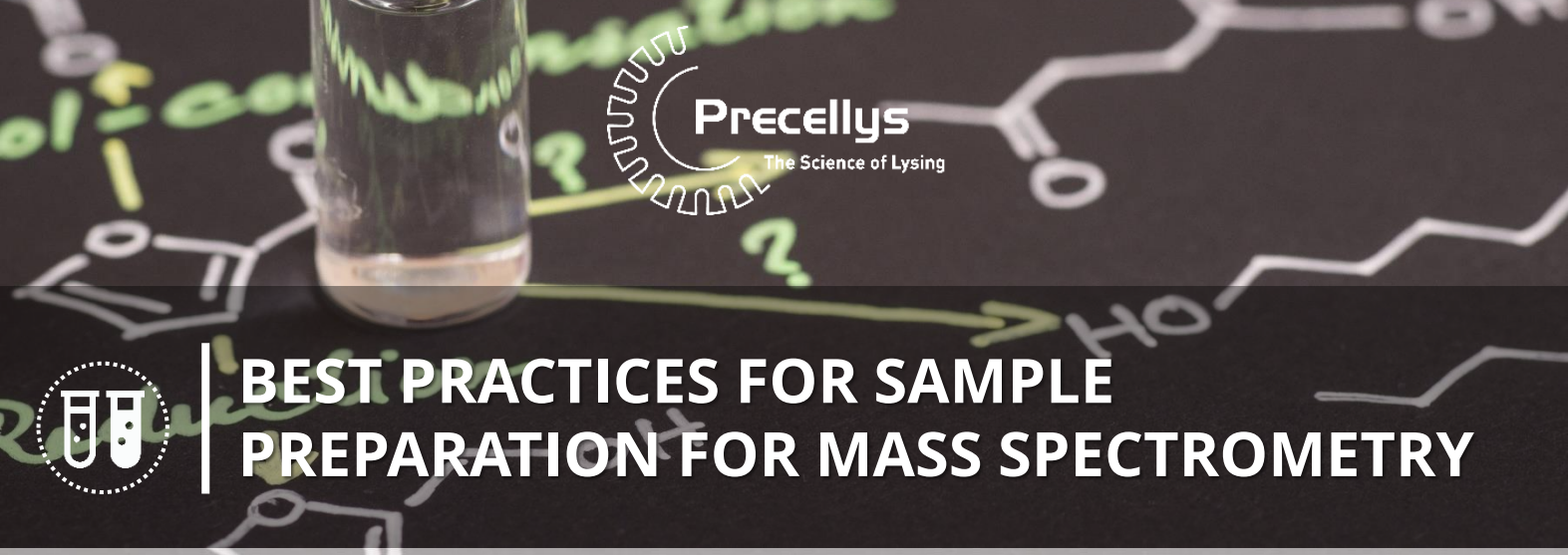


BEST PRACTICES FOR SAMPLE PREPARATION FOR MASS SPECTROMETRY

Mass spectrometry (MS) is one of the fastest developing analysis techniques in biology. It has become one of the methods of choice to identify and quantify various molecules, such as proteins, peptides, metabolites, and drugs in complex samples. When coupled with chromatography techniques, mass spectrometry offers high sensitivity, specificity, and flexibility.

A proper sample preparation protocol is critical for MS-based analysis workflows. Indeed, the quality and reproducibility of the protein, drug, or metabolite extraction can strongly influence MS results. Whether in the field of proteomics or metabolomics, it is important to obtain the highest yield and purity possible following the sample preparation step. This requires choosing an optimal protocol for the sample step.

The 3D-beating technology is considered the gold standard for sample homogenization. For this reason, Bertin Technologies has chosen 3-dimensional bead-beating technology to power its range of homogenizers, the Precellys®.

In this white paper, we present optimized protocols to help researchers get high-quality molecules for their MS analysis workflows.

DISCOVER OPTIMIZED HOMOGENIZATION PROTOCOLS FOR MASS SPECTROMETRY

SUMMARY

bertin health & life sciences

CONTACT
sales-life@bertin.group
www.bertin-technologies.com

DRUG EXTRACTION FROM WHOLE BRAIN RAT WITH MINILYS®

PharmOptima, Portage MI, USA

/ CONTEXT

PharmOptima focuses on in vivo and in vitro DM&PK and Discovery Services, providing IND & NDA-enabling research protocols. High quality DM&PK and Ocular PK research are a part of the laboratory's daily activities.

PharmOptima lab currently uses Precellys® to homogenize a variety of different animal tissues. In this study, Minilys® and probe sonicator have been compared to current method (Precellys®). Advantages & drawbacks are discussed.

/ MATERIAL

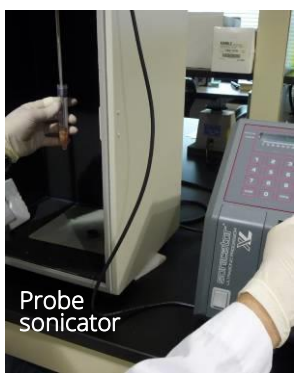
- Minilys® vs Precellys® Dual homogenizer vs Probe sonicator.
- Precellys® kit ceramic beads 7mL vial (réf.0904-01) +2 beads (Réf.03961-1-106).
- Sample: 1 thawed whole brain rat (~1.7 g) into 7mL vial (Minilys® & Precellys® trials) or into 15mL vial (Probe sonicator trial).

/ PROTOCOL

- Minilys® & Precellys®: 5000 rpm, 2x45sec (15sec break).
- Probe sonicator: several seconds.
- Analysis: liquid-liquid extraction; LC-MS/MS system.


Whole brain rat 7mL vial


Minilys


Probe sonicator

/ RESULTS

The analyte levels by samples are equivalent. No difference involving the three homogenizing techniques are detectable (See Figure 1). Despite one sample prep in 7mL vial, Minilys® is preferred rather the probe sonicator method which is fastidious and time consuming with cleaning steps and potential contamination between samples.

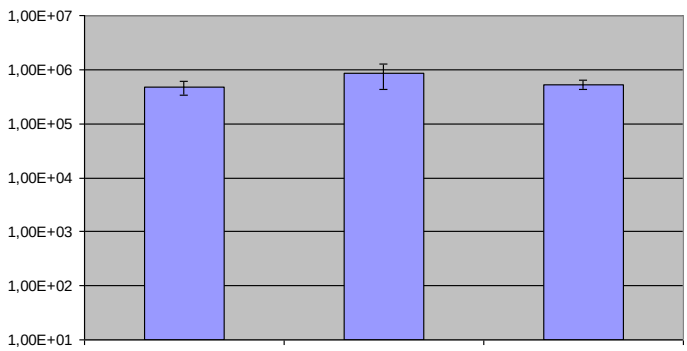


Figure 1: LC-MS/MS analysis from Precellys Dual, Minilys, and Sonicator sample.

/ CUSTOMER

PharmOptima
DRUG DISCOVERY & OPTIMIZATION

Precellys® Dual and Minilys® are suitable and reliable systems to extract Drug from animal tissues for DM&PK testing thanks grinding whole organs as brain rat with a fast process, cross-contamination free, high reproducibility and comparable drug levels extracted.

Minilys® homogenizer is a real alternative for standardization of the sample preparation for laboratories with a low or medium throughput of sample.

03712-210-DU055

THE PRECIPITATION OF PROTEINS AND LIPID EXTRACTION FROM COMPLETELY HOMOGENIZED RAT SKIN TISSUES

/ CONTEXT

In this study, protein precipitation and bioactive lipid extraction from rat skin tissues is reported. The combination Solid Phase Extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) approach was used to measure concentrations of lipid mediators in these tissues. Rat skin tissues were homogenized using CKMix50_7mL Precellys® Lysing Kit and Precellys® Evolution Touch tissue homogenizer combined with Cryolys® cooling unit. In addition, temperatures of homogenates were measured to investigate if temperature of complete homogenate of rat skin tissues remained below 40°C.

/ MATERIALS

- Automated Homogenizer: Precellys® Evolution Touch and Cryolys® cooling unit
- Lysing Kits: Tissue grinding CKMix50_7mL (Cat #: KT03961-1-306.7)
- Tissue Samples: Rat skin tissues (dorsal and ventral hindpaw)
- Homogenization Buffer: Dry ice-cold methanol

/ PROTOCOL

Samples: A total of 20-90 mg of adult rat skin tissues (dorsal and ventral) were obtained following standard practice and stored at -80°C until use.

Homogenization: Tissues were homogenized in 500 µL methanol in 7 mL Precellys® tubes containing a mix of 2.8 mm and 5.0 mm ceramic beads. Tissues were homogenized by running 5 cycles of 10 sec at 8,000 rpm, with a 120 sec break between cycles.

Cryolys® cooling unit: Samples were homogenized once the temperature of cooling unit homogenization chamber reached 5°C.

Temperature measurements: Immediately after sample homogenization, sample temperatures were measured using temperature probe.

/ RESULTS

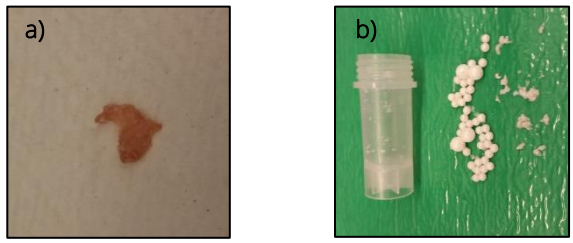


Fig. 1 Images of a whole rat hindpaw tissue sample (a), and homogenized tissue (b) using Precellys® Evolution touch.

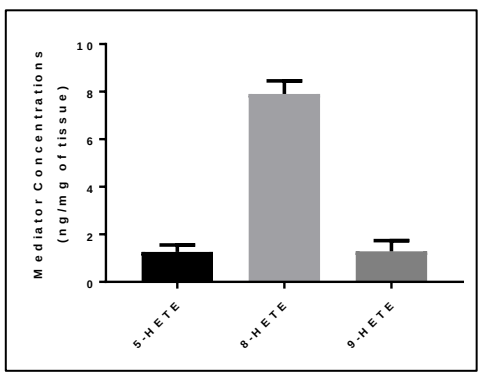


Fig. 2 Bioactive lipid mediators of the Arachidonic acid cascade were successfully measured, by LC-MS/MS, after lipids were extracted from the homogenate by solid phase extraction. Concentrations of 5-HETE, 8-HETE and 9-HETE were measured to be 1.3 ±0.3, 7.9±0.6 and 1.3 ± 0.4 ng/mg of tissue, respectively.

/ CUSTOMER

A.F. Domenichiello, B.C. Wilhite and C.E. Ramsden
Lipid Mediators, Inflammation and Pain Unit,
Laboratory of Clinical Investigation, National Institute on Aging

The rat hindpaw skin is considered to have thicker epidermis than the back skin, thus it is more challenging to obtain complete homogenate when working with these tissues. The combination of Precellys® lysing kit matrix and homogenization settings using Precellys® Evolution Touch tissue homogenizer, allowed for the successful generation of a rat hindpaw whole tissue sample homogenate. The study also showed that Cryolys® cooling unit was able to maintain sample temperatures below 40°C.

SPPP-810-DU130

DRUG ANALYSIS IN HAIR WITH PRECELLYS®

The National Institute of Criminalistics and Criminology

/ CONTEXT

Within the context of drug analysis in hair new protocols have been developed to establish the toxicological profile of a patient. Those techniques involve constraints as low volumes and concentrations and absence of crosscontamination. Before the analysis by HPLCMS/MS, the samples had to be reduced in powder form. See also the publication 1. Journal of Analytical Toxicology, Vol. 29, Oct 2005 2. Anal Bioanal Chem DOI 10.1007/s00216-007- 1297-9

/ MATERIALS

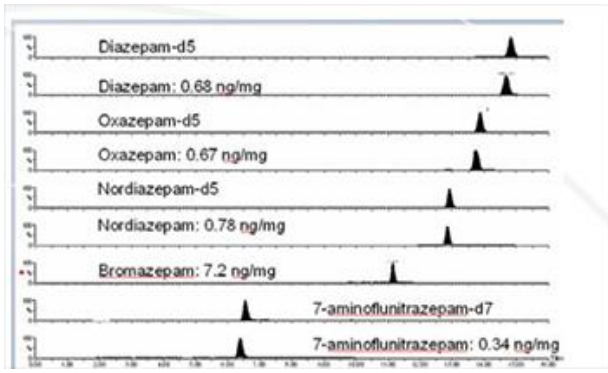
- Precellys® 24 Touch equipment.
- Precellys® kit MK28 (metal beads).
- Sample : 1cm hair segments.
- Buffer : empty.

/ PROTOCOL

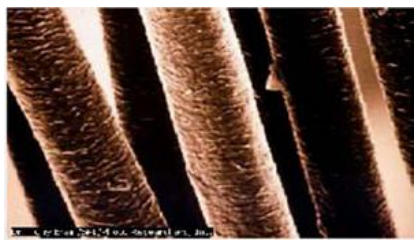
Precellys® 24 Touch parameters: 6500 rpm, 2x50 sec., 15 sec. Break.

/ RESULTS

Analysis of an authentic hair sample by HPLC-MS/MS with the following steps : 1. Decontamination (Dichloromethane) 2. Grinding (Precellys® 24 Touch) 3. Incubation (Methanol) 4. Centrifugation 5. Evaporation 6. Filtration 7. Analysis by HPLC-MS/MS



NICC, Belgium January 2005



/ CUSTOMER

Precellys®24 Touch is successfully evaluated on hair grinding, which is considered a difficult and challenging sample. Results of toxicology tests in crime labs are now faster.

SPPP-810-DU130

PROTEIN EXTRACTION EFFICIENCY OF SOFT AND HARD SEEDS USING THE PRECELLYS® LYSING KITS

Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA

/ CONTEXT

Sample preparation for protein extraction is a critical first step to achieve reliable analytical results. In this study we compared different bead kits using two different suspension liquids and two kinds of plant organ, soft and hard seeds, namely peanut and rice, respectively.

/ MATERIALS

- Precellys® 24 Touch protocol: 6500rpm, 3x60s, 20s pause in between cycles
- Precellys® lysing kits: CK14, CK28-R, MK28-R, CKMix50-R 2mL tubes
- Soft seed: One cotyledon of peanut (410 mg)
- Hard seed: ten seeds of rice (220 mg)
- Suspension media: saline buffer (1M NaCl, 50 mM Tris, pH 8.5) vs. ddH2O (control), 1ml/tube
- Centrifugation at 18000rpm, 25min, 4°C (Microfuge®18 Beckman) separated the top oil phase from the fiber/beads pellet (bottom phase), leaving a medium aqueous phase, which was used for protein quantification (analyzed by the BCA assay). Mean values were derived from triplicate samples.

/ RESULTS

Lysing Kits	Peanuts (%)	Rice (%)	Number of proteins identified from rice using ESI-MS-MS**
MK28-R	36.98	6.41	212 IDs
CK28-R	38.55	6.11	202 IDs
CKMix50-R	41.96	8.03	178 IDs
CK14	42.08	8.25	151 IDs

Table 1. Protein extraction efficiency as a percentage of total protein content, using saline extraction buffer. Extraction efficiency is based on the theoretical amount of protein, 25.8g, 6.12g/100g of peanut, rice respectively. (USDA National Nutrient Database for Standard Reference Release 26, Feb 7, 2014. http://ndb.nal.usda.gov/ndb/foods).

**A MudPIT method for LC-MS/MS was used to identify proteins from rice (Delahunty CM, Yates JR. MudPIT: multidimensional protein identification technology.

Biotechniques 2007; 43: 563-569).

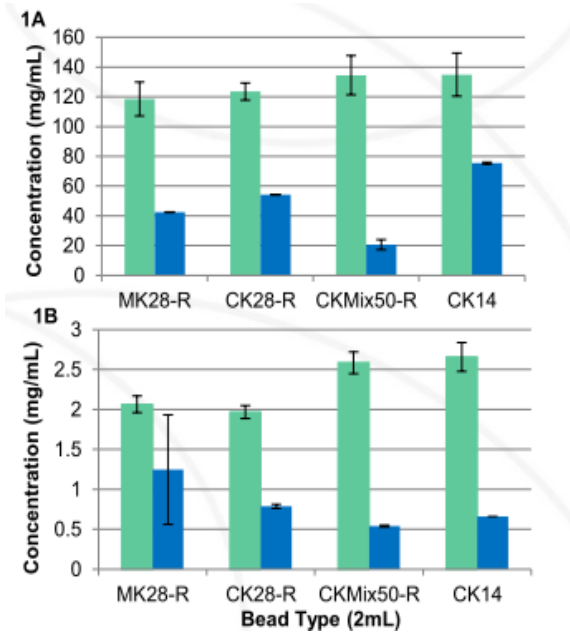


Figure 1. Protein concentration was quantified in the medium aqueous phase after peanut (A) and rice (B) homogenization in saline buffer (green bars) versus ddH2O (blue bars) using 4 different lysing matrices.

Protein extraction efficiency in peanuts and rice was compared after bead-beating with 4 different lysing kits. The use of saline buffer increased protein extraction efficiency as expected (Robert LS, Nozzolillo C, Altosaar I. Homology between legumin-like polypeptides from cereals and pea. Biochem J 1985; 226: 847-852). The CK14 beads gave the best protein yields compared to the other lysing beads for both hard and soft seeds.

Protein extraction efficiency from rice and peanuts was validated using ceramic (CK14, CK28-R, CKMix50-R 2mL) and stainless steel (MK28-R 2mL) lysing beads. The smallest bead diameter (CK14, 1.4 mm) correlated with the highest protein yields. The Precellys®24 Touch is a high-throughput homogenizer that can generate high quality extracts for proteomics when coupled with the right lysing kit.

03712-810-DU093

DROSOPHILA HOMOGENIZATION FOR LIPID ANALYSIS

Department of Developmental Biology, University of Regensburg, Germany

/ CONTEXT

Our research aim is to establish Drosophila as a model for Friedreich´s ataxia. This human neurological disorder is produced by the lack of the mitochondrial protein frataxin. Frataxin depletion results in a mitochondrial dysfunction and metabolic problems. We wanted to study weather reduction of frataxin in Drosophila also induced some metabolic responses such as loss of lipid homeostasis. In this work we have found that ubiquitous and glialtargetted reduction of frataxin expression leads to an increase in fatty acids [1].

/ MATERIALS

- Precellys® 24 Touch homogenizer.
- Precellys® lysing kit: 03961-1-002 (ceramic beads 2.8mm)
- Sample: Drosophila L3 larvae (15) or Drosophila adult heads (80).
- Buffer: Water.

/ PROTOCOL

Precellys® 24 Touch: 5500 rpm, 2x25 sec, 10s break.

Centrifugation steps: 5000 rpm

60s.

Analysis of lipid content: Samples were delipidated according to Bligh and Dyer, 1959 for thin layer chromatography studies and gas chromatography coupled with mass spectrometry (GC/MS) was carried out after FA methyl ester derivatization according to Ecker et al., 2010

/ RESULTS

Quality and quantity of extracted lipids from Drosophila samples using Precellys® 24 Touch technology was sufficient on the one hand to have reliable and reproducible results from different biological replica (not illustrated) and on the other hand to observe clear differences between control flies and frataxindeficient individuals (Figure1). Frataxin deficiency increases the amount of each fatty acid. In conclusion, loss of frataxin affects lipid metabolism and catabolism provoking an accumulation of fatty acids. Moreover, triacylglicerides and other neutral or phospholipids are not so affected.

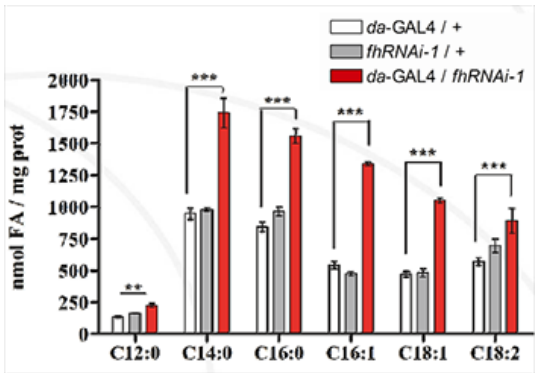
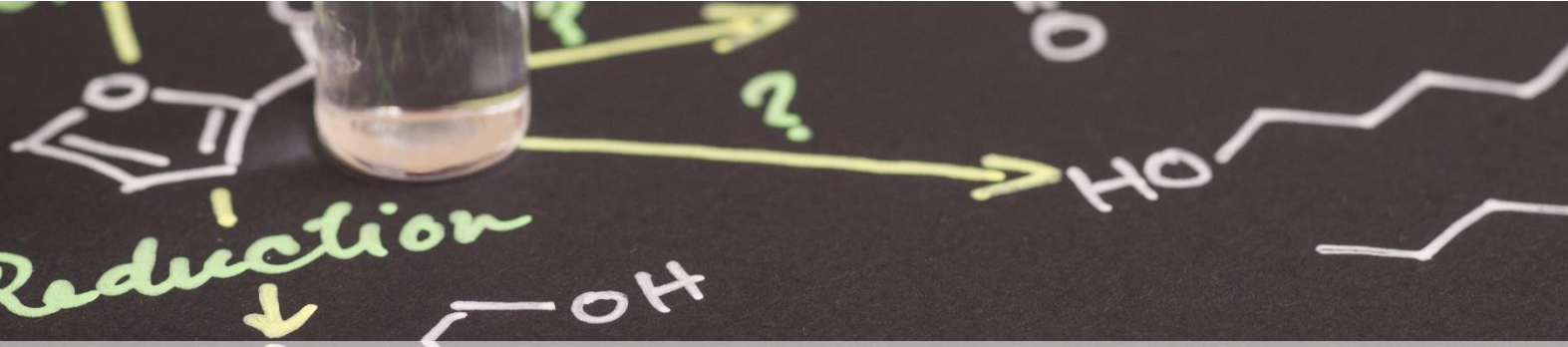


Figure 1: GC/MS analysis of fatty acids from Drosophila L3 larvae (Myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1) and linoleic acid (C18:2)).

/ CUSTOMER

Precellys®24 Touch provided us a complete fly homogenate containing lipid in the right range of both amount and purity, in order to carry out our experiments. Sample preparation is not only easy but cross contamination free.

03712-810-DU038



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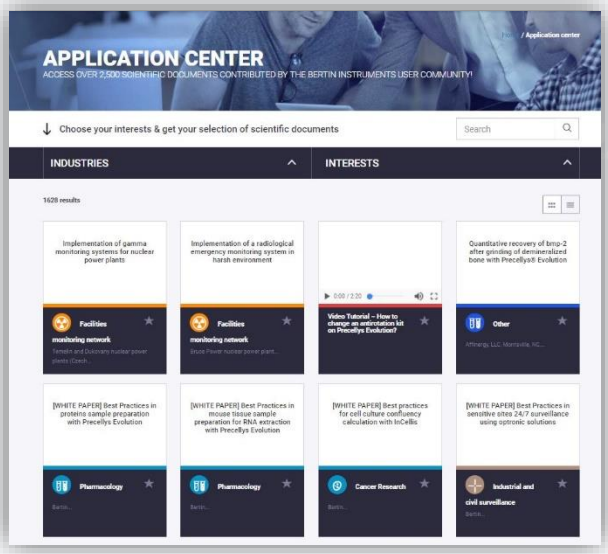

Precellys® Evolution Touch is the most advanced homogenizer gathering high efficiency and versatility for all sample preparation needs:

- **Flexibility:** 24 x 2mL (or 0,5mL), 12 x 7mL, 6 x 15mL and 96 well-plate format
- **Efficiency:** up to 10 000 rpm speed to grind any type of sample
- **Integrity:** protect your molecules with the Cryolys® Evolution cooling unit