Mass-directed Purification of Steroids with APCI and CombiFlash® Purlon

TELEDYNE ISCO
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Chromatography Application Note AN102

Abstract

Steroids are an important class of compounds that include sex hormones, corticosteroids, anabolic steroids, and anti-inflammatory compounds such as dexamethasone. This class of compounds is not generally detected with electrospray ionization (ESI) but is easily seen with atmospheric pressure chemical ionization (APCI).

This application note demonstrates the use of APCI for detection and purification of crude ergosterol as well as the use of dichloromethane (DCM) as a carrier solvent for very non-polar compounds in APCI detection.

Experimental and Results

All experiments were run on a Teledyne Isco CombiFlash Rf⁺ PurIon L system (PN 68-523-0049) with an APCI probe unless otherwise noted.

Crude ergosterol (monoisotopic mass 396.3 Da) was dissolved in dichloromethane and directly injected into the PurIon L system using the method development screen; methanol containing 0.1% formic acid was used as the carrier solvent. No molecular ion peak or fragment was observed under ESI+ or ESI- (Figure 1).

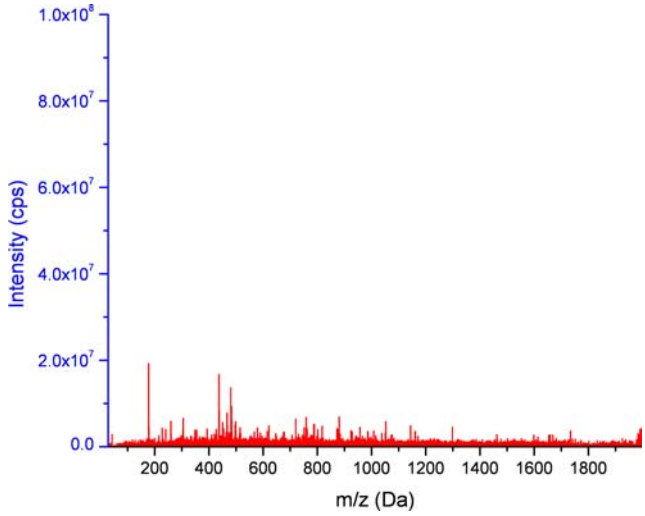

Figure 1: ESI+ spectrum of crude ergosterol

The ESI probe was changed for an APCI probe and the sample was injected using the same carrier as for the ESI experiment. APCI+ showed a strong peak at 379 Daltons (Da).

The use of the Ion Finder feature (Figure 2) suggested the base peak for ergosterol to be 379 Da after loss of water from the $[M+H]^+$ ion. Loss of water is a common fragmentation pathway of sterol ions under APCI[1]. The 379 Da peak was selected as the detection ion in the Ion Finder window and this value was automatically transferred to the method editor for the column.

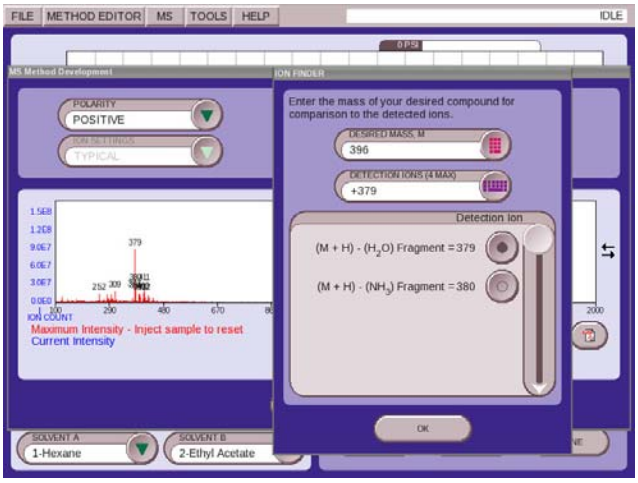

Figure 2: Ion Finder used to determine fragments of ergosterol with APCI

To purify the ergosterol, 0.4943 g crude ergosterol was dissolved in dichloromethane and mixed with 2.5 g silica gel (PN 60-3874-091); the mixture was rotary evaporated to dryness and placed in a 5 g empty solid load cartridge (PN 69-3873-235). The purification was run on a 40 g RediSep Rf Gold silica column (PN 69-2203-347) using the standard Gold Resolution method using a hexane/ethyl acetate gradient. Compounds were collected by mass directed fractionation using 379 Da (as selected in the Ion Finder).

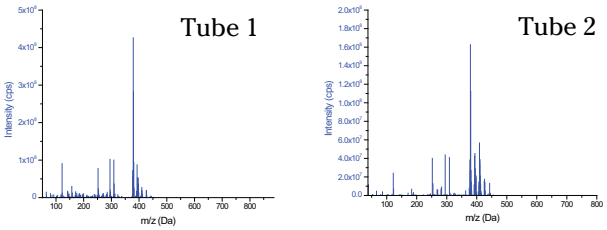

Figure 3: Purification of crude ergosterol with mass spectrometer and UV detection. Mass spectra show the contents of each collection fraction are the same.

1. Lagarda, M.J.; Garcia-Llatas, G.; Farre, R. Analysis of phytosterols in foods. Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 1486-1496

A peak detected at 379 Da was collected into two tubes; the Fraction Tube Composite Mass Spectrum feature (Figure 4) in PeakTrak confirmed that both fractions contained the same material; a difference in the mass spectrum would suggest an impurity in one of the fractions. This feature is useful to determine which fractions to combine to reduce impurities in the final product. The fractions were combined and evaporated to yield 0.1274 g (26% yield).

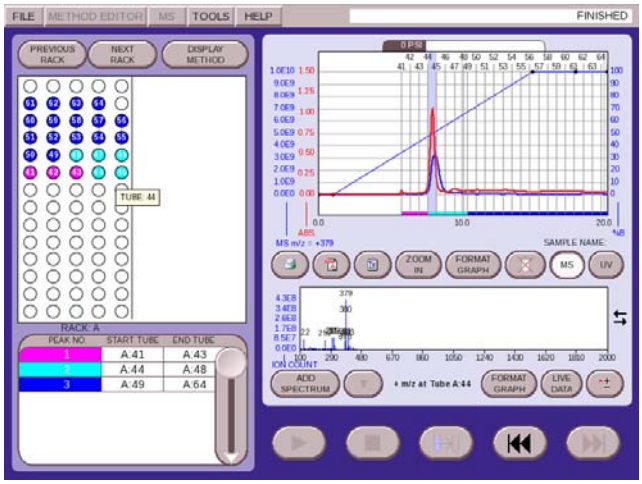

Figure 4: Fraction Tube Composite Mass Spectrum is seen by selecting a tube on the touchscreen (tube 44 in this example)

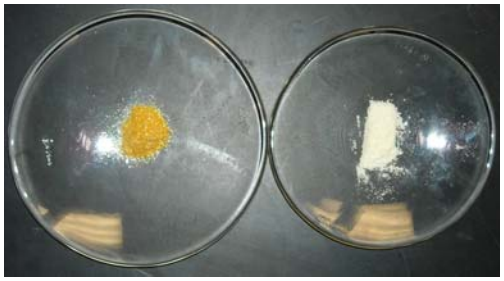

Figure 5: Crude ergosterol (left) and purified (right)

Many compounds suitable for APCI detection are very non-polar and may not dissolve in carrier solvents suitable for ESI such as acetonitrile or methanol. Carrier solvents such as ether and hexane should be avoided because they are flammable. However, DCM is non-flammable and readily dissolves non-polar compounds.

If a compound is found to elute in a non-polar solvent system such as hexane/ether, hexane/toluene, or similar non-polar solvent systems, the compound is a candidate for APCI detection and a DCM carrier solvent.

The MS Method Development window in PeakTrak was used to determine whether the compounds could be detected. A mixture of stigmasterol (monoisotopic mass 412.4 Da; $[M+H-H_2O]^+$=395 Da) and cholesterol (monoisotopic mass 386.4 Da; $[M+H-H_2O]^+$=369 Da) were injected using DCM as a carrier solvent; for both compounds, the $[M+H-H_2O]^+$ ion was detected for both compounds. The Ion Finder was used to enter the detected ions as described earlier (Figure 6).

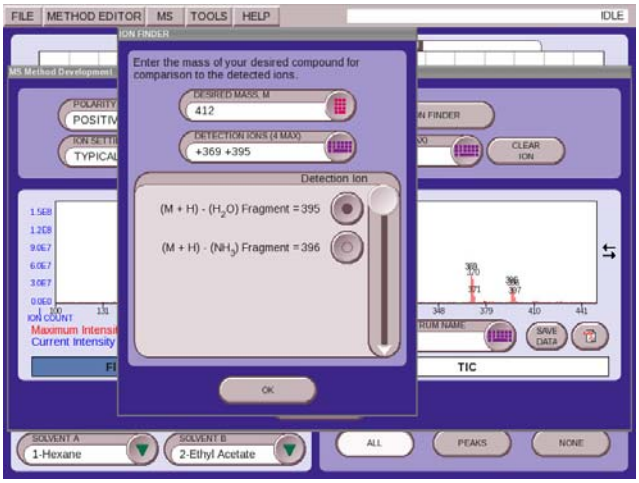

Figure 6: Ion Finder used for multiple compounds

Cholesterol and stigmasterol (0.3 g each) were dissolved in DCM and adsorbed on 2.6 g silica as previously described and placed in a 5 g empty solid load cartridge. The compounds were eluted with a gradient of toluene (A solvent) and diethyl ether (B solvent); the gradient was run to 75% B with a on a 40 g RediSep Rf Gold silica column (PN 69-2203-347). Detection with mass spectrometer used the ions at 395 and 369 Da as entered using the Ion Finder. The carrier solvent was DCM.

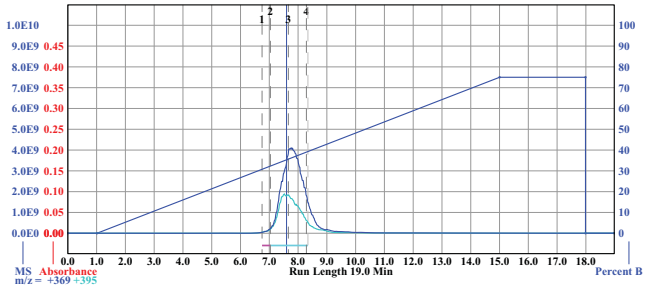

Figure 7: Ion Finder used to determine fragments of ergosterol with APCI

Even though both compounds co-eluted, the mass spectrometer data clearly shows that both compounds overlapped. Using other detectors, one could question whether one compound was still potentially held up on the column. The mass spectrometer shows whether or not peaks are pure, and that compounds actually eluted from the column.

Conclusion

The CombiFlash Rf$^+$ PurIon system with an APCI probe is able to detect compounds that systems only using ESI probes are not able to detect. These are generally relatively non-polar compounds.

The PeakTrak MS Method Development and Ion Finder windows allow confirmation that compounds ionize; they also help the chemist to identify fragments that can be used for detection at times when the $[M+H]^+$ (or $[M-H]^-$ for negative ionization) are not present. The Ion Finder is also useful for finding adducts of sodium, potassium, and also solvents such as methanol and acetonitrile if these are used.

When using non-polar solvent systems, DCM is useful as a carrier solvent because, in APCI, it is more compatible with these compounds. The CombiFlash Rf$^+$ PurIon system can also provide information about co-eluting peaks, impurities, and confirmation of compound elution from a column.

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