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Fully Automated Determination of Cannabinoids on a Biomek i7 Hybrid Workstation Using Positive Pressure Solid Phase Extraction

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Abstract

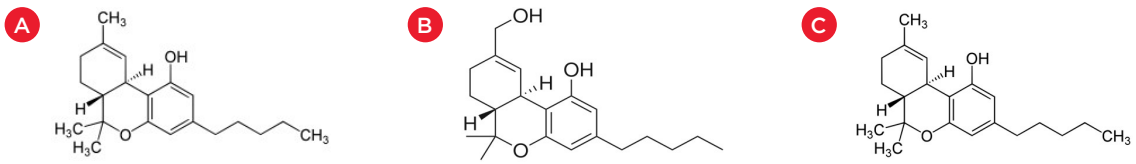
Tetrahydrocannabinol [THC, or (-)-Δ^9-trans-tetrahydrocannabinol] is the active chemical in cannabis, one of the most widely known hallucinogenic drugs. The therapeutic potential of cannabinoids has been the subject of scientific research for many years. The most common testing methods are LC-MS based methods, which require a time-consuming sample preparation. Automation of the sample preparation procedure can improve reproducibility while reducing both human error and active bench-time. In this application note we highlight the automation of sample preparation for THC measurement on a Biomek i7 hybrid workstation.

Introduction

Cannabis is one of the most well-known drugs in the world and it originates from the *Cannabis* plant of the hemp family. The primary psychoactive agent in Cannabis, Δ-9-tetrahydrocannabinol (THC), has a naturally occurring inactive precursor tetrahydrocannabinolic acid, often referred to as THC acid. Heat and drying will decarboxylate the inactive precursor, forming the psychoactive drug THC. Cannabinoids refers to a group of substances causing numbing and intoxicating effects [Figure 1(A)]. Cannabinoids have been used in traditional medicine for many years to relieve pain.

In recent years, these drugs attracted considerable interest as a potential therapeutic to treat numerous pathological conditions. In contrast to opioids, cannabinoids do not lead to life-threatening respiratory depression or a suppression of the important defense function against infectious germs, even when overdosed. Therefore, THC is used as dronabinol for the relief of cytotoxic-induced vomiting and for the treatment of anorexia with weight loss in AIDS patients[2]. Nabiximols is recommended to relieve spastic attacks in multiple sclerosis patients. According to conservative estimates, around 192 million people (≈ 3.9% of the world's population) use cannabis as an intoxicant[2].

Tetrahydrocannabinol [THC, or (-)-Δ^9-trans-tetrahydrocannabinol] is the active chemical in cannabis. The analytical determination of Δ^9-THC and its metabolites is increasingly important, as cannabis-containing products are increasingly coming onto the market and researchers need to identify the dosage-specific effects of the drug. Rapid cannabis tests do not provide quantitative determination in saliva or urine. Precise examination in saliva, blood, urine or breathing air requires extensive analytical procedures. using LC/MS/MS[3] or GC/MS[4].



Δ^9Tetrahydrocannabinol (Δ^9-THC)

11-OH-Δ^9-THC

11-nor-9-COOH-Δ^9-THC (THC-COOH)

Figure 1. Classical Cannabinoids.

The analytical determination of THC and its metabolites from serum or plasma requires a labor-intensive sample preparation method. This usually includes protein precipitation and extraction processes, and in some cases, even evaporation processes for a liquid exchange prior to the analytical measurement are necessary. In this application note, we developed a solid phase extraction—based, automated cannabinoid sample preparation method on a Biomek i7 Hybrid workstation from Beckman Coulter Life Sciences.

Materials and Methods

A solid-phase extraction method was used for the determination of Δ^9-THC-D_3, 11-OH-Δ^9-THC and 11-nor-9-Carboxy-Δ^9-THC in serum. Pig blood serum was used for the method development and evaluation. Materials used in the test procedures were purchased from different vendors prepared in different stock solutions (See Materials Table 3: Reagents used). A classical protein precipitation was followed by a solid-phase extraction realized using Strata® X-C µElution 96-well SPE plates (Phenomenex, Torrance, USA). By using a small elution volume, a higher concentration is achieved without needing an additional evaporation and reconstitution step.

The detailed sample preparation protocol is described in Table 1. In brief, the serum samples were transferred to 1.5 mL Eppendorf safe-lock vials (Eppendorf, Hamburg, DE). Proteins were precipitated by the addition of methanol and zinc sulfate, and internal standard solution was added to the serum samples. The solid-phase extraction was carried out on the integrated Positive Pressure Unit (amplius GmbH, Rostock, DE) using Strata®X-C µElution plates (Phenomenex, Torrance, USA). The finalized samples were analyzed by injecting 10 µL of the sample into an LC/Q-TOF-MS system (Agilent Technologies, Santa Clara, USA) with a flow rate of 0.5 mL/min. The system was calibrated according to the internal standard method in the range 0.2-100 ng/mL.

Step	Description
1	Transfer 500 µL MeOH to precipitation vial
2	Transfer 200 µL $ZnSO_4$ solution (0.2 M) to precipitation vial
3	Transfer 50 µL internal standard (ISTD) to precipitation vial
4	Transfer 200 µL serum sample to precipitation vial
5	Mix/shake for 1 min
6	Centrifuge for 4 min with 3,000 rpm
7	Condition the Strata® X-C µElution 96-well SPE plate with 200 µL MeOH
8	Equilibrate the Strata® X-C µElution 96-well SPE plate with 200 µL H_2O
9	Transfer 400 µL of sample from step 5 onto the Strata® X-C µElution 96-well SPE plate
10	Wash the Strata® X-C µElution 96-well SPE plate with 200 µL 0.1N HAc in H_2O
11	Wash the Strata® X-C µElution 96-well SPE plate with 200 µL 30% ACN 0.1N HAc in H_2O
12	Dry the Strata® X-C µElution 96-well SPE plate 10 min
13	Elute the cannabinoid metabolites twice with 25 µL 2% HAc in ACN
14	Transfer 50 µL H_2O to measurement vial for dilution
15	Transfer 25 µL eluate to measurement vial
16	Mix sample
17	Measurement of samples using LC/MS

Table 1. Sample processing protocol for the THC determination using positive pressure solid-phase extraction.

A fully automated workflow was achieved by integrating devices such a centrifuge (VSpin, Agilent Technologies, Santa Clara, USA; on the left side of the Biomek), a Positive Pressure Unit (amplius, GmbH, Rostock, DE; on the right side of the Biomek), and a Self-Refilling Quarter Reservoir (amplius GmbH, Rostock, DE; on-deck) on the Biomek i7 hybrid workstation (Figure 2). To ensure proper centrifugation, a suitable counterweight is placed close to the centrifuge. A special adapter was developed to hold 24 precipitation vials on the Biomek, (see Figure 3). The deck layout was optimized for the processing of up to 96 samples (Figure 2). To minimize the solvent evaporation, a Self-Refilling Quarter Reservoir (amplius GmbH, Rostock, DE) was integrated on the deck and was used to provide only the required amount of solvent at a time (Figures 4 & 5).

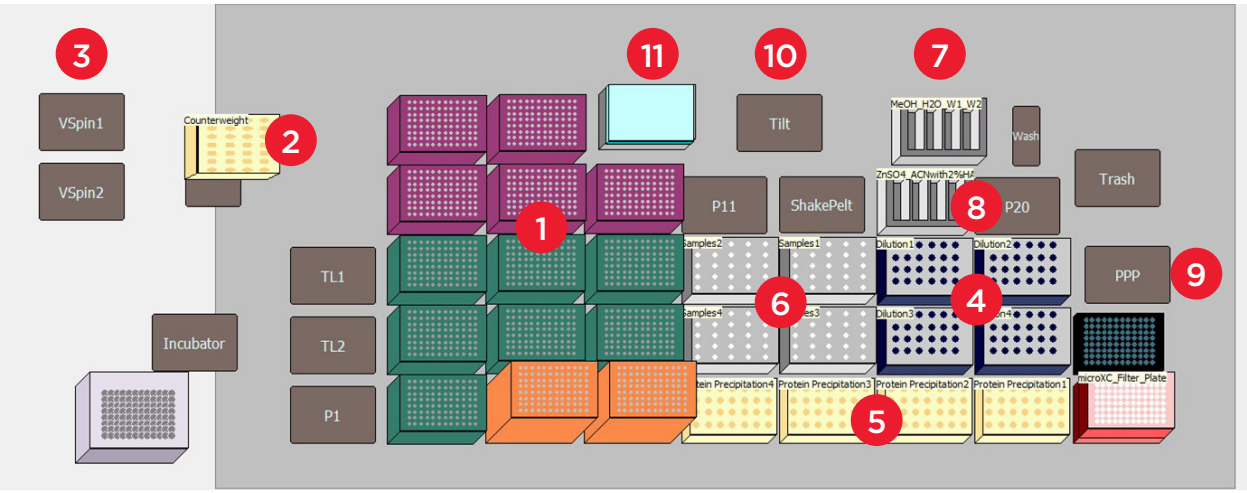


Figure 2. Deck layout for THC determination –**(1)** Tip boxes, **(2)** Counterweight, **(3)** Centrifuge, **(4)** Adapter for internal standard and dilution vials made of aluminum, **(5)** Vials for protein precipitation, **(6)** Serum samples in Eppendorf vials, **(7)** Quarter Self-Refilling reservoir, **(8)** Additional solvent reservoir, **(9)** Positive Pressure Processor, **(10)** 3D Tilting ALP, **(11)** Cooling Peltier ALP with internal standard in aluminum adapter.

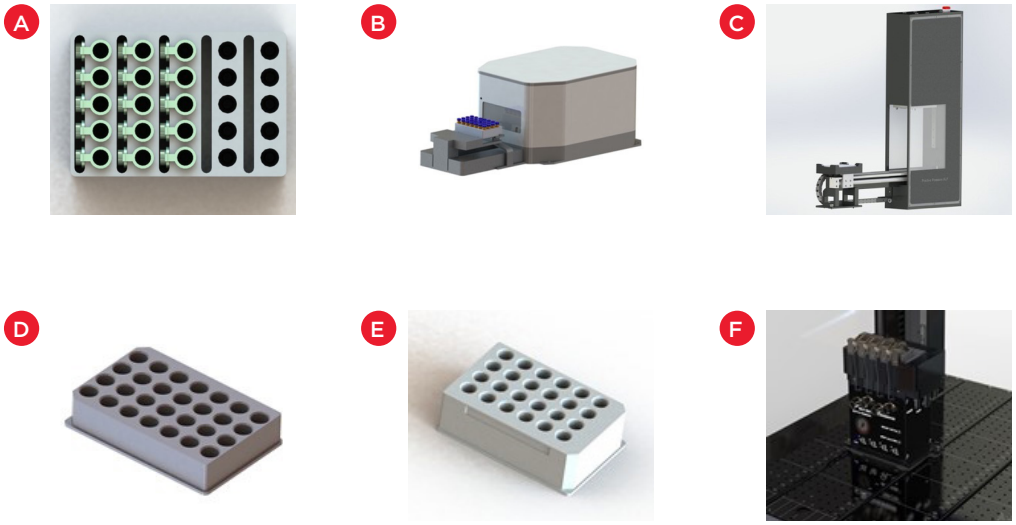


Figure 3. (A) storage adapter for sample vials (CELISCA, Rostock, DE), **(B)** Special adapter for 24 precipitation vials on VSpin centrifuge (CELISCA, Rostock, DE), **(C)** Positive Pressure Unit (amplius GmbH, Rostock, DE), **(D)** storage adapter for protein precipitation vials (CELISCA, Rostock, DE) **(E)** Adapter made of aluminum (CELISCA, Rostock, DE) **(F)** Self-Refilling Quarter Reservoir (amplius GmbH, Rostock, DE).

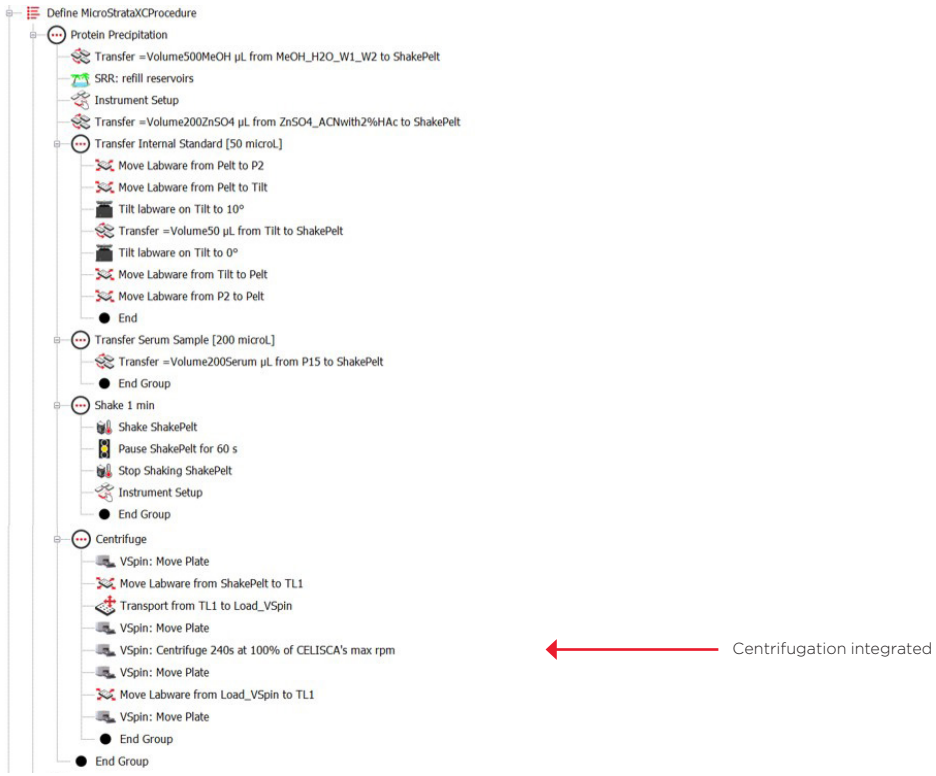


Figure 4. Biomek i7 Hybrid method for cannabinoid determination – protein precipitation with integrated centrifugation.

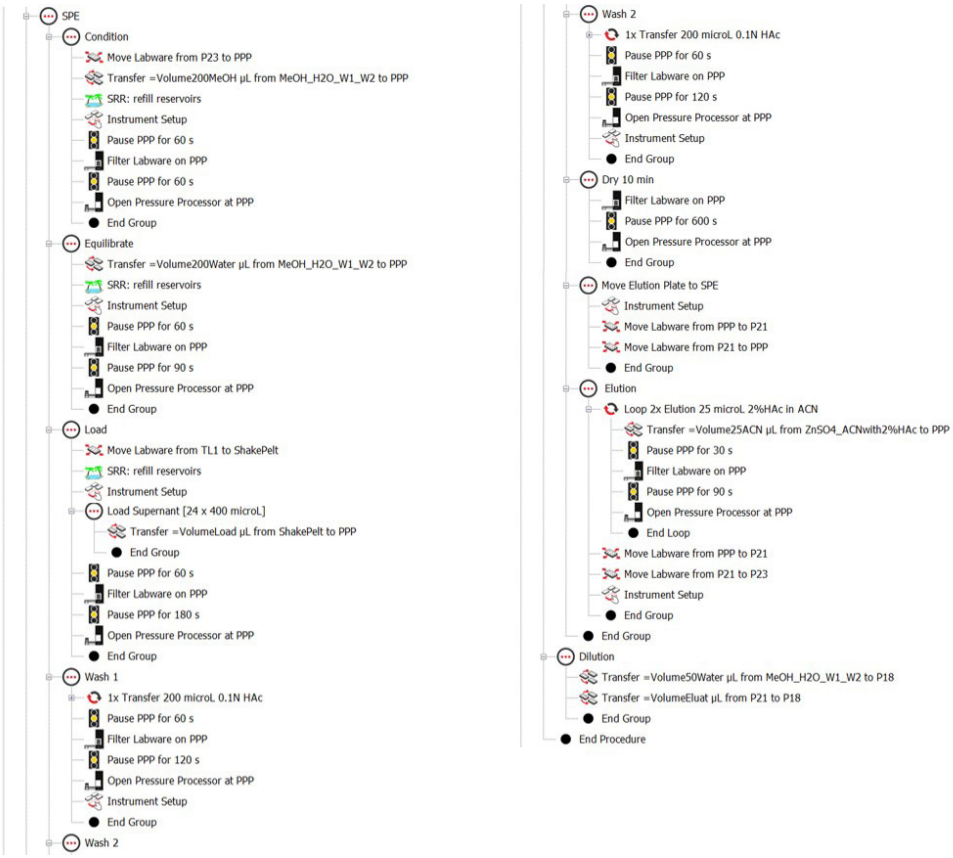


Figure 5. Biomek i7 method for THC determination – Sample cleanup using SPE.

Results

Figure 6 shows the calibration curves of Δ^9-THC-D_3, 11-Hydroxy-Δ^9-THC and 11-nor-9-Carboxy-Δ^9-THC. The recovery rates determined with the automated method were between 96.41% and 104.15%. The repeatability was determined with 12 sample replicates and the results showed a maximum coefficient of variation (CV) of 0.53%. For the determination of the within-laboratory precision, the experiment was repeated on 5 days with 10 samples each resulting in a CV between 0.44% and 3.11%. Limits of detection (method) were determined in a range of 0.625 ng/mL for Δ^9-THC-D_3 and 3.593 ng/mL for 11-nor-9-Carboxy-Δ^9-THC. The limit of detection for 11-Hydroxy-Δ^9-THC was 1.511 ng/mL. Limits of quantification (method) ranged from 0.704 ng/mL for Δ^9-THC-D_3, 3.957 ng/mL for 11-nor-9-Carboxy-Δ^9-THC and 1.38 ng/mL for 11-Hydroxy-Δ^9-THC.

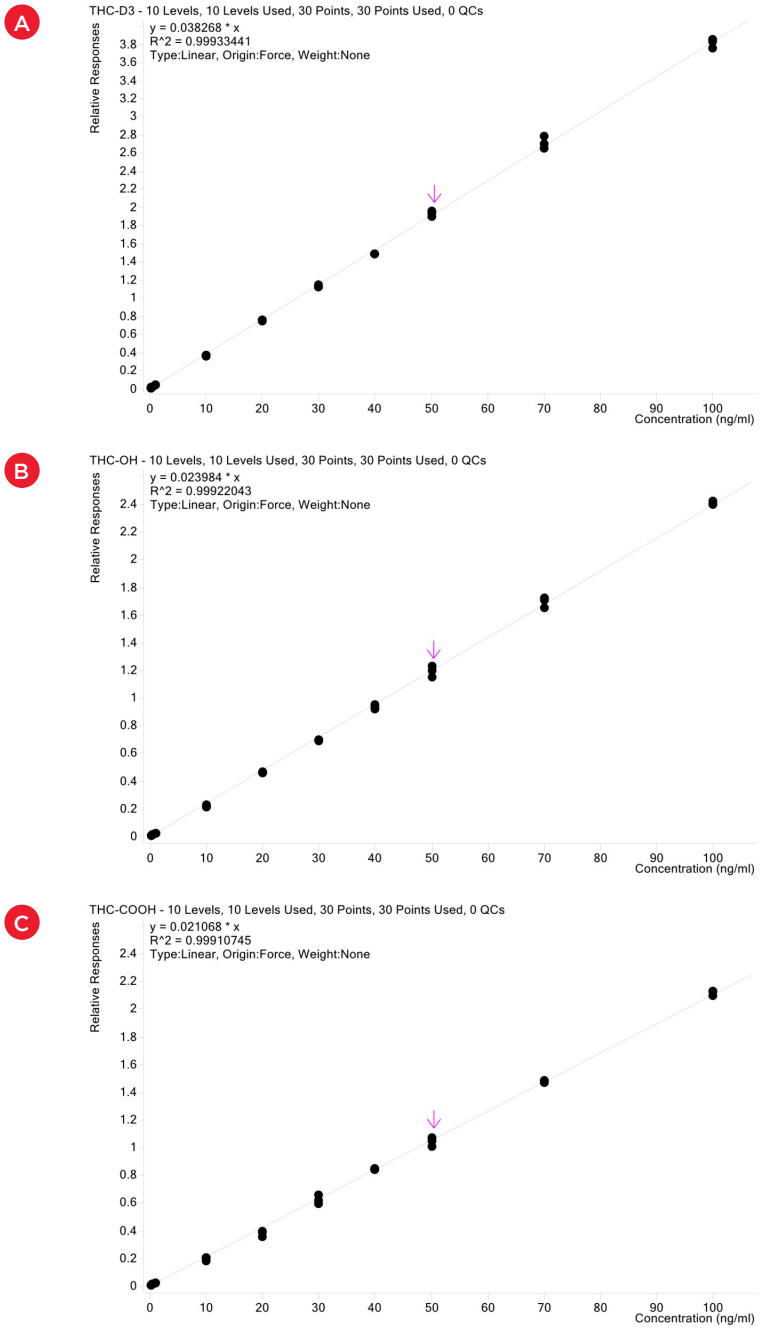


Figure 6. Calibration curves of **(A)** Δ^9-THC-D_3, **(B)** 11-Hydroxy-Δ^9-THC and **(C)** 11-nor-9-Carboxy-Δ^9-THC.

Summary

We developed a fully automated workflow for cannabinoid sample preparation on a Biomek i7 Hybrid workstation. The automated workflow showed excellent repeatability. All three types of cannabinoids used in the workflow (Δ^9-THC-D_3, 11-Hydroxy-Δ^9-THC and 11-nor-9-Carboxy-Δ^9-THC) showed low variation between replicates (<0.53%). The multiday precision values for all three analytes were <3.11% CV.

References

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2. Whiting, P., Wolff, R., Deshpande, S.: Cannabinoids for Medical Use: A Systematic Review and Meta-analysis, *Journal American Medical Association* **313(24)**, 2456-2473 (2015).

3. Teixeira, H., Verstraete, A., Proença, P., Monsanto, P., Vieira, D. N.: Validated method for the simultaneous determination of Δ^9-THC and Δ^9-THC-COOH in oral fluid, urine and whole blood using solid-phase extraction and liquid chromatography–mass spectrometry with electrospray ionization. *Forensic Science International* **170(2–3)**, 148-155 (2007).

4. Yonamine, M., Tawil, N., de Moraes Moreau, R. L., A., Silva O.: Solid-phase micro-extraction–gas chromatography–mass spectrometry and headspace-gas chromatography of tetrahydrocannabinol, amphetamine, methamphetamine, cocaine and ethanol in saliva samples, *Journal of Chromatography* B **789(1)**, 73-78 (2003).

Materials

Instruments used

Equipment	Manufacturer
Biomek i7 Workstation	Beckman Coulter Life Sciences, Indianapolis, USA
Shaking Peltier for Biomek 4000, FXp, NXp	INHECO Industrial Heating & Cooling GmbH, Martinsried, Germany
Static Peltier for Biomek 4000, FXp, NXp	INHECO Industrial Heating & Cooling GmbH, Martinsried, Germany
Positive Pressure Unit	amplius GmbH, Rostock, Germany
VSpin	Agilent Technologies, Santa Clara, USA
3D Tilt ALP	amplius GmbH, Rostock, Germany
Self-Refilling Quarter Reservoir	amplius GmbH, Rostock, Germany
LC-Q-TOF MS system	Agilent Technologies, Santa Clara, USA

Table 2. Instruments used.

Reagents used

Reagent	Manufacturer	Part Number
Methanol	Carl Roth GmbH, Karlsruhe, D	7342.1
2 M zinc sulfate	Sigma Aldrich, St. Louis, USA	83265
Deionized water	Sigma Aldrich, St. Louis, USA	W4502-10L
Acetonitrile	Carl Roth GmbH, Karlsruhe, D	8825.2
Acetic Acid	Carl Roth GmbH, Karlsruhe, D	3738.2
11-nor-9-Carboxy-Δ^9-THC-D_3	Sigma Aldrich, St. Louis, USA	T-004
11-Hydroxy-Δ^9-THC-D_3	Sigma Aldrich, St. Louis, USA	T-006
11-nor-9-Carboxy-Δ^9-THC	Sigma Aldrich, St. Louis, USA	T-006
11-Hydroxy-Δ^9-THC	Sigma Aldrich, St. Louis, USA	H-026
Δ^9-THC-D_3	Sigma Aldrich, St. Louis, USA	T-003
Pig blood serum	State Office for Agriculture, Food Safety and Fishing (LALLF, Rostock, DE)	-

Table 3. Reagents used.

Consumables used per 96 samples

Consumables	Number	Manufacturer	Part Number
Biomek i-Series tips 90 µL	480	Beckman Coulter Life Sciences, Indianapolis, USA	B85881
Biomek i-Series tip 230 µL	580	Beckman Coulter Life Sciences, Indianapolis, USA	B855903
Biomek i-Series tip 1070 µL	192	Beckman Coulter Life Sciences, Indianapolis, USA	B85971
GC Vials 2 mL	96	Agilent Technologies, Santa Clara, USA	5182-0716
GC Vials fixed insert	96	Agilent Technologies, Santa Clara, USA	9301-0978
Vial Caps	-	Agilent Technologies, Santa Clara, USA	5182-0731
Eppendorf Vials 1.5 mL	96	Eppendorf AG, Hamburg, D	EP0030121880
Strata® X-C µElution 96-well SPE plate	1	Phenomenex, Torrance, USA	8M-S029-4GA
96-Well Collection Plate, 350 µL Conical	1	Phenomenex, Torrance, USA	AH0-7192

Table 4. Consumables used.

Reusable Consumables and Adapters

Consumables	Number	Manufacturer/Vendor	Part Number
Quarter Reservoir Inserts and Frame	6	Beckman Coulter Life Sciences, Indianapolis, USA	372788
GC Vial Adapter used for protein precipitation	5	amplius GmbH, Rostock, Germany	-
Aluminum Adapter with cavities for GC Vials	5	amplius GmbH, Rostock, Germany	-
Eppendorf Vial Adapter	4	amplius GmbH, Rostock, Germany	-
Greiner Multiwell Plate Lid	1	Sigma Aldrich, St. Louis, USA	L4537
Greiner 96 MTP 0.2 mL klar round	1	Greiner 96 MTP 0.2 mL klar round	391-3605

Table 5. Reusable Consumables and Adapters.

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