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

A. Use of dry injection and modern flash columns

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

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

3. Rapid and efficient purification of natural products: gradient transfer 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

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

C. Mass guided purification of a chosen compound

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.

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6. Reference