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Explorative Solid-Phase Extraction for Accelerated Natural Products Discovery and Purification

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When purifying a natural product, the more specific information available on the target compound prior to purification, the more effective the purification (1,2). If no prior knowledge about the target compound(s) is available (e.g. from dereplication), a purification strategy is normally developed on the go or by standard fractionation methods. However, for extracts containing mainly unknown compounds, or when targeting bioactive compounds this lack of strategy beforehand can result in poor recoveries and, at worst, a permanent loss of activity in the extract.

Therefore, we at Centre for Microbial Biotechnology have developed a so called Explorative Solid-Phase Extraction (E-SPE) kit consisting of a set of different SPE columns with orthogonal selectivities, which in a fast and easy way will indicate the optimum purification strategy on a small scale in the exploratory stage of the discovery process before moving on to a semi-preparative or preparative scale. This will allow a more rational approach to the purification process. When linked to a bioassay system, E-SPE can potentially reveal information about the active compound that can help the ensuing purification, for example by suggesting a purification step that removes the main part of inactive extract components or a step that selectively captures the active compound. By using different types of stationary phases, the different functionalities of the molecules can be exploited to obtain pure compounds in the fewest possible chromatographic steps. When using an elution matrix (3) for visualisation of the bioactivity, the extract can be easily evaluated.

The method has been validated (in triplica) on 25 different marine bacteria with antibacterial effects, such as growth inhibition of *Vibrio anguillarum* and *Staphylococcus aureus* or quorum sensing inhibition. Further 8 filamentous fungi with anticancer effects have been included in the study. The E-SPE kit has proven it-self to be fast, easy and reproducible in use and has therefore been implemented as a standard screening procedure at CMB when dealing with new extracts.

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