Application Note

Microsaic Systems

HPLC-MS applications of the Microsaic 4000 MiD®

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INTRODUCTION

Liquid chromatography coupled with mass spectrometry (LC-MS) is one of the most powerful analytical tools for organic compound analysis, combining the outstanding separation resolution of liquid chromatography with the exceptional capabilities of mass spectrometry.

Various types of mass spectrometers used as complementary LC detectors are available but they are bulky, expensive to run and power hungry for use in every laboratory. These limitations have led to compromises in the choice of detectors for LC with limited performances.

The Microsaic 4000 MiD® is a versatile mass spectrometer that anyone can use in the laboratory. The MS is designed to be compatible with a wide range of chromatographic and non-chromatographic analyses so perfectly match your analytical needs. The Microsaic 4000 MiD® is the world's first integrated mass spectrometer in which all the components used for ion generation, transmission and analysis are microengineered and therefore gives the chemist the ability to have a quadrupole mass spectrometer at the bench or fume hood for more immediate access to sensitive and specific analysis.

Integrating all the vacuum pumps, fluidic connections and computer into a compact enclosure the size of a typical desktop PC, the Microsaic 4000 MiD® provides the smallest footprint, energy consumption and good quality data requiring minimal training and time.

Here we report the HPLC-MS analysis of pesticides, peptides, carbohydrates and ibuprofen to demonstrate the excellent versatility and simplicity of the Microsaic 4000 MiD® in several areas of application, such as environmental monitoring, food safety, forensics and pharmaceuticals.

METHOD

HPLC-MS analysis of pesticides, peptides, carbohydrates and ibuprofen has been carried out, using different ionisation modes (positive and negative) and scan types (full scan and selected ion monitoring, SIM). The Microsaic 4000 MiD® uses a microflow electrospray ionisation ion source called the spraychip® which operates at flow rates from 0.2 to 2 µL/min. The instrument, however, can be used at flow rates up to 2 mL/min using its split flow interface (SFI) which contains an in-built passive split which diverts a small proportion of the input flow to the spraychip®; the remaining flow can be sent to waste or collection. For lower flow rates (0.2 to 2 µL/min) the user has the option of using the direct flow interface (DFI) which sends the full input flow to the spraychip®. Here, an HPLC (Agilent 1100) was coupled to the Microsaic 4000 MiD® using an SFI with a 1:500 split ratio.

Chemicals and reagents

Pesticides: Pesticides standard solution, PESTANAL® grade, containing the following components at 10 ng/ L each in acetonitrile: atrazin, atrazin-desethyl, cyanazin, sebuthylazin, simazin, terbuthylazin, hexazinon, chlortoluron, diuron, isoproturon, linuron, methabenzthiazuron, metobromuron, metoxuron, monolinuron, metazoachlor, metolachlor.

Peptides: HPLC peptide mixture containing the following components at 10 µg/ L each: angiotensin II, Gly-Tyr, Leu-enkephalin, Met-enkephalin and Val-Tyr-Val; and Met-Arg-Phe-Ala acetate salt.

Carbohydrates: Sucrose, -Lactose, D-(+)-Xylose, D-(+)-Glucose, 1,2-O-Isopropylidene- -D-glucofuranose.

Ibuprofen

HPLC grade water, HPLC grade acetonitrile, LC-MS grade formic acid, LC-MS grade ammonium hydroxide, HPLC grade ammonium acetate.

All the chemicals and reagents were purchased from Sigma-Aldrich (Poole, UK).

Sample preparation

For the analysis of pesticides, an aliquot of the standard mixture at 10 ng/ L was diluted 1:10 in 90:10 (v/v) water:acetonitrile and then loaded into an autosampler vial.

Peptides were analysed from a mixture of 6 peptides at a final concentration of 10 g/mL. The solution was prepared by spiking an aliquot of HPLC peptide standard mix with Met-Arg-Phe-Ala acetate salt in 95:5 (v/v) water:acetonitrile.

Carbohydrates were analysed from a standard mix solution of 10 µg/mL. The solution was prepared by mixing an appropriate aliquot of Sucrose, ß-Lactose, D-(+)-Xylose, D-(+)-Glucose, 1,2-O-Isopropylidene- -D-glucofuranose in 90:10 (v/v) water:acetonitrile with 0.1 % ammonium hydroxide.

For the ibuprofen analysis, standards were prepared over the range of 0.1–100 µg/mL by spiking an appropriate volume of stock solution into 80:20 (v/v) water:acetonitrile with 0.1 % ammonium hydroxide.

LC/MS Conditions

Table 1 shows the standard recommended MS conditions, and Tables 2,3,4, and 5 shows the HPLC and MS conditions used for the analysis of pesticides, peptides, carbohydrates and ibuprofen, respectively.

Gas flow	2500 mL/min
Vacuum interface	50 V
Tube lens	10 V
Plate lens	5 V
Ion guide	1 V

Table 1: Standard MS conditions used throughout experiments

HPLC	Agilent 1100
Column	Supelco Ascentis Express C18; 100 mm x 4.6 mm; 2.7 µm particle size
Mobile phase A	Water with 0.1% formic acid
Mobile phase B	Acetonitrile with 0.1% formic acid
Gradient table	0 min – 20% B 10 min – 35% B 15 min – 60% B 20 min – 80% B A post-run equilibration time of 5 min was used
Column temperature	45 °C
Injection volume	10 µL
MS	Microsaic 4000 MiD®
Scan mode	Full scan
Mass Range	m/z 175 to m/z 300
Scan Rate	0.8 s/scan
Step size	m/z 0.2
Ion mode	Positive
Tip voltage	850 V

Table 2: HPLC and MS conditions for the analysis of pesticides

HPLC	Agilent 1100
Column mm; 2.7 µm particle size	Supelco Ascentis Express C18; 100 mm x 4.6
Mobile phase A	Water with 0.1% formic acid
Mobile phase B	Acetonitrile with 0.1% formic acid
Gradient table	0 min – 5% B
20 min – 65% B	
A post-run equilibration time of 5 min was used	
Column temperature	45 °C
Injection volume	20 µL
MS	Microsaic 4000 MiD®
Scan mode	Full scan
Mass Range	m/z 85 to m/z 800
Scan Rate	1 s/scan
Step size	m/z 0.2
Ion mode	Positive
Tip voltage	850 V

Table 3: HPLC and MS conditions for the analysis of peptides

HPLC	Agilent 1100
Column mm x 3 mm; 3 µm particle size	Phenomenex Luna NH2 (aminopropyl); 150
Mobile phase A	Water with 0.1% ammonium hydroxide
Mobile phase B hydroxide	Acetonitrile with 0.1% ammonium
Gradient table	0 min – 90% B
10 min – 30% B	
A post-run equilibration time of 10 min was used	
Column temperature	45 °C
Injection volume	10 µL
MS	Microsaic 4000 MiD®
Scan mode	SIM
Mass	m/z 149.2 (Xylose)
m/z 179.2 (Glucose)	
m/z 219.2 (1,2-O-Isopropylidene-α-D-glucofuranose)	
m/z 341.3 (ß-Lactose and Sucrose)	
SIM Count Time	150 ms per channel
Ion mode	Negative
Tip voltage	–750 V

Table 4: HPLC and MS conditions for the analysis of carbohydrates

HPLC	Agilent 1100
Column mm; 2.7 µm particle size	Supelco Ascentis Express C18; 100 mm x 4.6
Mobile phase A	5mM ammonium acetate
Mobile phase B hydroxide	Acetonitrile with 0.1% ammonium
Gradient table	0 min – 20% B
10 min – 70% B	
A post-run equilibration time of 5 min was used	
Column temperature	45 °C
Injection volume	10 µL
MS	Microsaic 4000 MiD®
Scan mode	SIM
Mass	m/z 205.3
Scan Rate	250 ms per channel
Ion mode	Negative
Tip voltage	–750 V

Table 4: HPLC and MS conditions for the analysis of ibuprofen

RESULTS AND CONCLUSIONS

Figure 1 shows the base peak chromatogram (BPC) for the pesticide analysis, generated using Microsaic's bespoke Masscape® software. The BPC shows 15 peaks corresponding to, in order of elution, atrazin-desethyl at 5.10 min (1), metoxuron at 8.23 min (2), hexazinon at 8.45 min (3), simazin at 9.35 min (4), cyanazin at 10.15 min (5), methabenzthiazuron at 12.36 min (6), chlortoluron at 13.01 min (7), atrazin at 13.50 min (8), monolinuron, isoproturon and diuron at 14.22 (9), metobromuron at 14.99 min (10), metazoachlor at 15.46 min (11), sebuthylazin at 15.90 min (12), terbuthylazin at 16.64 min (13), linuron at 16.93 min (14) and metolachlor at 18.80 min (15).

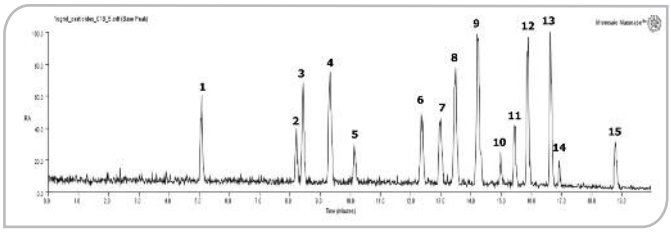

Figure 1: Base peak chromatogram (full scan) of the pesticide mix (10 ng each on column)

Figure 2 shows the mass spectrum generated from peak 9 in Figure 1 which represents a clear example of three co-eluting compounds which could not be distinguished using a UV detector alone. Characteristic isotopic patterns for the presence of one chlorine (monolinuron) and two chlorines (diuron) are also displayed.

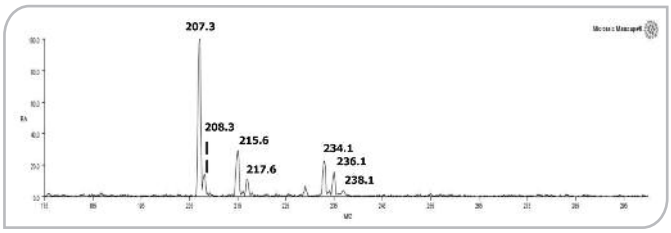

Figure 2: Mass spectrum of isoproturon at m/z 207.3, monolinuron at m/z 215.6 and diuron at m/z 234.1

Figure 3 shows the total ion chromatogram (TIC) acquired from the peptide analysis. The TIC shows 6 peaks corresponding to Gly-Tyr at 2.43 min (1), Met-Arg-Phe-Ala at 5.30 min (2), Val-Tyr-Val at 5.84 min (3), angiotensin II at 7.26 min (4), Met enkephalin at 7.84 min (5) and Leu enkephalin at 7.72 min (6).

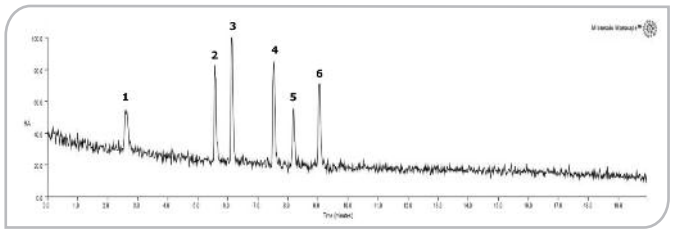

Figure 3: Total ion chromatogram of the peptide mix (200 ng each on column)

Figure 4 shows the mass spectrum generated from peak 2 showing singly- (m/z 524.4) and doubly-charged species (m/z 262.6) for Met-Arg-Phe-Ala.

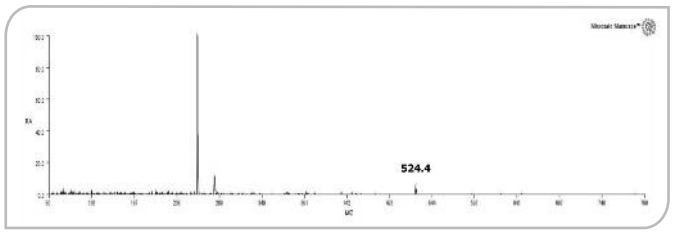

Figure 4: Profile mass spectrum of Met-Arg-Phe-Ala with single protonated ion at m/z 524.4 and double charged ion at m/z 262.6.

Figure 5 shows the 4 SIM chromatograms from the carbohydrate analysis, using m/z 219.2 (1,2-O-Isopropylidene-α-D-glucofuranose), m/z 149.2 (D-(+)-Xylose), m/z 179.2 (D-(+)-Glucose) and m/z 341.3 (Sucrose and ß-Lactose) as SIM channels.

The SIM traces show 5 peaks corresponding to 1,2-O-Isopropylidene-α-D-glucofuranose at 1.36 min (1), D-(+)-Xylose at 2.82 min (2), D-(+)-Glucose at 3.76 min (3), Sucrose at 4.49 min (4) and ß-Lactose at 4.79 min (5).

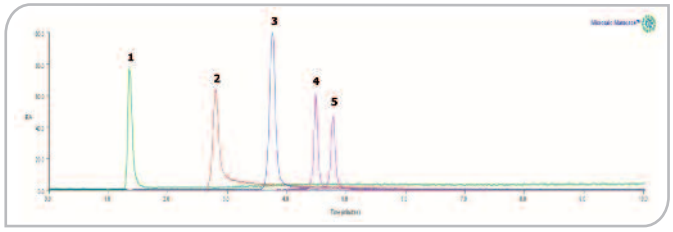

Figure 5: SIM traces for the carbohydrate mix (100 ng each on column)

Figure 6 shows the SIM chromatogram of ibuprofen selecting m/z 205.3 as SIM channel. The signal-to-noise ratio (S/N), calculated using a peak-to-peak approach, was 3.77 when 100 ng/mL of ibuprofen was injected (1 ng on-column). This concentration level was, therefore, considered to be the limit of detection (LOD) for ibuprofen using this method.

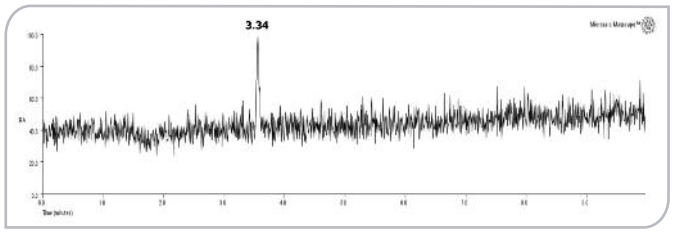

Figure 6: SIM total ion chromatogram of ibuprofen (1 ng on column) having S/N of 3.77.

Figure 7 shows the linearity for ibuprofen over the range of 0.25–100 µg/mL, using a least-squares linear regression analysis and 1/x2 as weighting factor. Each standard level was injected in duplicate and the concentration was back-calculated from the linear regression curve equation.

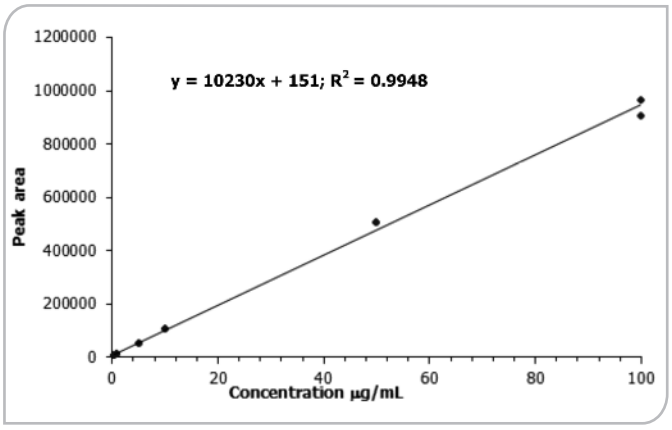

Figure 7: Least-squares linear regression of ibuprofen across the range 0.25–100 µg/mL.

Table 6 shows accuracy (calculated as relative error – RE%) and precision (expressed in terms of coefficient of variation – CV%) values for the back-calculated concentrations. The observed data is in accordance with the acceptance criteria published by FDA (±15% RE and CV), clearly demonstrating excellent linearity across the concentration range tested.

	Ibuprofen concentrations (µg/mL)						
	0.25	0.50	1.00	5.00	10.0	50.0	100
Mean (µg/mL)	0.24	0.52	1.06	4.99	10.4	49.3	94.5
SD (µg/mL)	0.02	0.02	0.03	0.32	0.17	0.06	4.02
RE (%)	3.46	-3.72	-6.19	0.22	-3.62	1.32	8.52
CV (%)	8.99	3.40	3.14	6.44	1.61	0.12	4.40

Table 6: Back-calculated concentrations of ibuprofen from standard solutions run in duplicate across the range 0.25–100 µg/mL.

SUMMARY

The compact nature of the Microsaic 4000 MiD® makes it an ideal upgrade for users wishing to extend the capability of their existing HPLC instrument to LC-MS. With the smallest footprint on the market and no external vacuum pumps the Microsaic 4000 MiD® can be easily located in an LC stack or within a gap no bigger than a PC monitor. The instrument has been designed to give the chemist all the versatility to have an easy-to-use quadrupole mass spectrometer for a wide range of bench-top LC-MS applications. Detection of pesticides, carbohydrates, peptides and quantification of a pharmaceutical drug have been reported in this note showing how some of the most common applications carried out in analytical laboratories can be easily and rapidly developed using Microsaic 4000 MiD®.