Mass spectrometry guided purification for efficient isolation of natural products at semi- and preparative scale

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1. Introduction

In natural product research the isolation of compounds from crude extracts is a key element. An increase in the efficiency of the purification process, by improvement of instrumental and methodological approaches, remains a crucial point. In this respect, modern Flash Chromatography, has evolved and uses 15 µm spherical particles for efficient separation at moderate pressures. Flash C-18 was tested for the rapid isolation of various plant secondary metabolites of interest. The separation of target molecules from the crude extracts was optimized by the application of a linear gradient at analytical level. The gradient was then geometrically transferred to Flash chromatography by a gradient transfer method based on the calibrations of the chromatographic system (measurement of dwell volume and extra column volume) [1]. UV and MS monitoring were performed at the preparative level for a comprehensive detection of the various compounds present in the extract. In particular, a single quadruple mass spectrometer coupled with the Flash-Prep chromatographic system (Puriflash® - MS) was evaluated for an efficient MS-guided isolation of four standard natural products from a synthetic mixture.

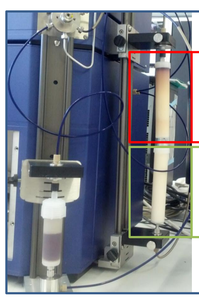
2. Isolation of natural products by modern Flash chromatography

Micro-fractionation of the methanolic extract of *Hypericum perforatum* L. (Hypercaceae)

A. Use of dry injection and modern flash columns

12 g dry load cartridge:

3g of MeOH extract
+
7g of C18 (40-60µm)
+
2g of sand



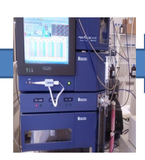
Combination of two 35g flash columns:

C18,15µ, HC Column(High Capacity): for improving the loading capacity, reducing the backpressure and reaching high flow rate

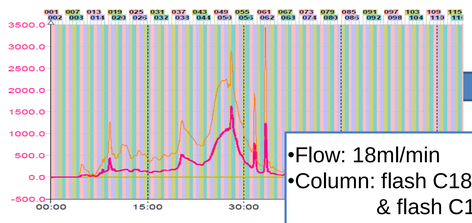
C18,15µ,HQ Column(High Quality): for improving resolution and efficiency of the purification

B. Micro-fractionation of *H. Perforatum* MeOH extract by Flash Chromatography

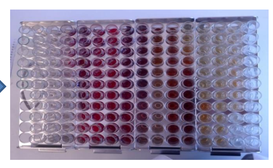
H. Perforatum MeOH extract



Flash Chromatography micro-fractionation

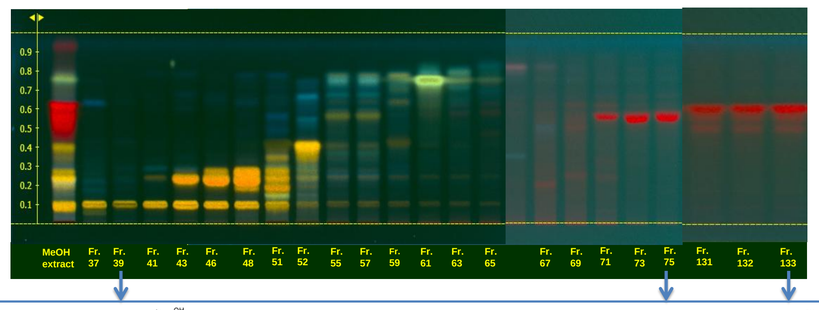


- Flow: 18ml/min
- Column: flash C18 HQ & flash C18 HC
- A:Water+0.1% F.A.
- B:Acetonitrile+0.1% F.A.
- 70 min. gradient method

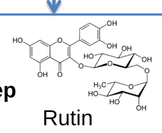


137 fractions collected
3 one-step isolated compounds
10 ml/fraction
all fraction rapid dried in 10h under vacuum
using a centrifugal evaporator

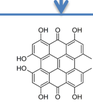
C. HPTLC-UV control of the fractions obtained by Flash Chromatography



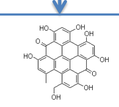
Compounds purified in one single step



Rutin

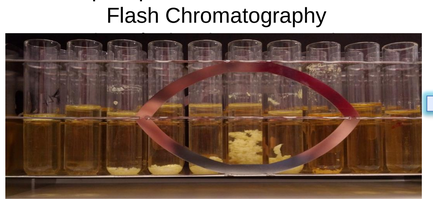


Hypericin



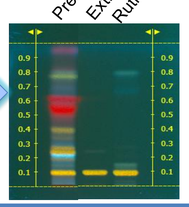
Pseudohypericin

Rutin precipitation was observed after Flash Chromatography



Collection of precipitate

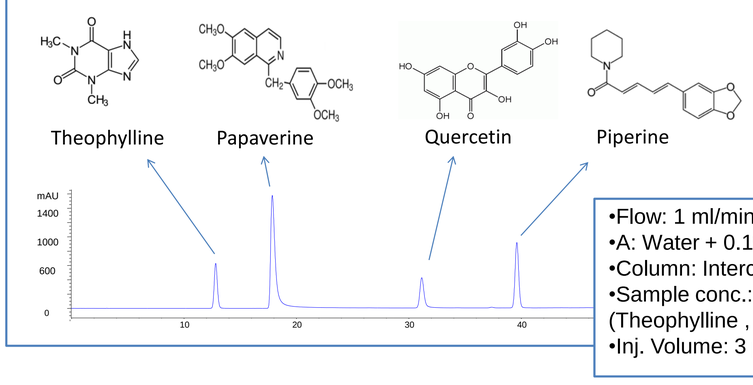
HPTLC-UV control



3. Rapid and efficient purification of natural products: gradient transfert from HPLC to FLASH CHROMATOGRAPHY-APCI/MS

Isolation of four compounds by reversed-phase Flash Chromatography-APCI/MS

A. HPLC analysis of a 4 Natural Products mixture

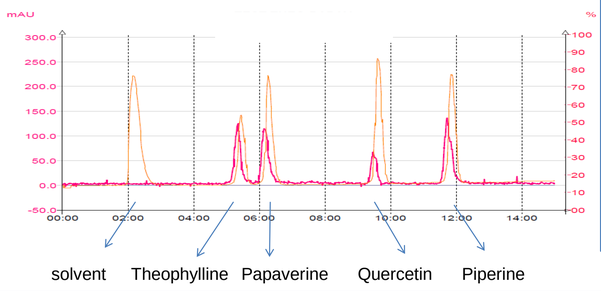


- Flow: 1 ml/min
- A: Water + 0.1% F.Acid B:MeOH + 0.1% F.Acid
- Column: Interchim 4.6mmx250mm C18HQ 15µm
- Sample conc.: 2 mg/ml for each standard (Theophylline , Papaverine , Quercetin , Piperine)
- Inj. Volume: 3 µl (6 ug loaded on column)

B. From HPLC analytical level to FLASH level by gradient transfer method [1]

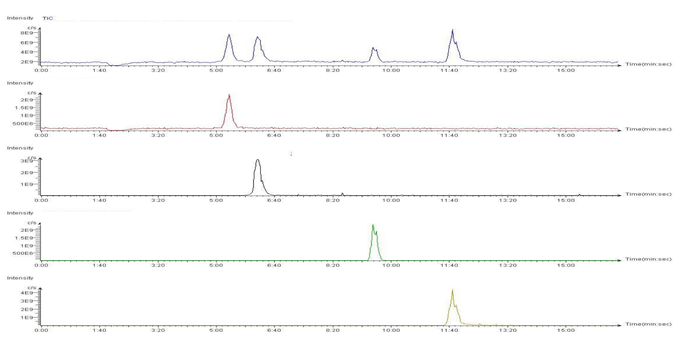
Orange trace: UV (SCAN 200-600 nm)

Purple trace: TIC



- Flow: 20 ml/min
- A:Water+0.1% F.A. B: MeOH+0.1%F.A.
- Column: Puriflash Column C18HQ 15µm 35g
- Sample conc.: 3 mg/ml for each standard (Theophylline , Papaverine , Quercetin, Piperine)
- Inj. Volume: 500 µl (1.5 mg loaded on column)
- Make-up flow:500ul/min
- Eluent make-up flow: MeOH+0.1%FA
- MS Ionization Method:APCI
- MS scan time:1000 ms
- MS Scan Range: m/z 125 – m/z 700

TIC and XIC overview



TIC

Theophylline (m/z 181)

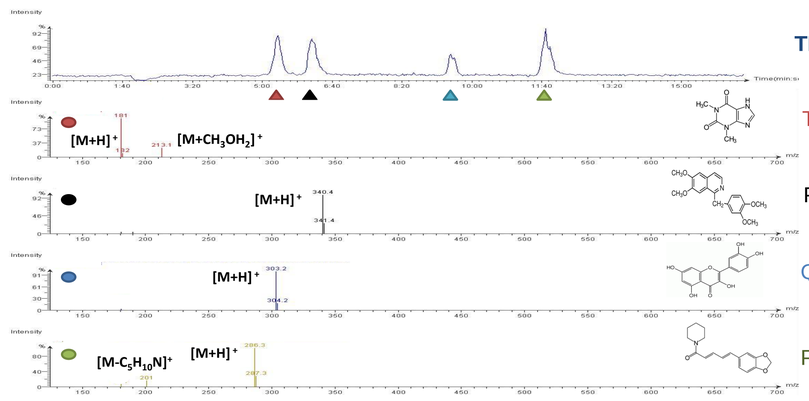
Papaverine (m/z 340.4)

Quercetin (m/z 303.2)

Piperine (m/z 286.3)

C. Mass guided purification of a chosen compound

m/z overview



TIC

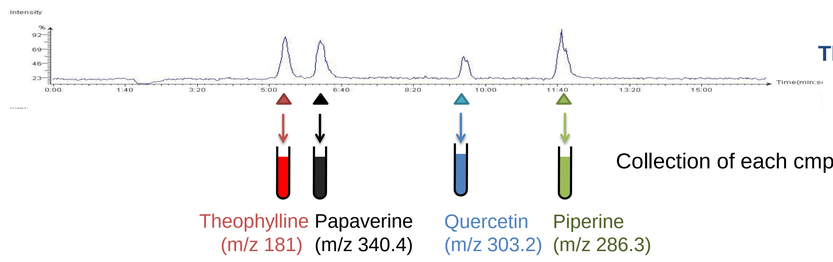
Theophylline (m/z 181)

Papaverine (m/z 340.4)

Quercetin (m/z 303.2)

Piperine (m/z 286.3)

Mass guided purification

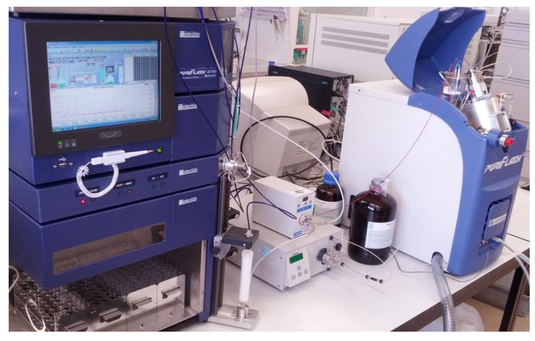


TIC

Collection of each cmpound triggered by m/z

Theophylline (m/z 181) Papaverine (m/z 340.4) Quercetin (m/z 303.2) Piperine (m/z 286.3)

A Flash-Prep chromatographic system coupled to a single quadrupole mass spectrometer(Puriflash® - MS) was used for the MS triggered isolation of each constituent.
Optimization of the splitting in the MS detector, provided an accurate collections of the compounds of interest, that can be efficiently monitored by MS without problem of overloading.



Flash Chromatography-APCI/MS system

4. Conclusion

The two-step chromatographic procedure, based on the geometric transfer of the gradient from analytical to Flash level and combined with an MS guided purification process, represents a powerful strategy, not only for the isolation of compounds that lack a UV chromophore, but also for the efficient targeted MS triggered isolation of compounds of interest in complex mixture. This rational approach has a high potential for the purification of biomarkers identified by UHPLC-MS metabolomics and dereplication process.

5. Acknowledgments

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6. Reference

[1] Davy Guillarme, Dao T.T. Nguyen, Serge Rudaz, Jean-Luc Veuthey, *Eur. J. Pharma. Biopharma. 2008, 68, 430*