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## ACCEPTED MANUSCRIPT

Dried blood spots combined to an UPLC-MS/MS method for the simultaneous determination of drugs of abuse in forensic toxicology

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#### **Highlights**

- A DBS-UPLC-MS/MS method is proposed for drugs of abuse analysis in forensic toxicology.
- The method shows small run time, high sensitivity, very good precision and accuracy.
- The storage stability of all the substances in the DBS was demonstrated.
- Real samples analysis revealed a good correlation between DBS and WB results.
- The method developed emphasizes the potential of using the DBS for forensic analysis.

#### **Abstract**

A method for the simultaneous determination of 11 illicit drugs, using the dried blood spot (DBS) sampling technique combined with the UPLC-MS/MS technology was developed to study its applicability within the forensic toxicology. The DBS samples, prepared from a blood volume of 50 μL and using the Whatman® BFC 180 bloodstain cards, were extracted with a methanol/acetonitrile mixture. The chromatographic separation was performed using an Acquity UPLC® HSS T3 column (100 mm x 2.1 mm, 1.8 μm) and an acetonitrile/2 mM ammonium formate (0.1% formic acid) gradient. The detection was accomplished with a TQ Detector, operating in the ESI+ and MRM modes. The method was validated in terms of selectivity, matrix effect, extraction recovery (42% to 91%), carryover, LOD and LOQ (0.5 to 1 ng/mL and 1 to 5 ng/mL, respectively), linearity (LOQ to 500 ng/mL), intraday and interday precision (3.8 to 14% and 5.3 to 13%, respectively), accuracy (-9.3% to 7.9%) and dilution integrity. An eight months stability study at room temperature, 2 to 8°C and -10°C, was also performed, with the best results obtained at -10°C. The procedure was applied to 64 real samples (92 positive results for substances included in this study). The results were compared with the methodologies routinely applied in the laboratory and the statistical analysis allowed to establish an acceptable correlation. This study permitted to determine that the DBS can represent an alternative or a complement to conventional analytical and sampling techniques, responding to some of the present issues concerning the different forensic toxicology applications.

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