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Automated Solid Phase Extraction Based Determination of Cannabinoids in Saliva using Biomek i7 Hybrid Workstation

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Abstract

Cannabinoids have variety of effects on consumers and therefore the effects of cannabinoids are being studied widely in research. Tetrahydrocannabinol [THC, or (-)-Δ^9-trans-tetrahydrocannabinol] and other cannabinoids can be measured in saliva providing a non-invasive sampling in contrast to blood samples. 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 in saliva on Biomek i7 hybrid workstation.

Introduction

The consumption of cannabis products is widespread, but it has numerous effects on the users. The type and extent of the effects and side effects depend on various factors such as duration of use, dose and habituation to cannabis. The acute psychoactive effects of cannabinoids can cause poor memory, reduced psychomotor or cognitive performance and impaired perception of temporal processes. Cannabis use reduces mental performance and has a negative effect on the ability to concentrate. Long-term use of cannabis usually results in tolerance, which is the cause of developing psychological addiction. In rare cases, prolonged use of cannabis can lead to schizophrenic psychosis. Cannabis can therefore lead to a schizophrenic illness or to the onset of psychosis earlier. Effects on female and male sex hormones are also known [1].

Due to these of adverse effects, it has become important to study the use short-term and long-term effects of cannabis usage. Cannabis is detected classically in blood. In addition, the determination in saliva is also possible. This enables an easy non-invasive collection of samples [2, 3, 4]. The automation of the process can lead to significantly higher sample throughputs.

Materials and Methods

For the automated sample preparation of Δ^9-THC-D_3, 11-OH-Δ^9-THC and 11-nor-9-Carboxy-Δ^9-THC in saliva a solid phase extraction method was used. Extraction and preconcentration were carried out using Strata® X-C µElution 96-well SPE plate (Phenomenex, Torrance, USA). A high concentration was achieved by using a very small elution volume, without the requirement of additional evaporation and reconstitution steps. Artificial saliva was used for the method development and evaluation. Materials used in the test procedures were purchased from vendors to prepare stock solutions (see Materials Table 3: Reagents used).

To automate the solid phase extraction steps a Positive Pressure Unit (amplius, GmbH, Rostock, DE) was integrated on the right side of the Biomek i7 Hybrid workstation. The deck layout was optimized for the processing of up to 96 samples and is shown in Figure 1. To reduce strong evaporation and to save solvents, 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. To protect the highly volatile internal standards supplied in small quantities from evaporation, lidded standards were placed in a specially developed cooling aluminum adapter (amplius GmbH, Rostock, DE) on a cooling position (INHECO Industrial Heating & Cooling GmbH, Martinsried, Germany, Figure 2).

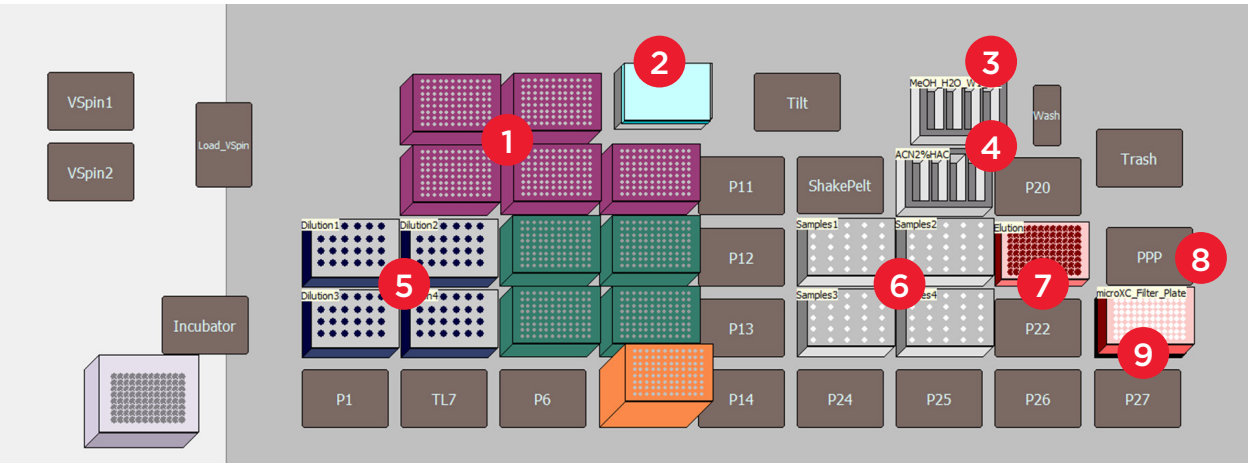

Figure 1. Deck layout for THC determination – **(1)** Tip boxes, **(2)** Cooling Peltier ALP with internal standard in aluminum adapter, **(3)** Quarter Self Refilling reservoir, **(4)** Additional solvent reservoir, **(5)** Adapter for internal standard and dilution vials made of aluminum, **(6)** Serum samples in Eppendorf vials, **(7)** Elution Plate, **(8)** Positive Pressure Processor, **(9)** Strata®X-C µElution plates (Phenomenex, Torrance, USA).

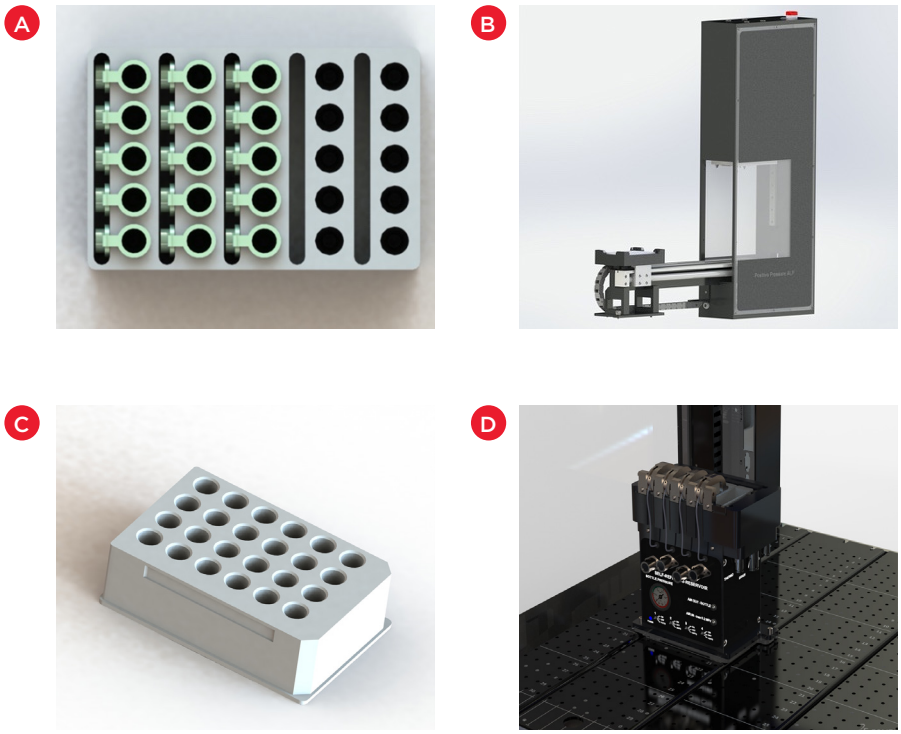

Figure 2. (A) storage adapter for sample vials (CELISCA, Rostock, DE), **(B)** Positive Pressure Unit (amplius GmbH, Rostock, DE), **(C)** Adapter made of aluminum (CELISCA, Rostock, DE) **(D)** Self Refilling Quarter Reservoir (amplius GmbH, Rostock, DE.

The detailed sample preparation protocol is described in Table 1. In brief, the saliva samples were transferred to 1.5 mL Eppendorf safe-lock vials (Eppendorf, Hamburg, DE). 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 internal standard was added during the load step of the solid phase extraction and mixed with the saliva sample by in-pipette-mixing. After elution, dilution and another labware change for supplying finalized samples in standard 1.5 mL vials, samples were analyzed by injecting 10 µL of the sample into a LC/Q-TOF-MS system (Agilent Technologies, Santa Clara, USA) with a flow rate of 0.4 mL/min. The system was calibrated according to the internal standard method in the range 0.2-330 ng/mL. All liquid handling steps were carried out using the Span8 pipetting head integrated in the Biomek i7.

To achieve higher throughputs, the liquid handling steps can be parallelized using the Multichannel Head. Using the Multichannel Head instead of the Span 8, allows all 96 wells of the Strata®X-C µElution plate to be filled simultaneously. In this case, the additional solvent reservoir can be replaced by a full solvent reservoir to enable the Multichannel Head to transfer the liquid. When processing 96 samples the turnaround times are reduced by 10 minutes. The modified deck layout is shown in Figure 3.

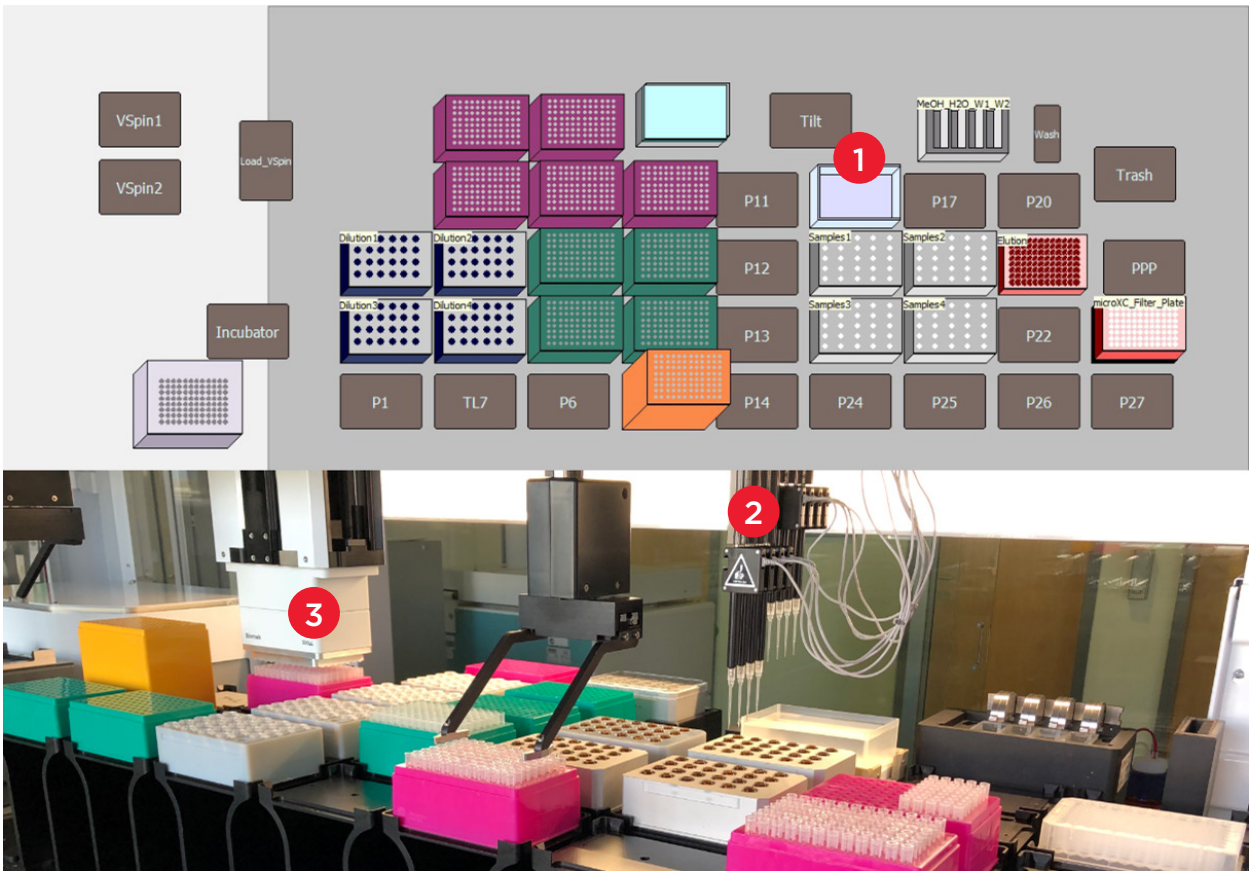

Figure 3: Modified Deck layout for THC determination using Biomek i7 Hybrid (Span-8 and MC) – **(1)** full solvent reservoir and **(2)** Span-8 and **(3)** Multichannel head of the Biomek i7 Hybrid workstation working on saliva samples to extract cannabinoids.

Step	Description
1	Condition the Strata® X-C µElution 96-well SPE plate with 200 µL MeOH
2	Equilibrate the Strata® X-C µElution 96-well SPE plate with 200 µL H_2O
3	Transfer 400 µL of saliva sample onto the Strata® X-C µElution 96-well SPE plate
4	Transfer 50 µL internal standard
5	Mix sample
6	Wash the Strata® X-C µElution 96-well SPE plate with 200 µL 0.1N HAc in H_2O
7	Wash the Strata® X-C µElution 96-well SPE plate with 200 µL 30% ACN 0.1N HAc in H_2O
8	Dry the Strata® X-C µElution 96-well SPE plate 10 min
9	Elute the cannabinoid metabolites twice with 55 µL 2% HAc in ACN
10	Transfer 40 µL H_2O to measurement vial for dilution
11	Transfer 40 µL eluate to measurement vial
12	Mix sample
13	Measurement of prepared samples using LC/MS

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

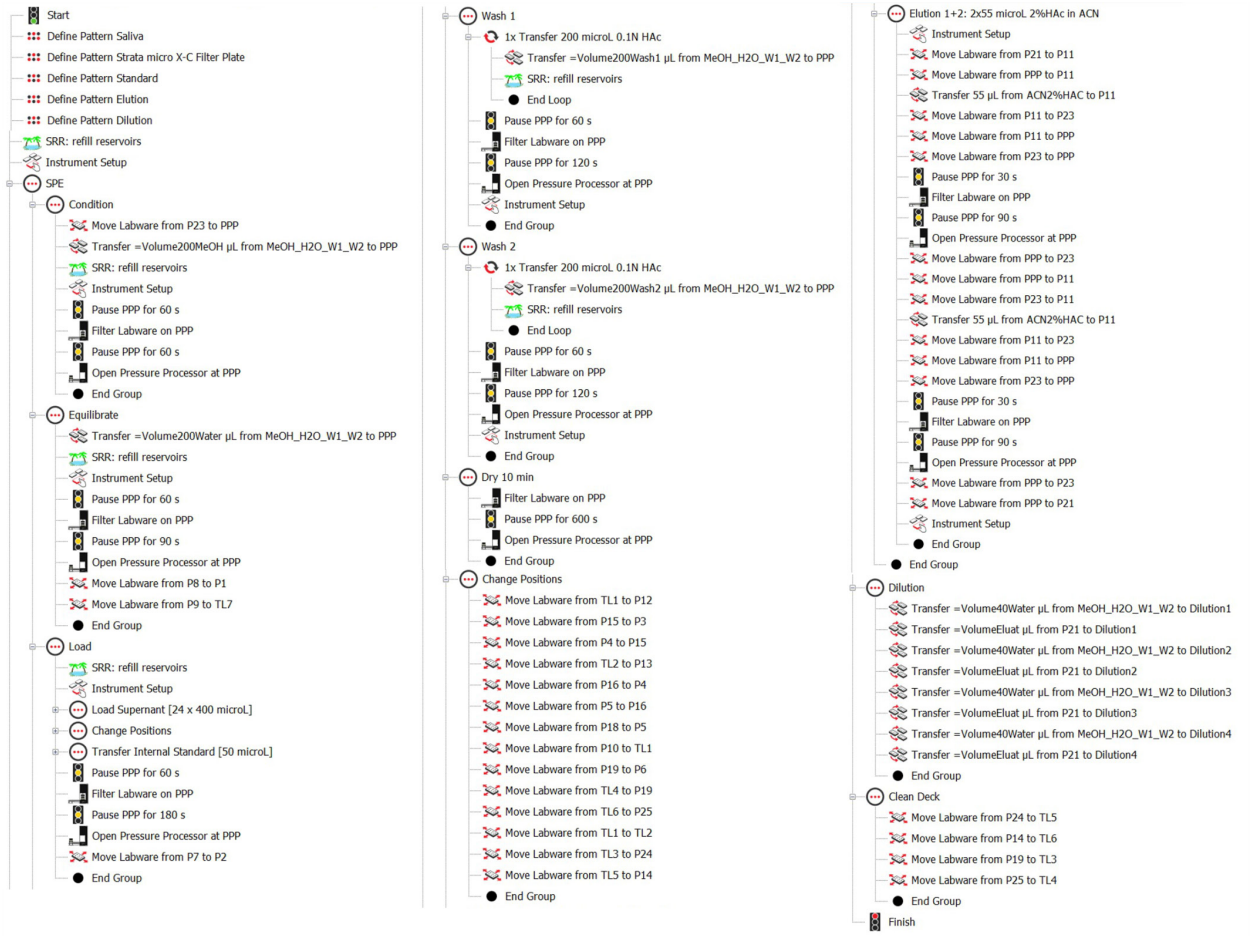

Figure 4: Biomek i7 method (Span-8) for THC determination – Sample clean up using SPE.

Results

Figure 5 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 ranged between 89.62% and 108.34%. The repeatability was determined with 10 samples and the results showed a maximum coefficient of variation (CV) of 0.78%. For the determination of the within-laboratory precision, the sample preparation was repeated for 5 days with 10 samples each resulting in a CV between 1.32% and 3.97%. Limits of detection (method) were determined in a range of 0.037 ng/mL for Δ^9-THC-D_3 and 0.942 ng/mL for 11-nor-9-Carboxy-Δ^9-THC. The limit of detection for 11-Hydroxy-Δ^9-THC was 0.328 ng/mL. Limits of quantification (method) ranged from 0.082 ng/mL for Δ^9-THC-D_3, 2.247 ng/mL for 11-nor-9-Carboxy-Δ^9-THC and 0.773 ng/mL for 11-Hydroxy-Δ^9-THC.

The most used cannabinoid for detecting marijuana in saliva is THC. The detection limits published in the literature are in the low ng/mL range between 0.05 and 1.1 ng/mL [5, 6]. Cutoff levels for THC and THC-OH defined by SAMHSA (Substance Abuse and Mental Health Service Administration) or DRUID (Driving under the influence of Drugs, Alcohol and Medicines) are 2 ng/mL or 1 ng/mL, respectively [2]. THC-COOH is less commonly used because THC-COOH is only present in the pg/mL range in saliva. Methods exist reaching this range (2 -8 pg/mL); however, this requires more extensive sample preparation with concentration by evaporation [7], derivatization [8], 2D chromatography techniques [9], or additional negative mode ionization [7, 10] is required to enhance signal intensity.

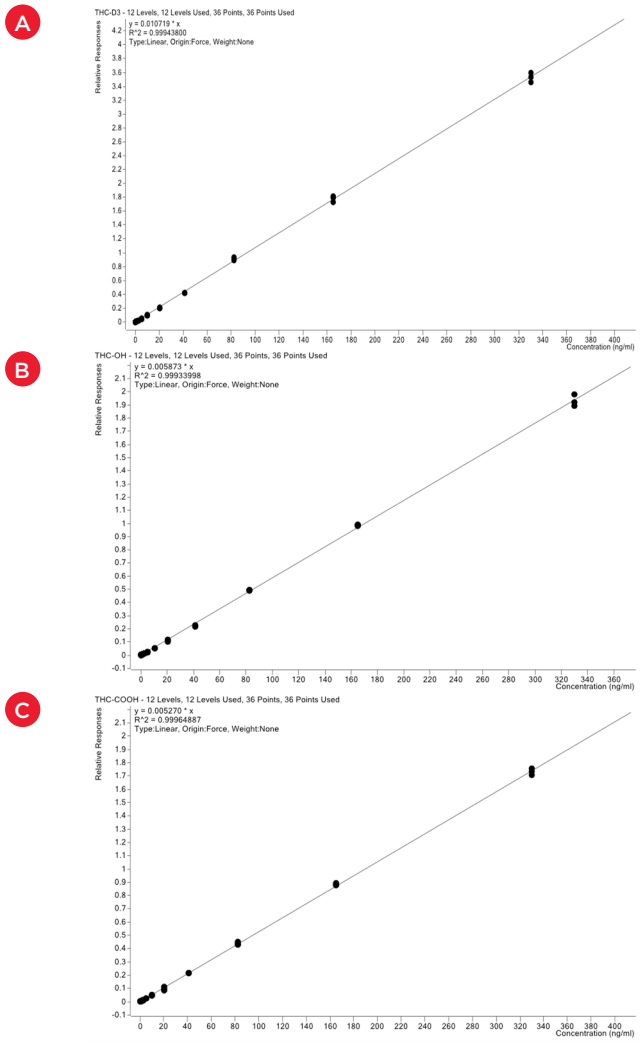

Figure 5: Calibration curves of **(A)** Δ^9-THC-D_3, **(B)** 11-Hydroxy-Δ^9-THC and **(C)** 11-nor-9-Carboxy-Δ^9-THC.

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Materials

Instruments used

Equipment	Manufacturer
Biomek i7 Hybrid (MC+Span-8)	Beckman Coulter Life Sciences, Indianapolis, USA
Static Peltier for Biomek 4000, FXp, NXp	INHECO Industrial Heating & Cooling GmbH, Martinsried, Germany
Positive Pressure Unit	amplius GmbH, Rostock, Germany
Self Refilling Quarter Reservoir	amplius GmbH, Rostock, Germany
LC-QTOF 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
Deionized water	Sigma Aldrich, St. Louis, USA	W4502
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
Artificial Saliva for pharmaceutical research	Sigma Aldrich, St. Louis, USA	SAE0149

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	384	Beckman Coulter Life Sciences, Indianapolis, USA	B855903
Biomek i-series tip 1070 µL	96	Beckman Coulter Life Sciences, Indianapolis, USA	B85971
Vials, 1.5 mL High Recovery, amber	4	Agilent Technologies, Santa Clara, USA	5183-2073
Vials, with fixed inserts	96	Agilent Technologies, Santa Clara, USA	5188-6592
Vial Caps	96	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 + Frame	5	Beckman Coulter Life Sciences, Indianapolis, USA	372788
Full Reservoir 150 mL	1	VWR International, Radnor, USA	1200-1300
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	Sigma Aldrich, St. Louis, USA	391-3605

Table 5: Reusable Consumables and Adapters.

Biomek i-Series Automated Workstations are not intended or validated for use in the diagnosis of disease or other conditions.

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