APPLICATION NOTE

Dry and wet grinding human hair samples with the Omni Bead Ruptor Elite bead mill homogenizer.

Summary

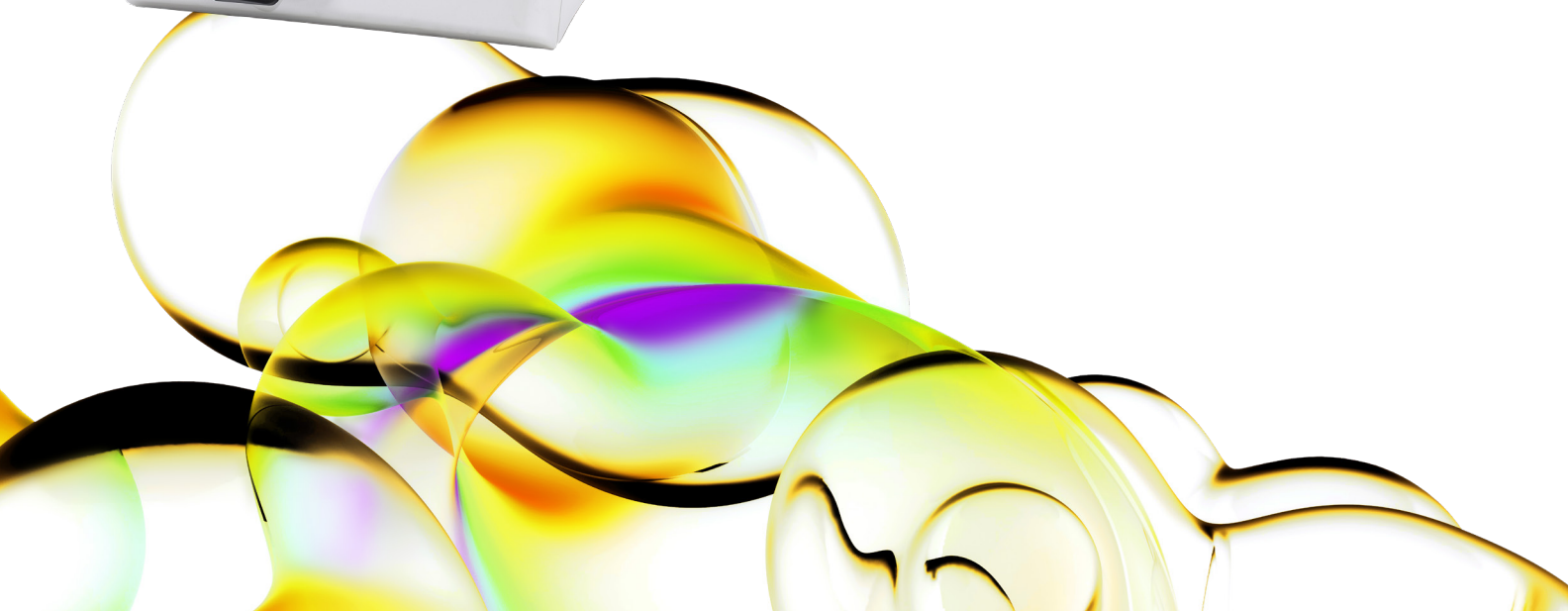
Hair is a sample matrix studied widely in forensic, racing anti-doping, and clinical research laboratories. Hormone and drug metabolites are easily extracted from hair samples for analysis using GC-MS or LC-MS/MS procedures to gain insights as well as for research exploring historic ingestion or metabolism of drugs and other small molecules of interest. DNA can be extracted from hair both to confirm an individual's identity as well as to screen for the presence of bacterial, fungal or viral pathogens.

Hair is a difficult matrix to grind, traditional mortar and pestle methods prove ineffective. To extract compounds from hair, it must be shaken in a solvent for long periods of time, sometimes with the application of heat. Hair can also be chemically digested. Either scenario is inefficient and may degrade analytes of interest.

The Omni Bead Ruptor Elite™ bead mill homogenizer can be used to grind hair in solution or dry, in less than 10 minutes. After dry or wet grinding, hair samples can be further processed using the Omni Bead Ruptor Elite bead mill homogenizer to increase extraction efficiency. Using the Omni Bead Ruptor Elite bead mill homogenizer, up to 360 hair samples can be processed in 1 hour, with no risk of carryover and no cleaning procedures in between samples.

For research use only. Not for use in diagnostic procedures.

Omni Bead Ruptor Elite bead mill homogenizer



Materials and methods

Materials

- Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- Omni Bead Ruptor Elite 2 mL or 7 mL Tube Carriage (Cat # 19-373 or 19-374)
- Hard Tissue Homogenizing Mix 2.8 mm Ceramic (2 mL or 7 mL) (Cat # 19-628 or 19-678)

Methods

Wet Grind

Small masses of human hair can be milled in the presence of diluents. Grind the hair sample using Omni Bead Ruptor Elite bead mill homogenizer with 2 mL tube carriage kit. Weigh up to 20 mg of hair into 2 mL Hard Tissue Homogenizing Mix (Cat # 19-628). Add 1 mL methanol to each hair sample. This can be an internal standard or buffer solution if required for your downstream sample preparation and analysis. Process for 3 cycles of 5.3 m/s x 180 seconds, with a 20 second dwell. Centrifuge tubes for 10 minutes at 13,300 rpm. Samples are ready for downstream sample preparation procedures such as solid phase or liquid extractions.

Dry Grind

Grind the hair sample using Omni Bead Ruptor Elite bead mill homogenizer with 2 mL tube carriage kit. Weigh 10 - 50 mg of hair into mL Hard Tissue Homogenizing Mix tubes (Cat # 19-628). Process for 3 cycles, 5.3 m/s x 60 seconds with a 20 second dwell. Remove an aliquot for downstream sample preparation and analysis, including supported liquid extraction (SLE) procedures. For liquid extraction or SLE sample preparation, add up to 1 mL of diluent, such as methanol or water, to samples, as well as internal standards where necessary. Process on the Omni Bead Ruptor Elite bead mill homogenizer for 3 cycles of 5.3 m/s cycle x 60 seconds, with a 20 second dwell, to extract analytes into diluent solution. Centrifuge tubes for 10 minutes at 13,300 rpm.
or

For large samples weighing 50 - 100 mg, use 7 mL Hard Tissue Homogenizing Mix (Cat # 19-678). Process for 60 seconds at 5.3 m/s, for 3 cycles, with a 60 second dwell.
or

For very large samples weighing 100 - 250 mg, use 7 mL Hard Tissue Homogenizing Mix (Cat # 19-678). Process for 60 seconds at 6.0 m/s, for 3 cycles, with a 60 second dwell.

Table 1: Sample homogenization summary

Sample type	Bead kit	Diluent volume	Speed (m/s)	Time (sec)	Cycles	Dwell time (sec)
Hair, 10 – 20 mg	19-628	1 mL	5.3	180	3	20
Hair, 10 – 50 mg	19-628	Dry grind	5.3	60	3	20
Hair, 50 – 100 mg	19-678	Dry grind	5.3	60	3	20
Hair, 100 – 200 mg	19-678	Dry grind	6.0	60	3	20

Results

Wet grinding procedures produce a fine particle suspended in solution, ready for downstream sample preparation or analysis. Dry grinding procedures result in a uniform fine powder. Both preparations are suitable for use in Biotage ISOLUTE® SLE+ protocols for analysis of hormones and toxicology metabolites, as shown in Figures 2 - 3, Table 2.

Dry and wet grinding human hair samples with the Omni Bead Ruptor Elite bead mill homogenizer.

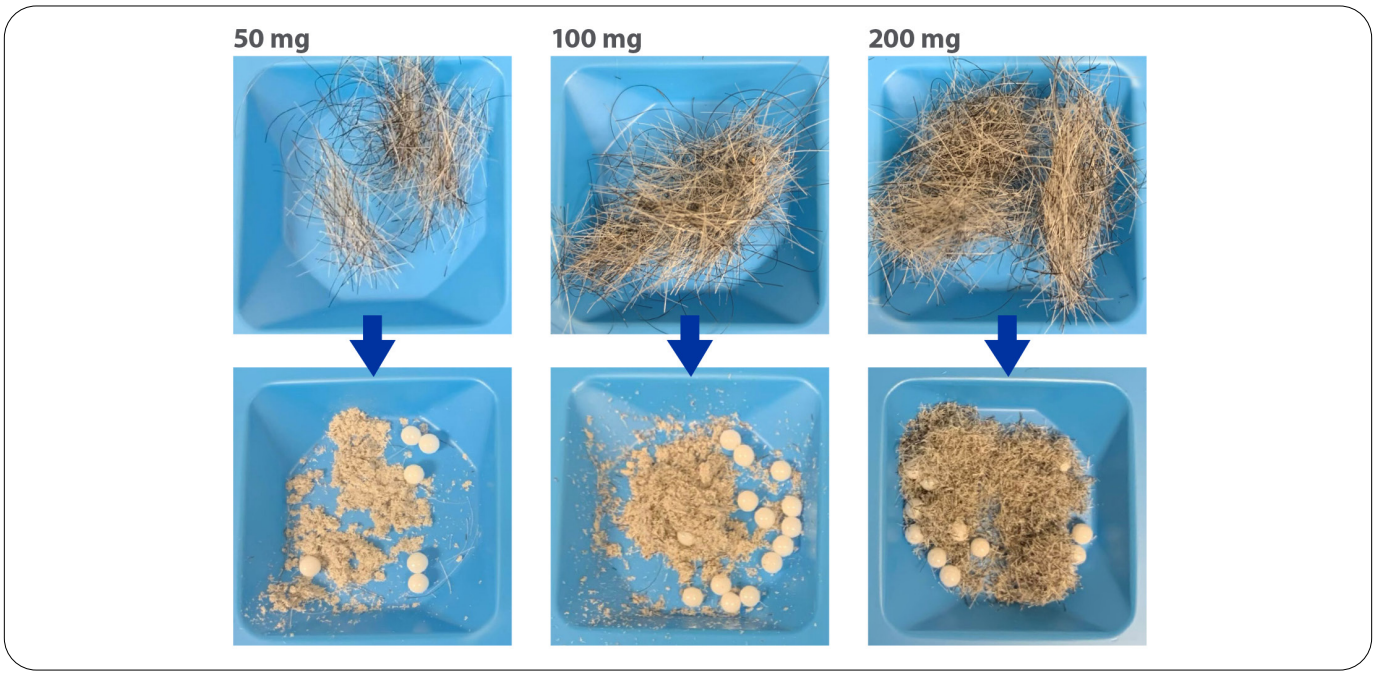


Figure 1: Hair processing before and after, 50 - 200 mg

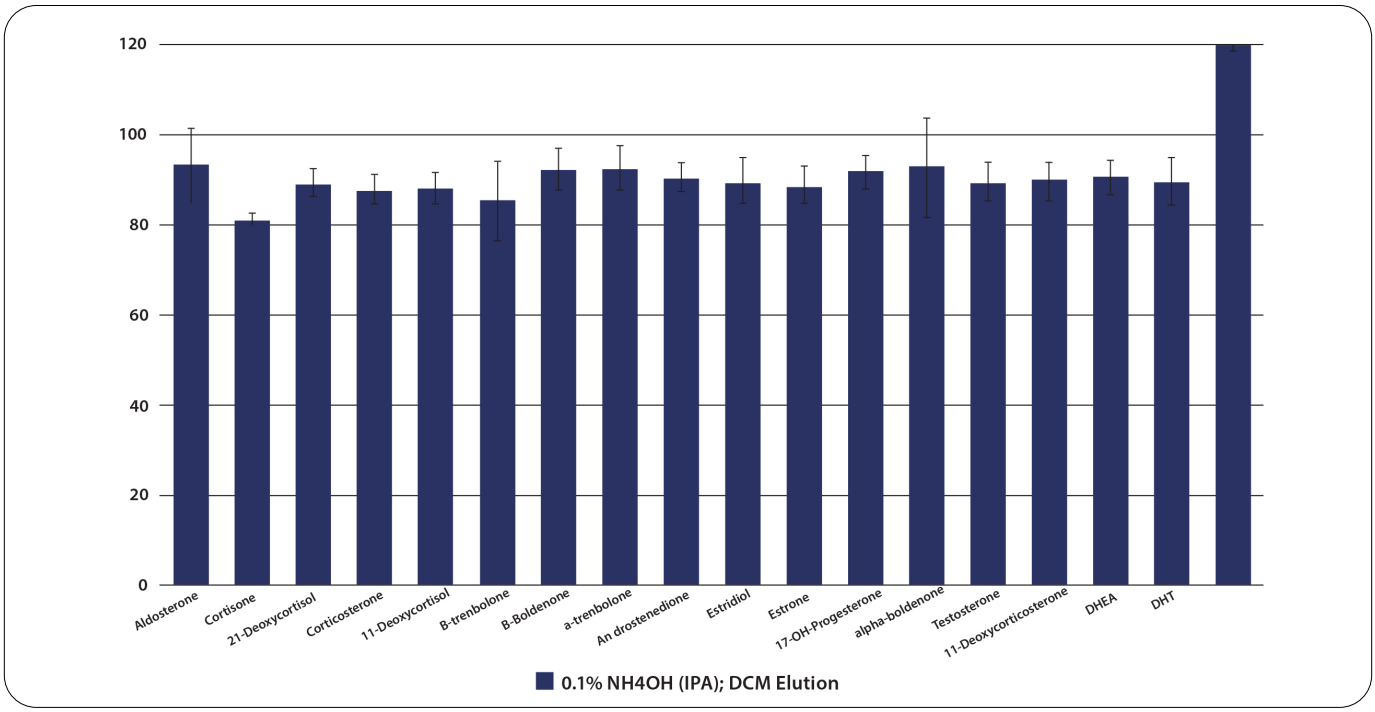


Figure 2: Hormone metabolite recovery from hair using the Omni Bead Ruptor Elite bead mill homogenizer – Biotage ISOLUTE SLE+ protocol

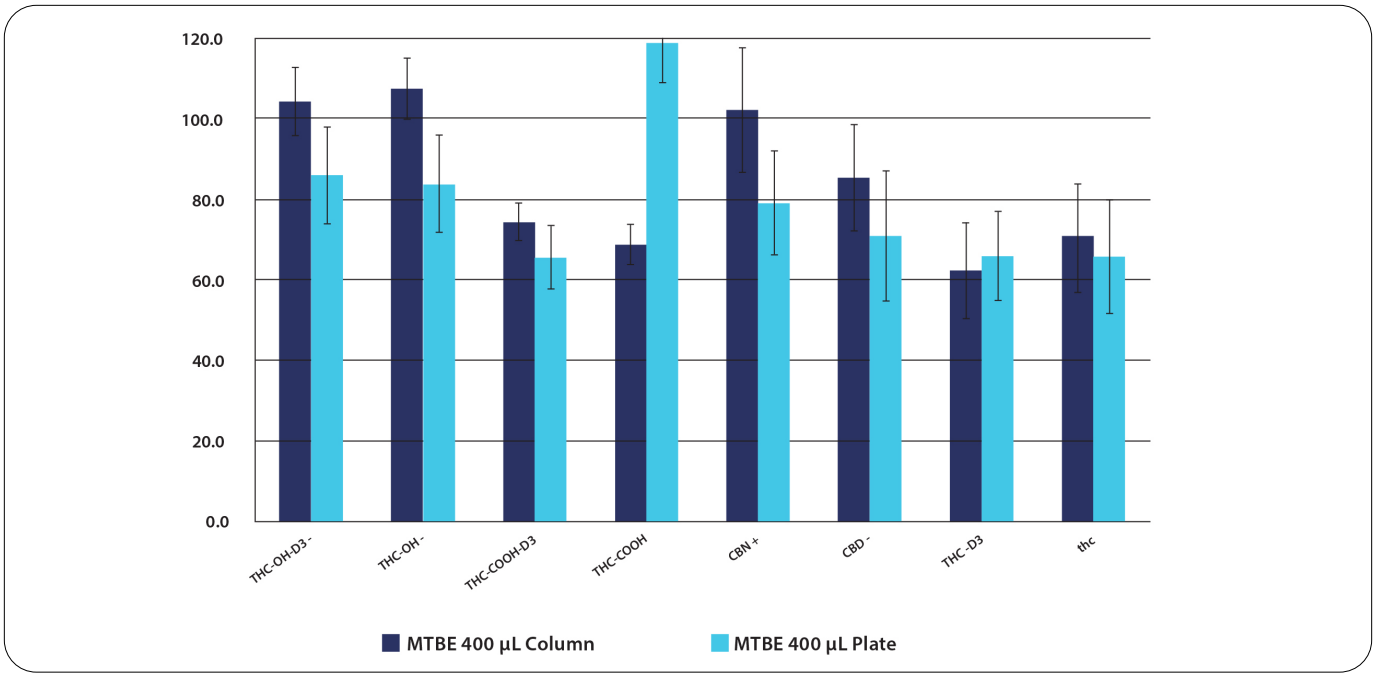


Figure 3: THC metabolite recovery from hair using the Omni Bead Ruptor Elite bead mill homogenizer – Biotage ISOLUTE SLE+ protocol

Table 2: Typical detection limits of common toxicology metabolites in hair using the Omni Bead Ruptor Elite bead mill homogenizer – Biotage ISOLUTE SLE+ protocol

Drug analyte	LLOQ pg/mg	Drug analyte	LLOQ pg/mg
Morphine	0.5	α-OH Triazolam	2.5
Oxymorphone	0.5	α-OH Alprazolam	12.5
Hydromorphone	0.5	Estazolam	0.5
Dihydrocodeine	0.5	Triazolam	0.5
Codeine	0.5	2-OH-Et-flurazepam	2.5
Oxycodone	0.5	Lorazepam	5
Hydrocodone	0.5	Alprazolam	0.5
6-MAM	0.5	Oxazepam	0.5
Amphetamine	1.25	Temazepam	0.5
MDA	1.25	Nordiazepam	2.5
MDMA	0.5	Diazepam	0.5
Methamphetamine	0.5	BZE	0.5
MDEA	0.5	Cocaine	0.5
EDDP	1.25	Nor-ketamine	0.5
Methadone	5	Ketamine	0.5
Mephedrone		Norfentanyl	0.5
7-amino Clonazepam	0.5	Fentanyl	0.5
7-amino Flunitrazepam	0.5	Zopiclone	0.5

Table 2 continued: Typical detection limits of common toxicology metabolites in hair using the Omni Bead Ruptor Elite bead mill homogenizer – Biotage ISOLUTE SLE+ protocol

Drug analyte	LLOQ pg/mg	Drug analyte	LLOQ pg/mg
Midazolam	1.25	Zolpidem	0.5
Flurazepam	0.5	Zaleplon	0.5
Bromazepam	2.5	Norbuprenorphine	25
Nitrazepam	2.5	Buprenorphine	1.25
Clonazepam	0.5	PCP	1.25
Flunitrazepam	2.5	LSD	1.25

Collaborators

These protocols were generated in collaboration with Biotage AB, https://biotage.com/. Experiments were carried out by scientists at Biotage GB (Cardiff, UK) or at Revvity.

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