



## **A Qualitative LC-MS/MS Method for Simultaneous Screening of 15 commonly used Antibiotics from dried Blood and Urine spots**

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**Abstract :** A simple, fast, sensitive and selective qualitative liquid chromatography with tandem mass spectrometry (LC-MS/MS) method for identification of fifteen (15) antibiotics in dried blood spot (DBS) and dried urine spot (DUS) was developed and validated. Amoxicillin, ampicillin, penicillin G, penicillin V, cloxacillin, cephalexin, sulfamethoxazole, trimethoprim, erythromycin, ciprofloxacin, tetracycline, clarithromycin, metronidazole, chloramphenicol, and azithromycin were identified in DBS and DUS samples. DBS and DUS samples were prepared by applying blood or urine (50  $\mu$ L) to filter papers and dried at room temperature for 3 hours in the dark. The whole diameter disk (containing 50  $\mu$ L of blood or urine) was cut out from each DBS and DUS and extracted using methanol and acetonitrile (20:80). The extracted sample was chromatographed without further treatment using an LC-MS/MS instrument equipped with C18 column, (Agilent ZORBAX C-18 Eclipse Plus 2.1  $\times$  150 mm, 1.8  $\mu$ m (p/n 959759-902)). The mobile phase was A) Water + 0.1 % formic acid + 0.1% ammonium formate, B) 80% acetonitrile + 20% methanol + 0.1% formic acid. The run time was 25 minutes and post run time was 2 minutes. Two multiple reaction monitoring (MRM) transitions were selected for all target compounds to ensure selectivity and robustness. The following method parameters were validated; limit of detection (LOD), selectivity (SLR), sensitivity (SNR), reliability (RLR), false positive rate (FPR) and false negative rate (FNR). The method has been applied for the detection of the 15 antibiotics in DBS and DUS from blood and urine of volunteers who were administered the antibiotics.

**Keywords :** Qualitative; antibiotics; LC-MS/MS; dry blood spot (DBS); dry urine spot (DUS).

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