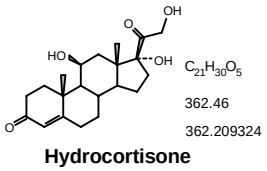
Waters Integrity System Applications

The Advantages of ThermaBeam LC/MS in Separations Development

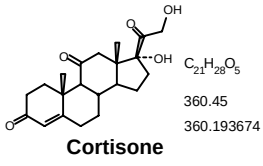
Highlights: The elution behavior of four chemically similar corticosteroids was observed under different isocratic solvent compositions using the Waters Integrity LC/MS System. This system creates classical Electron Ionization (EI) spectra which yield reproducible ion intensity "fingerprints" that are useful in structural confirmation and which were also used to monitor chromatographic elution order and coelution of specific sample components. Specificity of this mass detection method allowed a detailed examination of k' behavior as a function of solvent composition.

The process of method development usually consists of multiple trials with variations in column types and mobile phase compositions until the most positive result is achieved. During these trials, changes in elution order and peak coelutions are inevitable. This gives rise to ambiguity in peak assignment. Although photodiode array detection successfully detects peak coelution, absolute peak identification based upon UV spectral comparisons is not possible. EI mass spectra generated by a ThermaBeam LC/MS system provide a powerful means of selective detection. EI produces a number of fragment ions which may be used to identify a peak either by library matching or by interpretation of the mass spectrum. In this study, separation of a mixture of four chemically similar corticosteroids, Cortisone, Hydrocortisone, Prednisone and Prednisolone, was achieved through the trial-and-error development of a ternary (ether:anhydrous alcohol:isooctane) solvent, normal phase separation.

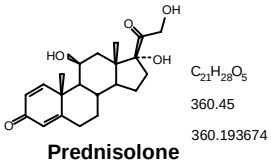
Analytical Conditions:
Column: Waters 2.0 X 150 mm Nova-Pak Silica at ambient temperature
Flow rate: 0.25 mls/min
Sample Prep: Dissolved in THF at a conc. of 1.0 mg/ml. Inj vol: 1-2 ul
Isooctane, Anhydrous alcohol, Ethyl ether were the reagents used
Optimum Gradient Conditions: See results.



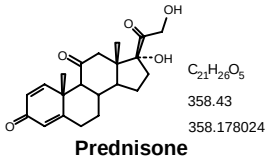
$C_{21}H_{30}O_5$
362.46
362.209324
Hydrocortisone



$C_{21}H_{28}O_5$
360.45
360.193674
Cortisone



$C_{21}H_{28}O_5$
360.45
360.193674
Prednisolone



$C_{21}H_{26}O_5$
358.43
358.178024
Prednisone

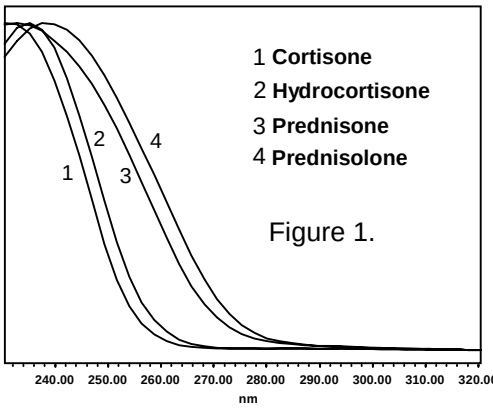
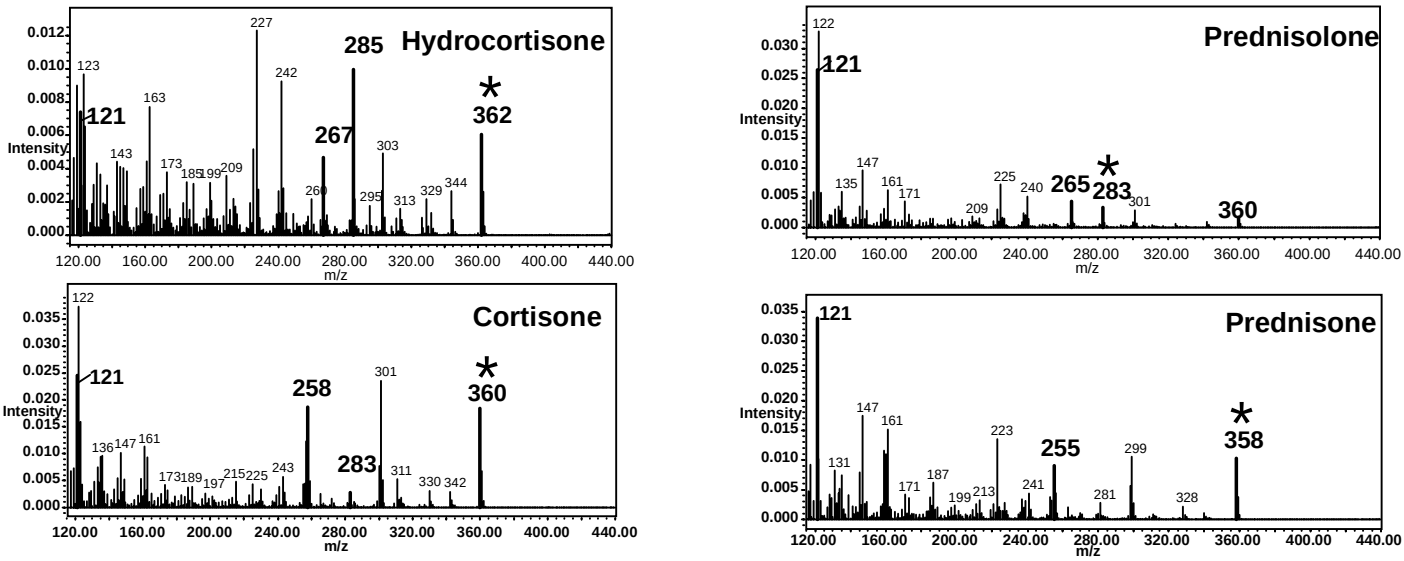


Figure 1.

These chemically similar corticosteroids do not yield unique UV spectra (Figure 1.). Therefore, selective detection based upon unique chromophores for each compound is not possible.



Unlike the UV spectra of each of these compounds, their mass spectra are easily distinguishable (see above), and the identity of isolated peaks can be confirmed using this data. In order to trace the retention behavior in overlapping peaks, one unique ion per compound is extracted from each mass spectrum thereby generating "Single Ion Chromatograms". Although all four of these steroids show molecular ions, Cortisone and Prednisolone have the same molecular weight. Therefore, one of the fragment ions was monitored for Prednisolone (m/z=283).

m/z= 362, Hydrocortisone

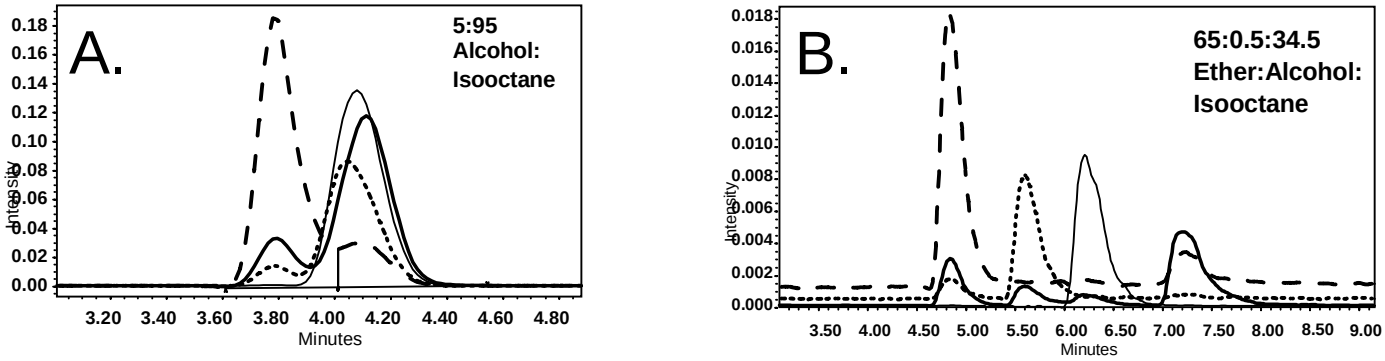
m/z= 360, Cortisone

m/z= 358, Prednisone

m/z= 283, Prednisolone

Figure A. shows that a binary mixture of isooctane and alcohol (or ethyl ether) failed to separate these components. Here, three out of four compounds coelute. However, even with this poor separation, the identification and retention behavior of each steroid can be clearly demonstrated using the Single Ion Chromatograms.

After much trial and error, Figure B. shows that the mobile phase composition of 65:0.5:34.5 ether:alcohol:isooctane offers a fast, nearly baseline separation of the mixture. The Single Ion Chromatograms uniquely illustrate the elution order of each compound thereby greatly assisting in the method development process.



EI LC/MS using the Integrity ThermaBeam System provides an extremely effective means of selective detection of sample components. With LC/MS, the analyst can easily obtain the necessary data to study chromatographic separation parameters and arrive at an optimized separation. This example also illustrates the continuing utility of normal phase chromatography where its unique selectivity can be of value.