## **Abstract**

The aim of this work is to develop and to optimize HPLC method for separation of a set of four tetracycline antibiotics - tetracycline, chlortetracycline, oxytetracycline, and doxycycline. Four different reversed octadecyl-silica stationary phases in various mobile phase compositions were examined in isocratic elution. The baseline resolution of all the analytes was obtained by using two columns - Astec C18 and Atlantis C18 I. The optimized separation system consisted of Atlantis C18 I. column, ACN/0.1% formic acid 15/85 (v/v) as the mobile phase and flow rate of 2 ml/min. The baseline resolution was achieved and the analysis time did not exceed 27.5 minutes.