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An open-source smart fraction collector for isocratic preparative liquid chromatography

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A R T I C L E I N F O

Keywords:
Sample preparation
Microextraction
Lab automatization
Chemist robots
Chromatography

A B S T R A C T

Preparative liquid chromatography is a technique for separating complex samples or isolating pure compounds from complex extracts. It involves eluting samples through a packed column and selectively collecting or isolating the separated bands in a sequence of fractions. Depending on the column length and the sample complexity, a large number of fractions may be obtained, making fraction collection a laborious and time-consuming process. Manual fraction collection is also tedious, error-prone, less reproducible, and susceptible to contamination. Several commercial and lab-made solutions are available for automated fraction collection, but most systems do not synchronize with the instrument detector and collect fractions at fixed volumes or time intervals. We have assembled a low-cost Arduino-based smart fraction collector that can record the signal from the UV–vis detector of the chromatography instrument and enable the automated selective collection of the targeted bands. The system consists of a robot equipped with position sensors and a 3-way solenoid valve that switches the column effluent between the waste or collection positions. By proper programming, an Arduino board records the detector response and actuates the solenoid valve, the position sensors, and the stepper motors to collect the target chromatographic bands.

Specifications table

Hardware name	*Open-Source smart fraction collector*
Subject area	• Chemistry and biochemistry Medical (e.g., pharmaceutical science) Biological sciences (e.g., microbiology and biochemistry) Environmental, and agricultural sciences Educational tools and open-source alternatives to existing infrastructure General
Hardware type	• Sample handling and preparation Automated sample preparation

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2468-0672/

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Hardware name	*Open-Source smart fraction collector*
Closest commercial analog	• FRC-40/FRC-10A; Thermo BS-30 ngc Fraction Collector Fraction Collector Foxy R1; DBS-160 160pcs 5 mL Automatic Fraction Collector; NANBEI digital sample automatic fraction collector
Open-source license	BSD-2
Cost of hardware	USD$ 350
Source file repository	DOI 10.17605/OSF.IO/KX3A2 *https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d*

1. Hardware in context

Preparative liquid chromatography (prep-LC) is a highly valuable technique used for fractionating complex samples and isolating pure compounds from intricate extracts [1]. It serves as a powerful separation tool extensively employed in natural products, organic synthesis, and analytical chemistry to selectively separate target analytes from other molecular entities within the sample. With its critical role in both laboratory and industrial settings, preparative liquid chromatography has emerged as an indispensable process for the separation, extraction, and purification of various pharmaceuticals and pharmaceutical intermediates, including pure enantiomers. Additionally, preparative HPLC holds significant importance in biotechnological applications, particularly in the purification and isolation of peptides and proteins [2].

In brief, prep-LC relies on the percolation of the sample through a preparative packed column (stationary phase), with the aid of a solvent of specific composition (mobile phase). Sample constituent separates according to their differential affinity by the stationary and mobile phases. A comprehensive overview of various modalities of preparative chromatography, their applications in sample treatment, and a discussion on diverse separation mechanisms, available columns, and separative stationary phases, along with a guide for solvent selection and separation modes, can be found in a recent book chapter by Kevin Robards and Danielle Ryan [3].

Preparative liquid chromatography (prep-LC) encompasses various modalities, including low-pressure or gravity-flow column chromatography. This type of chromatography, first introduced by Mikhail Tswett in 1906, relies on the percolation of the sample and mobile phase through a glass column packed with a specific stationary phase, driven by gravity [4]. In this technique, samples are loaded at the top of the column, and the eluent is collected in fixed-volume fractions at the column outlet. Depending on the column's length, dimensions, packing, and sample complexity, the elution process can take several hours, yielding a significant number of fractions. As a result, this procedure can be tedious, labor-intensive, and time-consuming.

Gravity-flow column chromatography, although slow and low in resolution, has paved the way for faster and more efficient separations achieved through high-performance preparative liquid chromatography (prep-HPLC). In prep-HPLC, highly efficient columns are utilized, and the sample, along with the mobile phase, is propelled through the column using high-pressure pumps. This technique harnesses advanced instruments equipped with large-scale injectors, high-pressure reciprocating piston pumping systems, and large-scale spectrometric detectors, enabling real-time monitoring of the separation process. Based on the real-time recorded chromatogram, the analyst can selectively collect or discard the column effluent according to their specific interests.

Kevin Robards and Danielle Ryan recently published a comprehensive review that explores the fundamentals and operational principles of prep-HPLC [5]. In their work, they discuss the distinct rules and priorities involved in preparative HPLC compared to analytical HPLC, with a specific focus on method development and the optimization of crucial parameters such as speed, purity, and yield [5]. Prep-HPLC offers significantly improved speed and efficiency compared to low-pressure LC. However, depending on the sample mass and column dimensions, repetitive prep-HPLC runs may be required. In such cases, the manual collection becomes a bottleneck, making the process tedious, error-prone, less reproducible, and susceptible to contamination.

Automated fraction collectors have become indispensable tools for laboratories involved in preparative liquid chromatography. Nowadays, most manufacturers of HPLC instruments offer automated fraction collectors specifically designed for preparative liquid chromatography. Although available in different formats, commercial fraction collectors are typically cartesian robots, providing at least three degrees of freedom and accommodating formats of up to 96-well plates. They are versatile enough to be used with both low-pressure LC and prep-HPLC. However, commercial fraction collectors can be expensive, making them challenging to acquire for research groups with a limited budget.

In recent years, open-access resources have emerged as profitable alternatives for implementing low-cost robotic and automation technologies in analytical chemistry laboratories worldwide [6,7]. 3D printing makes the fabrication of tailored hardware affordable, while open-source electronics and software facilitate the development of customized circuits and programs for the automated performance of specific laboratory tasks [8]. Chromatography laboratories also have benefited from that "open-source movement," and widely diverse lab-made robots have been introduced in recent years [9]. For example, our research group introduced robotic platforms [10,11] for the automatization of both sorbent- [12] and solvent-based [13,14] miniaturized sample preparation techniques. Those systems have even allowed the automated combination of microextractions with liquid chromatography or mass spectrometry instruments. Moreover, a wide diversity of lab-made robots are currently available in the literature, including autosamplers [15], liquid handling systems [16], robotic arms [17], and hybrid on-flow/robot systems [18], among others.

Fraction collectors have become more accessible to analytical chemists, thanks to the availability of open-source tools and the introduction of cost-efficient prototypes. For instance, Caputo and colleagues introduced the LEGO MINDSTORMS Fraction Collector [19], which is a cartesian robot based on the LEGO MINDSTORMS system's "intelligent brick." It is controlled using the EV3

Programming Software (https://www.youtube.com/watch?v=LzYv31-Kuns). Similarly, Booeshaghi et al. developed a low-cost, modular, and automated fluid sampling device for scalable fluidic applications [20]. Their device is a simple 3D-printed scaffold equipped with a corkscrew rotative rack, actuated by a stepper motor, and controlled by an Arduino Uno board (https://www.youtube.com/watch?v=yG7ECh5GO0o). In another example, Ficarro and colleagues developed an open-source fraction collector/MALDI spotter robot for capillary/nanoLC proteomics [43]. This system features four NEMA-17 stepper motors, belt/pinion actuators, and is controlled by an Arduino (flashed with GRBL 0.8.3c) using g-code commands transmitted by a Raspberry Pi (model 3B) running Raspian Linux. The researchers successfully demonstrated the robot's ability to collect capillary/nanoLC fractions into 96-well plates and spot microliter volumes onto 384-well MALDI plates, showcasing its potential for conducting multidimensional capillary/nanoLC/electrospray- and LC/MALDI-based proteomic experiments.

The previously introduced prototypes offer cost-effective and versatile alternatives for automated fraction collection. However, these systems lack the capability for intelligent fraction collection. Lab-made prototypes of this nature do not establish communication with the chromatography instrument's detectors and instead collect fractions sequentially at fixed intervals or volumes. Consequently, the entire column effluent is collected, resulting in a large number of fractions that require analysis, regrouping, drying, and re-purification. To address this limitation, we present an open-source "smart" fraction collector operated by an Arduino board. This innovative system can monitor the response of the HPLC detector and selectively collect the desired chromatographic peaks, enhancing efficiency and reducing the need for extensive fraction processing.

2. Hardware description

We present a low-cost, open-source fraction collector that can record the HPLC detector signal, thereby enabling automated and selective collection of chromatographic peaks. Unlike previous open-source fraction collectors, our system is specifically programmed to track the UV–Vis detector response. It is equipped with a 3-way solenoid valve that allows for precise direction of the column effluent to either a collection vial or the waste receiver in synchronization with the recorded chromatogram (Fig. 1A).

The setup includes a vertical actuator and a horizontally positioned rotative rack. The vertical actuator smoothly inserts or retrieves the effluent tube into/from the collection flask, and in coordination with the rotative rack, automatically adjusts the position of the vial during the collection of each chromatographic peak. To ensure accurate movement, position sensors are integrated into the system, which control the vertical and horizontal motors. Overseeing the entire operation is an Arduino Uno board, running a dedicated control program developed in the Arduino IDE. This program allows for continuous recording of the detector response and synchronized operation of all components of the fraction collector.

By utilizing an HPLC instrument with a UV–Vis detector featuring external recorder outputs, our Arduino code effectively monitors the chromatograms by reading the detector response through an RCA cable connected between the detector output recorder and an analog port of the Arduino.

Fig. 1B presents a schematic representation of the fluidic connection between the fraction collector and an HPLC instrument. The column effluent is directed to the UV–vis detector, enabling the real-time recording of eluting bands. The output hose from the detector is connected to the central part of the 3-way solenoid valve in the fraction collector. By default, the valve directs the detector effluent to the waste reservoir. However, when the fraction collector program detects a chromatographic peak, the valve switches, enabling the collection port, and the vertical actuator inserts the effluent tube into the collection flask. The UV–vis detector signal continues to be recorded, and once the peak has fully eluted, the solenoid valve switches back to the waste position. Subsequently, the vertical actuator retrieves the effluent tube, and the vial rack rotates to the next position.

To summarize, we have designed and assembled a cost-efficient, affordable, and easily implementable smart fraction collector. This

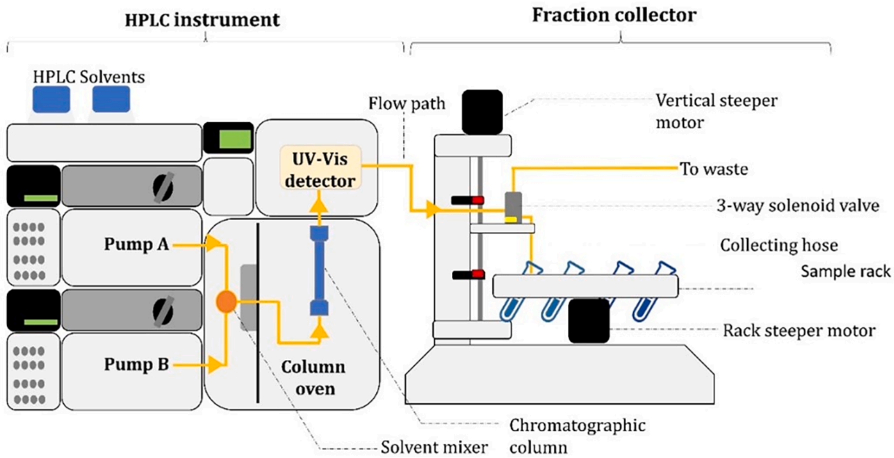

Fig. 1. Brief representation of the developed prototype. **A)** A general picture of systems, **B)** a Schematic representation of the fluidic connection of the developed fraction collector with an HPLC instrument.

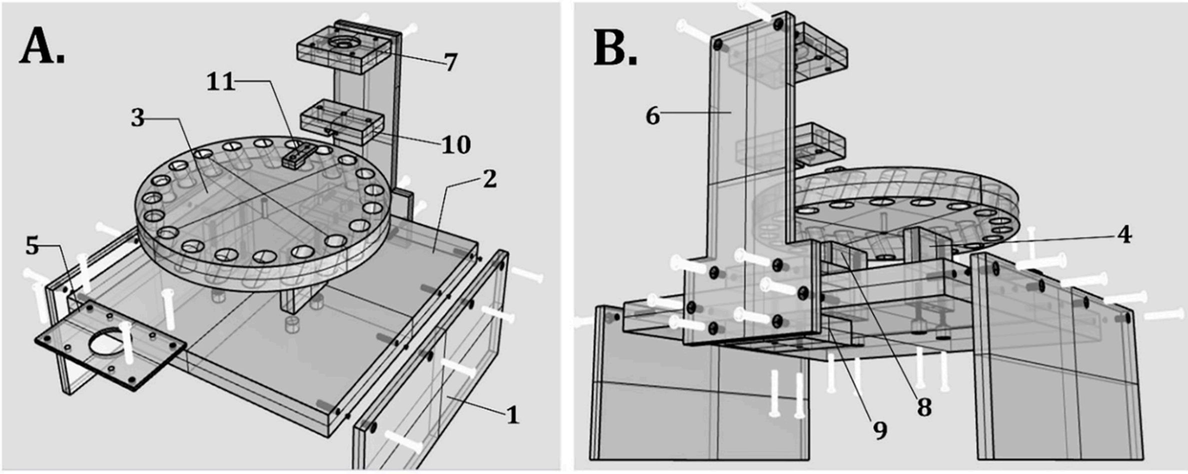

Fig. 2. Overview of the POM structure assembly. **A)** Anterior and lateral view; **B)** lateral and posterior view.

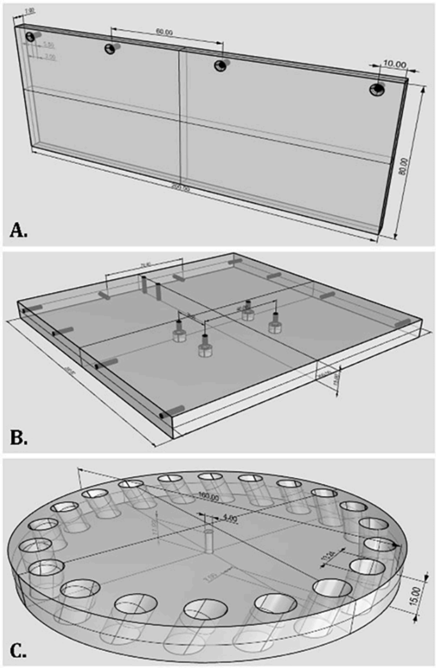

Fig. 3. Main parts of the horizontal base frame. **A)** Vertical plate 1; **B)** Horizontal plate 2; and **C)** Circular rack (3) (measures in mm).

system can compete with commercial versions and provide the advantage of being easily adaptable and customized to specific applications.

3. Design files

Design file name	File type	Open-source license	Location of the file
Individual control of each function	*Arduino firmware package*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
Continuous operation coupled to HPLC	*Arduino firmware package*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
Complete prototype	*CAD (.Step)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*

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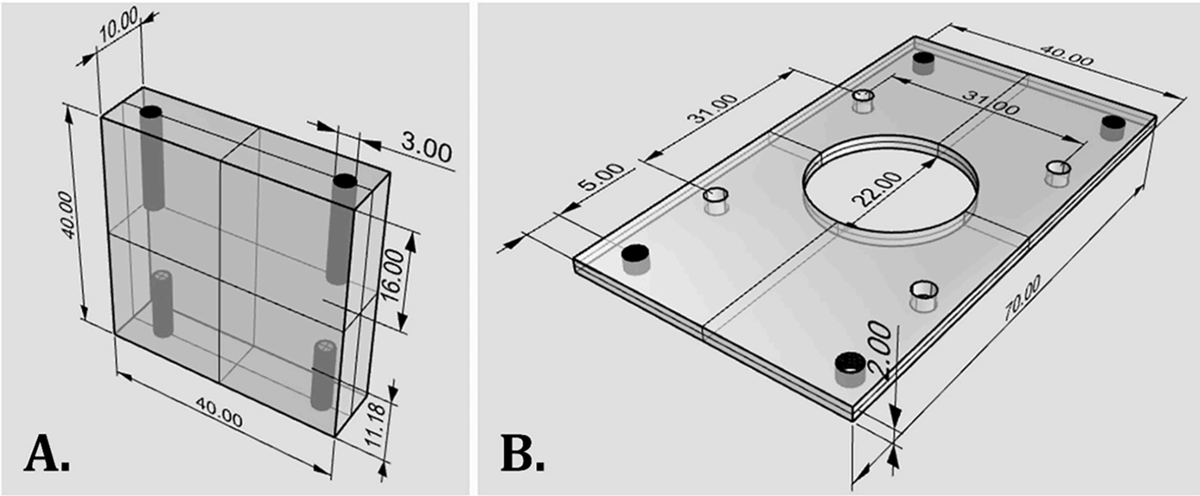

Fig. 4. Pieces for the attachment of the rack actuating motor to the horizontal base. **A)** vertical plates 4; **B)** motor holder plate 5.

(*continued*)

Design file name	**File type**	**Open-source license**	**Location of the file**
(1) Vertical POM plate	*CAD (.Step)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
(2) horizontal square POM plate	*CAD (.Step)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
(3) 20 positions Rack	*CAD (.Step)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
(4) Attaching motor rack plate	*CAD (.Step)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
(5) Motor X holding plate	*CAD (.Step)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
(6) T shaped vertical holder	*CAD (.Step)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
(7) Motor Z housing plate	*CAD (.Step)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
(8) Bottom plate of the vertical actuator	*CAD (.Step)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
(9) Lower supporting-attachment plate	*CAD (.Step)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
(10) Pushing Block	*CAD (.Step)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
(11) Collection tubing holder	*CAD (.Step)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
Breadboard Circuit	*Fritzing (.fzz)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*
Schematic circuit diagram	*Fritzing (.fzz)*	*CC BY 5.0*	*DOI https://doi.org/10.17605/OSF.IO/KX3A2 (https://osf.io/kx3a2/?view_only=ac67498e7abc4f24be764f5d13ee715d)*

4. Bill of materials summary

Designator	**Component**	**#**	**$/unit**	**Total cost**	**Source of materials**	**Material type**
3.1 Mechanical and structural project (Numerical designators in Figs. 2–8)						
3.1.1 Plastic parts						
1.	POM rectangular plates of 200x70x7 mm	2	R$ 60	R$ 120	Lab-made - machining	-Polymer
2.	Square POM plate of 200x200x15 mm	1	R$ 60	R$ 60	Lab-made - machining	-Polymer

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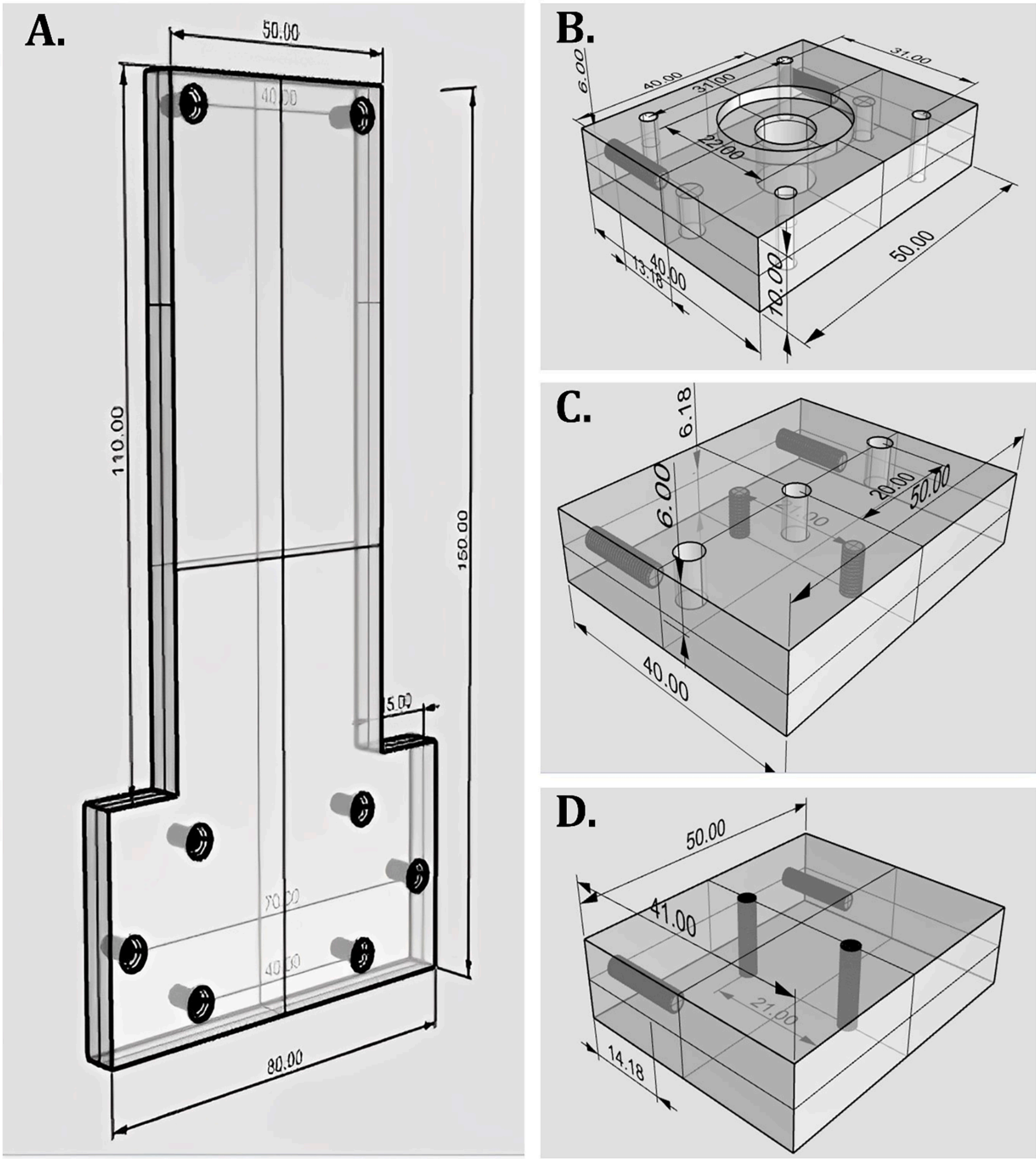

Fig. 5. Pieces of the vertical actuator holder. **A)** Vertical T-shaped holder; **B)**. Vertical actuator motor holding plate 7; **C)** bottom plate of the vertical actuator (8); and **D)** Lower supporting/attachment plate 9.

(*continued*)

Designator	Component	#	$/unit	Total cost	Source of materials	Material type
3.	Circular 20-position rack of polyacetal r = 8.0 cm	1	R$ 60	R$ 60	Lab-made - machining	-Polymer
4.	POM plates of 40x40x10 mm	2	R$ 20	R$ 40	Lab-made - machining	-Polymer
5.	Motor holder plate 70x40x2.0 mm	1	R$ 20	R$ 20	Lab-made - machining	-Metal or polymer

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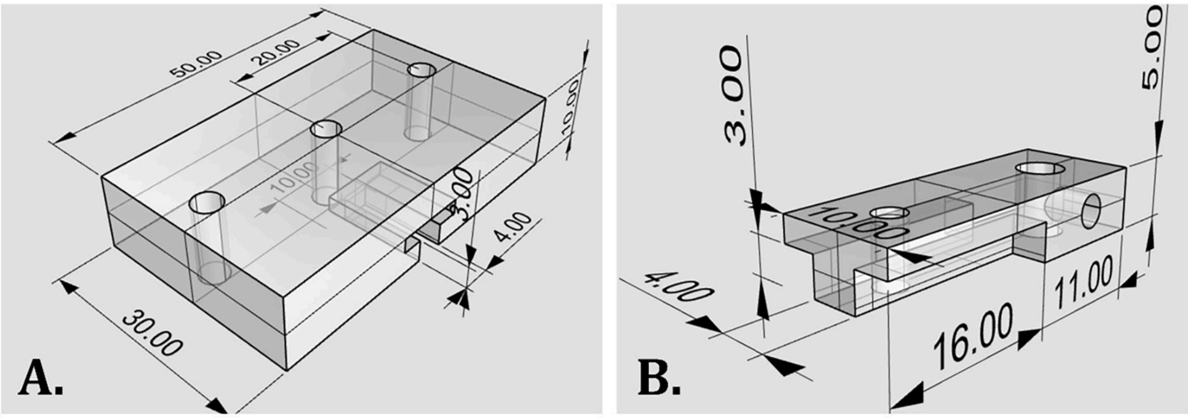

Fig. 6. Pieces of the collecting tube driver. **A)** Pucher block of the vertical linear actuator; **B)** collecting tube holder (11).

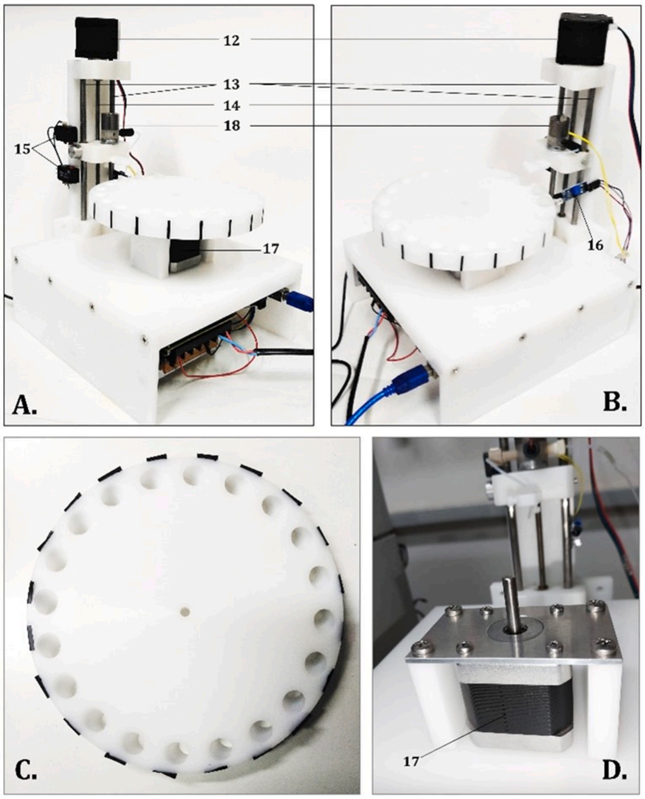

Fig. 7. General overview of the complete prototype of the fraction collector. **A)** right view of the prototype; **B)** left view of the prototype. **C)** inferior view of the rack, showcasing the central hole for shaft motor coupling; **D)** view of the attachment of the actuating rack stepper motor.

(*continued*)

Designator	Component	#	$/unit	Total cost	Source of materials	Material type
6.	T-shaped polyacetal vertical holder	1	R$ 40	R$ 40	Lab-made - machining	-Polymer
7.	Motor housing plate	1	R$ 20	R$ 20	Lab-made - machining	Polymer
8.	Bottom plate of the vertical actuator	1	R$ 20	R$ 20	Lab-made - machining	Polymer
9.	Lower supporting/attachment plate	1	R$ 20	R$ 20	Lab-made - machining	Polymer
10.	pusher block	1	R$ 20	R$ 20	Lab-made - machining	Polymer
11.	Collecting tube holder	1	R$ 20	R$ 20	Lab-made - machining	Polymer

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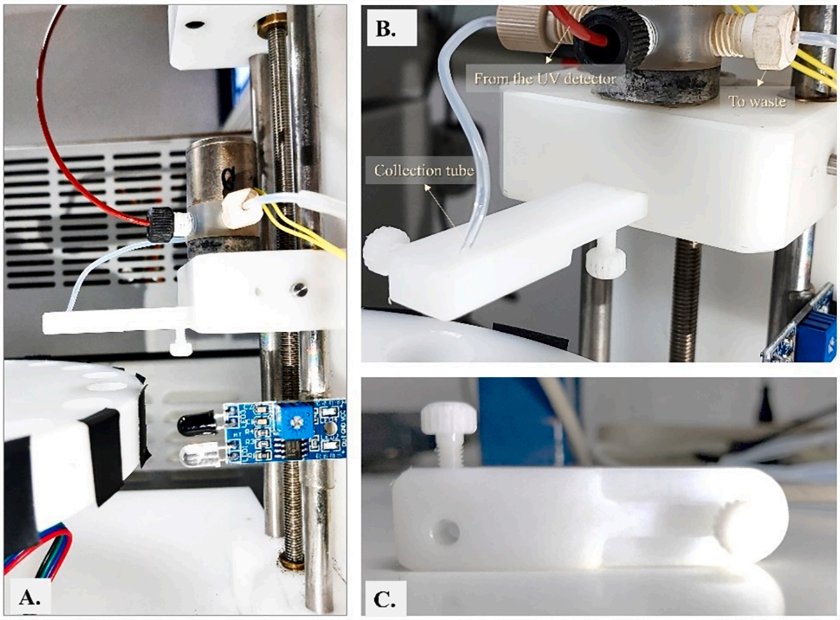

Fig. 8. Images depicting the solenoid valve and collection tube holder system. **A)** Illustration showing the positioning and connection of the solenoid valve. **B)** Image demonstrating the fluidic connection of the solenoid valve and attachment of the collection tube holder and the pusher block. C) Close-up detail of the bottom part of the collection tube holder.

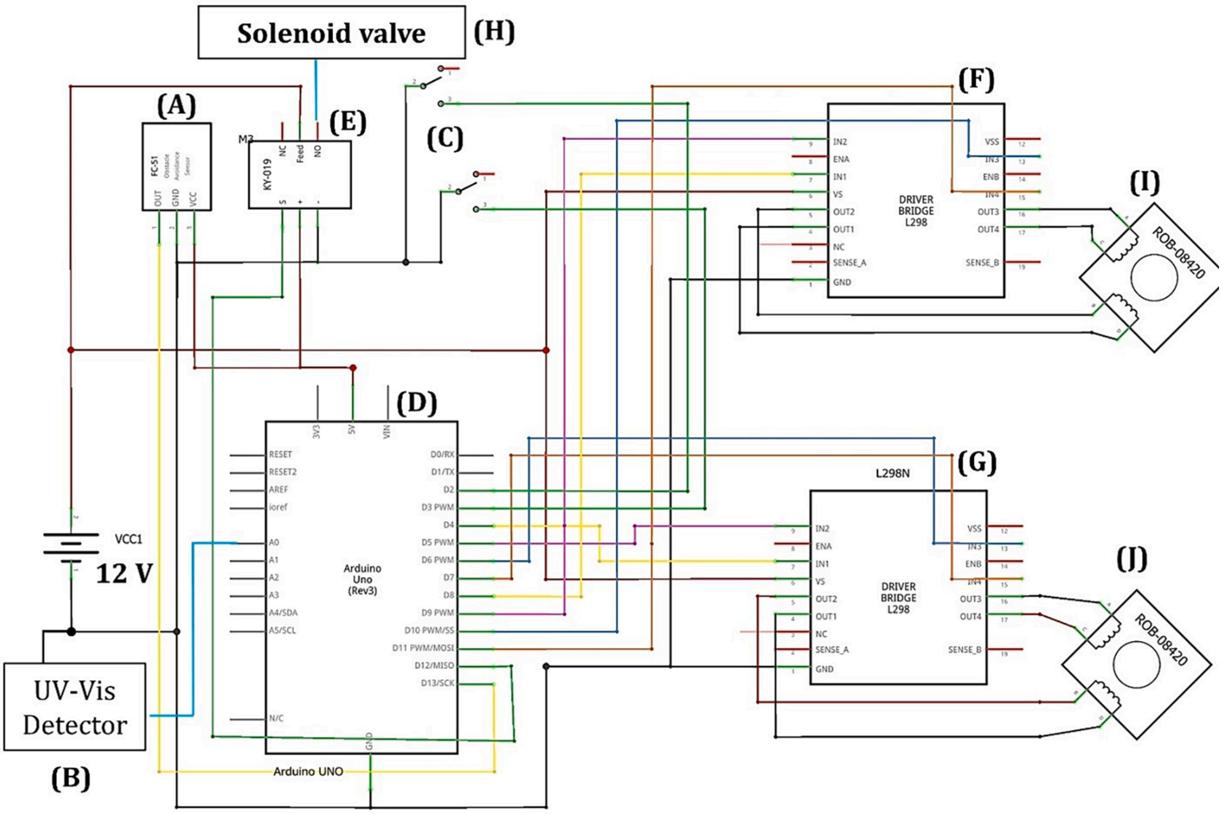

Fig. 9. Schematic of the Electronic Control Setup for the Developed Prototype.

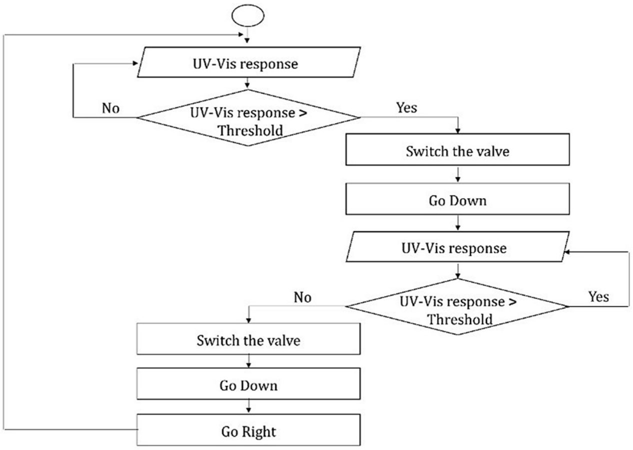

Fig. 10. Flowchart outlining the underlying principles for writing Arduino code that enables the operation of the fraction collector.

(*continued*)

Designator	Component	#	$/unit	Total cost	Source of materials	Material type
3.1.2 Additional mechanical components						
12, 17	NEMA 17 stepper motor	2	R$ 120	R$ 240	Ca & Ma – São Carlos	
13	Linear guides,	2	R$ 20	R$ 40	Aço Mix Metais -Sao Carlos	-Metal
14	endless screw	1	R$ 20	R$ 20	Aço Mix Metais -Sao Carlos	-Metal
18.	Integral Luer look (PTFE) 3-way solenoid valve (161 T031) (17)	1	USD$ 133	USD$ 133	https://www.nresearch.com/	Metal and polymer
3.2 Electronic project (alphabetic designators in Fig. 9)						
–	12 V 10 A DC Switching Power Supply	1	R$ 130	R$ 130	Ca & Ma – São Carlos	–
a. (15)	Infrared reflective sensor, with a LM393 voltage comparator	1	R$ 40	R$ 40	Ca & Ma – São Carlos	–
b.	RCA Cable for connection Arduino-UV–vis detector	1	R$ 10	R$ 10	Ca & Ma – São Carlos	–
c. (14)	End-stop switches KW11-3Z-2 3 T	2	R$ 10	R$ 20	Ca & Ma – São Carlos	–
d.	Arduino Uno Board	1	R$ 150	R$ 150	Ca & Ma – São Carlos	–
e.	Songle® SRD-05VDC-SL-C relay	1	R$ 30	R$ 30	Ca & Ma – São Carlos	–
f,g.	L298N double-H	2	R$ 40	R$ 40	Ca & Ma – São Carlos	–

5. Build instructions

5.1. Mechanical and structural project

Mechanically, the prototype is constructed with a polyacetal (POM) frame that offers robust structural support. It incorporates a linear actuator for precise positioning of the tubing collection, stepper motors for the movement of the rack and actuator, and a solenoid valve that facilitates the redirection of the detector effluent to either the collection tubes or the waste reservoir.

Fig. 2 presents an overview of the complete POM structure and the assembly of its various components. In our case, the structural frame was assembled from POM pieces manufactured using Computer Numerical Control (CNC) machining. However, these pieces can be replicated using alternative materials or technologies, such as 3D printing. To assist with this, we have included Computer-Aided Design (CAD) files of each component in the supplementary file repository (Section 3. Design files), along with a brief description of the assembly process that follows.

The POM structure consists of a horizontal base and a vertical actuator support. The horizontal base is created by assembling two vertical plates measuring 200x80x7.0 mm (1) and securing them to a horizontal square plate measuring 200x200x15 mm (2) using M3 × 15 mm screws. This base provides a sturdy foundation for the circular rack (3) that accommodates the 20 collection vials. The circular rack was manufactured from a POM piece with a diameter of 160 mm and a thickness of 15 mm. It incorporates evenly spaced circular holes with a diameter of 13 mm, positioned at intervals of 1.8° each. Additionally, these holes are inclined at an angle of 25° to ensure that, during the collection process, the effluent tubing remains clear of the vial walls. This well-thought-out design promotes efficient volume uptake and prevents the transfer of hanging drops between the vials. A hollow with a diameter of 0.5 cm and a depth of 1.5 cm was etched at the center of the bottom part of the rack to facilitate its secure connection to the stepper motor shaft through pressure (Fig. 3).

The rack actuating motor is secured to the horizontal base using a pair of vertical plates measuring 4.0x4.0x10 mm (4). These plates are bolted to the horizontal base using M3x20 mm screws and attached to a motor holder plate measuring 70x40x2.0 mm (5). In our case, we machined the motor holder plate from aluminum, but it can also be fabricated from a polymeric material through 3D printing. The motor holder plate features a central hole with a diameter of 22 mm, and four holes with a diameter of 3 mm spaced 31 mm between, for motor mounting (Fig. 4).

The vertical actuator support consists of a POM T-shaped holder (6) equipped with a vertical actuator motor housing plate 7 and attached to the horizontal base using two additional POM plates 8 and 9. The T-shaped vertical holder is machined from a 150x80x7 mm piece, with cutouts made at 15 mm and 40 mm from the edge (Fig. 5A). It is secured to the horizontal base using a pair of M3x25 mm screws.

The motor housing plate is connected to the T-shaped holder using two M3x25 mm screws. This plate measures 40x50x10 mm and features two concentric holes with diameters of 22 mm and 10 mm for motor and screw adjustment, respectively. The motor is attached to the holder through four 3.0 mm diameter holes, spaced 3.1 mm apart. Additionally, the plate has two 3.0 mm diameter holes that are 10 mm deep on the inner side to accommodate metallic linear guides (Fig. 5B).

To complete the vertical actuator, an additional POM plate is installed at the bottom (8). This plate features three 3.0 mm diameter holes designed to accommodate the linear guides and the endless screw of the vertical actuator (Fig. 5C). Additionally, it includes two horizontal M3 holes, each with a depth of 15 mm, to facilitate attachment to the T-shaped holder. Moreover, there are two vertical M3 holes provided for attaching the vertical support to the horizontal base.

A lower supporting POM plate is utilized to secure the attachment of the vertical support to the horizontal base (9). This plate is positioned underneath the square horizontal plate and is connected to the T-shaped holder, the horizontal base, and the bottom plate of the vertical actuator using M3 screws, as depicted in Fig. 5D.

The kit of plastic components is complemented by a pusher block (10) and a collecting tube holder (11), both driven by the vertical actuator. The pusher block is a 30x50x10 mm piece with two 3.0 mm diameter holes to accommodate the linear guides and a central 4.0 mm hole for attaching the nut of the vertical actuator's endless screw. It enables the vertical displacement of the collection tube (Fig. 6A). Additionally, to adjust the horizontal positioning of the collection tube, the pusher block features a T-shaped cavity where the collection tube holder can be adjusted using an M2x10 mm screw. The collection tube holder is a T-shaped component measuring 27x10x5 mm, featuring a front hole with a 3 mm diameter through which the collection tube extends. It is secured in place using an M2x10 mm screw (Fig. 6B).

Once the POM frame was finished, the structure was completed by adding additional mechanical parts. Fig. 7 provides a general overview of the complete prototype of the fraction collector, showcasing all the additional mechanical components. The vertical linear actuator was assembled by adding a NEMA 17 stepper motor (12), two 5.0 mm diameter and 120 mm length linear guides (13), and an M5 endless screw equipped with a 5 mm to 5 mm bore rigid shaft coupling (14). The linear guides and the endless screw are coupled to the motor holding plate, protruding through the pusher block and resting on the bottom plate of the linear actuator. Moreover, a pair of end-stop switches (15) was incorporated to control the displacement of the pusher block and the collection tube, while an infrared sensor (16) was added to regulate the rack positioning. These components were integrated into the lateral part of the T-shaped holder.

This rack is actuated by an additional NEMA 17 stepper motor (17) attached by pressure in the central hole of the inferior part of the rack. To enable accurate detection of the collection vial's position just below the effluent tubing, black frames have been painted on the edge of the rack. Initially, black frames with a thickness of 3 mm were tested. However, to ensure the proper functioning of the sensor, frames with a thickness of 1.0 cm were implemented, and the positioning was adjusted through programming.

To facilitate the effluent collection, a 3-way solenoid valve (18) was installed on a pusher block, which is driven by the vertical actuator. The solenoid valve's central part was connected to the UV–Vis detector effluent tube, the left port to the collection tube, and the right port to the waste conducting tube. The collection tube is secured in place by inserting it into a 4 mm diameter etched hollow and tightening it with an M2 × 1.0 cm POM screw (28) at the collection tube holder. Similarly, the collection tube holder is attached to the pusher block using an additional M2 × 1.0 cm POM screw (Fig. 8).

5.2. Electronic project

A copy of the breadboard and schematic design circuits is available in the supplementary repository files (Section 3. Design files). Fig. 6 illustrates the electronic configuration of the prototype control system, and a brief description of the circuit assembly is provided in the following sequence.

The entire circuitry was powered by a 12 V DC Switching Power Supply (RS-100–12, Mean Well, Taipé, Taiwan). An Arduino Uno board (d) captured the UV detector signal (b) and actuates two NEMA 17 stepper motors (i,j) driven by two L298N double-H bridge drivers (f, j). Additionally, the circuit included an infrared reflective sensor equipped with an LM393 voltage comparator (a), two end-stop sensors SWITCH KW11-3Z-2 3 T (c), and a 3-way solenoid valve (h) controlled via a Songle® SRD-05VDC-SL-C relay module (e).

The UV detector response was monitored by connecting the two terminals of the RCD cable to the A0 and GND pins of the Arduino. For carousel positioning surveillance, the IR sensor module was powered by connecting it to the 5 V + and GND pins, and its output was recorded through the D13 pin of the Arduino. The control of the carousel stepper motor involved connecting the in1, in2, in3, and in4 terminals of the first L298N driver to the D4, D5, D6, and D7 pins of the Arduino, respectively. Similarly, for controlling the vertical actuator stepper motor, the in1, in2, in3, and in4 terminals of the second L298N driver were connected to the D8, D9, D10, and D11 pins of the Arduino. To limit the range of motion for the vertical actuator, the common pins of the end-stop switches were connected to GND, while the Normally Open (NO) pins were connected to the Arduino ports D2 (lower switch) and D3 (upper switch). To control the effluent collection, the solenoid valve was actuated using the Normally Open (NO) port of the relay module, with the common port connected to the 12 V supply. Once powered (Vcc and GND), the IN port of the relay module was linked to the D12 pin of the Arduino.

5.3. Software and control

5.3.1. Arduino sketch

Two Arduino sketches were developed to control the autosampler: i) one for individual control and execution of each autosampler function, and ii) another with integrated functions in the void loop for regular operation, connected to the HPLC. A copy of both Arduino sketches can be found in the supplementary repository files (See section 3. Design files). In the first case, the void loop remains empty, and specific functions such as valve switching, plate spinning, or collection hose movement are executed through serial communication using the void serialEvent() command. This code is primarily used for specific movements or tasks, particularly for adjustment purposes when needed. In the second sketch, the collector operates in continuous mode, where the main code is placed within the void loop sketch. This enables the collector to continuously monitor the detector and collect peaks. This code is used for the regular operation of the fraction collector.

Fig. 7 illustrates a flowchart outlining the simplified principles underlying the second written Arduino code, enabling the routine operation of the fraction collector. In summary, the software functions by defining integer variables to read the state of position sensors and record the HPLC detector signal. Specific functions for stepper motor movements are also established. Arduino acquires two sets of ten analog measurements of the detector output, with a 200 ms interval between them, and calculates their mean values. A peak is detected when these mean values exceed the predefined threshold. Arduino then switches the solenoid valve to the collection position and moves the vertical carousel and vertical actuator to position the effluent tube inside the first collection vial. While the sum of the two mean values remains above twice the threshold, the effluent continues to be collected in vial 1. Once this condition is no longer met, the peak concludes. Arduino switches the solenoid valve to the waste position, lifts the effluent tube, and moves the carousel to the next collection position. Two new sets of ten analog measurements of the detector output are obtained, and their mean values are calculated. If either of the mean values falls below the preestablished threshold, a valley is recorded, and the effluent is directed to the waste receiver. When this final condition is no longer met, the program returns to the beginning of the loop, checks if a new peak has been detected, and proceeds with its collection.

6. Operation instructions

1. Turn on the main switch.
2. Download the Arduino sketch and open it on the HPLC computer.
3. Plug the USB cable from the Arduino into a USB port of the computer.
4. Compile and load the sketch into the Arduino board.
5. Plug the RCA cable into the detector recording output (Fig. 11a).
6. Turn on your HPLC system for normal operation, including the detector lamp.
7. Set the detector parameters according to the previous calibration. We use a Shimadzu SPD-20A UV–Vis detector. In this case, the best responses were obtained by adjusting the auxiliary range to 0.5 AU/V and the record range to 0.001 (Fig. 11b). Figure XC shows an example of a chromatogram reconstructed from the data acquired by Arduino.
8. You can monitor the detector lectures and the fraction collector state through the Arduino serial monitor.
9. Set your threshold for peak collection in the Arduino code (in the threshold variable at the beginning of the code).
10. Calculate and set your delay collection time in the code (CollectionDelay variable at the begging of the code). This should be calculated based on the dimensions of the tubing connecting the collector to the detector output, the collection tube, and the flow rate used. For example, using a tube with an inner diameter of 0.130 mm and a total length (connection + collection) of 400 mm, the dead volume of this part of the system is 1.7 μL. Operating at a flow rate of 200 μL/min, the effluent peak will reach the tip of the collection tube with a delay of 340 ms.
11. Inject a sample and monitor the lectures in the Arduino serial monitor. Set your threshold in the Arduino sketch according to the required detectability.
12. Program your HPLC method and inject your samples.

7. Validation and characterization

The functionality of the developed prototype was evaluated by connecting it to a 20A Prominence HPLC instrument (Shimadzu, Japan). The HPLC instrument consisted of two LC-20AD pumps, a SIL-20A autosampler, a CTO-20A column oven, and a UV/Vis SPD detector 20A. Separation was conducted using a Kinetex Core-Shell analytical column (150 mm × 3.0 mm i.d., 2.7 μm particle size) in isocratic elution mode. The mobile phase employed was a mixture of water and methanol (60:40), and the flow rate was set to 0.25 mL/min. Model samples were prepared using aqueous solutions of anthracene (1.0 mg/L) and a mixture of five parabens (methylparaben, ethylparaben, propylparaben, butylparaben, and benzyl paraben) at a concentration of 5 mg/L. For analysis, 5 μL of the sample was injected.

The UV/Vis SPD detector 20A is equipped with two signal output connectors: [RECORDER] and [INTEGRATOR] (integrating data processor) connectors. These connectors are used to record the detector signal using analog recorders, which were the primary method of capturing chromatograms before the emergence of modern HPLC software. According to the manufacturer's guidelines, the [INTEGRATOR] connector is used to connect a variable range recorder, while the [RECORDER] connector is designed for a fixed range recorder. The fixed range recorder allows adjustment of the recording range by utilizing the instrument's recorder range setting functions ([AUX RANGE] and [RANGE]). It is important to note that these functions also impact the recording of the signal detector for

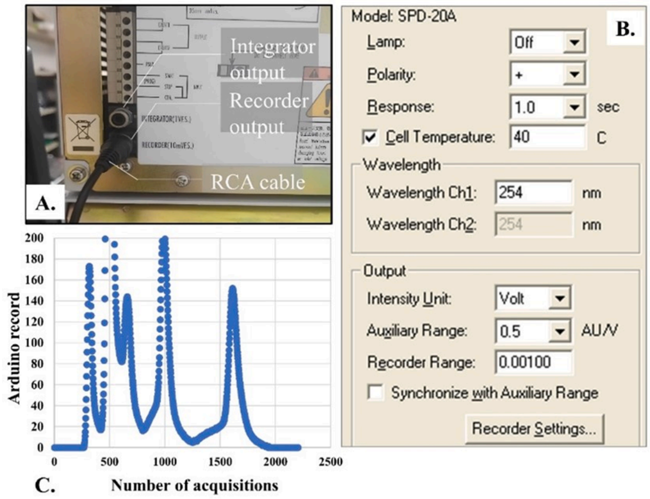

Fig. 11. Setting parameters for recording the UV–vis signal using Arduino. A) Connection of the RCA cable to the detector recorder output; B) parameter of the detector acquisition.; C) example of one chromatogram, reconstructed from the signal recorded by Arduino.

our specific application using Arduino.

The [AUX RANGE] function is responsible for assigning different units of absorbance to measured voltage and has a significant impact on the appearance of the chromatogram in the HPLC software. For instance, when injecting the same solution of anthracene, we observed a peak maximum high in the software at 260 mV, 129 mV, and 52 mV when the [AUX RANGE] values were set to 0.5 UA/V, 1.0 UA/V, and 2.5 UA/V, respectively. Thus, our objective is to achieve maximum detectability in the software instrument.

On the other hand, the [RANGE] function assigns different units of absorbance at the full scale (AUFS) and directly affects the output voltage of the [INTEGRATOR] and [RECORDER] connectors. To illustrate this, when injecting the same anthracene solution and recording the voltage output from the [RECORDER] using a multimeter, we observed peak voltages of 1.0 V, 250 mV, and 125 mV when the [RANGE] values were set to 0.001 AUFS, 0.005 AUFS, and 0.01 AUFS, respectively. Therefore, we set the [RANGE] to the minimum acceptable value for the instrument, which is 0.0001 AUFS, to ensure maximum voltage outputs.

Significant differences were observed in the voltages measured at the [INTEGRATOR] and [RECORDER] outputs. When injecting the anthracene solution, a voltage of 250 mV was measured at the maximum peak height in the [INTEGRATOR] output. In contrast, the [RECORDER] output registered a voltage of 1.0 V at approximately 25% of the maximum peak height, which remained constant while the peak intensity exceeded that level. Therefore, our objective is to achieve better resolution at the beginning and end of the chromatographic peak. This can be accomplished by utilizing the [RECORDER] output to monitor the detector signal using the Arduino UNO board.

The values set for [AUX RANGE] and [RANGE] also have an impact on the values recorded through the analog port of the Arduino board. For instance, if the [AUX RANGE] is set to 4.0 UA/V or the [RANGE] is set to 0.1, the analog reading of the [RECORDED] output using the Arduino port will be zero, regardless of the intensity of the chromatographic peak observed in the instrument software.

By setting the [AUX RANGE] and [RANGE] to 0.5 UA/V and 0.0001 AUFS, respectively, we assessed the Arduino's capability to reproduce the chromatogram using the signal from the UV–Vis detector recorded at the Arduino analog port. Initially, a small Arduino code was developed to read the A0 port and print the measured values on the serial port every 100 ms. We utilized a processing code, available at https://mundoprojetado.com.br/arduino-como-salvar-dados-em-txt/, to save the data received from the Arduino serial port into a.txt file.

Fig. 8c illustrates the chromatogram obtained by plotting the data recorded through the Arduino's analog port after injecting 5 μL of a mixture of five parabens (methylparaben, ethylparaben, propylparaben, butylparaben, and benzyl paraben) at a concentration of 5 mg/L. In this particular case, the maximum value recorded by the Arduino was 205, resulting in the ethylparaben (2) and propylparaben (4) peaks being truncated in the reconstructed chromatogram. It is important to note that the Arduino UNO has a resolution of 10 bits, and its analog reads are interpreted by the Arduino as values between 0 and 1023 (2^{10} digital words). As by default reference voltage of the Arduino analog port is 5.0 V, the recorded value of 205 is the result of the analog-to-digital conversion (ADC) when the [RECORDER] output provides its maximum response (1.0 V), following the expression (1):

$$ADC = \frac{V_{in}*1024}{V_{ref}} \tag{1}$$

In Equation (1), ADC represents the value recorded in the Arduino serial port (205), V_{in} represents the input voltage through the analog pin (1.0 V), and V_{ref} represents the reference voltage of the analog pin (5.0 V).

The limited range of values recorded by the Arduino (0–205) can potentially hinder the sensitivity of the fraction collector, thus imposing limitations on its applicability. To address this issue, we incorporated the command "analogReference(INTERNAL)" into the void setup of the code, enabling the use of the internal reference of the Arduino. This adjustment ensures that the analog ports of the

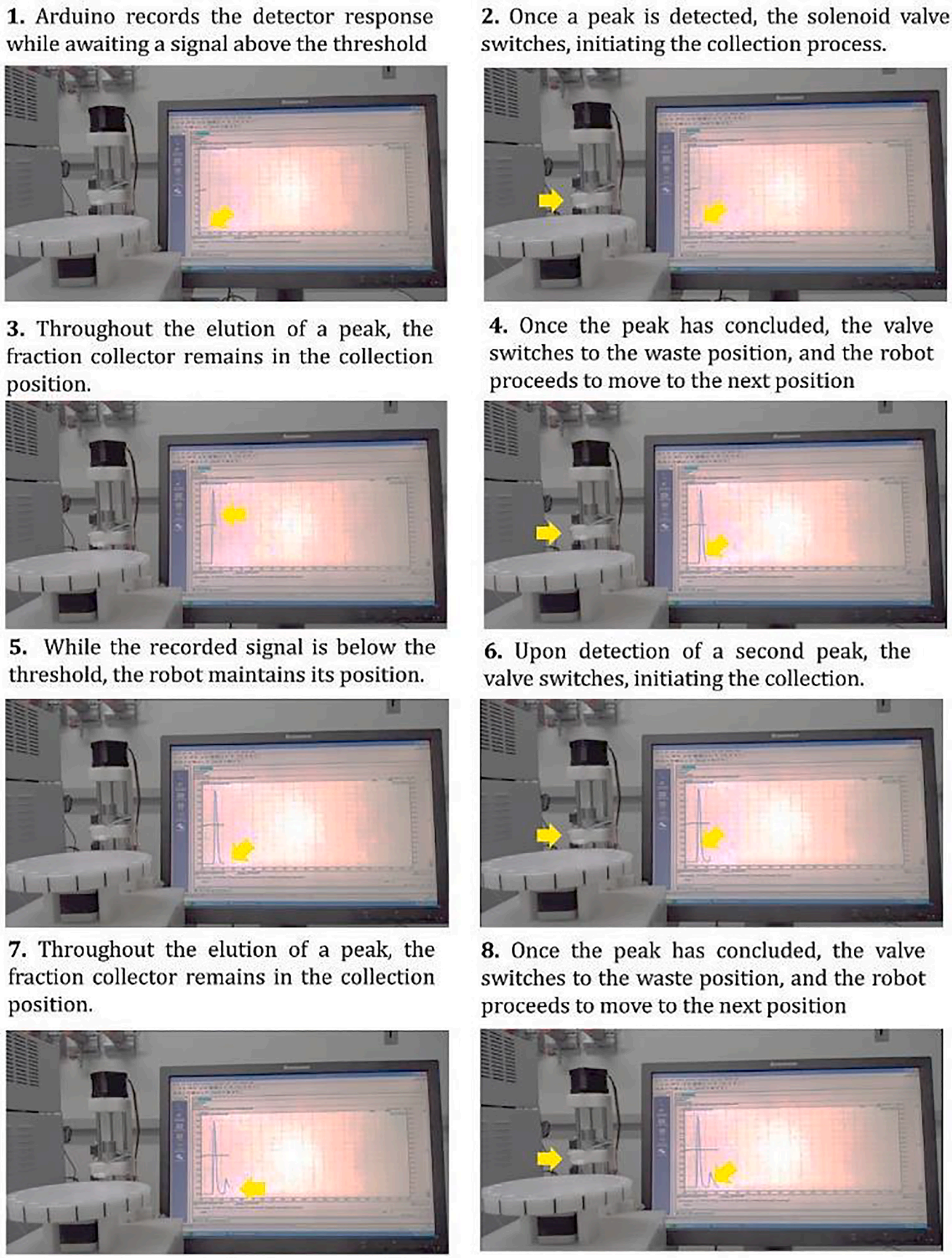

Fig. 12. Illustration of the sequence of the collection process of the peaks eluting from the injection and separation of a mixture of methyl and butylparaben.

Arduino employ a reference of 1.1 V during the analog-to-digital conversion. Consequently, an [RECORDER] output of 1.0 V corresponds to an analog read value of 1000 in the Arduino serial port. By utilizing this setup, the baseline noise and chromatographic peaks can be distinguished with improved resolution or a lower significant bit (LSB) of 1.1/1024 V.

Finally, all the above-discussed detection parameters were integrated into the code with the additional operation function of the sample collector. To illustrate the prototype operation, we set the threshold value in the Arduino code at 50 and injected a mixture of ethyl and butylparaben in water, at 5 μg/L of concentration, and the fraction collector was allowed to monitor the chromatogram, detect the eluent peaks, start the collection, and change the position once the peak has completely eluted. A video illustrating the performance of the fraction collector during a chromatographic run is provided in the following YouTube link: https://youtu.be/zOyQt5cTPA4. Fig. 12 illustrates the collection procedure.

Finally, it is crucial to emphasize that peak detection occurs when the recorded signal exceeds a specific threshold above a baseline. Therefore, this approach is particularly effective when operating under isocratic conditions where a flat baseline is maintained. Although isocratic elution is the preferred choice for conducting preparative LC separations and compound purification using preparative high-performance liquid chromatography (prep-HPLC) is typically performed in isocratic mode, gradient preparative LC separations can also be a valuable tool for obtaining pure compounds from complex samples. Exploring the feasibility of fraction collection in gradient mode has the potential to significantly enhance the capabilities of our prototype. However, pursuing this avenue of investigation requires further study and development as it involves distinct programming considerations that take into account the slope of the baseline resulting from the gradient. Future studies could focus on developing an improved version of the fraction collector by addressing these programming requirements.

Ethics statements

The authors declare no conflict of interest related to this work.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT in order to check grammatic, and writing to improve readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

Deyber Arley Vargas Medina: Conceptualization, Methodology, Software, Validation, Writing – original draft. **Asdrubal Lozada-Blanco:** Software, Writing – original draft. **Julie Paulin García Rodríguez:** Validation, Writing – original draft. **Fernando Mauro Lanças:** Conceptualization, Supervision, Writing – review & editing. **Álvaro José Santos-Neto:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ohx.2023.e00462.

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