Method Validation

Linearity and range

A stock solution of isoconazole nitrate (500 µg mL^{-1}) was prepared in triplicate using the mobile phase as solvent, and each solution was serially diluted with the mobile phase for use as standard solutions with concentrations in the range of 25 - 75 µg mL^{-1}. Each set of stock and standard solutions wasanalysed using the optimised chromatographic conditions,generating three calibration plots. The calibration curves were constructed by plotting the peak area of IN (*y*) against the concentration of IN (*x*);thedata were then subjected to linear regression analysis.

Precision

The precision of the method was evaluated based on intra-assay variation and intermediate precision (inter-assay variation),expressed as the relative standard deviation. Six injections of drug at a theoretical concentration of 50 µg mL^{-1} were performed on the same day, and the relative standard deviation was calculated to determine the intra-day precision. These studies were also repeated on different days to determine the inter-day precision.

Accuracy

The accuracy study was performed using concentrations corresponding to 80%, 100% and 120% of the concentration listed on the label of the IN cream; results were expressed as % recovery.

Robustness

To evaluate the robustness of the proposed method, deliberate modifications of the sample preparation method and of the chromatographic conditions were investigated. To evaluate the robustness of the HPLC method, the flow rate, composition of the mobile phase, column lot and oven temperature were all varied. The variables changed during sample preparation were the type of filter used (regenerated cellulose, nylon, PTFE - Teflon®or polyvinylidene fluoride),and the stability of the samples after 24 h.

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) values were calculated based on the slope of the calibration curve, and the signal-to-noise ratio was also determined. The LOD was 3:1, and the LOQ was 10:1.

Selectivity

The method selectivity was verified by comparing the chromatogram of the pharmaceutical formulation reference sample against the chromatograms of the reference standard solution and the blank (inactive ingredients). The method selectivity was also evaluated through comparison of the chromatogram of isoconazole nitrate with the chromatograms ofother structurally similar compounds (i.e., miconazole).