Macedonian Pharmaceutical Bulletin, 72 (Suppl 1) 243 - 244 (2026)
Online ISSN: 1857-8969
DOI: 10.33320/maced.pharm.bull.2026.72.03.116
Short communication

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. It is widely used for anaerobic and protozoal infections (Edward, 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. It is commonly used for vulvovaginal candidiasis and superficial fungal infections (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 behavior. *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.

The aim of this study was 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 and reference products.

Materials and methods

Dissolution method development and comparative dissolution testing versus referent product were performed according to the general principles described in USP <711> Dissolution. Apparatus 2 (paddle method) was used throughout the study. Dissolution testing was conducted in 900 mL of medium maintained at 37 ± 0.5 °C with a paddle rotation speed of 75 rpm. Samples were withdrawn at 15, 30, 60, 120, and 240 minutes over a total testing period of 240 minutes. Method development was focused on the selection of a suitable dissolution medium capable of providing adequate release and discrimination for both active pharmaceutical ingredients. Considering the poor aqueous solubility of clotrimazole, four surfactant-containing media were evaluated to ensure appropriate dissolution conditions and sink behavior.

The investigated media included: 0.05 M phosphate buffer adjusted to pH 4.5 containing 0.5% *w/v* Tween 80; 0.05 M phosphate buffer adjusted to

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pH 4.5 containing 2.0% *w/v* Tween 80; 0.05 M acetate buffer adjusted to pH 4.5 with 1.0% w/v sodium lauryl sulfate (SLS); and 0.05 M phosphate buffer adjusted to pH 4.5 containing 1.0% *w/v* SLS.

Dissolution samples were analyzed using a suitable validated analytical method for quantification of both metronidazole and clotrimazole. The following reagents were used: 85% orthophosphoric acid and sodium hydroxide pellets obtained from VWR Chemicals; trifluoroacetic acid and potassium dihydrogen phosphate from Thermo Fisher Scientific; potassium phosphate monobasic and sodium acetate trihydrate from Carlo Erba Reagents; sodium lauryl sulfate from Sigma-Aldrich; and Tween 90 from Mosselman (Belgium). Metronidazole Certified Reference Material (CRM) and Clotrimazole CRM were purchased from Sigma-Aldrich, USA.

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

Results and discussion

Dissolution method development was performed to evaluate and optimize suitable surfactant-containing media for metronidazole and clotrimazole pessary formulations, with comparative dissolution testing between test and reference products used to assess the suitability of the selected conditions. Comparative dissolution studies were performed for metronidazole and clotrimazole pessary formulations, on three batches manufactured by Replek Farm, as test product, versus reference product, using different surfactant-containing dissolution media at pH 4.5. Dissolution profiles showed comparable release between test and reference products, with all f2 values ≥ 50.

In phosphate buffer containing 1% sodium lauryl sulfate (SLS), f2 values ranged from 52.09 to 59.95 for metronidazole and from 53.30 to 62.14 for clotrimazole. In acetate buffer containing 1% SLS, similar results were obtained, with f2 values of 53.71 for metronidazole and 51.12 for clotrimazole.

Higher similarity values were observed in phosphate buffer containing Tween 80, where f2 values reached 70.70–76.16 for metronidazole and 62.85–66.01 for clotrimazole, indicating more favorable and reproducible dissolution conditions for both active substances.

Surfactant type and concentration significantly influenced the dissolution behavior of both active ingredients, highlighting the importance of medium optimization for poorly water-soluble compounds such as clotrimazole. Based on dissolution performance, phosphate buffer containing Tween 80 was identified as the most suitable medium for further development and comparative testing of metronidazole and clotrimazole pessaries.

Conclusion

The study demonstrated that surfactant-containing media are essential for the in vitro evaluation of metronidazole and clotrimazole pessaries due 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.

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