIDAZOLE AND CLOTRIMAZOLE VAGINAL PESSARIES

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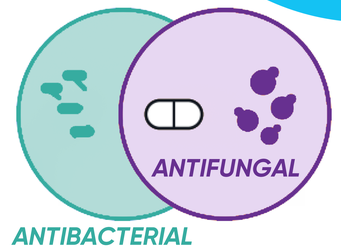
INTRODUCTION

Metronidazole is a nitroimidazole antimicrobial agent active against anaerobic bacteria and protozoa. It acts through reduction under anaerobic conditions, forming reactive intermediates that damage microbial DNA, leading to cell death (Edward DI, 1980).

Clotrimazole is an imidazole antifungal agent with broad activity against dermatophytes, yeasts, and molds. It inhibits ergosterol synthesis, disrupting fungal cell membrane integrity and function, and is commonly used for vulvovaginal candidiasis (Haller I, 1985).

The combination of metronidazole and clotrimazole provides broad antibacterial and antifungal coverage and is used in mixed vaginal infections. Current pharmacopeial quality control of pessaries includes uniformity of mass, content uniformity, disintegration, and microbiological quality - but does not assess drug release behaviour. In vitro dissolution testing can provide important information on formulation performance, particularly for poorly water-soluble compounds such as clotrimazole. However, limited information exists on suitable dissolution conditions for combined pessary formulations.

Aim: to develop and optimize an in vitro dissolution method for metronidazole and clotrimazole pessaries by selecting an appropriate dissolution medium and test conditions, and evaluating test versus reference products.



MATERIALS & METHODS

Dissolution method development and comparative dissolution testing versus the reference product were performed according to the general principles described in USP <711> Dissolution.

Dissolution test conditions

Parameter	Condition
Apparatus	USP Apparatus 2 (paddle)
Paddle speed	75 rpm
Medium volume	900 mL
Temperature	37 ± 0.5 °C
Sampling times	15, 30, 60, 120, 240 min (total 240 min)

Dissolution Media Investigated

	Medium composition (pH 4.5)
1	Phosphate buffer **+ 0.5% w/v** Tween 80
2	Phosphate buffer **+ 2.0% w/v** Tween 80
3	Acetate buffer **+ 1.0% w/v** sodium lauryl sulfate (SLS)
4	Phosphate buffer **+ 1.0% w/v** SLS

Method development focused on selecting a medium capable of adequate release and discrimination for both APIs. Given the poor aqueous solubility of clotrimazole, four surfactant- containing media were evaluated to ensure appropriate sink conditions. Samples were analyzed using a validated analytical method for simultaneous quantification of both actives.

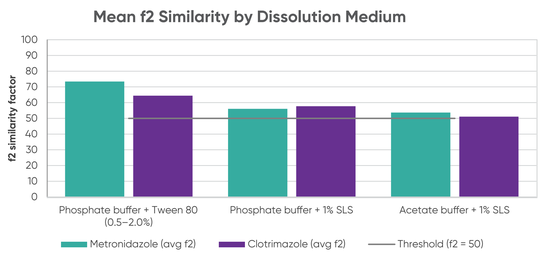
Test pessaries (metronidazole/clotrimazole 500 mg/200 mg) were manufactured by Replek Farm Ltd., Skopje, N. Macedonia; the reference product (Zifex Duo) was obtained from Antibiotice S.A., Romania.

RESULTS & DISCUSSION

Comparative dissolution studies were performed on three batches manufactured by Replek Farm (test) versus the reference product, using the surfactant-containing media at pH 4.5. Dissolution profiles showed comparable release between test and reference products, with all f2 similarity values ≥ 50.

f2 similarity factor, test vs. reference product (range across batches / surfactant concentrations)

Dissolution medium	Metronidazole	Metronidazole	Metronidazole	Clotrimazole	Clotrimazole	Clotrimazole	Threshold
Phosphate buffer +	f2 Min	f2 Max	f2 Avg	f2 Min	f2 Max	f2 Avg	(f2 = 50)
Tween 80 (0.5–2.0%)	70,7	76,2	73,45	62,9	66	64,45	50
Phosphate buffer + 1% SLS	52,1	60	56,05	53,3	62,1	57,7	50
Acetate buffer + 1% SLS	53,7	53,7	53,7	51,1	51,1	51,1	50

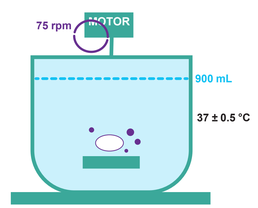


Surfactant type and concentration significantly influenced dissolution behaviour of both APIs. Phosphate buffer containing Tween 80 gave the highest and most reproducible f2 values for both metronidazole (70.7–76.2) and clotrimazole (62.9–66.0), identifying it as the most suitable medium for further method development and comparative testing.

CONCLUSION

Surfactant-containing media are essential for the in vitro evaluation of metronidazole and clotrimazole pessaries, owing to clotrimazole's poor aqueous solubility.

Phosphate buffer with Tween 80 provided the best dissolution performance and reproducibility - confirming the importance of dissolution medium optimization for reliable testing of combined vaginal pessary formulations.



USP Apparatus 2 (paddle method) — 900 mL medium, 75 rpm, 37 ± 0.5°C

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