Enhanced Performance of a Liquid Microjunction Surface Sampling Probe using ESI and APCI

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OVERVIEW

- **Purpose:**
  - Evaluate and improve the performance of a Liquid Microjunction Surface Sampling Probe (LMJ-SSP)
- **Methods:**
  - All experiments were performed on an AB/MDS SCIEX 4000 QTRAP® mass spectrometer using a LMJ-SSP fabricated from a MicroIonSpray II®
- **Results:**
  - Turbo gas heaters and the normal ESI interface with LMJ-SSP typically improved signal levels by a factor of 6-10 compared to using the nanoelectrospray interface on the 4000 QTRAP®, when operating at ≥ 5 µL/min
  - The LMJ-SSP was used for
    - Automated high throughput spot sampling – 20s/spot
    - Detection of tryptic peptides separated on RP-C8 HPTLC plates
    - Detection and relative quantitation of drugs and metabolites sampled from thin tissue sections of drug-dosed small animals

INTRODUCTION

- Over the last several years we have been researching and developing a LMJ-SSP that can be coupled with most any atmospheric pressure liquid introduction ionization source for examination of analytes in/on a variety of surfaces [1-8]
- In this poster, we discuss our work with the LMJ-SSP using ESI for
  - Automated high throughput spot sampling – 20s/spot
  - Detection of tryptic peptides separated on RP-C8 HPTLC plates
  - Detection and relative quantitation of drugs and metabolites sampled from thin tissue sections of drug-dosed small animals
- We also discuss recent changes to the system that provide enhanced signal levels
  - These changes may allow the system to use ESI and APCI as secondary ionization sources
    - This is currently undergoing investigation and not reported on here

EXPERIMENTAL

- All experiments were performed on an AB/MDS SCIEX 4000 QTRAP® mass spectrometer (MDS Analytical Technologies, Concord, Ontario, Canada). Details of the setup, surface control and automation, and data analysis software have been described in detail elsewhere [1-8].

Figure 1. LMJ-SSP Mounted with the Nanoelectrospray and Standard ESI Interface on 4000 QTRAP®

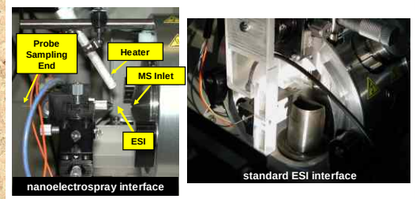


Figure 2. LMJ-SSP Signal Level with Standard Interface

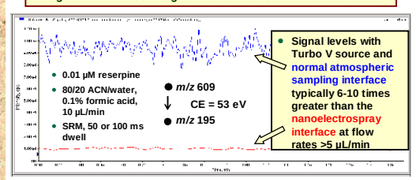
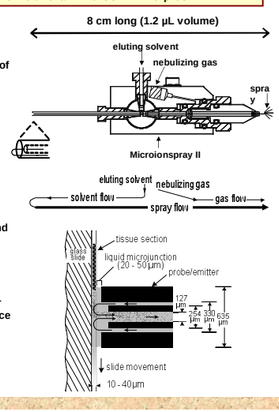


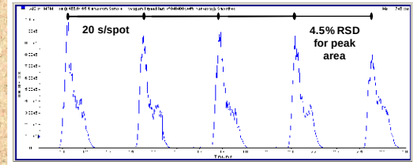
Figure 3. General LMJ-SSP Principles

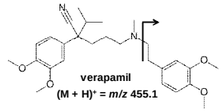
- Solvent pumped towards the surface through the annulus of the sampling and solvent delivery capillaries
- Solvent forms liquid microjunction with surface
- Material dissolved in solvent is aspirated from the surface through inner sampling capillary and sprayed
- Applicable to all species that can be dissolved in solvent and ionized by ESI or other ionization source in use



RESULTS AND DISCUSSION

Figure 4. High Throughput Spot Sampling with LMJ-SSP



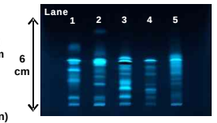


- m/z 455 → CE = 35 eV → m/z 165

- 1.5 ng verapamil spotted on and sampled from a s.s. MALDI target plate along with 4.5 µg of the matrix α-cyano-4-hydroxy cinnamic acid
- Eluting solvent 80/20 (v/v) ACN/water (0.1% formic acid) at 10 µL/min
- *Approximately 20 s/spot maximum throughput under conditions used*
- Higher solvent flow rate or shorter probe would increase throughput
- *15 s/spot easily achievable*
- See also Poster MPII #206 on spot sampling with LMJ-SSP

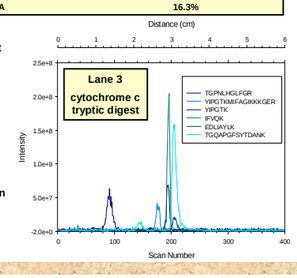
Figure 5. LMJ-SSP Analysis of Tryptic Peptides Separated on RP C8 TLC Plates

- Stationary phase: HPTLC RP-C8 (200 µm thick), F254
- Development solvent: 70/30 (v/v) methanol/water, 0.1 M ammonium acetate
- Stain: fluorescamine
- Plate loadings: 7 µL of 2 mg/L of each protein digest (14 ng protein)



Lane	Protein Tryptic Digest	Sequence Coverage using LMJ-SSP/ES-MS
1	lysozyme	39.5%
2	myoglobin	71.9%
3	cytochrome c	53.9%
4	beta casein	9.4%
5	BSA	16.3%

- Lanes scanned at 100 µm/s (10 min/lane)
- Eluting solvent 70/30 (v/v) water/ACN (0.1% formic acid)
- Full scan "EMS" spectra recorded (m/z 250 - 2000)
- Peak identification software and database matching used to identify peptides

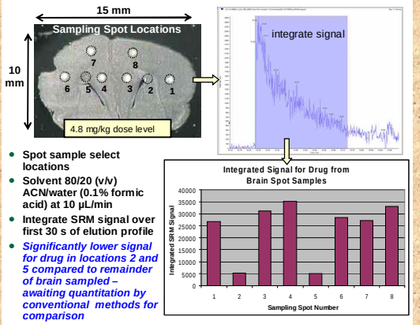


Surface Sampling Probe Examination of Guinea Pig Brain Thin Tissue Sections

- Hartley guinea pig brain thin tissue sections (20 µm thick) mounted on glass slides
- Animals dosed subcutaneous with 1 (0.48), 3 (1.6), 5 (2.4), or 10 (4.8 mg/kg) µmol/kg, sacrificed after 60 min, brains removed and frozen, and sections taken through the striatum
- Sections were kept at -80 °C until examined
- Standard solution (0.1 µM) in 80/20 (v/v) ACN/water (0.1% formic acid) aspirated through SSP to optimize 4000 QTRAP for SRM detection

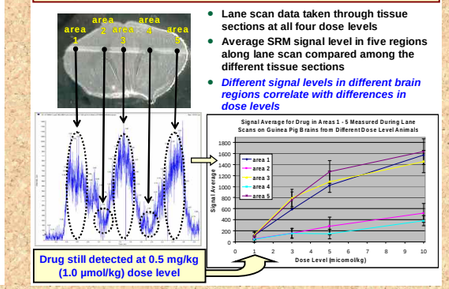
- m/z 482 → CE = 50 eV → m/z 98

Figure 6. LMJ-SSP Spot Sampling of Guinea Pig Brain Sections



- Spot sample select locations
- Solvent 80/20 (v/v) ACN/water (0.1% formic acid) at 10 µL/min
- Integrate SRM signal over first 30 s of elution profile
- *Significantly lower signal for drug in locations 2 and 5 compared to remainder of brain sampled – awaiting quantitation by conventional methods for comparison*

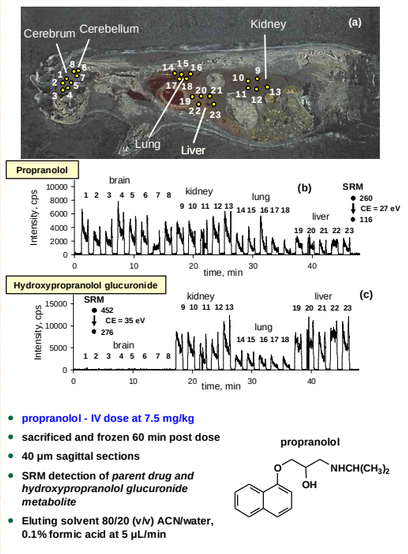
Figure 7. LMJ-SSP Lane Scans of Guinea Pig Brain Sections



- Lane scan data taken through tissue sections at all four dose levels
- Average SRM signal level in five regions along lane scan compared among the different tissue sections
- *Different signal levels in different brain regions correlate with differences in dose levels*

Drug still detected at 0.5 mg/kg (1.0 µmol/kg) dose level

Figure 8. Detection of Dosed Propranolol and Major Metabolite in a Whole-Body Thin Tissue Section



- propranolol - IV dose at 7.5 mg/kg
- sacrificed and frozen 60 min post dose
- 40 µm sagittal sections
- SRM detection of *parent drug and hydroxypropranolol glucuronide metabolite*
- Eluting solvent 80/20 (v/v) ACN/water, 0.1% formic acid at 5 µL/min

propranolol

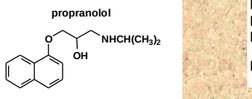
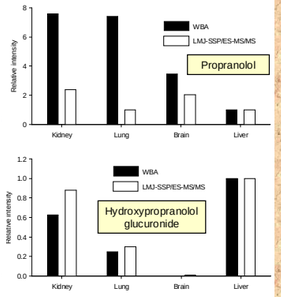


Figure 9. Relative Quantitation of Dosed Propranolol and Major Metabolite in a Whole-Body Thin Tissue Section

- Relative comparison of LMJ-SSP and whole-body autoradiography (WBA) determined levels for drug and metabolite
- See Poster TPAA #035 for WBA details
- Normalized to level of drug in liver, drug levels in lung and kidney by LMJ-SSP appear low compared to WBA
- Normalized to level of metabolite in liver, metabolite levels in all tissues comparable for both techniques



CONCLUSIONS

- The LMJ-SSP/ESI-MS can be used to sample, ionize and mass analyze analytes from a variety of surfaces in different sampling modes
- In this poster, we discussed
  - Automated high throughput spot sampling – 20s/spot shown
    - 15s/spot can easily be achieved
  - Detection of tryptic peptides separated on RP-C8 HPTLC plates
    - Use of a larger area sampling probe would more effectively sample from the large chromatographic bands
  - Detection and relative quantitation of drugs and metabolites sampled from thin tissue sections of drug-dosed small animals
    - More experiments with "calibrated" tissues are required to judge the quantitative capabilities
- Signal improvement approaching a factor of 10 was accomplished using turbo gas heaters and the normal ESI interface versus the nanoelectrospray interface
  - None of the application data presented here was acquired using this enhanced interface

LITERATURE CITED

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