

## Comparison of three different methods of trimethoprim determination in extemporaneous pharmaceutical oral suspensions (1% w/v)

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### **Purpose.**

To develop an alternative method to the official time-consuming HPLC (BP 2005) for quantitation of trimethoprim (TMP)

### **Methods.**

In order to get a higher retention time and better resolution, the proportions of mobile phase constituents of the BP HPLC method were modified to 1:1:1. This method was compared to a colorimetric and a microbiological assay. The first was based on the quantitative extraction of TMP from the suspension with dichloromethane and spectrophotometric determination (420nm) of the yellow chromophor formed in the presence of bromothymol blue. ATCC 10536 (more sensitive to TMP than ATCC 11105) *E. coli* strain was used as the test organism for the microbiological assay since it is the most prevalent microorganism in urinary infections, usually treated with TMP. This assay was performed both by incorporation and plate streaking of *E. coli* into nutrient agar. The inhibition zone was measured using a calliper (mean of vertical, horizontal and diagonal measurements) after incubation at 37°C for 24h.

### **Results.**

Both colorimetric and microbiological assays were found to be more sensitive (3-18 and 8-5000µg/ml, respectively) than the HPLC method (50-800µg/ml). Calibration curves for colorimetric and HPLC methods showed a better correlation coefficient (0.9997) than incorporation and plate streaking (0.9963 and 0.9934, respectively). The optimal conditions for the colorimetric method included sonication of the TPM suspension with dichloromethane for 20min. The 10°C increase of temperature due to sonication was considered irrelevant for TMP stability and lead to an increased recovery of 48%. The reproducibility between day (n=4) showed a coefficient of variation of 0.17% and the chromophor was stable for at least 24h. The microbiological incorporation assay showed a greater recovery (Post Hoc Test, Tukey and Scheffe) than the colorimetric and HPLC methods (99.1±12.17%, 90.0±0.72%, 89.0±3.18%, respectively). The plate streaking method showed a recovery of 95.0±0.74% similar to the others.

### **Conclusion.**

The colorimetric method was found to be a good alternative to HPLC, showing similar accuracy but better sensitivity. The microbiological assay, although very dependent on the expertise of the operator, is the only one that guarantees the preservation of the antibiotic effectiveness.