

Digestion of Various Cannabis Samples with PDC

The huge variety of cannabis-based products requires fast and easy-to-use acid digestion prior to element analysis in order to fulfill governmental requirements for quality control.

Multiwave 7000 and its pressurized digestion cavity (PDC) offers the opportunity to digest diversified cannabis samples for subsequent analysis even within one run.



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 as well as 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 country or 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 huge variety of matrices of cannabis products result in unique challenges for sample preparation prior element analysis. Samples like oil, extract or flower produce significant amounts of reaction gases during digestion which limits the sample amount of hermetically closed vessels. The pressurized digestion cavity (PDC) of the Multiwave 7000 offers the opportunity to digest different reactive organic samples within one single run. The PDC can operate up to 199 bar allowing the digestion of huge amounts of organic samples and the combination of different matrices in one run. This enables high sample throughput and fast method development.

In order to demonstrate the suitability of the Multiwave 7000 and its PDC for sample preparation of various cannabis products within one run prior to element analysis the recovery rates of spiked samples were determined for As, Cd, Hg, Pb.

2 Instrumentation

The digestions were performed in Mutliwave 7000 together with Rack 18 with disposable glass vials and the concentrations were determined with by ICP-MS.



Figure 1: Multiwave 7000



Figure 2: Racks for Multiwave 7000

3 Experimental

3.1 Samples

Five types of cannabis products were digested:

- Flower
- Oil
- Extract
- Cookie
- Lotion

3.2 Digestion Procedure

Prior digestion flower and cookie were milled to homogenize the sample and the extract was preheated to 50°C to achieve a suitable viscosity. All samples were first covered with water before acid was added in order to suppress any initial reaction.

For the unspiked samples, approximately 0.5 g of the respective sample was weighed into the disposable 18 mL glass vial. 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, 75 µL for mid-level and 350 µL for high level) of a mixed standard solution (As, Cd, Hg and Pb each 10 µg/mL) to reach the concentrations as mentioned in Table 1. The chosen spike levels are based on the action levels for different cannabis products mentioned in the California State regulations. Prior digestion 5 µL of a stabilization solution (100 ppm gold in 2% HCl) was added to stabilize mercury and arsenic.

All solutions were covered with 1 mL of H₂O and 5 mL of conc. HNO₃.

Metal	Spike Low	Spike Mid	Spike High
As, Cd, Hg, Pb	1	15	70

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

The filled vials were put into Rack 18 and closed with plug-on caps. The fully loaded rack was put into the liner already filled with load solution. The preinstalled method "Reactive.HIGH" was used as temperature program and is given in Table 2.

3.3 Temperature Program

- Starting pressure: 40 bar
- Cooling temperature: 80 °C
- Pressure release rate: 10 bar/min

Step	Ramp [min]	Temperature [°C]	Hold [min]
1	35:00	250	--
2	--	250	15:00

Table 2: Temperature Program of the method "Reactive.HIGH"

After digestion 1 mL of conc. HCl was added in order to stabilize mercury and arsenic and the resultant solution was transferred to a sample tube. After final dilution to 50 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 method "Reactive.HIGH" was chosen as it can handle the high reactivity of oils and the needed high temperatures for extracts allowing digestion of all kind of samples within one run.

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% for certified reference materials. 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%. Despite the recovery of mercury of extract (spike high) and cookie (spike mid) which are slightly above California State regulation, all individual values lie well within given

specifications with recovery rates between 84.5% and 122.2% and a mean recovery of 105.1%. The relative standard deviations are lower than 5.54% and result in an overall mean of 1.4%. These results demonstrate compliance with California State regulations and relevant pharma specifications given in the US Pharmacopoeia.

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

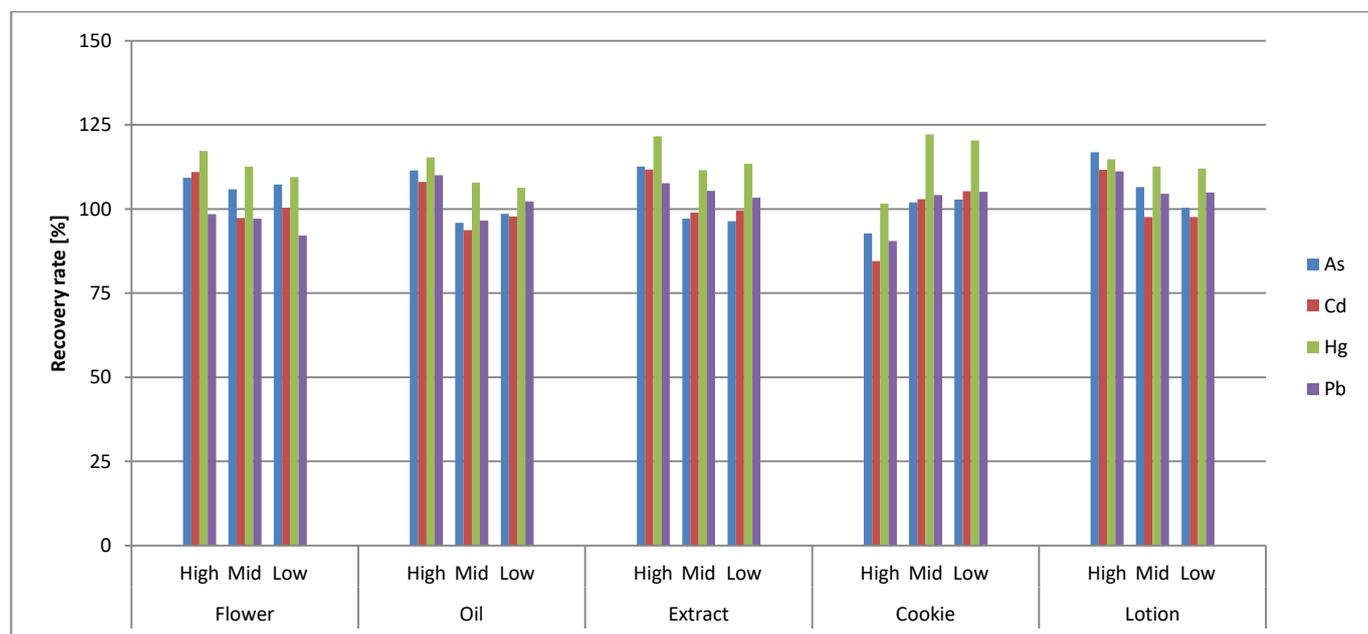


Figure 4: Recovery rates for As, Cd, Hg and Pb of all analyzed samples

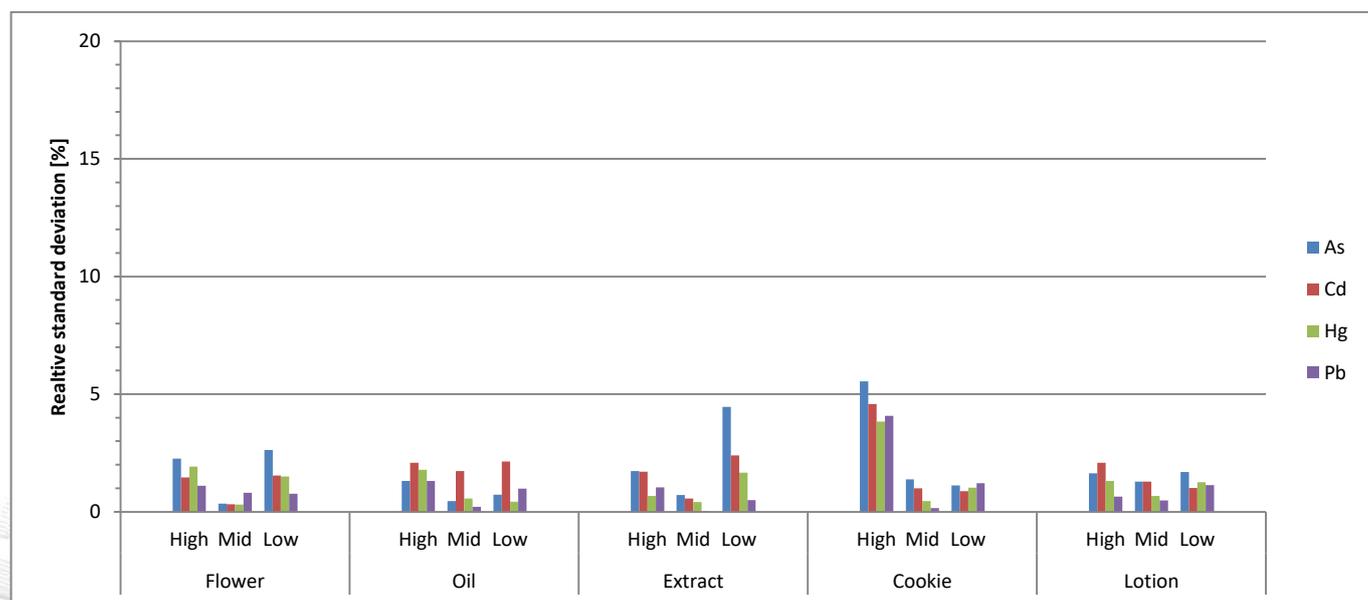


Figure 5: Relative standard deviations for As, Cd, Hg and Pb of all analyzed samples

Flower		As	Cd	Hg	Pb
Spike High	Measured value [ppb]	76.50	77.70	82.07	68.93
	Recovery [%]	109.29	111.00	117.24	98.47
	RSD [%]	2.26	1.46	1.92	1.10
Spike Mid	Measured value [ppb]	15.88	14.60	16.89	14.57
	Recovery [%]	105.87	97.35	112.58	97.15
	RSD [%]	0.34	0.32	0.30	0.81
Spike Low	Measured value [ppb]	1.07	1.00	1.09	0.92
	Recovery [%]	107.30	100.32	109.43	92.07
	RSD [%]	2.62	1.54	1.50	0.77

Oil		As	Cd	Hg	Pb
Spike High	Measured value [ppb]	78.00	75.60	80.75	77.02
	Recovery [%]	111.43	108.00	115.36	110.03
	RSD [%]	1.31	2.08	1.79	1.31
Spike Mid	Measured value [ppb]	14.38	14.06	16.17	14.48
	Recovery [%]	95.88	93.73	107.83	96.52
	RSD [%]	0.46	1.73	0.56	0.21
Spike Low	Measured value [ppb]	0.99	0.98	1.06	1.02
	Recovery [%]	98.60	97.80	106.34	102.23
	RSD [%]	0.72	2.14	0.43	0.98

Extract		As	Cd	Hg	Pb
Spike High	Measured value [ppb]	78.81	78.23	85.14	75.35
	Recovery [%]	112.58	111.76	121.63	107.65
	RSD [%]	1.73	1.70	0.67	1.04
Spike Mid	Measured value [ppb]	14.57	14.84	16.73	15.81
	Recovery [%]	97.12	98.95	111.56	105.40
	RSD [%]	0.71	0.57	0.41	0.02
Spike Low	Measured value [ppb]	0.96	0.99	1.13	1.03
	Recovery [%]	96.36	99.47	113.45	103.44
	RSD [%]	4.46	2.40	1.66	0.50

Cookie		As	Cd	Hg	Pb
Spike High	Measured value [ppb]	64.94	59.15	71.13	63.32
	Recovery [%]	92.78	84.49	101.61	90.46
	RSD [%]	5.54	4.58	3.83	4.08
Spike Mid	Measured value [ppb]	15.30	15.43	18.33	15.62
	Recovery [%]	102.01	102.90	122.17	104.14
	RSD [%]	1.38	1.00	0.45	0.16
Spike Low	Measured value [ppb]	1.03	1.05	1.20	1.05
	Recovery [%]	102.82	105.32	120.36	105.15
	RSD [%]	1.12	0.87	1.03	1.21

Lotion		As	Cd	Hg	Pb
Spike High	Measured value [ppb]	81.84	78.18	80.37	77.82
	Recovery [%]	116.91	111.69	114.82	111.17
	RSD [%]	1.64	2.09	1.31	0.65
Spike Mid	Measured value [ppb]	15.97	14.64	16.89	15.67
	Recovery [%]	106.49	97.62	112.62	104.49
	RSD [%]	1.28	1.28	0.67	0.48
Spike Low	Measured value [ppb]	1.00	0.98	1.12	1.05
	Recovery [%]	100.33	97.59	111.99	104.92
	RSD [%]	1.69	1.01	1.26	1.14

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

5 Conclusion

Multiwave 7000 and its PDC demonstrated perfect suitability to digest various cannabis samples in one run. Combining highly reactive samples like CBD oil with less reactive ones works fine due to the special concept of the pressurized digestion cavity. This allows for high throughput and fast method development which is of special interest for testing labs.

The used method allows for combination of sample matrices in the same run and covers the high reactivity of oils and the required high temperatures of extracts. If samples are less reactive or do not require high temperatures like flower, edibles or beverages the methods can be optimized by reducing temperature and ramp time in order to reduce the overall process time.

The recovery rates of Class 1 elements which include the toxic heavy metals arsenic, cadmium, mercury and lead lie well between the defined acceptance criteria of USP <233>, Ph. Eur. 5.20 and ICH Q3D (70 to 150% for the recovery and not more than 20% for the RSD). This shows that microwave assisted digestion with a pressurized digestion cavity fulfills governmental regulations and pharmaceutical requirements for sample preparation of cannabis products.

Operators benefit from the easy handling of the equipment accelerating the workflow and reducing the possibility of errors. The use of disposable vials saves time consuming cleaning efforts.

6 References

We acknowledge Kaycha Labs, 4131 SW 47TH Ave, Suite 1408, Davie, FL, 33314-4036 greatly for support and instrumentation during the experiments.



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