Waters® Alliance™ LC/MS System

LC/MS API Methods Development:
Optimizing existing HPLC methods for acidic analytes in negative mode

Michael P Balogh, Waters Corporation, Milford, MA

Key words:
Botanicals, St. John's Wort, Method Conversion for LC/MS, Natural Products

Natural products analysis: hypericin and related compounds from plant matrix

Sometimes existing chromatographic methods are less than ideal for adaptation to LC/MS. In this case, a method proposed for the analysis of hypericin, (the suspected active ingredient in the natural antidepressant St. John's Wort prepared from the plant *Hypericum perforatum,* L.) was determined to be unsuitable. The gradient only separated two of the four currently known dianthrones and inhibited detection and quantitation through the use of a strong acid with the weakly anionic hypericin. The method cited here solves a number of problems and provides:

-reproducible quantitation
-resolution of all related dianthrones in the sample
-detection of the unique wavelengths associated with dianthrones
-improved limits of detection for the mass spectrometer

An improved method[1] was derived from published work[2] for the somewhat anionic hypericin molecule using TEA (triethyl ammonium acetate) resulting in:

- an improved UV profile for the unique dianthrone lambda max at 588 nm (Figure 1) and,
- highly accurate quantitation of the separated dianthrones (Figure 2).

Many current studies have adopted 300 mg therapeutic doses containing 0.3% hypericin (or 0.9 mg). Determining adulteration and substandard preparations is of primary importance. In addition, this method provides useful separation of the four currently known biosynthetic and dehydrated forms (Figure 3).

Chromatographic conditions:

Column: Waters Symmetry® C_8, 2.1 mm x 150 mm
Flow Rate: 0.3 mL/min
Mobile phase A: 100-mM triethylamine acetate, pH 7.0 (TEA acetate)
B: methanol
C: acetonitrile
Initial conditions: 30:39:31 (A:B:C) A 15-minute linear gradient to final conditions of 10:50:40 with a 10 minute reequilibration to initial conditions.

[1]Balogh, M.P. and Li, J.B., *Analysis of Hypericin with HPLC-PDA-MS: Advantages of Multispectral Techniques*, LCGC, in print spring 1999.
[2]Piperopolous, G., Lotz, R., Wixforth, A., Schmierer, T., Zeller, K.P., *J.Chrom. B*, 695, 309-316 (1997).

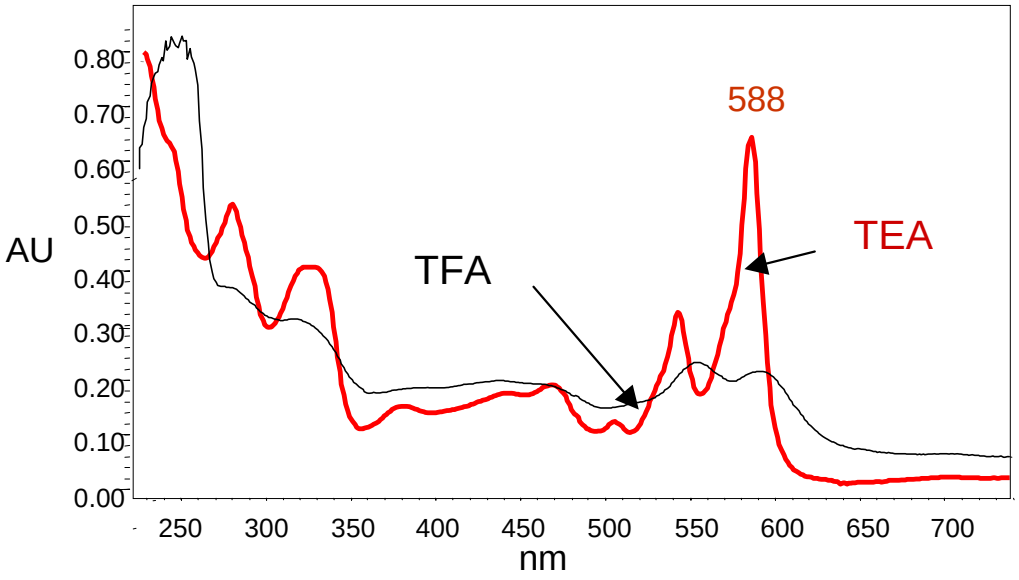

Figure 1 UV Profile of hypericin with 0.5% TFA mobile phase and 100 mM TEA mobile phase (lambda max 588 **nm)**

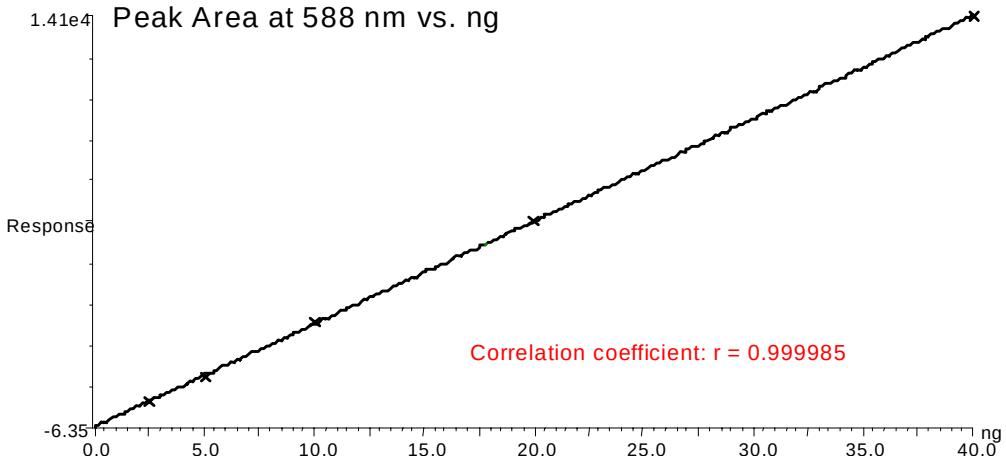

Figure 2 Calibration - 2 ng to 40 ng on-column

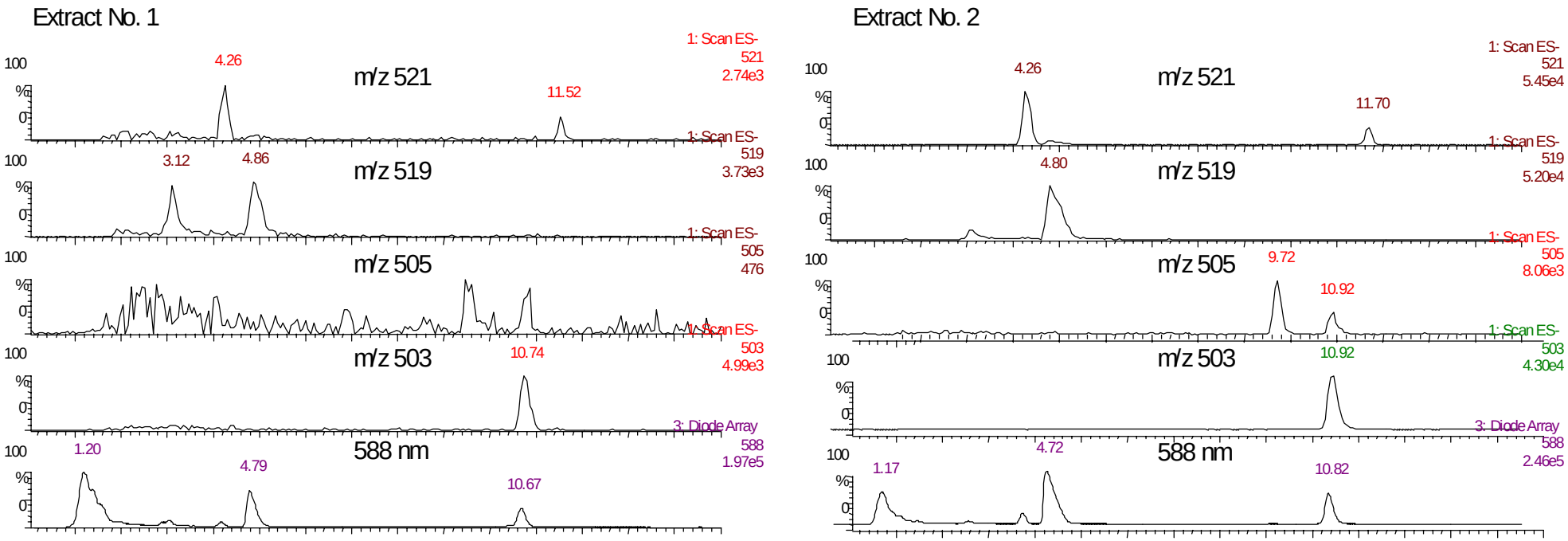

Figure 3 Negative mode electrospray acquisition for two samples of St. John's Wort Preparation:

- pseudoprotohypericin (521 m/z; biosynthetic precursor)
- protohypericin (519 m/z)
- pseudohypericin (505 m/z; biosynthetic precursor)
- hypericin (503m/z)

The PDA extracted wavelength (588 nm) is shown in the lower trace for reference.