A Reversed-Phase High-Performance Liquid Chromatography Method for the Determination of Cotrimoxazole (Trimethoprim/ Sulphamethoxazole) in Children Treated for Malaria

Rønn, Anita M.[*] ; Mutabingwa, Theonest K.[†] ; Kreisby, Solveigh[‡] ; Angelo, Helle R.[§] ; Fuursted, Kurt[‡]; Bygbjerg, Ib C.[*]

**Department of Infectious Diseases, Rigshospitalet/Centre for Medical Parasitology, Copenhagen, Denmark; †National Institute for Medical Research, Amani-Tanga, Tanzania; ‡Department of Microbiological Development, The State Serum Institute, Copenhagen, Denmark; §Department of Clinical Biochemistry, Bispebjerg Hospital, Copenhagen, Denmark*

Received March 31, 1999;

Accepted July 25, 1999.

Address correspondence and reprint requests to Anita M. Rønn, Department of Clinical Pharmacology, 7642, State University Hospital, Rigshospitalet, 2200 Copenhagen, Denmark.

Therapeutic Drug Monitoring: December 1999 - Volume 21 - Issue 6 - p 609

Abstract

Summary
A high-performance liquid chromatography (HPLC) method was developed for the simultaneous analysis of trimethoprim (TMP), sulphamethoxazole (SMX), and acetylsulphamethoxazole (AcSMX) in small amounts of blood. The method involved precipitation with 50 µL trichloracetic acid (1M) to 125 µL plasma or serum sample. 60 µL supernatant was added to 60 µL mobile phase, modified with 50 µL 1 M sodium hydroxide/mL. The mobile phase consisted of 20% acetonitrile and 80% phosphate buffer adjusted to pH 6.15. Using 125 µL of the sample, limits of quantitation were 0.1 µg/mL for TMP, 1.0 µg/mL for SMX, and 1.0 µg/mL for AcSMX. The precision of the method was 2% to 11% over the range of concentrations tested, 0.5–30 µg/mL for TMP, 5–300 µg/mL for SMX, and 2.5–150 µg/mL for AcSMX, respectively. No interference with other commonly used drugs was observed. The method is rapid, simple, specific, and sensitive enough for pharmacokinetic studies. The small amount of blood required makes it suitable for pediatric patients. The method was used to analyze samples from Tanzanian children aged 6–59 months participating in a cotrimoxazole (TMP/SMX)/chloroquine randomized trial for the treatment of uncomplicated malaria. Venous blood samples from 68 children were collected 2 hours after the first dose of TMP/SMX (4 mg/kg TMP/20 mg/kg SMX at two divided doses for 5 days) and again at treatment day 4. Individual variations in plasma concentrations of TMP, SMX, and AcSMX were considerable. The mean and SEM plasma concentrations (g/mL) of TMP, SMX, and AcSMX 2 hours after the first treatment dose were 2.0 ± 1.0 (range 0.5–6), 53 ± 22 (range 24–146), and 13.5 ± 12 (range 0–65), respectively. On the fourth day the attained plasma concentrations were not significantly different from samples collected after the first dose.