BIOTRANS
LA ROCHELLE 2023

N°502 / OC
TOPIC(s) : Enzyme discovery and engineering

Flow-Injection Mass Spectrometry: A Rapid and Versatile Method for Screening Enzymatic Reactions

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PURPOSE OF THE ABSTRACT

The screening of enzymatic reactions traditionally relies on photometric and fluorometric assays, which offer high throughput but are restricted to substrates with the required optical properties, or on chromatographic methods, which are very general but require analysis times of several minutes per sample. Mass spectrometry (MS) without chromatographic separation has the potential to bridge the gap between these two approaches, as it is broadly applicable and relatively fast (analysis times ≤1 min/sample). Powerful MS-based high-throughput screening methods have been reported in recent years,[1–3] but these rely on specialised or even custom-built equipment, which is accessible to only few research groups.

We have found that flow-injection mass spectrometry (FIA–MS) performed on a common instrumental platform, single-quadrupole HPLC–MS, can be used for the qualitative and quantitative analysis of diverse biotransformations. Samples are injected into the eluent flow of a liquid chromatograph with no column installed and are thus introduced into the electrospray MS with a throughput of 1 sample per minute. Common organic buffers (e.g., bicine, tricine, MOPS) present in the biotransformations can fulfil the function of an internal standard, allowing a linear quantification of analytes over 1–2 orders of magnitude in concentration. We demonstrate the application of FIA–MS to the screening of reactions catalysed by imine reductases, transaminases, methyltransferases, and a strictosidine synthase, using crude biocatalyst preparations (lysates or whole cells) and straightforward, plate-based liquid handling workflows. In each case, FIA–MS rapidly generated actionable insights into enzyme activity and selectivity that were readily confirmed by chromatographic re-analysis. We are, therefore, convinced that FIA–MS will become a useful additional tool for the screening of enzymatic reactions.

FIGURES

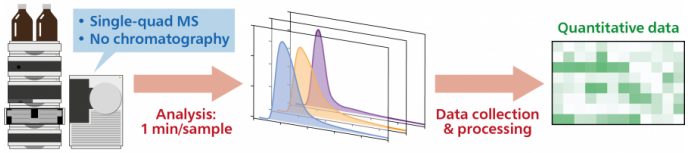

FIGURE 1

Figure 1

Screening of enzymatic reactions by flow injection analysis mass spectrometry

FIGURE 2

KEYWORDS

Flow Injection Analysis | Mass Spectrometry | High-Throughput Screening

BIBLIOGRAPHY

[1] E. E. Kempa, J. L. Galman, F. Parmeggiani, J. R. Marshall, J. Malassis, C. Q. Fontenelle, J.-B. Vendeville, B. Linclau, S. J. Charnock, S. L. Flitsch, N. J. Turner, P. E. Barran, JACS Au 2021, 1, 508-516.

[2] D. A. Holland-Moritz, M. K. Wismer, B. F. Mann, I. Farasat, P. Devine, E. D. Guetschow, I. Mangion, C. J. Welch, J. C. Moore, S. Sun, R. T. Kennedy, Angew. Chem. Int. Ed. 2020, 59, 4470-4477.

[3] I. Sinclair, M. Bachman, D. Addison, M. Rohman, D. C. Murray, G. Davies, E. Mouchet, M. E. Tonge, R. G. Stearns, L. Ghislain, S. S. Datwani, L. Majlof, E. Hall, G. R. Jones, E. Hoyes, J. Olechno, R. N. Ellson, P. E. Barran, S. D. Pringle, M. R. Morris, J. Wingfield, Anal. Chem. 2019, 91, 3790-3794.