**Supplementary Table** – Blood Steroid Profiling LCMSMS Methods used for Newborn Screening for CAH

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Method** | **Instrument** | **Chromatography Column** | **Spot Size** | **Extraction Solvent** | **Sample**  **Runtime** | **Internal Standards** | **Target Steroids** |
| Lacey J. et al 2004 (19) | SCIEX API 3000 | C18, 2.1 x 20mm | 4.8mm | DEE | 12.0 min | d8-17OHP | 17OHP, A4, F |
| Janzen N. et al 2007 (20) | Waters Ultima | C18 3μm, 2.1 x 20mm | 6.0mm | ACN/H2O (50:50) | 6.0 min | d8-17OHP, d2-F | 17OHP, A4, F, 21DF, 11DF |
| Dhillon et al 2011 (25) | SCIEX API 3000 | C18, 5μm, 2.1x50mm | 4.7mm | MeOH/ H2O (90:10) | 6.5 min | d8-17OHP, d4-F,  d7-A4 | 17OHP, A4, F |
| Janzen et al 2011  (26) | Waters Xevo | C18, 1.7μm, 2.1x50mm | 2x4.7mm | ACN/ACE (50:50) | 1.5 min | d8-17OHP, d8-21DF d2-F d2-11DF d7-A4 | 17OHP, A4, F, 21DF, 11DF |
| Magnisali et al 2011 (21) | SCIEX API 5000 | C18, 5μm, 2.1x50mm | 5mm | MeOH (followed by SPE) | 3.0 min | d8-17OHP, d4-F | 17OHP, A4, F, 11DF, E |
| Rossi et al 2011  (27) | Waters Xevo | C18, 1.8μm, 2.1x50mm | 2x3.0mm | ACN/ACE (50:50) | 3.5 min | d8-17OHP, d4-F  d7-A4 | 17OHP, A4, F |
| Kim et al 2015 (23) | Agilent 6490 | C18, 2.6μm, 2.1x50mm | 3.2mm | MeOH/ACN (50:50) | 20 min | d8-17OHP, d4-F  d2-11DF d8-21DF d7-A4 d8-B d8-11DOC | 17OHP, A4, 21DF, 11DF, F, B, 11DOC |
| Boelen 2016 (24) | Waters Xevo TQS | C18, 1.7μm, 2.1x50mm | 2x3.2mm | ACN/ H2O (80:20) | 6.5 min | d8-17OHP | 17OHP, A4, T, 21DF, 11DF, 11DOC, E, F, B |
| Lai 2020 (28) | Waters Xevo TQS | C18, 1.8μm, 2.1x50mm | 3.2mm | MeOH/ H2O (95:5) | 3.5 min | d8-17OHP, d4-F d7-A4 | 17OHP, F, A4 |
| Bialk 2019 (29) | SCIEX API 5000 | C18, 5μm, 2.1x50mm | 3.2mm | ACN/H2O (80:20) | 9.0 mins | d8-17OHP, d8-21DF d4-F d7-A4 | 17OHP, A4, 21DF, 11DF, F |
| Zhan 2022 (30) | Waters Xevo TQS | C18, 1.7μm, 2.1x50mm | 2x5mm | ACN/MeOH (50:50) | 8.0 mins | d8-17OHP, d4-F d2-11DF d3-T d3-A4 | 17OHP, A4, 21DF, 11DF, F, T |