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E-SPE : Exploratory Solid-Phase-Extraction for Accelerated Natural Product Discovery and Purification

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Background

Many NP labs have developed internal strategies for standardised extract screening and purification, only publishing parts of the overall setups.2 Nevertheless, many new bioactive compounds are being identified for the first time in a non-traditional context, indicating the need for new purification strategies. Accelerated Natural Product Discovery and Purification (A-NP) is a research strategy that aims to identify new bioactive compounds and their sources faster than traditional NMR characterization. This involves the selection of organism, extraction, preparative purification, dereplication, and final NMR characterization, as well as the development of new purification strategies.

Method setup

To obtain maximum complementarity, four different columns were selected. Together they provide information on size, charge and polarity of the active components. Each active fraction is subjected to comparative dereplication. A total of 15 fractions for bioassay is generated from each extract.

Method validation

The E-SPE method was tested on 25 different marine bacteria with antibacterial activities such as growth and quorum sensing inhibition. A few well-researched filamentous fungi were included as further validation. Each organism was tested in triplicates to ensure reproducibility. The method was found to be widely applicable on both bacterial and fungal samples, however very dependent on the matrix. Especially for marine samples, extensive desalting was necessary to ensure reproducibility. Further tests are necessary to investigate the robustness of the method as well.

Method implementation

As a proof-of-concept, a purification strategy was developed based on the E-SPE activity profile for Ps. luteoviolacea and carried out on an extract from 8 L of culture.

Conclusions

E-SPE is a successful method to reveal novel, bioactive natural products from both fungal and bacterial sources. The advantages of E-SPE are many:

- Finding a purification strategy on small amounts before engaging semi-preparative purification
- Identifying possible pit-falls (stability, synergy etc.)
- Reducing false positives (media components, salt etc.)
- Reducing number of candidates for dereplication (at CMB by LC-UV-HRMS)
- Unmasking potential candidates
- Enables target-guided isolation rather than bio-guided fractionation
- Standardising the analysis of samples (SOP)

Results obtained on a small scale (a single agar plate or 50 mL of culture) can readily be translated into bigger scale (200 plates, 10 L culture) for preparative results. The E-SPE strategy 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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References

7. Enzinger et al., in Methods in Biotechnology vol. 4: Natural Products validation, ed. ELF. Connell, 1998

Image 1: UV/visible spectra of extracted material from Ps. luteoviolacea, further analyzed by LC-MS. The known antibacterial compound Indolmycin was identified for the first time in a non-Staphylococcus strain. We are currently awaiting final NMR confirmation of a series of unknown compounds as well as the rest of the active components.