

Article

Turning a 3D Printer into a HPLC Fraction Collector: A Tool for Compound-Specific Stable Isotope Measurements

Matheus C. Carvalho ^{1,*}  and Joanne M. Oakes ²¹ Southern Cross Analytical Research Services, Southern Cross University, Lismore, NSW 2480, Australia² Faculty of Science and Engineering, Southern Cross University, Lismore, NSW 2480, Australia;
joanne.oakes@scu.edu.au

* Correspondence: mcarvalh@scu.edu.au

Abstract: Compound-specific isotope analysis (CSIA) can provide unique insights into the cycling of elements including carbon and nitrogen. One approach for CSIA is the use of high-performance liquid chromatography (HPLC) to separate compounds of interest, followed by analysis of these compounds using an elemental analyser coupled to an isotope ratio mass spectrometer. A key component of this technique is the fraction collector, which automatically collects compounds as they are separated by HPLC. Here, we present a fraction collector that is a simple adaptation of a 3D printer, and, thus, can be easily adopted by any laboratory already equipped for HPLC. In addition to the much lower cost compared to commercial alternatives, this adaptation has the advantage for CSIA that the 3D printer is able to heat the collected fractions, which is not true for many commercial fraction collectors. Heating allows faster evaporation of the solvent, so that the dried compounds can be measured by EA-IRMS immediately. The procedure can be repeated consecutively so that diluted solutions can have the compounds concentrated for analysis. Any computer-controlled HPLC can be integrated to the fraction collector used here by means of AutoIt.

Keywords: Arduino; AutoIt; carbohydrates; CSIA; IRMS; HPLC; liquid chromatography; stable isotopes



Citation: Carvalho, M.C.; Oakes, J.M. Turning a 3D Printer into a HPLC Fraction Collector: A Tool for Compound-Specific Stable Isotope Measurements. *Hardware* **2023**, *1*, 29–53. <https://doi.org/10.3390/hardware1010004>

Academic Editor: Peter C. Hauser

Received: 30 October 2023

Revised: 28 November 2023

Accepted: 1 December 2023

Published: 7 December 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Compound-specific isotope analysis is the name given to a group of analytical techniques that generally consist of isolating specific chemical compounds and then measuring them for their stable isotope composition [1,2]. The term is used to differentiate from isotope analysis performed without the initial separation step, which is known as bulk isotope analysis. Targets for compound specific isotope analysis include carbohydrates [3–5], amino acids [3,6–11], and fatty acids [3,11–14].

High-performance liquid chromatography (HPLC) is one technique that enables compound-specific isotope analysis (CSIA). HPLC is used to separate dissolved compounds in a liquid sample by passing the solution through a chromatographic column. The chromatographic column has stronger affinity for some compounds compared to others, and this makes compounds elute from the column at different times. There have been numerous studies in which samples separated by HPLC have been subsequently measured using isotope ratio mass spectrometry (IRMS), for a range of chemical elements [15–22]. HPLC–IRMS can be achieved using online HPLC–IRMS, where a HPLC pump is directly connected to an IRMS machine [23], but this approach has some limitations: it is limited to carbon isotopes only (while IRMS can measure many different elements); it can only operate using low-flow water as the mobile phase (in contrast, HPLC can operate using many other solvents, including organic solvents, which cannot be used for online HPLC–IRMS); and it demands the use of corrosive chemicals and fragile membranes/filaments, which make the operation of the system highly labor (and capital)-intensive [24]. Furthermore, on-line HPLC–IRMS demands specialized equipment that is not present in most IRMS laboratories [24]. An alternative to online HPLC–IRMS is offline HPLC–IRMS, where compounds

separated by HPLC are physically collected then subsequently analysed with an IRMS. While not as efficient as the online approach, offline HPLC–IRMS is arguably more flexible, robust, and accessible, because there is no limitation of solvent or target element, there is no need for specialized interfaces between the HPLC and the IRMS, and HPLC is a very common technique present in many laboratories.

A key element of offline HPLC–IRMS is the use of a fraction collector, which is a device that automatically collects the compounds as they elute from HPLC. Here, we show how a common 3D printer can be easily adapted to function as a fraction collector, with the ultimate aim of enabling stable isotope analyses for compounds separated using HPLC.

2. Design

2.1. Justification of the Design

Fraction collectors for HPLC typically consist of delivery tubing and a container tray. The delivery tubing (or the tray) is moved so that the liquid eluting from the HPLC, corresponding to specific peaks of the various compounds separated by the HPLC, can be delivered to the individual compartments within the tray. This allows separate collection of each of the desired compounds.

Fraction collectors can be seen as a kind of autosampler. This means that they are among the simplest instruments in a laboratory. Despite their simplicity, commercial fraction collectors are arguably overpriced, as is most commercial scientific equipment [25–27]. This has prompted scientists to build their own fraction collectors [28–31]. Adapting these designs, or some autosampler designs [32], could be an option for our purposes. However, we realized that a simpler approach would be to adapt commercially available 3D printers [33,34]. The main advantages of adapting a 3D printer to function as a fraction collector are: (1) low cost; (2) wide availability; (3) high similarity in function. This similarity consists of the ability of 3D printers to move the extruder (equivalent to the delivery tubing) and the printing bed so that positions in 3D space can be reached. Also, most current 3D printers have an advantage compared to autosamplers that might also be repurposed as fraction collectors: 3D printers are equipped with a heated bed, which is used to aid printing. A heated bed is convenient for a fraction collector because it allows the collected liquid to be simultaneously evaporated, so that only the non-volatile compounds remain in each container as dry powders. This way, the collected fractions can be immediately taken for diverse chemical measurements, including stable isotope analyses of carbon and nitrogen, which are our main interest.

2.2. 3D-Printer Modifications

The fraction collector used in the current study consists of a 3D printer (3D printer touch, Balco) that had its extruder removed and replaced with a simple tube holder for the polyether ether ketone (PEEK) tubing coming from the HPLC. A metal tray (PAL TR98, CTC analytics) was placed on the heat bed of the 3D printer using double-sided tape. A tube leading to a waste drum was attached to the side of the 3D printer body to allow for waste collection.

Instead of the original control board for the 3D printer, an MKS-GEN-L board (Maker-base) was connected to the stepper motors, the heat bed, and the power supply of the 3D printer. Replacing the control board is not strictly necessary, but we have familiarity with the MKS-GEN-L board, and, thus, opted for this approach. The Marlin firmware was uploaded to the board to allow the control of the stepper motors and heat bed. Stepper motor drivers (A4988) were installed on the board. A list of the components used is in the bill of materials (Table 1).

2.3. Bill of Materials

Table 1. Bill of materials.

Component	Source	Cost
3D printer Prusa I3 style ¹	https://www.ebay.com.au/itm/155077037136 (accessed on 30 November 2023)	AUD 232.00
MKS GEN L board	https://www.ebay.com.au/itm/354788810185 (accessed on 30 November 2023)	AUD 70.00
A4988 drivers (5 units)	https://www.ebay.com.au/itm/134438897517 (accessed on 30 November 2023)	AUD 11.00
PAL TR98 tray	https://www.palparts.com/product/pal-tr98/ (accessed on 30 November 2023)	AUD 1045.00
Male-female DuPont cable for stepper motors (5 units)	https://3dprintingperth.com/products/stepper-motor-cable-male-to-female-extension-dupont-wire?variant=39471376826481 (accessed on 30 November 2023)	AUD 15.00
Adapter model BT7242A for stepper motors	https://www.aliexpress.com/i/32881420875.html (accessed on 30 November 2023)	AUD 5.00
Waste tube	https://www.labfriend.com.au/bsafe-corrugated-tubing-pp-%C3%B8-65-x-100-mm-t-175-mm (accessed on 30 November 2023)	AUD 50.00
Cable holder for waste tube	https://www.ebay.com.au/itm/203860080384 (accessed on 30 November 2023)	AUD 4.00
M3 screw, nut, and space	Hardware store	Less than AUD 5.00
3D-printed tube holder	Appendix A	Less than AUD 1.00
Total		AUD 1438.00

¹ A model is suggested here, but any model with similar design can be used.

3. Build Instructions

The instructions presented here are for one specific model of 3D printer. However, they can be easily adapted for most other similar models available on the market.

The first step is to remove the filament extruder (Figure 1).

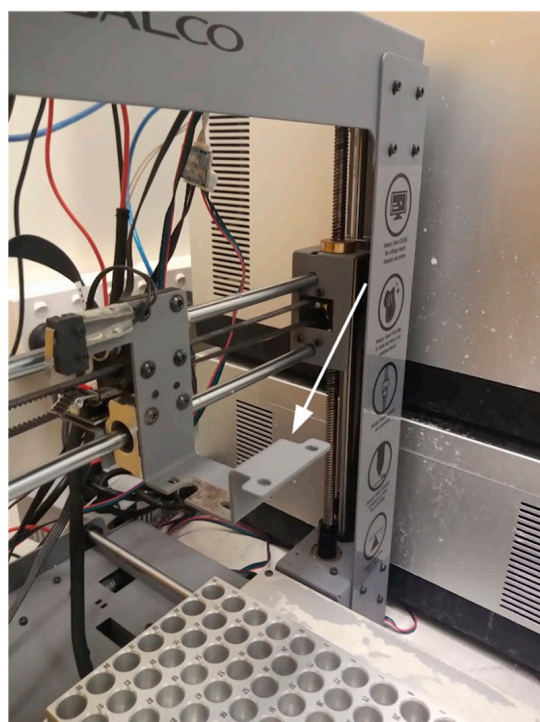


Figure 1. 3D printer without its filament extruder. Arrow points to the extruder support.

With the extruder removed, the tube holder can be attached to its place (Figure 2) using two M3 screws and nuts. The design file for the tube holder is available from <https://osf.io/d24ek/> (accessed on 30 November 2023). Note that this tube holder was designed specifically for this 3D printer and for the tube diameter (1.5 mm) used with the HPLC. Different dimensions will demand a different design.



Figure 2. Custom 3D-printed tube holder attached to the extruder support on the 3D printer.

The next modification is to attach a metal tray to the printing bed (Figure 3). Here, we used a PAL TR98 tray from CTC analytics. Metal trays are suitable to allow heating, and thereby concentration, of the samples upon collection. We used double-sided adhesive tape to attach the tray to the bed.

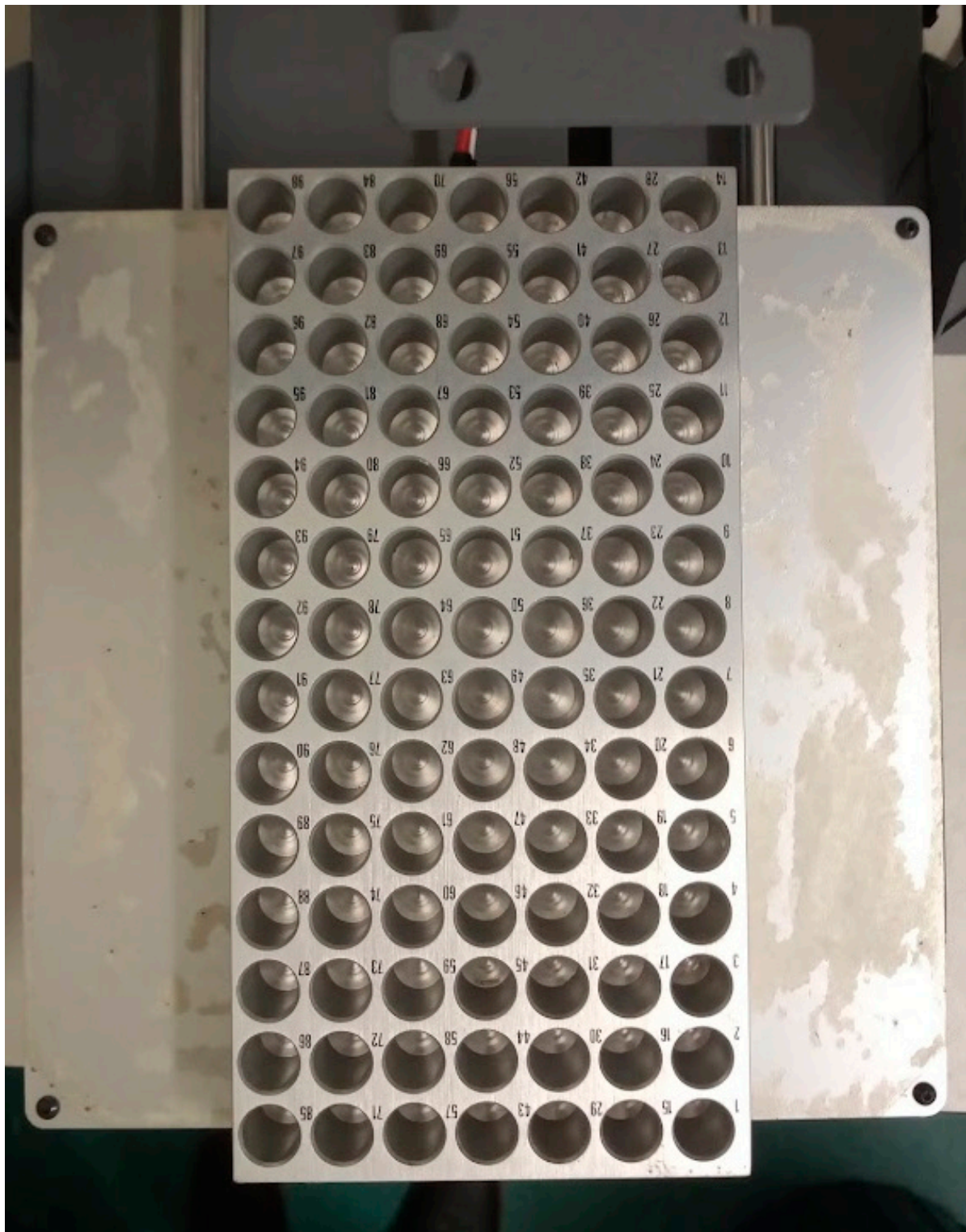


Figure 3. Sample tray attached to the printing bed.

The next steps involve re-wiring the printer so that a different control board is used instead of the one that comes with the printer. By turning the printer upside down and removing the lower cover, the cables become accessible. The cables for the stepper motors, for the heated bed, and for the power supply must be disconnected from the main board (Figure 4).

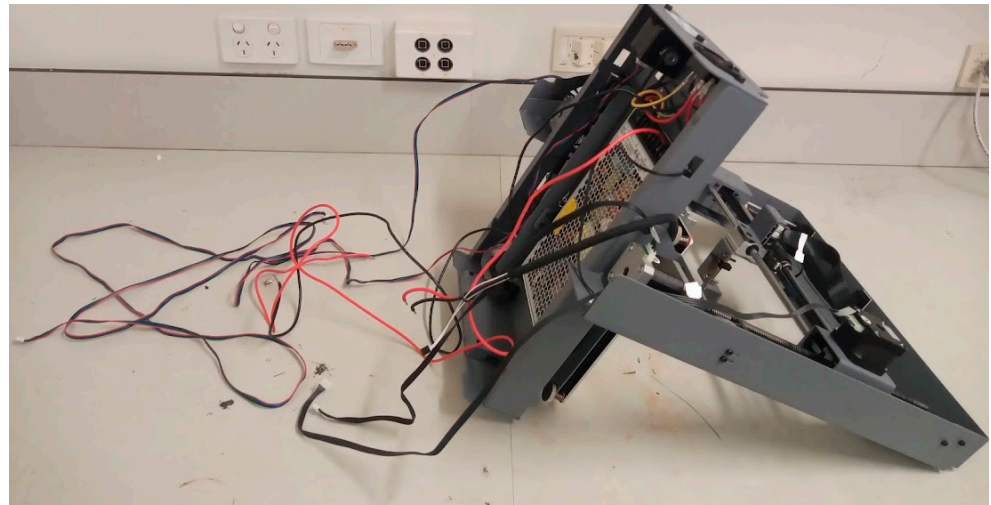


Figure 4. The 3D-printer turned upside down with the cables disconnected from the control board.

Instead of the original control board in the 3D printer, we used the MKS-GEN-L control board because of our familiarity with it [32]. The board needs to be prepared to be connected to the stepper motors. The first step is to upload the Marlin firmware [35] (download from <https://osf.io/d24ek/>, accessed on 30 November 2023) to the board. This can be achieved by using the Arduino IDE (download from <https://www.arduino.cc/en/software>, accessed on 30 November 2023). The board needs to be connected to a computer using a USB cable. The configuration parameters used in the firmware were optimized for this specific 3D printer model. They may need to be modified for different 3D printer models.

Once the board is disconnected from the computer, the stepper motor drivers can be connected to the board (Figure 5). Here, A4988 drivers were used.

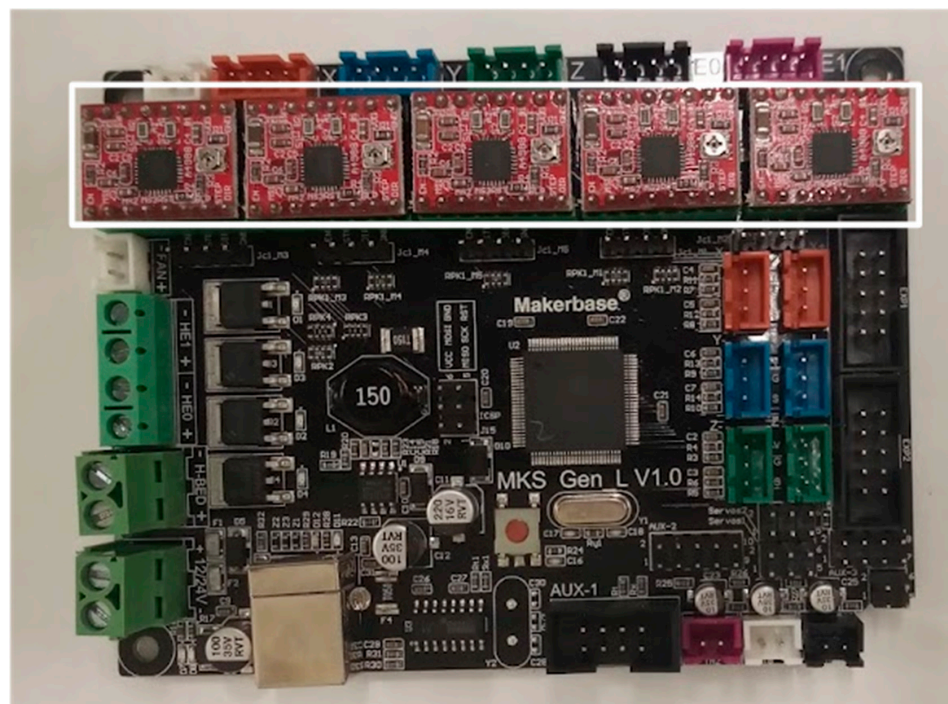


Figure 5. MKS-GEN-L board with A4988 stepper motor drivers (outlined in white in the figure) attached. The orientation of the drivers is important, as misplacement may lead to driver or board failure. Notice the small screws on each driver positioned to the right of the photo, and away from the power connections of the board.

Once the control board is prepared, it can be connected to the power supply, heat bed, and stepper motors (Figures 6–8). Notice that for the Z axis an adapter (model BT7242A) was used to enable use of two motors. Different 3D printer models may need different arrangements.

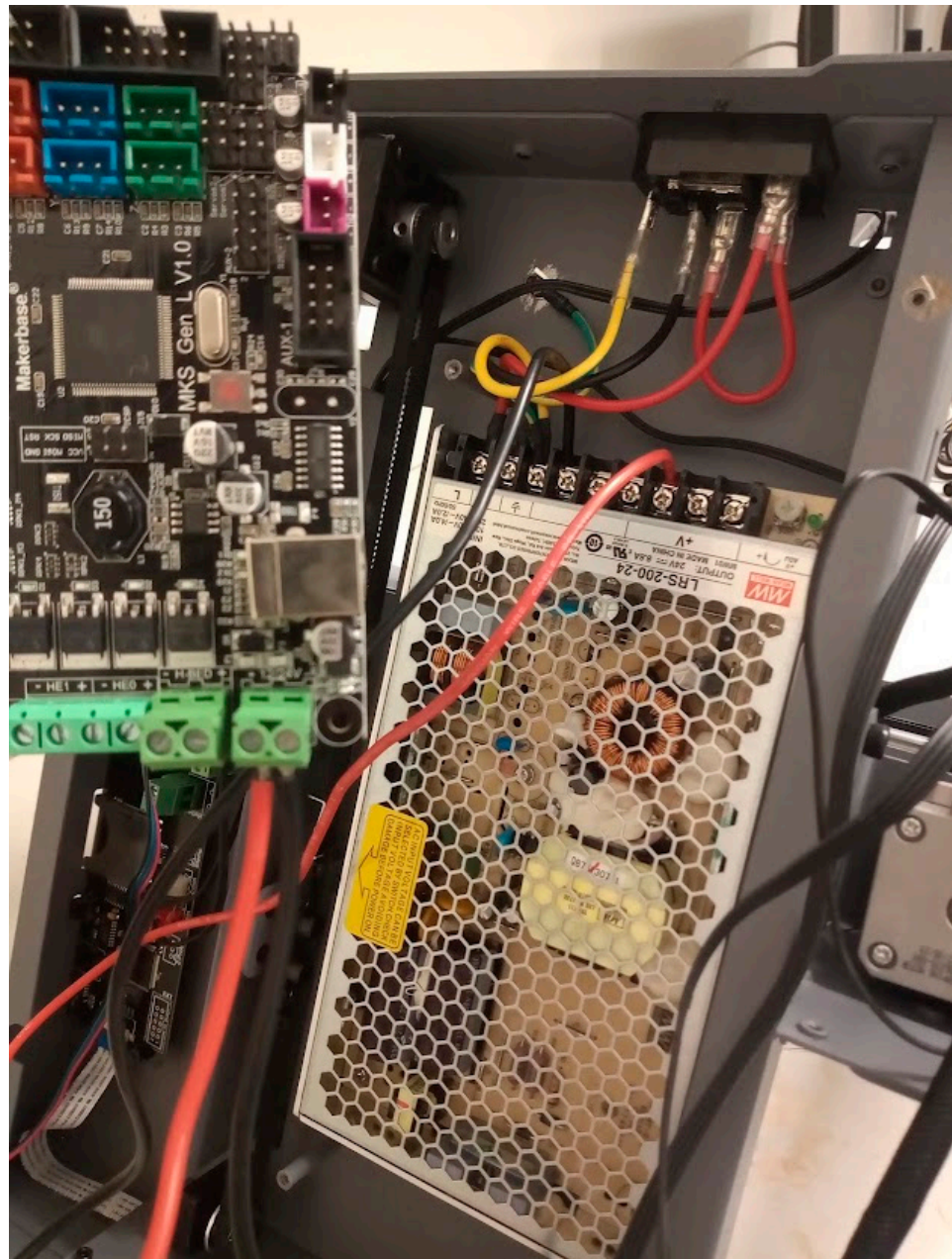


Figure 6. Power supply connected to the MKS-GEN-L control board.

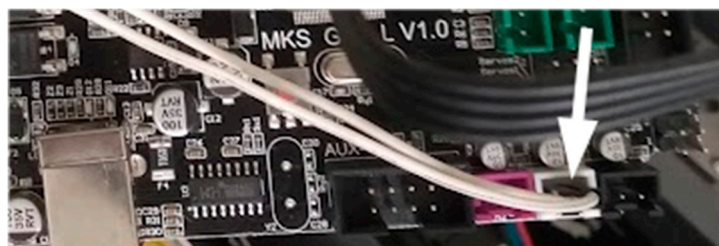


Figure 7. Heat bed connected to the MKS-GEN-L control board.

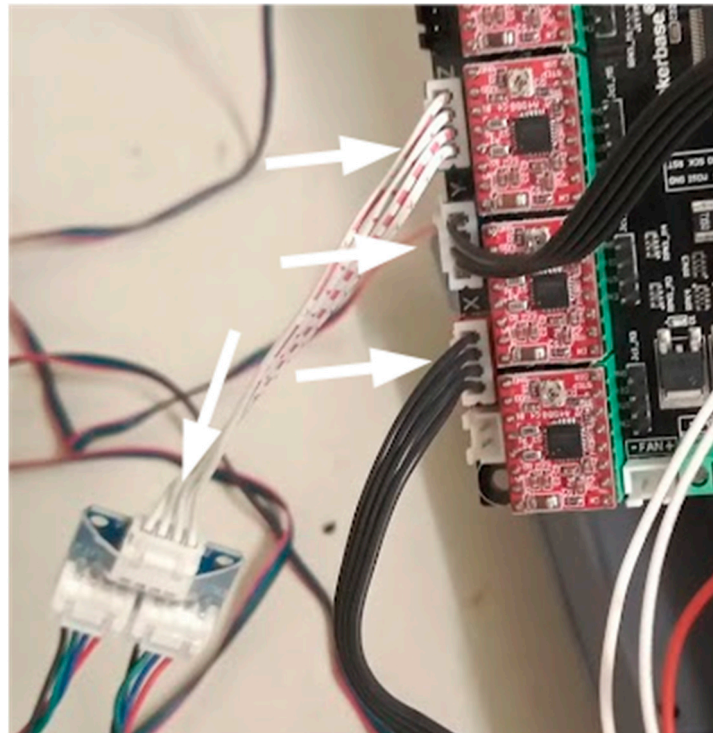


Figure 8. Stepper motors connected to the MKS-GEN-L control board. Connections indicated by arrows.

Once all cable connections are complete, the control board should be attached to the top of the 3D printer body using an M3 screw, nut, and a spacer (Figure 9).

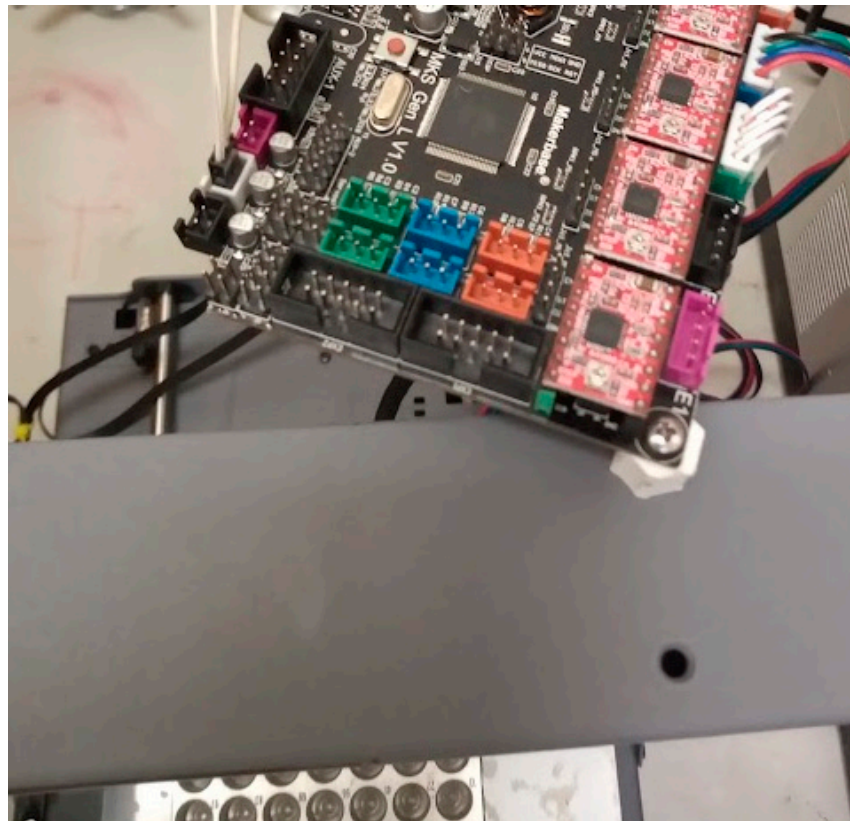


Figure 9. Control board attached to the top of the 3D printer body using a screw and a spacer.

Organize the cables at the back of the 3D printer, paying attention to their possible interference with the path of the moveable parts (Figure 10). Cable ties are useful for this purpose.

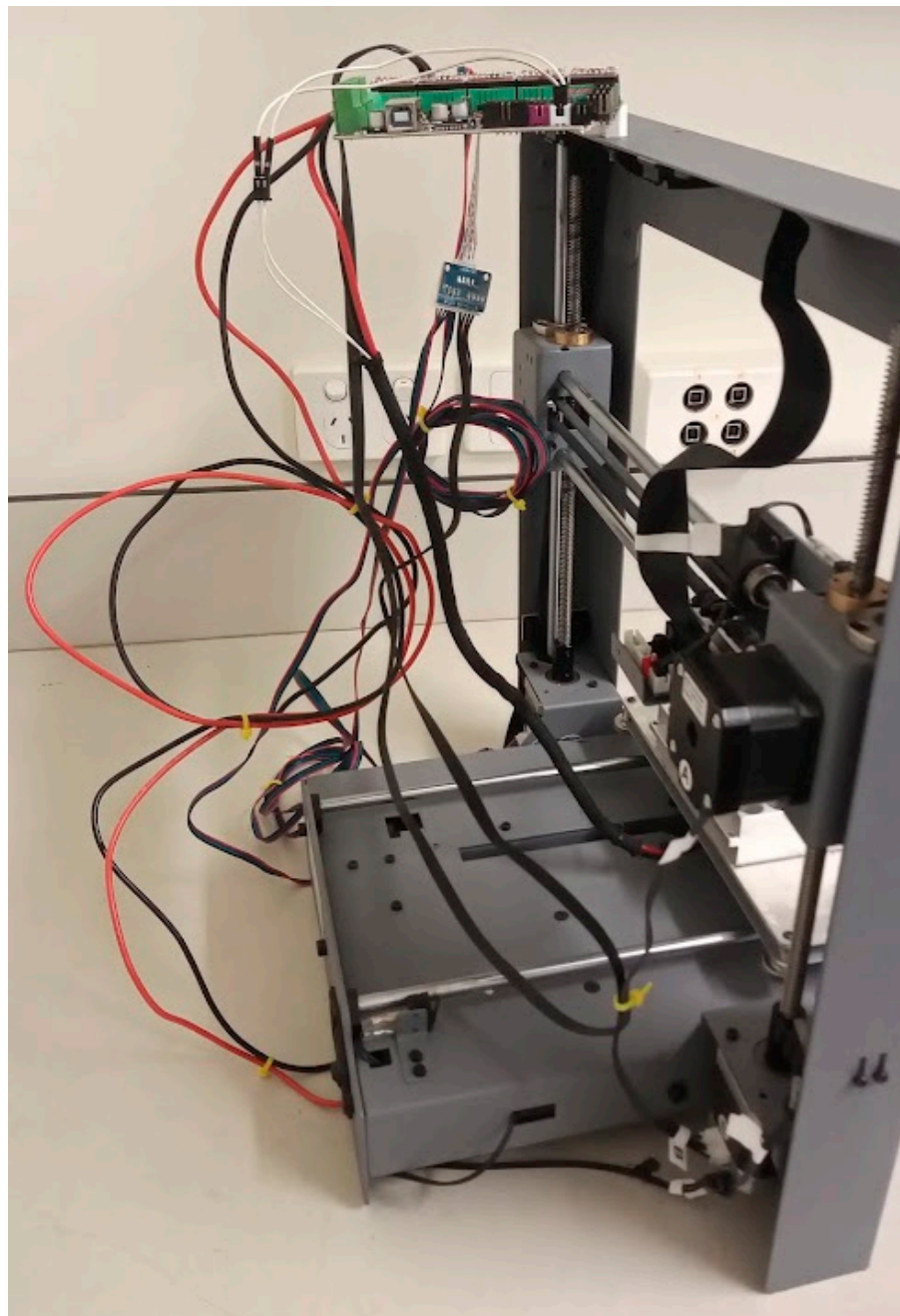


Figure 10. Cables at the back of the 3D printer.

Attach the waste tube to the side of the 3D printer body (Figure 11). It must be in a position that can be reached by the tube holder that will support the HPLC delivery line, but not on top of the tray where the samples will be collected. In our configuration, the tube was cut in half to allow waste to drip into the tube from above and was held in place using a metal tube. Using a waste tube connected to a waste drum allows for line purging and the discarding of undesired eluents.

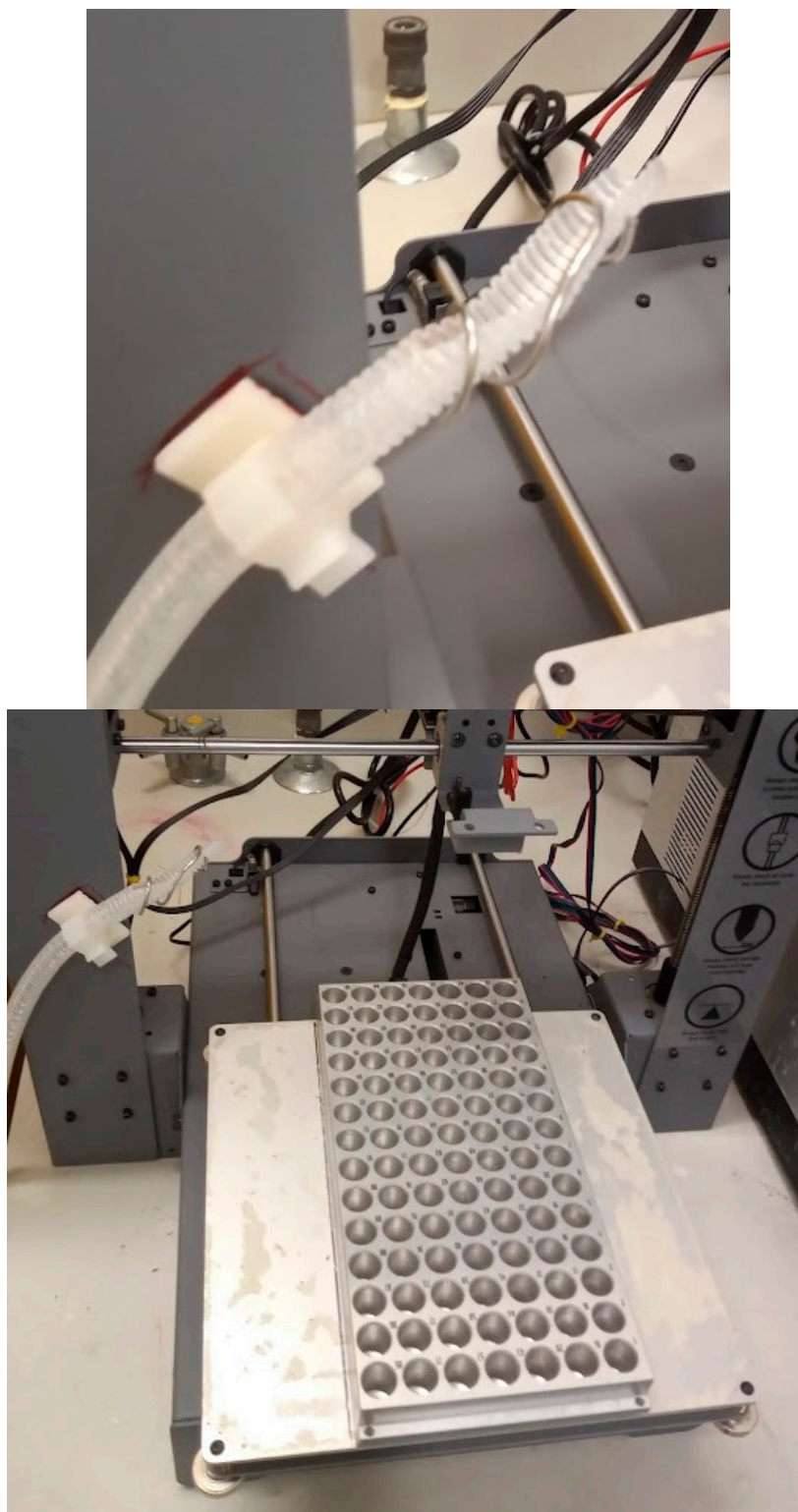


Figure 11. Waste tube attached to the 3D printer.

Attach the delivery line from the HPLC to the tube holder on the 3D printer (Figure 12). A portion of the tube (about 3 mm) should hang beneath the holder so that the sample solution falls without interference from the holder. The delivery line should be as short as possible to minimize dispersion of the HPLC eluents before collection.

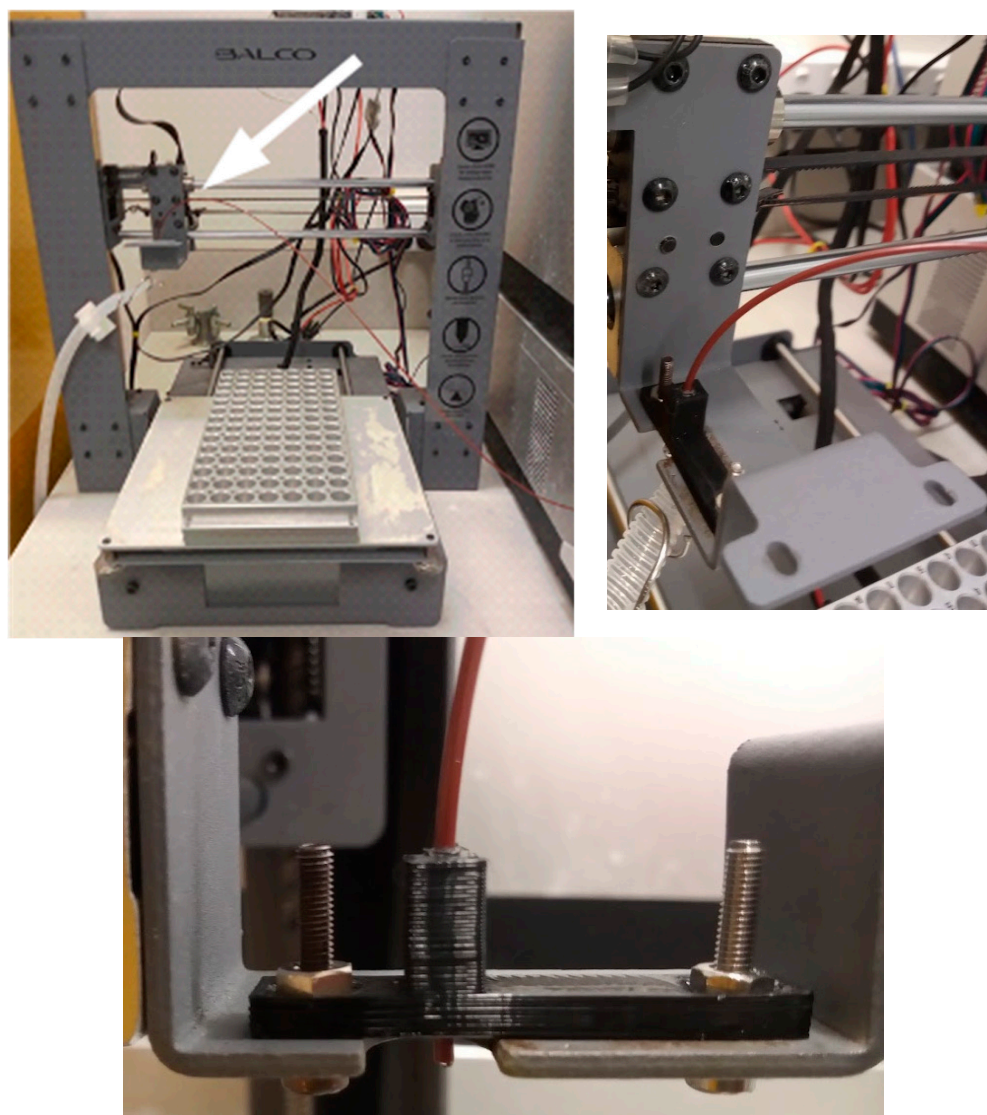


Figure 12. HPLC tube attached to the tube holder on the 3D printer.

Once the fraction collector is assembled, the sample tray can be loaded with tin (PN SC9428, Sercon) or silver (PN SC1295, Sercon) capsules (Figure 13). Silver capsules are needed if the eluent is corrosive. The capsule size (6 mm × 9 mm in diameter) was chosen to match the tray. A different tray would demand a different tin capsule size.



Figure 13. Sample tray loaded with tin capsules.

The control board is connected to the computer, and the power supply to the wall. When the power is turned on, the fraction collector will be ready to use. Of course, in addition to the fraction collector preparation, the HPLC system should also be prepared in accordance with the protocols used for the separation to be performed.

Finally, because the procedure takes many hours (often more than a full day) to complete, it is important that contamination (e.g., dust) is avoided. The system should be placed in a clean and quiet place, in a closed room, preferably with air conditioning.

4. Operating Instructions

4.1. Control Board Preparation

The fraction collector was controlled using the Hype!Terminal program (<https://osf.io/d24ek/>, accessed on 30 November 2023), which allows commands to be sent through COM ports (including USB, which connects the control board to the computer).

The first action when launching Hype!Terminal is to configure the COM port (Figure 14). Most parameters in the configuration do not need to be changed, except for the port number and the BAUD rate (bits per second). The port number needs to be known previously (e.g., by checking the device manager utility on the Windows operating system). Bits per second need to be changed to 115,200.

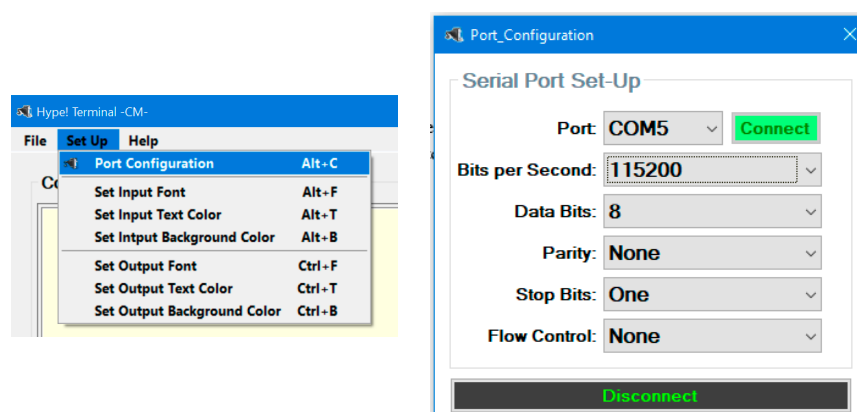


Figure 14. COM port configuration on Hype!Terminal.

Once the connection with the board is established, the command “M121” must be sent to allow the use of negative coordinates when sending commands to the stepper motors. This and the COM port configuration need to be performed every time the program is started.

4.2. Basic Commands

Once the control board is connected and negative coordinates can be used, the fraction collector can be controlled from the computer using Hype!Terminal. Movements are achieved using the command G1. For example, G1 × 1F500 moves the HPLC tube horizontally one unit with 500 speed units. G1Z2F500 moves it vertically two units. G1Y3F500 moves the heat bed (and consequently the tray) three units. More than a single axis can be used as input at any given time. For example, the command G1X3Y4F500 will move the HPLC tube and the tray simultaneously. The command M114 can be used to verify the current position. If it is desired that the current position becomes zero, the command is G92. For example, G92X0Y0Z0 forces all axes to have the value of zero.

The heat bed can be warmed using the command M140. Using M140S80 will warm the bed to 80 °C. It is useful to preheat the sample tray, as it can take some minutes to reach the final temperature. To turn off the heat bed, use M140S0.

4.3. Determining Important Positions

Stepper motors do not use absolute positioning, but instead rely on positions that are relative to arbitrary origin points. When the board is connected, it assumes all positions (X, Y, and Z) are equal to zero. It is, therefore, possible to determine all other relevant positions in relation to any initial position. However, it is more convenient to start from an easily reproducible position so that the other positions do not need to be redefined every time the machine is started. Here, the zero positions were determined as the leftmost possible position for X, the frontmost position for Y, and an arbitrary height for Z, at which the HPLC tube can move without touching the waste tube (Figure 15).

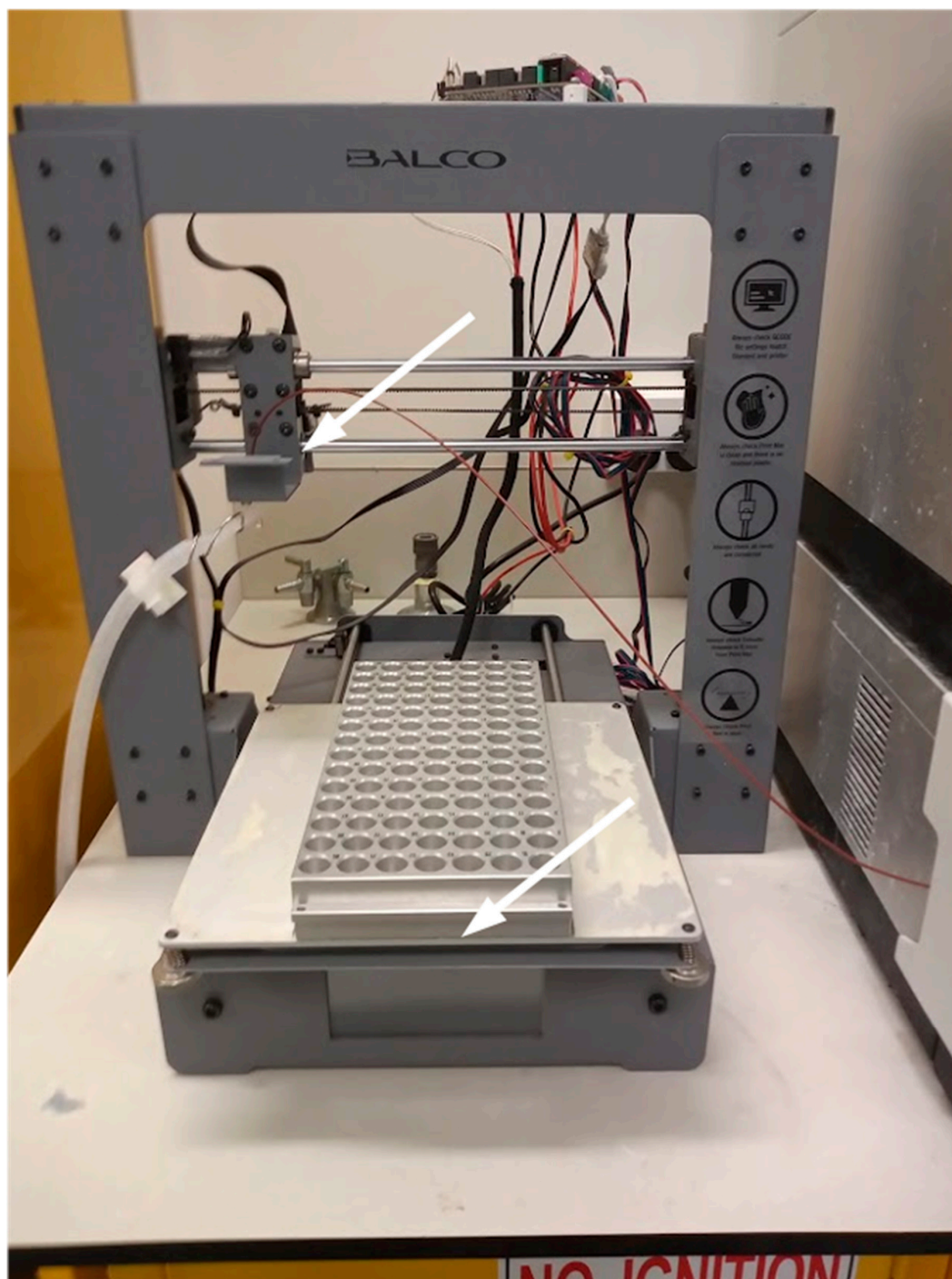


Figure 15. HPLC delivery line at the zero position.

The next important positions are the four corners of the tray that can be reached by the HPLC tube (Figure 16). Both the leftmost ($X = 2.30$; positions given here are likely to be different for different 3D printers and trays) and rightmost ($X = 5.95$) positions of the tray are accessible, but only 11 of the 14 back-to-front positions are accessible (Y from 0.30 to 7.05).

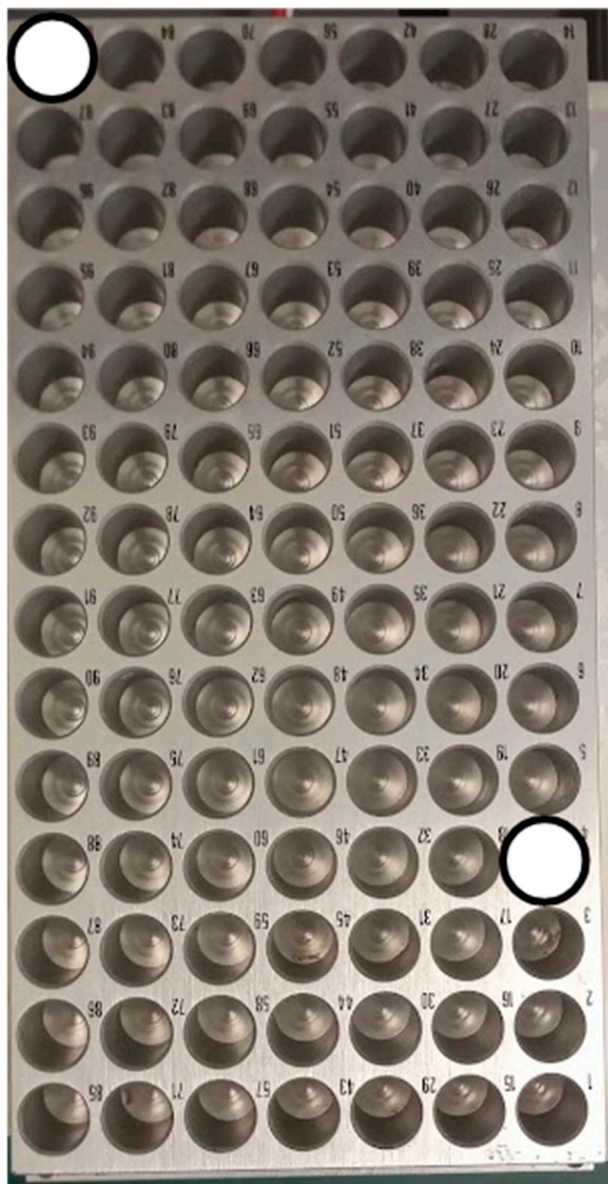


Figure 16. Sample tray with the two extreme positions that can be reached by the HPLC delivery line marked by white circles.

The remaining important positions are the vertical positions. For the waste, these were $Z = -1$. For moving above the tray, $Z = -17$, and for liquid delivery to the tray, $Z = -19$.

The determination of the positions normally only needs to be performed once. It may need to be repeated if the tray is removed and put back in place, for example.

4.4. Automated Control

The fraction collector and the HPLC were integrated using an AutoIt script [36]. AutoIt allows the integration of different programs using seamless control of the Windows elements, or mouse click and keyboard entries [36]. Here, we employed both approaches. To control Hype!Terminal, AutoIt sent seamless commands to the input field (Figure 17).

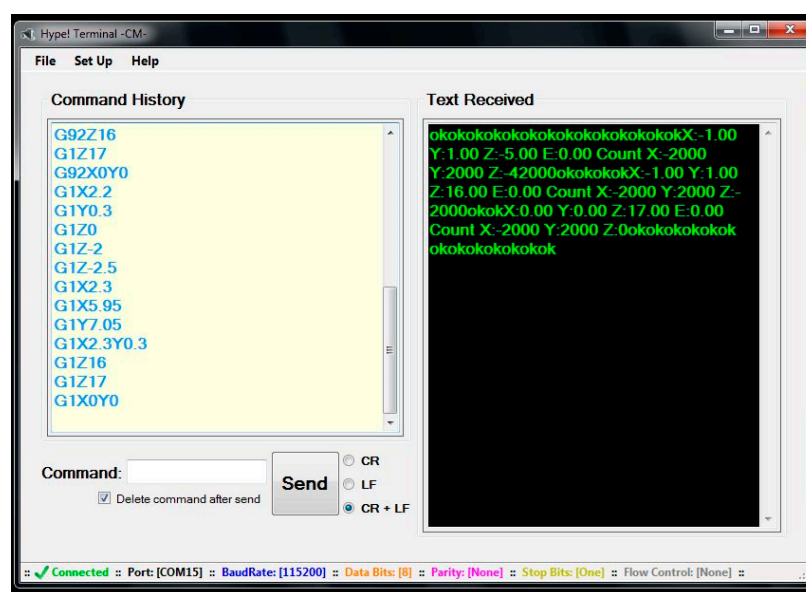


Figure 17. Hype!Terminal graphical interface. The input fields that received AutoIt instructions were the text box after “Command” and the “Send” button.

The fraction collector worked together with a HPLC system (Ultimate 3000, Thermo Fisher), controlled with Chromeleon 7 (Thermo Fisher). The system was equipped with a solvent pump, an autosampler, and a column compartment with temperature control.

To control the HPLC pump, seamless commands were sent to the input field for flow control (Figure 18). However, to control the HPLC autosampler, seamless commands did not work, and, thus, automated mouse and keyboard strokes were sent to the “Pos.” input box, and to the “Inject” button (Figure 19).

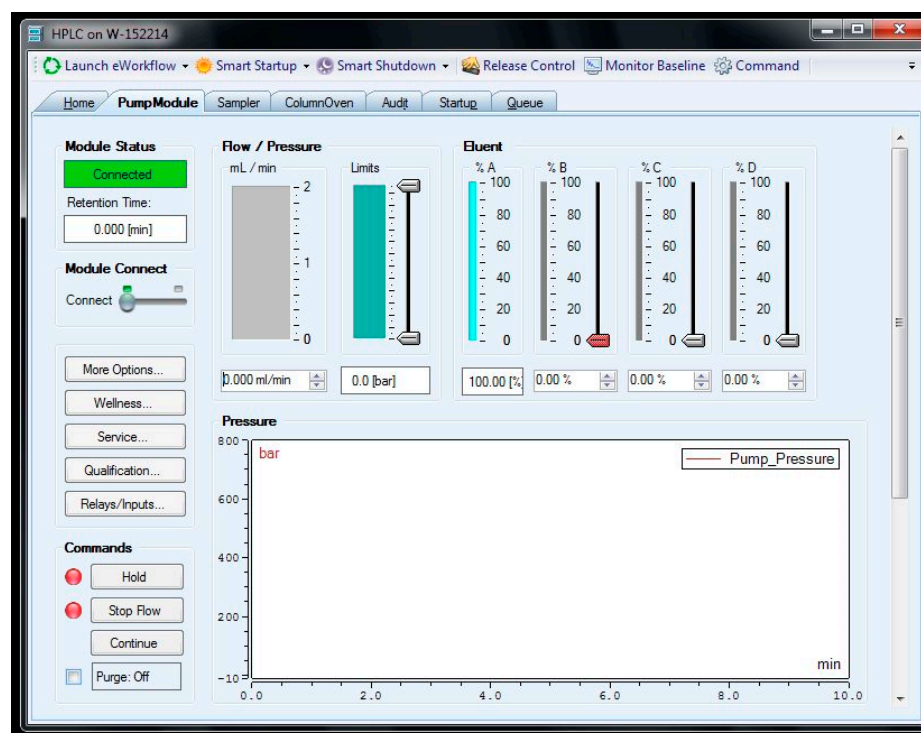


Figure 18. Chromeleon interface for pump control. Commands were sent to the “Flow/pressure” input box (it has a value of 0.000 in the figure).

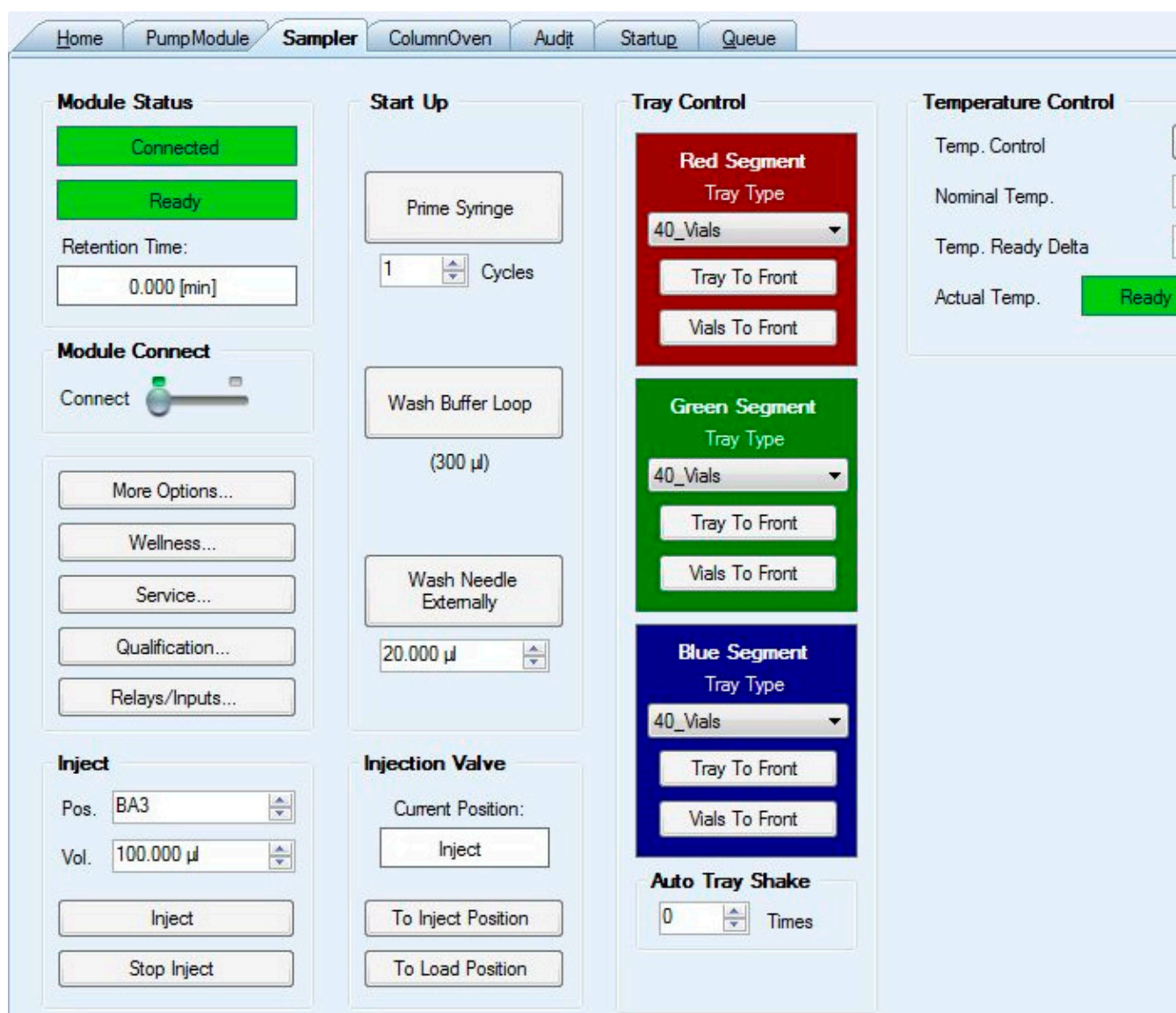


Figure 19. Chromeleon interface for autosampler control. Commands were sent to the “Pos.” input box, and to the “Inject” button.

A typical script would consist of the following instructions:

- (1) Turn on the HPLC pump (by sending a command to the Chromeleon pump control interface);
- (2) Inject a sample volume from a sample in the HPLC autosampler (by sending a command to the Chromeleon sampler control interface);
- (3) Wait a predetermined time until the compound of interest is at the exit of the tube in the fraction collector (this needs to be determined beforehand);
- (4) Move the HPLC tube to the first tin capsule (by sending commands to Hype!Terminal);
- (5) Wait for a predetermined time above the tin capsule so that the compound is completely collected in it;
- (6) Move to the next tin capsule (by sending commands to Hype!Terminal);
- (7) Repeat step 6 for as many compounds as are needed to be collected (by sending commands to Hype!Terminal);
- (8) Move to the waste tube (by sending commands to Hype!Terminal);
- (9) Turn the HPLC pump off (by sending a command to the Chromeleon pump control interface);

- (10) Wait until the collected liquid has completely evaporated;
- (11) Repeat all steps for multiple replicates of the sample, as required to collect sufficient material for elemental analysis coupled to isotope ratio mass spectrometry (EA–IRMS).

A script example can be found in Appendix B, and a video showing the setup working can be found as Supplementary Material Video S1 and on <https://youtu.be/uQRLIZQone4> (accessed on 30 November 2023).

5. Validation

Stable Carbon Isotope Analysis of Glucose

The fraction collector was tested for accuracy in the measurement of glucose in a water solution. The HPLC was equipped with a HyperRez XP Carbohydrate H+ (Thermo Fisher, Sydney, Australia) column kept at 75 °C in the column compartment. A guard column (also HyperRez XP Carbohydrate H+) was used, as well as a frit between connections to prevent column clogging. The frit was replaced every time pressure surpassed 40 bar to avoid deterioration of the column. This limit also dictated the maximum flow rate that could be used, which was 0.6 mL min^{−1}. Milli-q water was used as the solvent.

The solution of glucose (Sigma Aldrich, Sydney, Australia, 99.9% purity) was prepared using milli-q water, with a final concentration of 4 mM of carbon. This concentration is probably high compared to many natural samples, but was chosen as a simple starting point to demonstrate the viability of the fraction collector system. The glucose solution was transferred to 2 mL vials that were placed in the HPLC autosampler. The autosampler injected 0.1 mL of the glucose solution into the HPLC. At a flow rate of 0.3 mL min^{−1}, glucose eluted from the column after 22 min, whilst at a flow rate of 0.6 mL min^{−1}, glucose eluted at 11 min. These retention times were determined via preliminary injections with collections every minute. Based on these retention times, a narrow collection range, between 10 and 12 min for 0.6 mL min^{−1} and between 20 and 24 min for 0.3 mL min^{−1} was employed. Repeated injections were performed, and it was found that glucose was better collected using a flow rate of 0.6 mL min^{−1}; the time spent on collection was smaller, but more importantly the peak was sharper, and concentrated only in a single tin capsule, whilst with 0.3 mL min^{−1}, the peak was spread across two tin capsules (Figure 20). The $\delta^{13}\text{C}$ of glucose (-10.5 ± 0.1 mUr) was determined from previous measurements of the solid powder using EA–IRMS. Empty tin capsules would have low peak areas when analysed with EA–IRMS and their carbon stable isotope ratios ($\delta^{13}\text{C}$) would be different from the known value for glucose, while tin capsules containing glucose would have larger areas and $\delta^{13}\text{C}$ closer to the known value. The actual value for the compound (glucose, in this case) collected in the tin capsules is calculated by using $\delta^{13}\text{C}$ values for empty tin capsules as blanks, and applying a mass balance. It was found that for injections performed at 0.6 mL min^{−1}, the average value for glucose was -10.4 ± 0.25 mUr, $n = 7$, while for 0.3 mL min^{−1}, results were -10.4 ± 0.13 mUr, $n = 3$. In both cases, the results are very close to the known $\delta^{13}\text{C}$ for the glucose powder used to make the glucose solution that was tested.

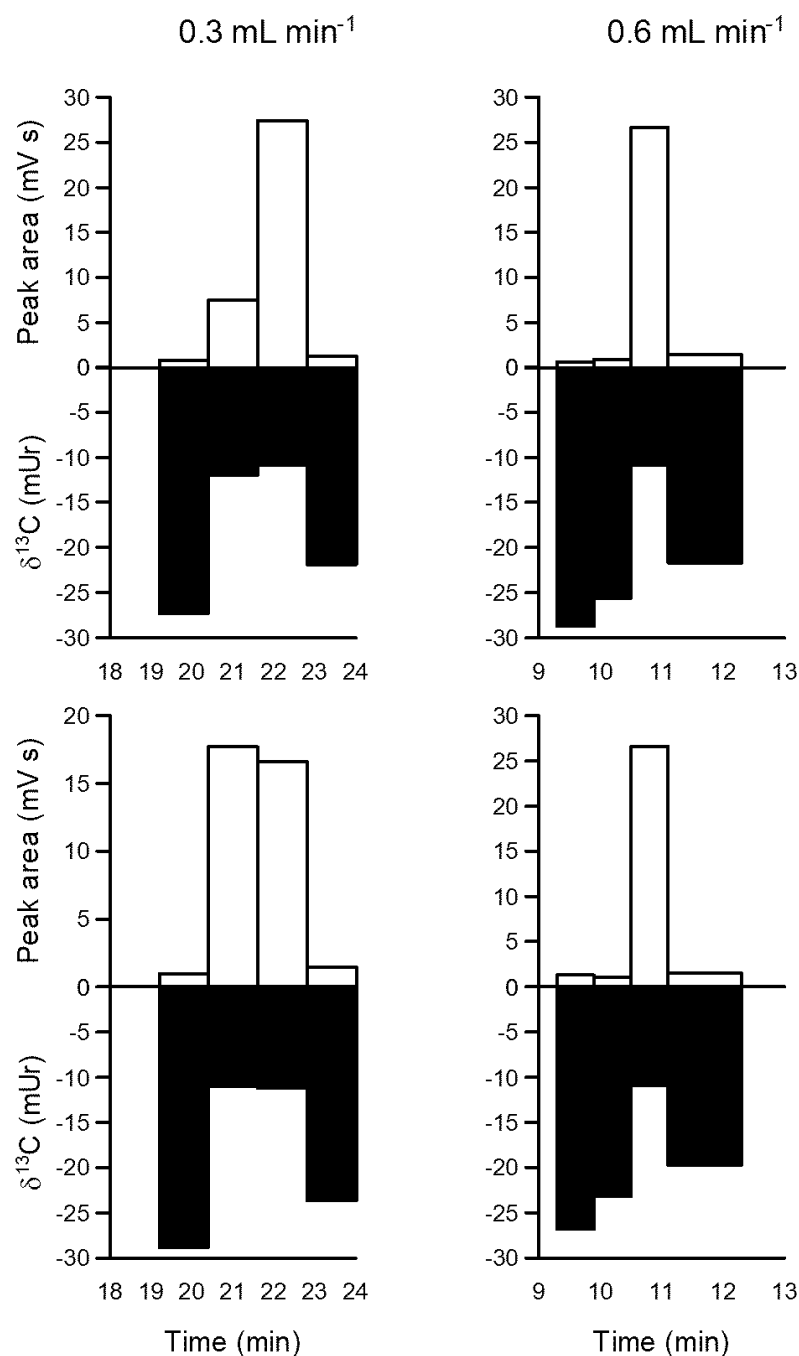


Figure 20. Typical results for glucose collected four times using the 3D printer fraction collector interfaced with an HPLC, and analysed using an elemental analyser coupled to an isotope ratio mass spectrometer (EA–IRMS). Peak areas are proportional to the amount of carbon in the tin capsule, while $\delta^{13}\text{C}$ represents the “delta” notation used to describe the abundance of ^{13}C compared to ^{12}C in a substance (SI units Ur).

6. Conclusions

The fraction collector presented here is a low-cost and easy-to-build solution to prepare compound-specific samples for stable isotope analyses. Compared to most other systems (open-source and commercial), it has the added advantage of concentrating the compounds via evaporation as the collection proceeds.

The 3D printer used here was based on a very popular template (Prusa I3), and so the adaptation of any 3D printer of this family should be as simple as for the one used here.

Most other 3D printer models should be also amenable to use, provided they are equipped with a heat bed.

The fraction collector performed very well without issues for three days uninterrupted, and there is no reason to doubt that it could function uninterrupted for much longer. Compared to the normal operation of a 3D printer, the fraction collector makes much less intensive use of the stepper motors, but equivalent or higher use of the heat bed.

Here, only water was used as a solvent. However, many HPLC methods employ corrosive solutions like acids or bases, or organic solvents like methanol, which is very toxic. If hazardous solvents need to be employed, modifications on the basic design provided here are necessary. An easy solution for volatile solvents is to place the fraction collector inside a fume hood. For corrosive solvents, instead of tin capsules, silver capsules may be necessary (e.g., SC1295, from Sercon).

Although the fraction collector was presented here as a solution for CSIA, it could be similarly employed for other analyses. A very simple adaptation would be, for example, replacement of tin capsules with small Eppendorf vials correctly positioned in the collection tray.

Here, the HPLC system was not equipped with sensors, which demanded that subsequent analysis using EA-IRMS was performed every time, even when the purpose of the operation was not really to measure the compounds, but simply to determine retention time, for example. This is clearly a sub-optimal approach, especially in the early phases of method development. A better solution would be to have the HPLC equipped with a sensor, so that tasks like optimization of retention time determination and peak separation could be performed without requiring EA-IRMS. By employing sensors, “smart” fraction collection could also be attempted, allowing for even further optimization [31].

Peak spreading (Figure 20) and other issues, such as co-elution of different substances at the same time, are known challenges for compound-specific isotope ratio mass spectrometry, which are detailed elsewhere [1]. Despite these problems, fraction collection is a technique with established scope in numerous scientific fields. We hope that the introduction of our 3D-printer-based fraction collector contributes to an even more widespread adoption of the fraction collection approach.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/hardware1010004/s1>, Video S1: FractionCollector.mp4.

Author Contributions: Conceptualization, methodology, software, and validation: M.C.C.; resources and writing: M.C.C. and J.M.O.; project administration and funding acquisition: J.M.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Australian Research Council, ARC LIEF grant number LE130100153, awarded to J.M.O.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Any data can be obtained by emailing the first author.

Acknowledgments: We thank Leslie Christidis and Graham Lancaster, both Southern Cross University, for their continuing support to open-source projects at the university.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Open Scad code for the tube holder.

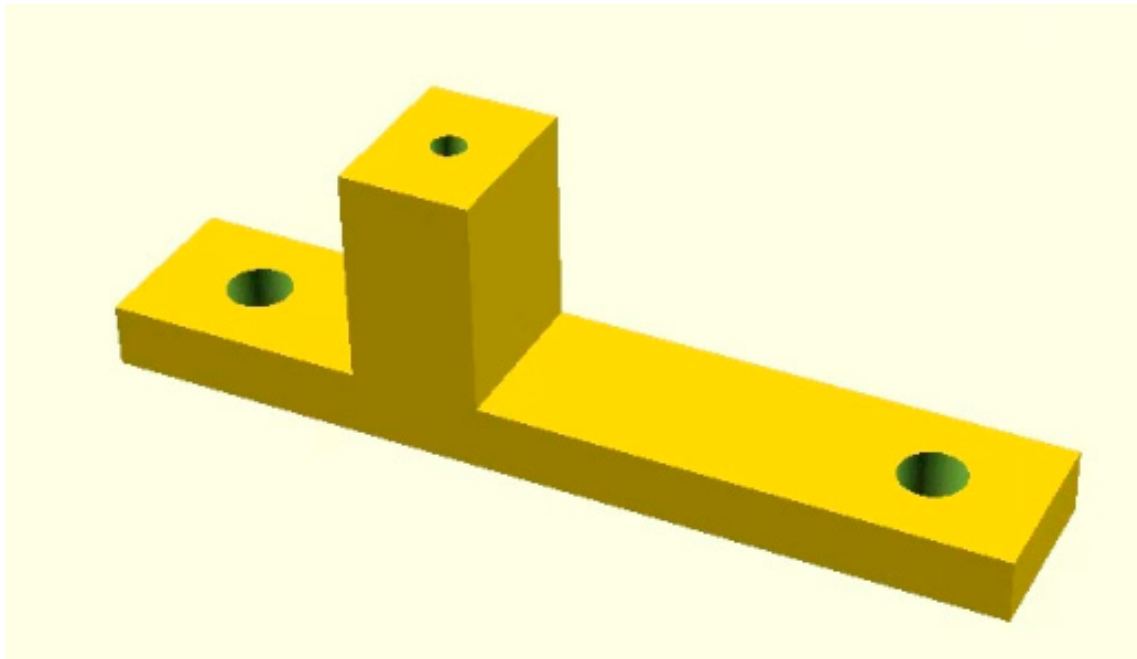


Figure A1. Three-dimensional drawing of tube holder.

```
//lowest part of the block
basex = 42;
basey = 8;
basez = 3;
basetx = 0;
basety = 0;
basetz = 0;

//hole
holer = 1.5;
holez = basez;
holetx = basetx+5;
holety = basety+4;
holetz = basetz;

//neck
neckx = 6;
necky = 8;
neckz = 10;
necktx = 12;
neckty = 0;
necktz = basez;

$fn=64;

difference(){
  translate([basetx,basety,basetz]){cube([basex,basey,basez]);} //base
  #translate([holetx,holety,holetz]){cylinder( holez, holer, holer, false);}
  #translate([holetx+10,holety,holetz]){cylinder( holez, 0.8, 0.8, false);}
  #translate([holetx+32,holety,holetz]){cylinder( holez, holer, holer, false);}
}
```



```

    }

    difference(){
    translate([necktx,neckty,necktz]){cube([neckx,necky,neckz]);} //base
    #translate([holetx+10,holety,holetz]){cylinder( 20,    0.8,    0.8, false);}

    }

```

Appendix B

Example of AutoIt script to enable the 3D printer to work as a fraction collector for HPLC. This script is strictly for the example provided here. Different equipment and software will demand a different script.

```
Opt("WinTitleMatchMode", 1)
```

```
$Hypecontrol = "[NAME:tb_cmd]"
```

```
$PumpFlowControl = "WindowsForms10.EDIT.app.0.282af8c4"
```

```
;important positions
```

```
$zs = 0.0 ;safe Z
```

```
;TIN
```

```
$tinxs = 2.3 ;first tin X
```

```
$tinxe = 5.95 ;last tin X
```

```
$tinys = 0.3 ;first tin Y
```

```
$tinye = 7.05 ;last tin Y
```

```
$tinxn = 7
```

```
$tinyn = 12
```

```
$ztin = -2.5
```

```
;WASTE
```

```
$xw = 0.0 ; waste X
```

```
$yw = 0.0 ;waste Y
```

```
$zw = 16 ; needle inside waste Z
```

```
;2D array for tin positions
```

```
Dim $tinxy[100][2]
```

```
$tin = 0
```

```
;Tin tray
```

```
For $y = 0 To $tinyn - 1
```

```

If Mod($tin, 2) = 0 Then
    For $x = 0 To $tinxn - 1
        $tinxy[$tin][0] = $tinxs + $x * ($tinxe - $tinxs) / ($tinxn - 1)
        $tinxy[$tin][1] = $tinys + $y * ($tinye - $tinys) / ($tinyn - 1)
        $tin = $tin + 1
    Next
Else
    For $x = $tinxn - 1 To 0 Step -1
        $tinxy[$tin][0] = $tinxs + $x * ($tinxe - $tinxs) / ($tinxn - 1)
        $tinxy[$tin][1] = $tinys + $y * ($tinye - $tinys) / ($tinyn - 1)
        $tin = $tin + 1
    Next
EndIf
Next

;sampling routine
$turns = 10
$bunch = 7
$samples = 3

For $turn = 1 To $turns
    ConsoleWrite("Starting turn " & $turn & " " & @HOUR & " " & @MIN & " " & @CRLF)
    SetPump(0.3)
    Sleep(0.5 * 60 * 1000)
    For $sample = 1 To $samples
        $tins = $bunch * ($sample - 1) + 1
        For $tino = $tins To $tins + $bunch - 1
            If Mod($tino - 1, $bunch) = 0 Then
                InjectSample($sample)
                Sleep(15.5 * 1.2 * 60 * 1000)
                LeaveWaste()
            EndIf
            ConsoleWrite("Starting tin " & $tino & " " & @HOUR & " " & @MIN & " " & @CRLF)
            GoToTin($tino)
            Sleep(1.2 * 60 * 1000)
        Next
        GoToWaste()
        Sleep(5 * 60 * 1000) ;Rinsing in between samples
    Next
    SetPump(0.0)
    Sleep(1 * 3600 * 1000) ;Waiting for samples to evaporate the solvent
Next

```

```
;Level 2
```

```
Func GoToTin($sample)
    Move("Z", $zs, 200, 1000, $Hypecontrol) ;go up
    MoveXY($tinxy[$sample - 1][0], $tinxy[$sample - 1][1], 1000, 1000, $Hypecontrol) ;goes to origin
    Move("Z", $ztin, 500, 500, $Hypecontrol) ;go down
EndFunc ;==>GoToTin
```

```
Func GoToWaste()
    Move("Z", $zw + 2, 200, 5000, $Hypecontrol) ;go up
    Move("X", $xw, 200, 5000, $Hypecontrol) ;go left
    Move("Z", $zw, 200, 5000, $Hypecontrol) ;go down
EndFunc ;==>GoToWaste
```

```
Func LeaveWaste()
    Move("X", $xw + 1, 200, 5000, $Hypecontrol) ;go right
EndFunc ;==>LeaveWaste
```

```
;Level 1
```

```
Func Move($direction, $position, $speed, $wait_time, $control)
    ControlSetText("Hype", "", $control, "G1" & $direction & $position & "F" & $speed)
    Sleep(1000)
    ControlSend("Hype", "", $control, "{ENTER}")
    Sleep($wait_time)
EndFunc ;==>Move
```

```
Func MoveXY($position1, $position2, $speed, $wait_time, $control)
    ControlSetText("Hype", "", $control, "G1X" & $position1 & "Y" & $position2 & "F" & $speed)
    Sleep(1000)
    ControlSend("Hype", "", $control, "{ENTER}")
    Sleep($wait_time)
EndFunc ;==>MoveXY
```

```
Func SetPump($Flow)
    WinActivate("HPLC")
    ControlSend("HPLC", "", $PumpFlowControl, "{DEL 50}{BACKSPACE 50}")
    Sleep(1000)
    ControlSend("HPLC", "", $PumpFlowControl, $Flow)
```

```

Sleep(500)
ControlSend("HPLC", "", $PumpFlowControl, "{ENTER}")
EndFunc    ;==>SetPump

Func InjectSample($sample)
    WinActivate("Chromeleon")
    Sleep(1000)
    MouseClick("left", 400, 550)
    Sleep(1000)
    Send("{DEL 50}{BACKSPACE 50}")
    Sleep(1000)
    Send("BA" & $sample & "{ENTER}")
    Sleep(2000)
    MouseClick("left", 400, 620)
EndFunc    ;==>InjectSample

```

References

1. Meier-Augenstein, W. GC and IRMS technology for ^{13}C and ^{15}N analysis on organic compounds and related gases. In *Handbook of Stable Isotope Analytical Techniques*; De Groot, H.J., Ed.; Elsevier: Amsterdam, The Netherlands, 2004; Volume 1, pp. 153–176.
2. Whiteman, J.P.; Elliott Smith, E.A.; Besser, A.C.; Newsome, S.D. A Guide to Using Compound-Specific Stable Isotope Analysis to Study the Fates of Molecules in Organisms and Ecosystems. *Diversity* **2019**, *11*, 8. [\[CrossRef\]](#)
3. Veuger, B.; van Oevelen, D.; Middelburg, J.J. Fate of microbial nitrogen, carbon, hydrolysable amino acids, monosaccharides, and fatty acids in sediment. *Geochim. Cosmochim. Acta* **2012**, *83*, 217–233. [\[CrossRef\]](#)
4. Moerdijk-Poortvliet, T.C.W.; van Breugel, P.; Sabbe, K.; Beauchard, O.; Stal, L.J.; Boschker, H.T.S. Seasonal changes in the biochemical fate of carbon fixed by benthic diatoms in intertidal sediments. *Limnol. Oceanogr.* **2018**, *63*, 550–569. [\[CrossRef\]](#)
5. Moerdijk-Poortvliet, T.C.W.; Stal, L.J.; Boshker, H.T.S. LC/IRMS analysis: A powerful technique to trace carbon flow in microphytobenthic communities in intertidal sediments. *J. Sea Res.* **2014**, *92*, 19–25. [\[CrossRef\]](#)
6. Oakes, J.; Riekenberg, P.; Eyre, B. Assimilation and short-term processing of microphytobenthos nitrogen in intertidal sediments. *Limnol. Oceanogr.* **2020**, *65*, 2377–2389. [\[CrossRef\]](#)
7. Riekenberg, P.; Oakes, J.M.; Eyre, B.D. Uptake of dissolved organic and inorganic nitrogen in microalgae-dominated sediment: Comparing dark and light in situ and ex situ additions of ^{15}N . *Mar. Ecol. Prog. Ser.* **2017**, *571*, 29–42. [\[CrossRef\]](#)
8. Riekenberg, P.; van der Heide, T.; Holthuijsen, S.J.; van der Veer, H.W.; van der Meer, M.T.J. Compound-specific stable isotope analysis of amino acid nitrogen reveals detrital support of microphytobenthos in the Dutch Wadden Sea benthic food web. *Front. Ecol. Evol.* **2022**, *10*, 951047. [\[CrossRef\]](#)
9. Ohkouchi, N.; Chikaraishi, Y.; Close, H.G.; Fry, B.; Larsen, T.; Madigan, D.J.; McCarthy, M.D. Advances in the application of amino acid nitrogen isotopic analysis in ecological and biogeochemical studies. *Org. Geochem.* **2017**, *113*, 150–174. [\[CrossRef\]](#)
10. Pollierer, M.M.; Larsen, T.; Popatov, A.; Bruckner, A.; Heethoff, M.; Dyckmans, J.; Scheu, S. Compound-specific isotope analysis of amino acids as a new tool to uncover trophic chains in soil food webs. *Ecol. Monogr.* **2019**, *89*, e01384. [\[CrossRef\]](#)
11. Hanz, U.; Riekenberg, P.; de Kluijver, A.; van der Meer, M.; Middelburg, J.J.; de Goeij, J.M.; Bart, M.C.; Wurz, E.; Colaço, A.; Duineveld, G.C.A.; et al. The important role of sponges in carbon and nitrogen cycling in a deep-sea biological hotspot. *Funct. Ecol.* **2022**, *36*, 2188–2199. [\[CrossRef\]](#)
12. Oakes, J.; Rysgaard, S.; Glud, R.N.; Eyre, B.D. The transformation and fate of sub-Arctic microphytobenthos carbon revealed through ^{13}C -labeling. *Limnol. Oceanogr.* **2016**, *61*, 2296–2308. [\[CrossRef\]](#)
13. Burian, A.; Nielsen, J.M.; Hansen, T.; Bermudez, R.; Winder, M. The potential of fatty acid isotopes to trace trophic transfer in aquatic food-webs. *Philos. Trans. R. Soc. B* **2020**, *375*, 20190652. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Twining, C.W.; Taipale, S.J.; Ruess, L.; Bec, A.; Martin-Creuzburg, D.; Kainz, M.J. Stable isotopes of fatty acids: Current and future perspectives for advancing trophic ecology. *Philos. Trans. R. Soc. Lond B Biol. Sci.* **2020**, *375*, 20190641. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Broek, T.A.B.; McCarthy, M.D. A new approach to $\delta^{15}\text{N}$ compound-specific amino acid trophic position measurements: Preparative high pressure liquid chromatography technique for purifying underivatized amino acids for stable isotope analysis. *Limnol. Oceanogr. Methods* **2014**, *12*, 840–852. [\[CrossRef\]](#)
16. Phillips, A.A.; Wu, F.; Sessions, A.L. Sulfur isotope analysis of cysteine and methionine via preparatory liquid chromatography and elemental analyzer isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **2020**, *35*, e9007. [\[CrossRef\]](#) [\[PubMed\]](#)

17. Sun, Y.; Ogawa, N.O.; Ishikawa, N.F.; Blattmann, T.M.; Takano, Y.; Ohkouchi, N. Application of a porous graphitic carbon column to carbon and nitrogen isotope analysis of underivatized individual amino acids using high-performance liquid chromatography coupled with elemental analyzer/isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **2023**, *37*, e9602. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Zhang, L.; Lee, W.-m.C.; Kreider-Mueller, A.; Kuhnel, E.; Baca, J.; Ji, C.; Altabet, M.A. High-precision measurement of phenylalanine and glutamic acid $\delta^{15}\text{N}$ by coupling ion-exchange chromatography and purge-and-trap continuous-flow isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **2021**, *35*, e9085. [\[CrossRef\]](#)
19. Yun, H.Y.; Won, E.-J.; Choi, J.; Cho, Y.; Lim, D.-J.; Kim, I.-S.; Shin, K.-H. Stable isotope analysis of residual pesticides via High Performance Liquid Chromatography and Elemental Analyzer–Isotope Ratio Mass Spectrometry. *Molecules* **2022**, *27*, 8587. [\[CrossRef\]](#)
20. Broek, T.A.B.; Walker, B.D.; Andreasen, D.H.; McCarthy, M.D. High-precision measurement of phenylalanine $\delta^{15}\text{N}$ values for environmental samples: A new approach coupling high-pressure liquid chromatography purification and elemental analyzer isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **2013**, *27*, 2327–2337. [\[CrossRef\]](#)
21. Mauve, C.; Bleton, J.; Bathellier, C.; Lelarge-Trouverie, C.; Guérard, F.; Ghashghaie, J.; Tchaplal, A.; Tcherkez, G. Kinetic $^{12}\text{C}/^{13}\text{C}$ isotope fractionation by invertase: Evidence for a small in vitro isotope effect and comparison of two techniques for the isotopic analysis of carbohydrates. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 2499–2506. [\[CrossRef\]](#)
22. Thompson, R.A.; Morello, D.R.; Panicker, S.; Toske, S.G.; Li, L. Carbon and nitrogen isotopic analysis of morphine from opium and heroin samples originating in the four major heroin producing regions. *Drug Test. Anal.* **2022**, *14*, 505–513. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Godin, J.-P.; Fay, L.B.; Hopfgartner, G. Liquid chromatography combined with mass spectrometry for ^{13}C isotopic analysis in life science research. *Mass Spectrom. Rev.* **2007**, *26*, 751–774. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Perini, M.; Bontempo, L. Liquid Chromatography coupled to Isotope Ratio Mass Spectrometry (LC-IRMS): A review. *Trends Anal. Chem.* **2022**, *147*, 116515. [\[CrossRef\]](#)
25. Pearce, J.M. *Open-Source Lab: How to Build Your Own Hardware and Reduce Research Costs*; Elsevier: Waltham, MA, USA, 2014; p. 271.
26. Pearce, J.M. Economic savings for scientific free and open source technology: A review. *HardwareX* **2020**, *8*, e00139. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Bravo-Martinez, J. Open source 3D-printed 1000 μL micropump. *HardwareX* **2018**, *3*, 110–116. [\[CrossRef\]](#)
28. Caputo, M.; Lyles, J.T.; Salazar, M.S.; Quave, C.L. LEGO MINDSTORMS Fraction Collector: A Low-Cost Tool for a Preparative High-Performance Liquid Chromatography System. *Anal. Chem.* **2020**, *92*, 1687–1690. [\[CrossRef\]](#)
29. Ficarro, S.B.; Alexander, W.M.; Tavarres, I.; Marto, J.A. Open source fraction collector/MALDI spotter for proteomics. *HardwareX* **2022**, *11*, e00305. [\[CrossRef\]](#)
30. Boeshaghi, A.S.; Kil, Y.; Min, K.H.; Gehring, J.; Pachter, L. Low-cost, scalable, and automated fluid sampling for fluidics applications. *HardwareX* **2021**, *10*, e00201. [\[CrossRef\]](#)
31. Medina, D.A.V.; Lozada-Blanco, A.; Rodrigues, J.P.G.; Lanças, F.M.; Santos-Neto, A.J. An open-source smart fraction collector for isocratic preparative liquid chromatography. *HardwareX* **2023**, *15*, e00462. [\[CrossRef\]](#)
32. Carvalho, M.C. Automated weighing in the stable isotope lab: When less is more. *MethodsX* **2023**, *10*, 102207. [\[CrossRef\]](#)
33. Baas, S.; Saggiomo, V. Ender3 3D printer kit transformed into open, programmable syringe pump set. *HardwareX* **2021**, *10*, e00219. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Zhang, C.; Wijnen, B.; Pearce, J.M. Open-Source 3-D Platform for Low-Cost Scientific Instrument Ecosystem. *J. Lab. Autom.* **2016**, *21*, 517–525. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Campbell, T.; Jones, J.F.X. Design and implementation of a low cost, modular, adaptable and open-source XYZ positioning system for neurophysiology. *HardwareX* **2020**, *7*, e00098. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Carvalho, M.C. *Practical Laboratory Automation Made Easy with AutoIt*; Wiley VCH: Hoboken, NJ, USA, 2016; p. 290.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.