

New Methods for the Analysis of Oxosteroids, Oxysteroids and other Oxygenated Lipids

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Neutral steroids have traditionally been analysed by gas chromatography – mass spectrometry following the necessary derivatisation. However, both liquid chromatography – electrospray (ES) mass spectrometry and nano-ES mass spectrometry offer advantages for the analysis of steroids from crude biological extracts. Unfortunately, neutral steroids are invariably poorly ionised by ES, leading to low sensitivity. To overcome this, derivatisation reactions can be performed to add an acidic, basic or charged functional group to the steroid. In this study we have investigated the derivatisation of ketone groups to imine and hydrazone groups. The ionisation and fragmentation chemistry of these derivatives has been explored.

A series of oxosteroids were derivatised with the following reagents:

- 1) hydroxylamine hydrochloride
- 2) hydroxylamine-O-sulphonic acid
- 3) carboxymethoxylamine
- 4) (carboxymethyl)pyridinium chloride hydrazide
- 5) (carboxymethyl)trimethylammonium chloride hydrazide.

The resulting imines (oximes) or hydrazones were analysed by nano-ES, LC-ES and tandem mass spectrometry.

3-oxo- and 17-oxo-steroids are readily derivatised with reagents 1 - 5 to give imines or hydrazones. 20-oxo groups are more difficult to derivatise due to steric hindrance. The resulting derivatives give intense $[M+H]^+$, $[M-H]^-$ or M^+ ions, which fragment in both the ion source and collision cell. Structurally informative information is obtained in both the high and low collision energy regimes.
