TECHNICAL NOTE 73878

Direct infusion of an analyte with an ISQ EC or ISQ EM single quadrupole mass detector

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Goal

Describe how direct infusion or infusion into the eluent flow is set up with the help of an external syringe pump.

Introduction

In LC-MS (liquid chromatography – mass spectrometry), the analysis method often needs to be adjusted to the specific compounds, both on the chromatography (LC) and the mass spectrometry (MS) side. For MS, the mass spectrometers need to be tuned and calibrated upfront to assure efficient ion transmission and adjust mass resolution and mass accuracy. Then, the source settings are adapted to the compound. The Thermo Scientific™ ISQ™ EC and EM single quadrupole mass detectors offer an Autospray functionality, which provides generic source settings for compounds based on the intended LC flow rate and compound and eluent properties such as thermal stability and volatility. This provides good sensitivity for most standard compounds.[1] If further optimization is desired, this can be done by infusing the analytes with a syringe pump into the mass spectrometer. The infusion can be done directly or into an eluent stream. The choice is determined by the intended final flow rate of the analysis method. If the flow rate is relatively high, then direct infusion becomes difficult since the syringe would be depleted in a short time span and thus, infusion into the HPLC eluent stream is the better option. In addition, infusion into the eluent stream takes the effect of the eluent on the MS analysis into consideration.

While infusion allows immediate adjustments to the source and acquisition settings it requires manual input. Automation of the optimization can be achieved with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software. A sequence of flow injections with short run times iterates through the source parameters using custom variables.[2] Afterwards, the best conditions can be used for the actual method.

In the following, the setup for direct fusion and infusion into the HPLC eluent stream will be described in detail.

Setup

Setting up a direct infusion

Place the syringe pump on top of the ISQ EC or EM or next to it and place the syringe into it. Then, cut a piece of PEEK tubing to a length to easily connect the syringe with the grounding union on the HESI source. For cutting the PEEK tubing, use the capillary cutter provided in the ISQ EC or EM installation kit. Use the one-piece fingertight fitting to connect the tubing to the grounding union. For connecting to the syringe, use the peek union. Tighten the PEEK capillary on one side of the PEEK union and the 3 cm Teflon™ tubing on the other side of it using both times a ferrule and a two-piece fingertight fitting. For infusion, the syringe needle can then be gently pushed into the Teflon tubing (Figure 1 and Table 1).

Do not connect the tubing to the inlet union of the ISQ EC or EM because it may cause a delay volume and may lead to contamination of the source fluidics.

For direct infusion, only the ISQ EC or EM is added to the system configuration in Chromeleon CDS software. Appropriate source settings are defined by entering the flow rate and MS acquisition settings in the ISQ EC or EM panel of the method editor of the CDS. The syringe is controlled manually. The syringe pump needs to run for a few minutes to flush the lines and push the sample liquid towards the source to create a good, stable MS signal.

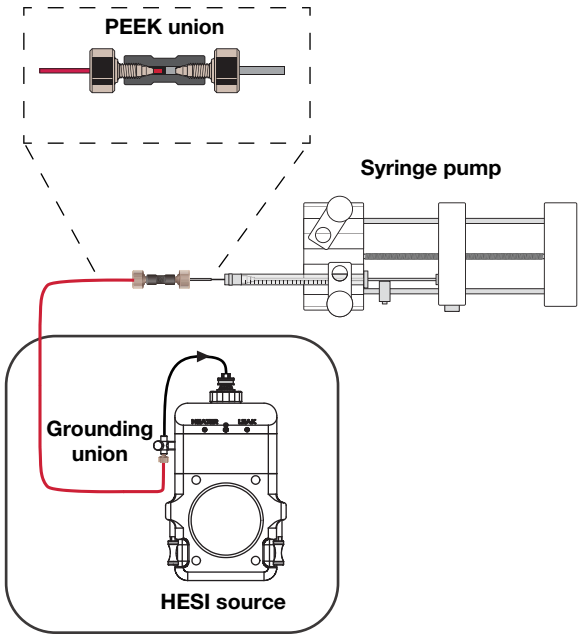

Figure 1. Direct infusion setup

Table 1. Required material for syringe pump-based analyte infusion

Item	Description	Part number
Syringe pump	Chemyx™ Fusion 100-X syringe pump	1R76402-6200
Syringe	Hamilton™ 1700 Series Gastight™ Syringes: RN (removable needle) Termination, 22G, Point Style 3 (blunt), 500 µL , 81265	00301-19016 (Thermo Scientific) or at Fisher Scientific
Syringe needle replacement set	Hamilton™ Large Hub Removable Needles, 22G, Point Style 3 (blunt), for 250 µL – 10 mL Syringes, pack of 6	77800-04 (Fisher Scientific)*
Fluidic connections for connection syringe to HESI source or tee piece	Syringe Adapter Kit (content see Table 2)	70005-62011 (Thermo Scientific)
Tee piece (only needed for infusion into eluent stream)	PEEK tee piece,1/16 inch orifice, 0.02 in thru-hole, 10-32, P-727	00101-18204 (Thermo Scientific) or at Fisher Scientific

* Please note part numbers at Fisher Scientific are country specific and therefore omitted. Please search with the description and the Hamilton or IDEX part number.

Table 2. Content of Syringe Adaptor Kit

Item	Description	Part number (Thermo Scientific)
Ferrule	Fingertight, natural PEEK, F-142, 2 pieces	00101-18196
Fitting (one-piece)	Fingertight, one-piece, natural PEEK, 10-32, F-120, 16 pieces	00109-99-00016
Fitting (two piece)	Fingertight, two-piece, natural PEEK, two wings, 10-32, F-300, 2 pieces	00101-18081
Tubing	Red PEEK, 1/16 inch OD, 0.005 inch ID, 1535XL, 0.6 m	00301-22912
Tubing	Teflon FEP, 1/16 inch OD, 0.030 inch ID, 1522, 3 cm	00301-22915
Union	PEEK union, 10-32, 0.01 inch thru-hole, P-742	00101-18202

Setting up an infusion into the eluent stream

Setting up the infusion into the eluent stream is like the setup described above. Instead of connecting the PEEK tubing to the grounding union, it is connected to the PEEK tee piece. So, the setup between tee piece and syringe is identical to above. The tee piece is connected to the capillary coming from the calibrant delivery system of the ISQ EC or EM on one side and the grounding union on the other side. The latter connection is done using a red PEEK tubing cut to an appropriate length and two one-piece fingertight fittings. The HPLC system is connected to the inlet union in the same way as in standard LC-MS setups (Figure 2 and Table 1).

For infusion into the eluent flow, the HPLC and ISQ EC or EM are added to the system configuration in Chromeleon CDS. The ISQ EC or EM can be linked to the pump in the instrument configuration, and the HPLC flow rate can be used for ISQ EC or EM source setting determination as long as the syringe pump flow rate is 10% or less of the flow rate delivered by the pump. Then, the source settings provided by the Autospray algorithm deviate very little from the ones of the actual flow rate. For example, if the syringe delivers 10 µL/min and the HPLC pump delivers 400 µL/min, then the deviation would be 2.5% (410 µL/min vs 400 µL/min). If the syringe pump flow rate is 10% or more of the pump flow rate, then the pump should not be linked to the ISQ EC or EM in the instrument configuration. Instead, the flow rate should be manually defined in the method editor pane for the ISQ EC or EM. The actual flow rate is the sum of the HPLC flow rate and the syringe pump flow rate. Then, the Autospray algorithm can determine adequate source parameters based on the total flow rate. The other MS acquisition settings are defined in the ISQ EC or EM pane of the method editor, too. The syringe is controlled manually. Again, the HPLC pump and syringe pump need to run for a few minutes to flush the lines and push the sample liquid towards the source to create a good, stable MS signal.

When loading sample into the syringe, make sure no air bubbles are pulled into the barrel of the syringe because these bubbles cause spray instability. If air bubbles are visible, turn the syringe with the needle facing upwards. Tap the barrel gently so that all air bubbles rise towards the needle and then gently push them out by pressing the plunger. Insert the syringe into the syringe pump and connect the needle to the capillary. Before MS analysis the pump flow needs to be set and started. The syringe should be turned on for 2–3 minutes to assure that the analyte has reached the source.

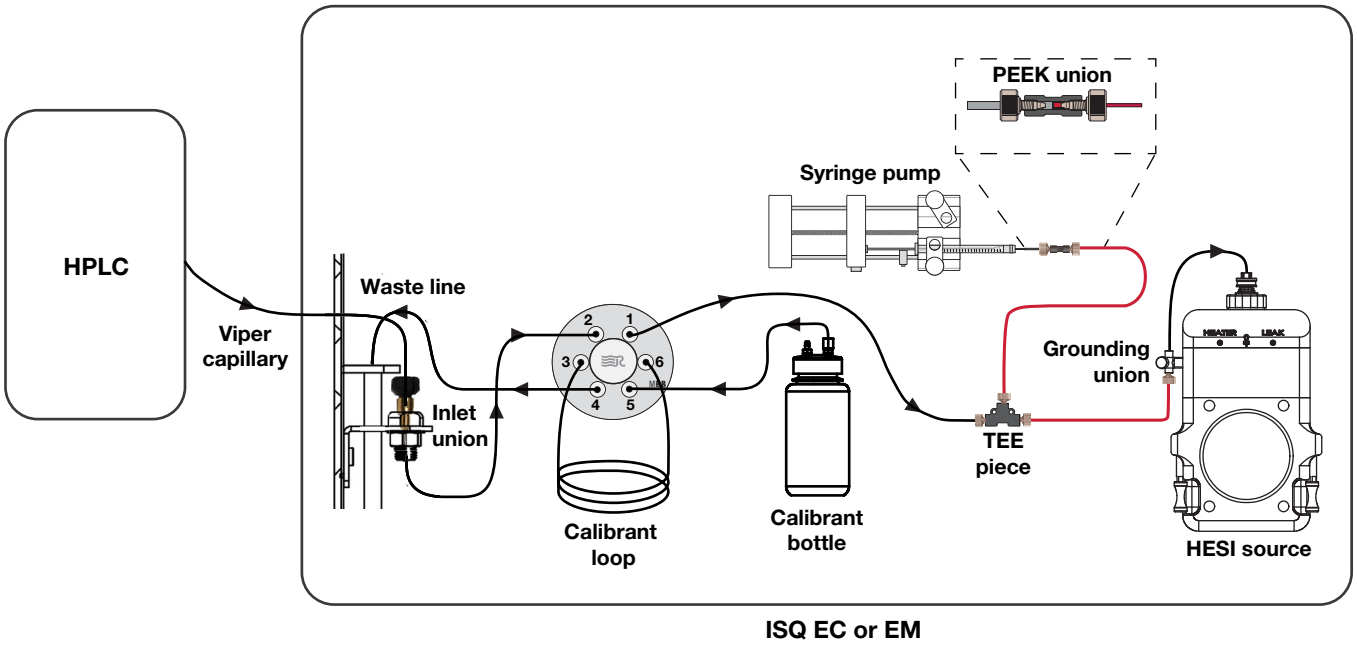

Figure 2. Infusion into the eluent stream

Data review and data acquisition

Brief online data review without recording the data can be done through real time scan. In this mode all parameters need to be manually defined. Data recording is done in Chromeleon CDS through standard acquisition of a sequence list. Blank injections are created with instrument methods having the appropriate source and acquisition settings. The acquisition time can be selected as needed. Usually 2 to 3 minutes should be enough to acquire stable MS data. The syringe pump is delivering sample throughout the sequence. Please note that some source settings need time to change, e.g., the vaporizer or ion transfer tube temperature. Chromeleon software will wait until the respective setpoints are reached. Therefore, a sequence may take longer than anticipated and the syringe may run out of sample during the sequence if the equilibration time is not considered.

Application examples

Myoglobin (20 pmol/µL in 50% methanol, 0.05% formic acid) was infused with a flow rate of 10 µL/min (Figure 3). Sheath gas was set to 8 psig, auxiliary gas to 1 psig, and sweep gas to 0 psig. The vaporizer temperature was switched off, and the ion transfer tube temperature was set to 250 °C. The source voltage was 3000 V. Acquisition was done in profile mode for 500–2000 *m/z* and 1 s dwell time.

The charge envelope of myoglobin could be clearly detected. In addition to myoglobin without the heme group, myoglobin with the heme group and the heme group itself were detected at lower abundance.

Next, myoglobin (20 pmol/µL in 50% methanol, 0.05% formic acid) was infused with a flow rate of 10 µL/min into an eluent flow (Figure 4). The eluent composition was 50% acetonitrile, 0.1% formic acid. The flow rate was 400 µL/min. Sheath gas was set to 42.9 psig, auxiliary gas to 4.8 psig, and sweep gas to 0 psig. The vaporizer temperature was set to 227 °C, and the ion transfer tube temperature was set to 250 °C. The source voltage was 3000 V. Acquisition was done in profile mode for 500–2000 *m/z* and 1 s dwell time.

The charge envelope of myoglobin could be clearly detected. In this case, only the myoglobin without the heme group was detected. The myoglobin with the heme group and the heme group itself were not detected, possibly because of the 40-fold sample dilution by the eluent.

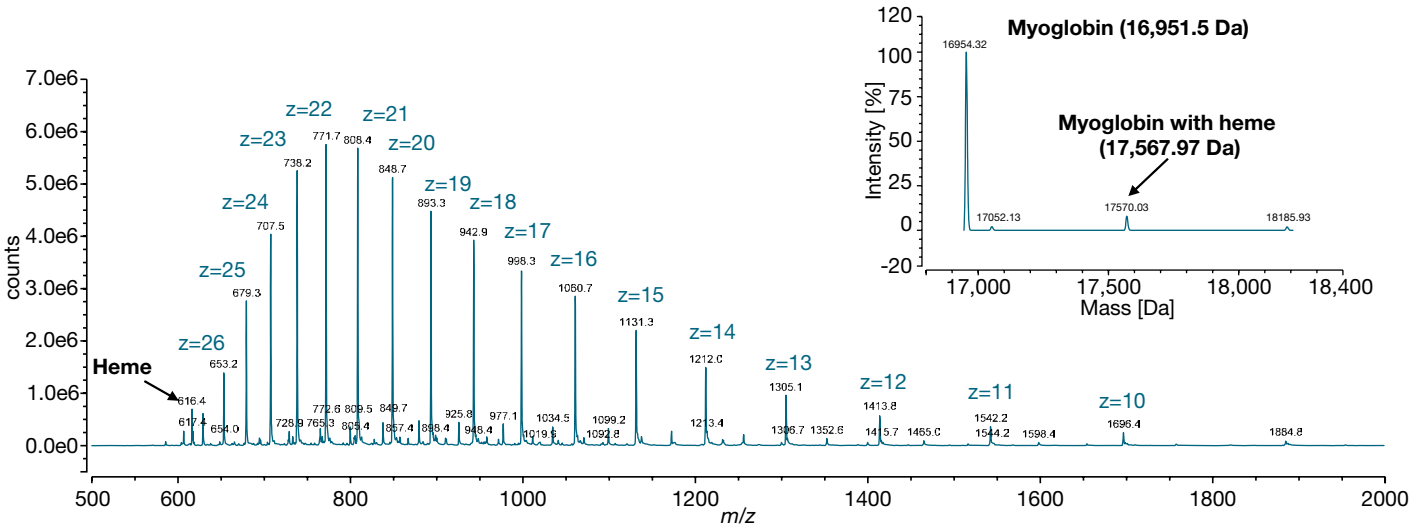

Figure 3. Direct infusion of myoglobin. The charge envelope of the protein can be clearly seen. The heme group and the protein with the heme group can be seen at lower abundance. Deconvolution of the protein signal (insert on top right) can be performed with the Intact Protein Deconvolution tool in Chromeleon CDS or in Thermo Scientific™ Biopharma Finder™ Software. Deconvolution provided the uncharged mass spectrum of the protein signal. In this case, myoglobin with and without the heme group was detected.

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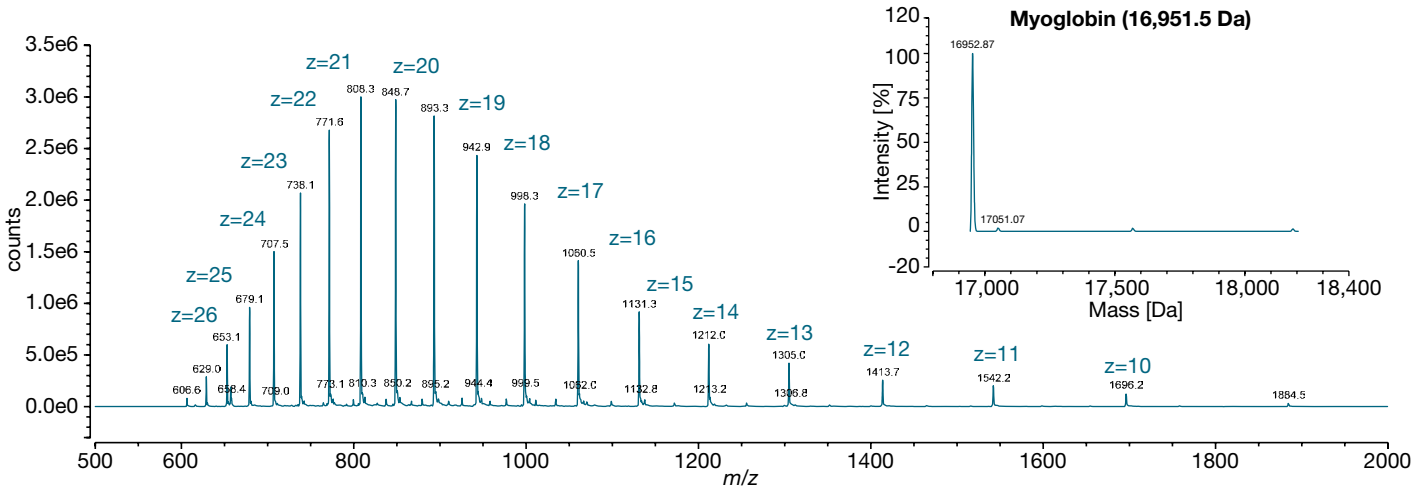

Figure 4. Infusion of myoglobin into the eluent steam. The charge envelope of the protein can be clearly seen. Deconvolution provided the uncharged mass spectrum of the protein signal. In this case, only myoglobin without the heme group was detected.

Conclusion

Here, we describe the simple and straightforward setup of infusions on the ISQ EC or EM using a syringe pump. It can be used for performing fast analyses or optimizing method settings.

References

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