Microwave-assisted Digestion of Various Cannabis Products with Pressure-activated Venting

Due to the legalization of cannabis and the associated legislation concerning trace metal analysis the demand for fast and easy-to-use acid digestion techniques is increasing.

HVT vessels with SMART VENT Technology enable the convenient and reliable digestion of cannabis-based products prior to spectroscopic analysis.

1 Introduction

Although prohibited in the early 20th century, cannabis is now undergoing resurgence in medical and recreational use. In recent years more and more countries legalized the use of medical marijuana and Uruguay, Canada, Georgia and some US states also legalized recreational use of cannabis. Compared to the well-established system of cultivation, processing and sales of cannabis products, the governmental regulations of quality control are far behind. There is a lack of standardized methods and each state is currently responsible for regulating the quality controls, leading to multiple different legal situations. However, to ensure consumer safety and product quality cannabis-based products and the plant material itself must be tested for the potency of active ingredients, the presence of pesticides and toxic heavy metals like arsenic, cadmium, mercury and lead. These metals are of special interest as they are hazardous to humans even at low concentrations.

During growth plants absorb trace elements from soil, fertilizers and pesticides which can accumulate in the plant material. This effect based on natural metabolism may lead to high concentrations of toxic heavy metals in cannabis plants which are transferred to the final products opening the possibility of harming the consumer. It is of governmental and producers interest to test for heavy metals content in order to ensure consumer safety and high product quality.

The industry provides a huge variety of cannabis-based products such as flower, edibles, inhalations, oils, tinctures, lotions and many more. All these different matrices cause unique challenges for sample preparation prior to analysis. Reactive samples like CBD oil or flower generate a huge amount of reaction gases during digestion which limits temperature and sample amount in hermetically closed vessels. A controlled release of reaction gases is ensured by HVT vessels with pressure-activated venting in order to guarantee high temperatures and excellent digestion quality of even larger sample sizes.

To demonstrate the suitability of HVT vessels with SMART VENT technology for sample preparation of cannabis-based products prior to element analysis the recovery rates of spiked samples were determined for As, Cd, Hg, Pb.

2 Instrumentation

Samples were digested in HVT50 vessels using rotor 12HVT50 in Multiwave GO and concentration values were determined with a Shimadzu ICPMS-2030.

Figure 1: Multiwave GO

Figure 2: Rotor 12HVT50

3 Experimental

3.1 Samples

Five types of cannabis products were digested:

- Flower
- CBD oil
- Gummy
- Chocolate
- Lotion

3.2 Digestion Procedure

For the flower samples water was added prior to acid to suppress an initial reaction. For CBD oil first 2 mL of acid were added, then vortexed and afterwards the remaining amount of acid and water were added to ensure homogenization of the oily sample. For the other samples the order of addition does not matter.

For the unspiked samples, approximately 0.5 g of the respective sample was weighed into the HVT vessels. For the spiked samples also approximately 0.5 g of the respective sample was taken. Additionally these samples were spiked with different volumes (5 µL for low level, 100 µL for mid-level and 350 µL for high level) of a mixed standard solution (As: 10 µg/mL, Cd: 10 µg/mL, Hg: 5 µg/mL, Pb: 20 µg/mL) to reach the concentrations as mentioned in Table 1. The chosen spike levels are representatively based on the action levels for different cannabis products mentioned in the California State regulations. All solutions were covered with 3 mL of H_2O and 7 mL of conc. HNO_3.

Metal	Low	Mid	High
As	2	40	140
Cd	2	40	140
Hg	1	20	70
Pb	4	80	280

Table 1: Concentration levels for the spiked samples given in parts per billion (ppb)

The filled vessels were placed in the Multiwave GO equipped with rotor 12HVT50 and digested according to the temperature program given in Table 2.

Step	Ramp [min]	Temperature [°C]	Hold [min]
1	25:00	195	15:00
2	---	70	---

Table 2: Temperature program, control mode AVG

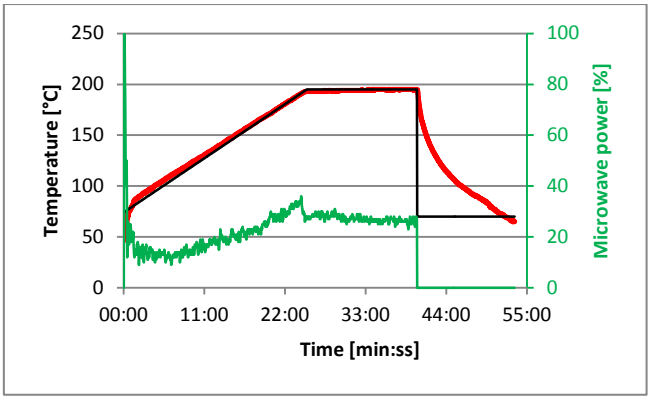

Figure 3: Representative run data (cannabis flower)

The resultant solutions were transferred to a sample tube and 1 mL of conc. HCl was added in order to stabilize mercury and arsenic. After final dilution to 25 mL with deionized water the samples were measured on the ICP-MS (n=3). For the preparation of all solutions trace metal grade acids were used.

The recovery rates were evaluated by calculating the measured values of the spiked samples (spiked sample minus unspiked sample) in relation to the theoretical spike level (see Table 1).

4 Results

All samples were completely digested as well as clear and colorless upon dilution.

The results were evaluated by reference to the relevant limits mentioned by the California State regulations as well as the United States Pharmacopoeia (USP), the European Pharmacopoeia (Ph. Eur.) and the respective guideline of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH).

California State regulations require recovery rates between 80% and 120%. USP <233> Elemental Impurities - Procedures, Ph.Eur. 5.20 and ICH Q3D state that the recovery rates of spiked samples have to be between 70% and 150% and the relative standard deviations (RSD) have to be not more than 20%.

Regardless of the respective kind of cannabis samples, all individual values lie well within the mentioned pharma specifications (recovery rates between 75.0% and 126.9%, RSDs below 11.2%).

On average the recovery rates lie at 102.1% for all elements which also demonstrates compliance with California State regulations.

The recovery rates and relative standard deviations are illustrated in Figure 4 and Figure 5 respectively. The data are listed in Table 3.

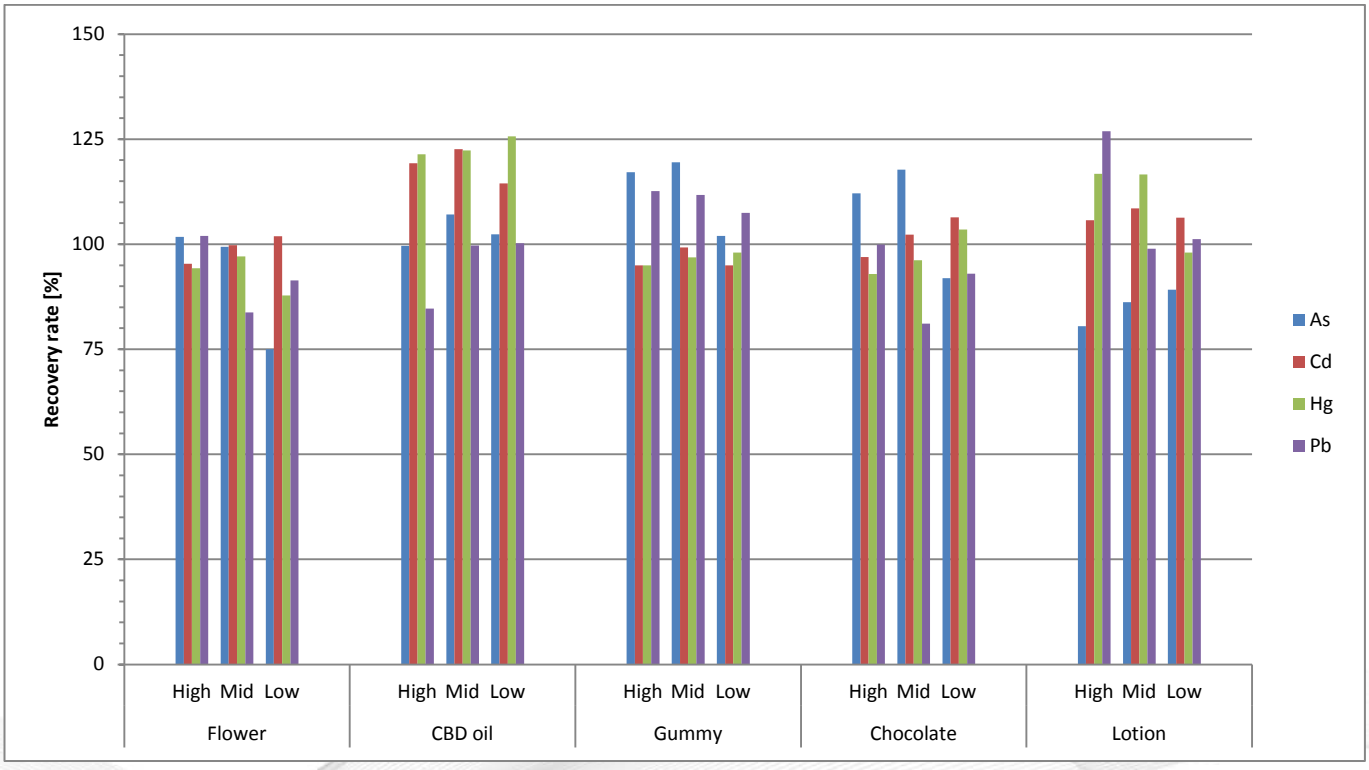

Figure 4: Recovery rates for As, Cd, Hg and Pb

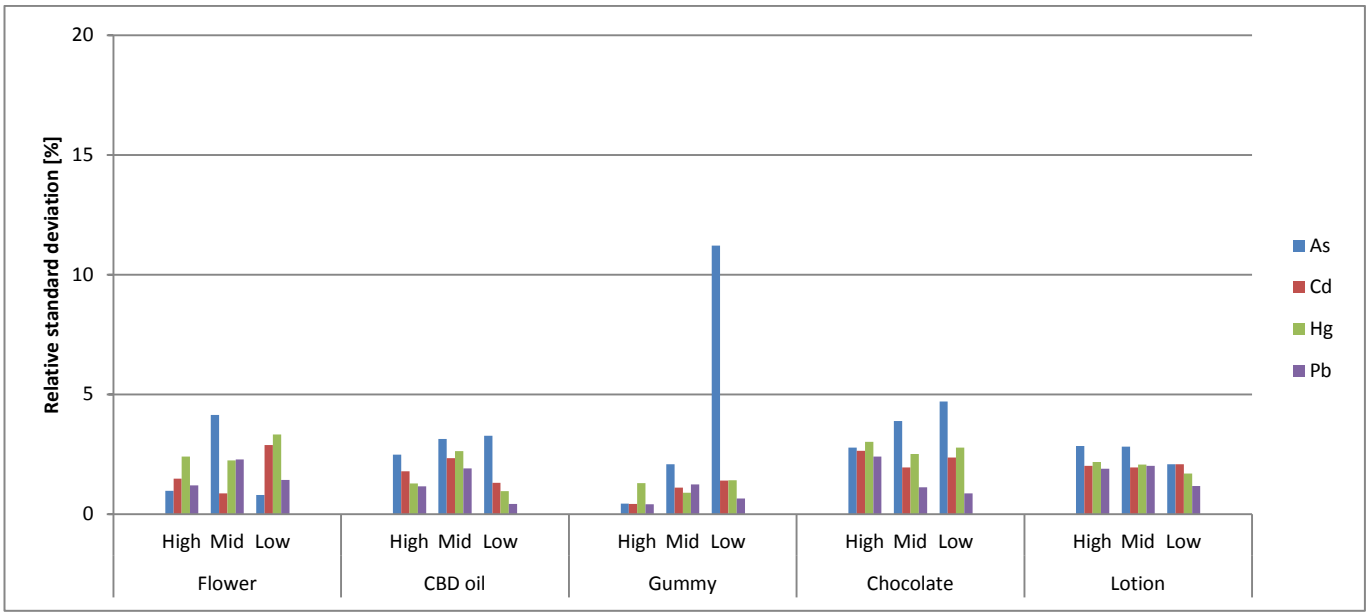

Figure 5: Relative standard deviations for As, Cd, Hg and Pb

Flower		As	Cd	Hg	Pb
Spike High	Measured value [ppb]	142.50	133.44	65.98	285.52
	Theoretical value [ppb]	140.00	140.00	70.00	280.00
	Recovery [%]	**101.8**	**95.3**	**94.3**	**102.0**
	RSD [%]	**1.0**	**1.5**	**2.4**	**1.2**
Spike Mid	Measured value [ppb]	39.75	39.89	19.43	67.02
	Theoretical value [ppb]	40.00	40.00	20.00	80.00
	Recovery [%]	**99.4**	**99.7**	**97.1**	**83.8**
	RSD [%]	**4.2**	**0.9**	**2.2**	**2.3**
Spike Low	Measured value [ppb]	1.50	2.04	0.88	3.66
	Theoretical value [ppb]	2.00	2.00	1.00	4.00
	Recovery [%]	**75.0**	**101.9**	**87.8**	**91.4**
	RSD [%]	**0.8**	**2.9**	**3.3**	**1.4**

CBD oil		As	Cd	Hg	Pb
Spike High	Measured value [ppb]	139.45	167.00	85.01	237.19
	Theoretical value [ppb]	140.00	140.00	70.00	280.00
	Recovery [%]	**99.6**	**119.3**	**121.4**	**84.7**
	RSD [%]	**2.5**	**1.8**	**1.3**	**1.2**
Spike Mid	Measured value [ppb]	42.85	49.05	24.46	79.74
	Theoretical value [ppb]	40.00	40.00	20.00	80.00
	Recovery [%]	**107.1**	**122.6**	**122.3**	**99.7**
	RSD [%]	**3.1**	**2.3**	**2.6**	**1.9**
Spike Low	Measured value [ppb]	2.05	2.29	1.26	4.01
	Theoretical value [ppb]	2.00	2.00	1.00	4.00
	Recovery [%]	**102.4**	**114.5**	**125.7**	**100.3**
	RSD [%]	**3.3**	**1.3**	**1.0**	**0.4**

Gummy		As	Cd	Hg	Pb
Spike High	Measured value [ppb]	164.00	133.00	66.48	315.49
	Theoretical value [ppb]	140.00	140.00	70.00	280.00
	Recovery [%]	**117.1**	**95.0**	**95.0**	**112.7**
	RSD [%]	**0.4**	**0.4**	**1.3**	**0.42**
Spike Mid	Measured value [ppb]	47.80	39.70	19.38	89.39
	Theoretical value [ppb]	40.00	40.00	20.00	80.00
	Recovery [%]	**119.5**	**99.3**	**96.9**	**111.7**
	RSD [%]	**2.1**	**1.1**	**0.9**	**1.2**
Spike Low	Measured value [ppb]	2.04	1.90	0.98	4.30
	Theoretical value [ppb]	2.00	2.00	1.00	4.00
	Recovery [%]	**102.0**	**95.0**	**98.0**	**107.5**
	RSD [%]	**11.2**	**1.4**	**1.4**	**0.7**

Chocolate		As	Cd	Hg	Pb
Spike High	Measured value [ppb]	157.00	135.71	65.04	279.71
	Theoretical value [ppb]	140.00	140.00	70.00	280.00
	Recovery [%]	**112.1**	**96.9**	**92.9**	**99.9**
	RSD [%]	**2.8**	**2.7**	**3.0**	**2.4**
Spike Mid	Measured value [ppb]	47.10	40.91	19.24	64.91
	Theoretical value [ppb]	40.00	40.00	20.00	80.00
	Recovery [%]	**117.8**	**102.3**	**96.2**	**81.1**
	RSD [%]	**3.9**	**2.0**	**2.5**	**1.1**
Spike Low	Measured value [ppb]	1.84	2.13	1.04	3.72
	Theoretical value [ppb]	2.00	2.00	1.00	4.00
	Recovery [%]	**92.0**	**106.4**	**103.5**	**93.0**
	RSD [%]	**4.7**	**2.4**	**2.8**	**0.9**
Lotion		**As**	**Cd**	**Hg**	**Pb**
Spike High	Measured value [ppb]	112.69	147.96	81.72	355.29
	Theoretical value [ppb]	140.00	140.00	70.00	280.00
	Recovery [%]	**80.5**	**105.7**	**116.7**	**126.9**
	RSD [%]	**2.9**	**2.0**	**2.2**	**1.9**
Spike Mid	Measured value [ppb]	34.49	43.41	23.32	79.13
	Theoretical value [ppb]	40.00	40.00	20.00	80.00
	Recovery [%]	**86.2**	**108.5**	**116.6**	**98.9**
	RSD [%]	**2.8**	**2.0**	**2.1**	**2.0**
Spike Low	Measured value [ppb]	1.78	2.13	0.98	4.05
	Theoretical value [ppb]	2.00	2.00	1.00	4.00
	Recovery [%]	**89.2**	**106.3**	**98.0**	**101.3**
	RSD [%]	**2.1**	**2.1**	**1.7**	**1.2**

Table 3: Measurement data, recovery rates and relative standard deviations of all samples

5 Conclusion

Microwave-assisted closed vessel digestion using HVT vessels with SMART VENT Technology works perfectly for cannabis flower and cannabis-based products. The suitability of SMART VENT Technology on Multiwave GO combined with ICP-MS was successfully verified on five different cannabis related matrices. Each sample was successfully digested within 40 minutes and due to fast cooling of the Multiwave GO each run was finished in less than 55 minutes.

The recovery rates of the toxic heavy metals As, Cd, Hg and Pb lie well between the defined limits of USP <233>, Ph. Eur. 5.20 and ICH Q3D (70 to 150% for the recovery and not more than 20% for the RSD). On average the recovery rates also comply with California State limits (80 to 120%). This demonstrates that microwave assisted digestion with HVT vessels fulfills current governmental regulations for cannabis products.

Operators benefit from the tool-free handling of HVT vessels to accelerate the workflow. The mentioned procedure can be easily transferred to Multiwave PRO using HVT vessels in Rotor 24HVT50 and 41HVT56 enabling even higher throughput with 24 or 41 samples per run, respectively.

6 References

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