

Simultaneous Determination of Sildenafil, Its N-Desmethyl Active Metabolite and Other Drugs in Human Urine by Gradient RP–HPLC Method

**by Irena Baranowska^{1,*}, Piotr Markowski¹, Jacek Baranowski²
and Jaroslaw Rycaj³**

¹ *Department of Analytical and General Chemistry, Silesian University of Technology,
ul. M. Strzody 7, 44-100 Gliwice, Poland*

² *Department of Clinical Physiology, University Hospital, SE-581 85 Linköping, Sweden*

³ *Department of Pediatric Cardiology, Silesian Centre of Heart Disease, Medical University of Katowice,
ul. Szpitalna 2, 44-800 Zabrze, Poland*

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A new, rapid, sensitive and accurate gradient reversed-phase high-performance liquid chromatography technique for simultaneous separation and analysis of sildenafil citrate (SC), its N-desmethyl active metabolite – N-desmethylsildenafil (UK–103,320) in the presence of different drugs in human urine was developed. The analysed drugs were extracted from urine by liquid-liquid extraction. Effective RP–HPLC separation of the examined drugs was performed using a Merck LiChroCART® analytical column (Purospher® STAR RP–18 endcapped, 125 × 3 mm, particle size 5 µm) with a gradient mobile phase system and diode array or fluorescence detector. Linear ranges of detection for SC and UK–103,320 were found to be 0.03–8.5 µg mL⁻¹ (r² = 0.9994) for both compounds. Linear ranges for other drugs (analgesic, antibiotic, diuretic and demulcent), which could exist in urine from patients treated with SC were also determined. Complete separation of all analytes was achieved below 25 min. The retention times for all studied analytes ranged from 4.76 to 18.84 min. The limits of detection and limits of quantification for both analysed compounds were calculated and recovery studies were also performed. The mean absolute recoveries of SC and UK–103,320 were > 94%. The new procedure was suitably validated and successfully applied for the analysis of SC, its active metabolite and other drugs in urine samples of patients with pulmonary hypertension.