

## Application Focus:

### *Preparative Grade purification of Hypericum perforatum L without the prep column*

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#### Introduction

High grade compound isolation from crude extract provides a number of challenges for the end user. Stainless steel preparative separations can be expensive and potentially damaging to the preparative column as well as logistically difficult to achieve, often requiring access to a dedicated purification group. Traditional Flash methods typically fall short due to both individual and collective system / Flash column chromatography weaknesses.

The method below was performed on an Interchim Generation 4 4250 250 bar system with accompanying MS detection utilising 15 micron Interchim puriFlash® cartridges and dry load. This is an expensive piece of equipment by most purification departmental standards. In reality, this same method could of been performed on our entry level Interchim XS420 20 bar Flash unit, the XS420 and its inherent dual piston pump tolerances, are perfectly adequate in this environment.

#### Application Overview

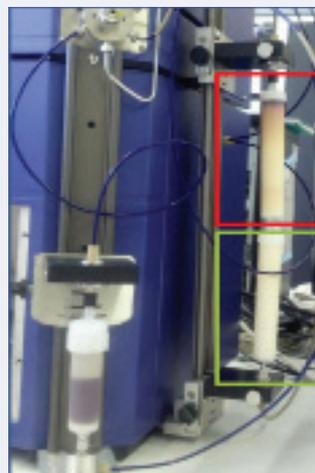
Hypericum perforatum L (St John's Wort) is ground and filtered in methanol. The extract was tested against a range of Interchim 15 micron chromatography media, housed in a Flash style cartridge (puriFlash®) for suitability of effective isolation of a number of plant secondary metabolites. Upon media selection, method was optimised for the separation of target molecules through analytical HPLC using a linear gradient. This gradient was then geometrically transferred to Flash chromatography by gradient transfer methodology based on the calibrations of the chromatographic system (measurement of the dwell volume and the extra column volume). UV and MS monitoring were used at the preparative level (puriFlash® 4250-MS) for comprehensive detection of four standard natural compounds from a synthetic mixture.

#### Method

2 gram of methanol extracted Hypericum perforatum L (St John's Wort) was loaded onto a 12 gram dry load cartridge consisting of 8 grams of C18 (20 -40 micron) + 2 grams of sand.



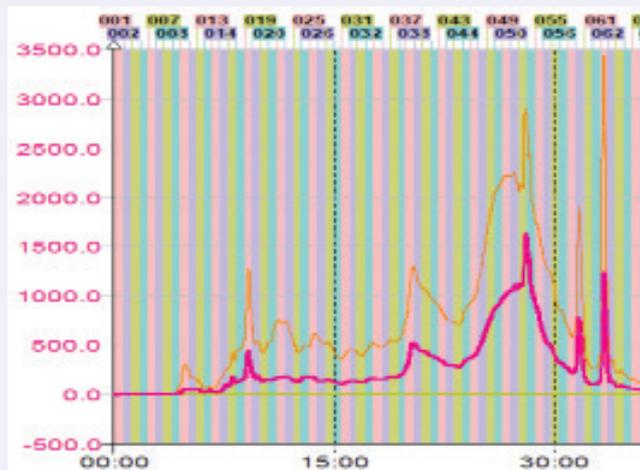
A combination of two 15 micron puriFlash® cartridges were selected for use in tandem for micro fractionation to provide optimal purification



*puriFlash® C18 15 micron HC  
facilitating maximum load  
capacity, reduced back  
pressure & high flow rate*

*puriFlash® C18 15 micron HQ  
facilitating improved resolution  
and purification efficiency*

Flow: 18ml / min  
 Column: 15µm C18 HC / 15µm C18 HQ in tandem  
 Solvent A: Water + 0.1% Formic acid  
 Solvent B: Acetonitrile + 0.1% Formic acid  
 Gradient Method, 70 min

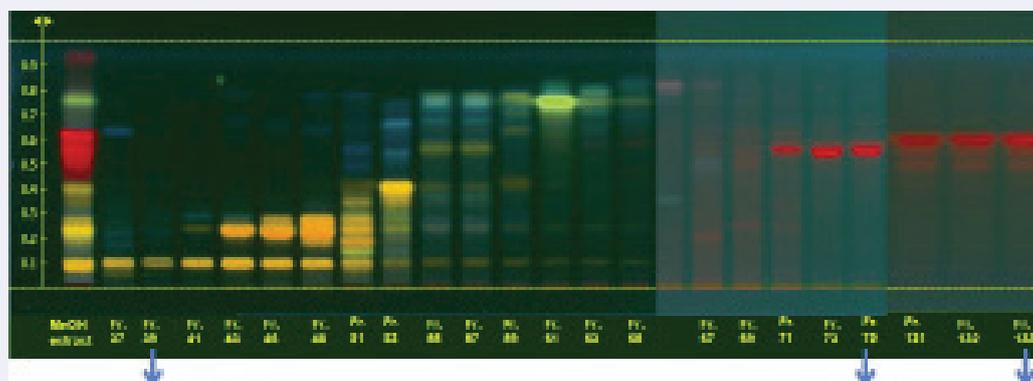


- 137 fractions collected
- 3 single step isolated compounds
- 10ml / fraction

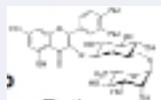
All fractions were dried under vacuum using a centrifugal evaporator within 10 hours.



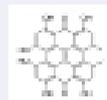
High Performance thin-layer chromatography (HPTLC) UV fraction control highlighted all fractions collected



Single Step compound purification



Rutin



Hypericin



Pseudohypericin



Rutin precipitation was observed after purification



Precipitate collection



HPTLC-UV control

Methanol Extract  
 Rutin precipitate  
 Rutin (commercial std)

## Conclusions

The HPTLC control demonstrates that the Rutin precipitate carries less impurity than the commercially purchased Rutin standard. The purity of Rutin is dependent upon a number of factors, these include:

- A well constructed and implemented purification methodology
- Effective 15 micron chromatography (at less than 20 Bar in what is deemed the traditional flash environment)
- Precise solvent management through the system's fluidics
- Precise solvent management through the system's dual piston pump, applying an accurate flow & gradient for sample management



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