New Capability:
Automated Surface Sampling Coupled to HPLC/MS
**dropletProbe™ – automated surface sampling coupled to HPLC/MS**

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In drug discovery, knowing the distribution of parent drug and metabolites in tissue sections is a crucial step. Presently, the particular molecular forms and quantities of the drug-related materials present must be determined from punched samples of areas of interest. Thin tissue sections or whole organ tissue homogenates are analyzed with conventional sample extraction, cleanup and high-performance liquid chromatography-mass spectrometry (HPLC-MS). While this conventional procedure can be fully automated, an automated direct surface sampling and analysis method capable of sample post-processing would save time and other resources by shortening the sampling and extraction steps of a quick first pass look for drug and metabolite distributions.

A PAL System autosampler coupled to HPLC/MS is capable of liquid-extraction based surface sampling as developed at the Oak Ridge National Laboratory. The system allows the analyst to deal with complex sample matrices and to identify isomeric compounds not distinguishable by direct surface sampling methods alone.

**Operation:**

First, the surface sample to be analyzed is mounted in a custom sample tray. Then an optical image of the mounted sample is acquired using a flatbed scanner controlled with the LMJ Points Plus© software developed at the Oak Ridge National Laboratory [1-5]. Following the selection of the sampling locations in the software, the PAL autosampler aspirates extraction solvent from a vial in a custom sample tray. This is followed by locking the needle guide with the needle protruding over the end of the guide using a needle lock hole [1-5]. Optionally, the laser sensor attached to the injection unit z-axis is positioned above the location to be analyzed where the laser sensor-to-surface distance is measured (Figure 1a). Using this measured distance (D), an appropriate z-axis value for the autosampler can be automatically determined for an optimal sampling needle-to-surface distance (d_{NS}, typically 100-300 µm) even for samples and sample locations of dramatically disparate height. This step can be avoided if analyzing a flat surface perpendicular to the z-axis. As illustrated in Figure 1b, this step is followed by moving the probe to the optimal d_{NS} sampling distance from the surface and a specific volume of extraction solvent (typically 0.5-5 µL) is dispensed onto the selected surface spot of interest creating a liquid microjunction between the needle and the surface (Figure 1c). After a given extraction time (typically 1-5 s) (Figure 1d), the extract is aspirated back into the syringe (Figure 1e). The dispense/aspirate extraction cycle can be repeated several times to achieve maximum analyte extraction. At the end of the extraction process, the entire sample is injected onto an HPLC column for subsequent HPLC/MS analysis. Figures 2a 

![Figure 1: Schematic of the surface sampling process](image1)

![Figure 2: Extraction liquid dispensed by the needle in locked position on](image2)
and b show sampling of a tissue biopsy and brain tissue of a whole body thin tissue section.

Features:
- software enables:
  - acquisition of optical image of surface for point-and-click selection of locations to be sampled
  - data analysis
  - heatmap generation
  - report keeping
- no hardware modification on the PAL System required for basic operation
- use of a cooled stack or an open tray
- <1 min sampling time
- method can be integrated into existing LC/MS workflow
- manual XYZ-axis control for complex samples (e.g. fungi)
- optional laser displacement sensor for unattended analysis of samples and sample locations of dramatically disparate height

Applications:
- drugs and metabolites [1-4] and macromolecules (proteins* and oligonucleotides**) from thin tissue sections
- drugs from tissue biopsy [4]
- metabolites from fungal cultures*
- small molecules from plant tissues (capsaicin from peppers, caffeine from coffee beans)**
- drugs from over the counter tablets**
- small molecule contamination on drywall**

References

* Submitted for publication.
** Unpublished data.
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