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Purification of peptides and small molecules using a preparative LC and Microsaic 4000 MiD® mass detector

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INTRODUCTION

Preparative liquid chromatography (Prep-LC) has become a major tool in the production of specialty chemicals. Prep-LC coupled with mass spectrometry (MS) is a well-established method for purifying target compounds. This technique allows collecting fractions with high purity and excellent recovery for target molecules by combining ultra violet (UV) and mass-based detection.

Various types of mass spectrometers used as complementary LC detectors are available but they are bulky, power hungry and expensive to purchase and run.

The Microsaic 4000 MiD® is a miniaturised single quadrupole mass detector. Integrating all the vacuum pumps and computer into a compact enclosure, the Microsaic 4000 MiD® provides a tiny footprint, low energy consumption and good quality data. The core technologies are chip-scale "plug & play" versions of traditional MS components which can be rapidly interchanged by the user for maximum application flexibility and deployability. The mass detector requires minimal training and maintenance making it ideal for bench chemists.

This application note reports the mass-based purification of bradykinin, a well-known vasodilator peptide used to lower the blood pressure, and verapamil, a calcium channel blocker used to treat hypertension, cardiac arrhythmia and cluster headaches, by using a Gilson Prep-LC platform coupled to a Microsaic Systems mass detector.

MATERIALS AND METHODS

Chemicals and reagents

Bradykinin, verapamil, chlorpheniramine, chloramphenicol, caffeine and probenicid.

Milli-Q water, acetonitrile CROMASOLV® Plus for HPLC (≥99.9%), methanol CROMASOLV® Plus for HPLC (≥99.9%), formic acid reagent grade (≥95%) and trifluoroacetic acid reagent plus grade (99%).

All the chemicals and reagents were purchased from Sigma-Aldrich (USA).

Sample preparation

For the peptide analysis, a standard solution of bradykinin at 1 mg/mL in water was used.

For the purification of verapamil, a test sample using 8 mg/mL each of chlorpheniramine and caffeine, 5 mg/mL of verapamil and 7 mg/mL of probenicid was prepared in methanol:water 50:50 (v/v).

System components and set-up

The automated purification of bradykinin and verapamil was carried out by coupling the Gilson Prep-LC platform to the Microsaic 4000 MiD® mass detector (Figure 1).

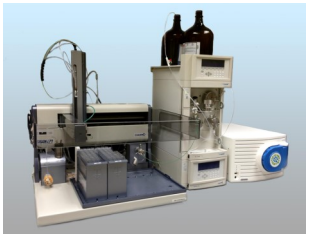

FIGURE 1. *Gilson preparative HPLC system coupled to the Microsaic 4000 MiD® mass detector.*

The instrumental set-up comprises two flow paths. The main flow leads from the binary pumps to the autosampler, column, active splitter and diode array detector before reaching the fraction collector. The secondary (or make-up) flow leads from the isocratic pump to the active splitter before reaching the mass detector. The active splitter sustains applications using flow rates too high to be routed directly into the electrospray ionization source and transfers a small aliquot of the main flow into the make-up flow, reducing sample losses.

Trilution LC v. 3.0 SP4 and Masscape v. 2.43 software packages where used for setting up conditional logic fractions collection and acquisition methods, respectively.

LC/MS conditions

Table 1 and 2 show the Prep-LC/MS conditions used for the purification of bradykinin and verapamil, respectively.

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Fraction collection settings

All the fractions were collected using a conditional logic method set up in Trilution by selecting primary UV channels using slope criteria and secondary MS channels employing target mass criteria. The mass spectral information was imported in Trilution via analogue.

TABLE 1. *Prep-LC and MS conditions for the analysis of bradykinin*

HPLC	Gilson 333/334 Prep Scale
Column	Phenomenex Luna C18; 21 x 50 mm; 5 µm
Mobile phase A	Water, 0.1% formic acid
Mobile phase B	Acetonitrile, 0.1% formic acid
Gradient	10 to 60% B in 6 min
Flow rate	15 mL/min
Injection volume	2.5 mL
MS	Microsaic Systems 4000 MiD®
Scan mode	Full scan
Mass range	*m/z* 220 to *m/z* 620
Scan rate	1 Hz
Ion mode	Positive
Make-up pump solvent	Methanol:water 80:20 (v/v), 0.1% formic acid
Make-up pump flow rate	1 mL/min
Attenuation	25:1

TABLE 2. *Prep-LC and MS conditions for the analysis of verapamil*

HPLC	Gilson 333/334 Prep Scale
Column	Phenomenex Luna C18; 21 x 50 mm; 5 µm
Mobile phase A	Water, 0.1% trifluoroacetic acid
Mobile phase B	Acetonitrile, 0.1% trifluoroacetic acid
Gradient	25 to 70% B in 7 min
Flow rate	15 mL/min
Injection volume	1.5 mL
MS	Microsaic Systems 4000 MiD®
Scan mode	Full scan
Mass range	*m/z* 100 to *m/z* 500
Scan rate	1 Hz
Ion mode	Positive
Make-up pump solvent	Methanol:water 80:20 (v/v), 0.1% formic acid
Make-up pump flow rate	1 mL/min
Attenuation	1000:1

RESULTS AND DISCUSSIONS

The isolation and purification of bradykinin was carried out loading the column with 2.5 mg of the peptide (exact mass of 1059.6 Da). The fraction was collected using conditional UV slope at 210 nm and doubly charged MS trace at m/z 531.2 (Figure 2).

Figure 3 shows the mass spectrum generated from the peak eluting at 2.64 minutes with doubly (m/z 531.2) and triply-charged (m/z 354.4) species for bradykinin.

The Prep-LC/MS system was also used to isolate and purify verapamil (exact mass of 454.3 Da), from a mixture containing chlorpheniramine, chloramphenicol, caffeine and probenicid. The fraction was collected using mass-based purification only, setting the MS level at m/z 455.6 (Figure 4).

Figure 5 shows the verapamil fraction collection spectrum generated from the peak eluting at 4.65 min with protonated verapamil at m/z 455.6.

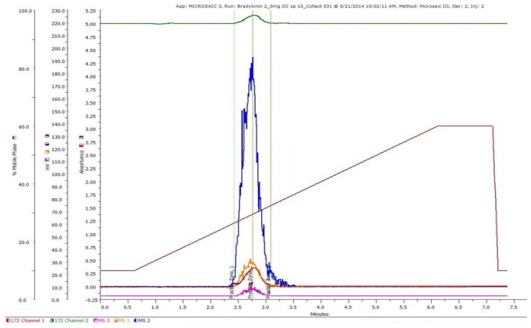

FIGURE 2. *Chromatogram for the bradykinin analysis with UV wavelength at 220 nm in green, UV wavelength at 210 nm in red, total ion chromatogram (TIC) from m/z 220 to m/z 620 in blue, extracted ion chromatogram (EIC) for doubly charged ion at m/z 531.2 in orange and EIC for triply charged ion at m/z 354.4 in pink.*

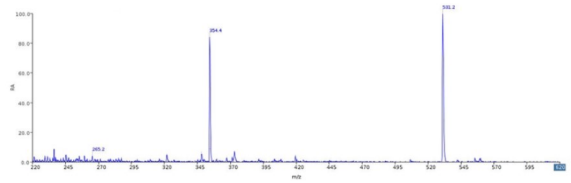

FIGURE 3. *Mass spectral data of bradykinin with doubly protonated ion at m/z 531.2 and triply charged ion at m/z 354.4.*

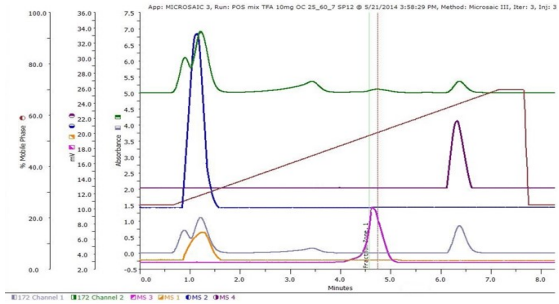

FIGURE 4. *Chromatogram for the purification of verapamil with UV wavelength at 220 nm in green, UV wavelength at 210 nm in blue-grey, EIC for chlorpheniramine at m/z 275.4 in orange, EIC for caffeine at m/z 195.2 in blue, EIC for verapamil (collected) at m/z 455.6 in pink and EIC for probenicid at m/z 286.2 in purple.*

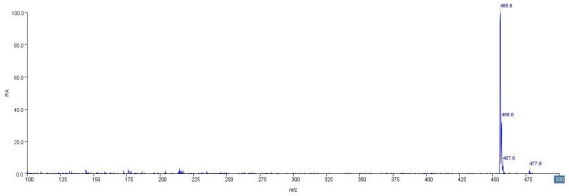

FIGURE 5. *Mass spectra of the protonated ions for verapamil at m/z 455.6.*

CONCLUSIONS

In this application note all the advantages of using the Microsaic 4000 MiD® mass detector for peptides and small molecule drugs purification in preparative scale were demonstrated. Prep-LC/MS purification, combining UV and mass-based detection for fraction collection, provides additional mass spectral data confirmation for the collected fractions of interest and offers the benefits of enhanced selectivity excluding unwanted fractions, overcoming the inherent limitations associated with purifying compounds using optical detection alone.

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