Detection of Polar and Nonpolar Compounds on TLC Plates Using Laser Desorption/Electrospray+Atmospheric Pressure Chemical Ionization/Mass Spectrometry (LD/ESI+APCI/MS)

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(2014 HPTLC Symposium)
Two ambient ionization methods are currently used in TLC/MS:

• (1) Electrospray Ionization (ESI)

• (2) Atmospheric Pressure Chemical Ionization (APCI)
ESI-based TLC/MS:

1. Solvent elution followed by ESI/MS

2. Desorption/ionization by charged droplets impact (DESI)

3. Over-run TLC followed by ESI/MS

4. Two-Step ionization: pulsed laser for sampling and ESI for ionization
(1) Automated interface for hyphenation of planar chromatography with mass spectrometry
(2) Thin-Layer Chromatography and Mass Spectrometry Coupled Using Desorption Electrospray Ionization (TLC-DESI/MS)


**DESI**
*(ESI-based)*
(3) Interfaces To Connect Thin-Layer Chromatography with Electrospray Ionization Mass Spectrometry

(4) Electrospray-assisted laser desorption/ionization mass spectrometry for direct ambient analysis of solids –**ELDI/MS**


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**Diagram Description:**
- **LB:** Laser Beam
- **ISC:** Ion Source Chamber
- **EC:** Electrospay Cone
- **SS:** Sample Surface
- **SP:** Sprayer Plate

**Figure (b):** Image showing the electrospray process.

**Figure (d):** Image showing dropletspray ionization process.

**Cytochrome c**

**Mass Spectrum:**
- Peaks at masses +11, +12, +13, +14, +15, +16, +17, +18, +19
- Mass range: 600 to 1200 Da
High Throughput TLC-ELDI/MS System

First Generation (Lego-made)  Shiea et al., Anal Chem. 2012, 84, 5864-8
High Throughput TLC-ELDI/MS System

Second Generation (Teflon-made)
High Throughput TLC-ELDI/MS System

Third Generation (Stainless Steel and Teflon made)
High Throughput TLC-ELDI/MS System
Using Laser-Induced Acoustic Desorption/ Electrospray Ionization Mass Spectrometry To Characterize Small Organic and Large Biological Compounds in the Solid State and in Solution Under Ambient Conditions – TLC-LIAD/MS

Shiea et al., Anal. Chem. 2009, 81, 868–874
APCI-based TLC/MS:

1. Solvent elution followed by APCI

2. Desorption/Ionization by charged APCI species impact (DART)

3. Over-run TLC followed by APCI/MS

4. Two-Step ionization: pulsed laser for sampling and APCI for ionization

5. Thermal desorption followed by APCI/MS
(1) Self-aspirating atmospheric pressure chemical ionization source for direct sampling of analytes on surfaces and in liquid solutions

**APCI system**
New coupling of planar chromatography with direct analysis in real time mass spectrometry


DART (APCI-based)

MS

TLC plate

DART source

isopropyl-9H-thioxanthen-9-one

![Graph showing chromatographic peaks with retention times and mass intensities.](image)
(2) TLC-DART/MS
(3) Rotation Planar Chromatography Coupled On-Line with Atmospheric Pressure Chemical Ionization Mass Spectrometry

(4) Thin Layer Chromatography/Plasma Assisted Multiwavelength Laser Desorption Ionization Mass Spectrometry for Facile Separation and Selective Identification of Low Molecular Weight Compounds (PAMLDI or LD-DART/MS)
(4) TLC combined with laser-induced acoustic desorption/dielectric barrier discharge ionization mass spectrometry (LIAD-DBDI/MS)
Shiea et. Al. (poster #28 in this symposium)

LIAD-DBDI
(APCI-based)

Gas flow
HV (AC)

APCI plume
Analysis ion
MS inlet

Neutral analyte
TLC plate
Glass slide

Pulsed laser beam

(b) EIC 168
MH+
carbazole

(c) EIC 263
MH+
triphenylphosphine

(d) EIC 202
M⁺
Fluoranthene
Thin-layer chromatography and mass spectrometry coupled using proximal probe thermal desorption with electrospray or atmospheric pressure chemical Ionization


TD-ESI or TD-APCI

- Corona needle
- ESI
- TLC plate
- computer controlled x,y,z positioner
- extension to cone electrode
- sample cone
- cone electrode
- modified window
- mini vacuum pump
- source block

Caffeine
Acetaminophen
Aspirin
Due to limitations in ionization capabilities, each of current ambient ionization technique can only ionize analytes with limited mass ranges and polarities: ESI: nonvolatile and polar compounds, APCI: small and less or non-polar compounds.
TLC combined with laser desorption and ESI+APCI/MS
(1) An ambient ionization source capable to generate primary ions (reacting species) from both ESI and plasma-APCI was developed.

(2) The analytes on the TLC plate were desorbed using a pulsed laser beam (LD).

(3) The desorbed analytes (polar, nonpolar, volatile, and nonvolatile) were simultaneously interacted with the primary ions to form analyte ions. The analyte ions were subsequently detected by an ion trap mass analyzer attached to the ESI+APCI source.
Configuration of ESI+APCI Source (patent pending)

- ESI System
- DBDI System
- Ring Electrode
- Nebulizing Gas Inlet
- ESI Solution Inlet
- Stainless Steel Capillary (I.D. 1.6 µm, O.D. 500 µm)
- Copper Rod
- Glass Tube
- Fused Capillary (O.D. 320 µm, I.D. 110 µm)
- Stainless Steel Tube (grounded)
- Glass tube
- Ring electrode (HV)
- N2
ESI+APCI source - ionization of polar/nonpolar and small/large molecules in one analysis

(a) ESI only

(b) APCI only

(c) ESI + APCI

(d) ESI + APCI

Gas flow

Solution

In

Gas flow

HV (AC)
Ionization Mechanisms Involved in ESI+APCI Ion Source
Penning Ionization, Ion-Molecule Reactions, Electrospray Ionization

**APCI only:**
\[ \text{He}^*(2^{3}S) + M \rightarrow M^{+} + \text{He} + e^- \]
1. \( \text{H}_2\text{O}^{+} + \text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^{+} + \text{OH}^{-} \)
2. \( \text{H}_3\text{O}^{+} + n\text{H}_2\text{O} \rightarrow [(\text{H}_2\text{O})_n]^+ \)
3. \( [(\text{H}_2\text{O})_n]^+ + M \rightarrow \text{MH}^+ + n\text{H}_2\text{O} \)

**ESI only:**
\( M_{(S)} + \text{H}^+, \text{H}_3\text{O}^+, \text{CH}_3\text{OH}_3^+ \rightarrow \text{MH}^+ \)
\( M_{(L)} + \text{Droplet}^{n+} \xrightarrow{\text{fusion ESI}} \text{MH}_{n}^{n+} \)

**APCI + ESI:**
\[ \text{He}^*(2^{3}S) + M \rightarrow M^{+} + \text{He} + e^- \]
\( M_{(S)} + \text{H}^+, \text{H}_3\text{O}^+, \text{CH}_3\text{OH}_3^+ \rightarrow \text{MH}^+ \)
\( M_{(L)} + \text{Droplet}^{n+} \xrightarrow{\text{fusion ESI}} \text{MH}_{n}^{n+} \)
TLC-LD/ESI+APCI/MS

Pulsed Laser

- pulsed laser beam
- sample
- ESI plume and Plasma

CW Laser

- CW laser beam
- sample
- ESI plume and Plasma
Pulse laser desorption/ESI+APCI/MS to simultaneously characterize Indole, Ferrocene, Lidocaine, and Angiotensin I
Rosemary Essential Oil – CW LD-ESI+APCI/MS

ESI only

ESI+APCI

APCI only

m/z

Relative intensity
TLC combined with LD/ESI+APCI/MS for sample analysis

Gas flow

ESI solution (HV DC)

Gas flow

HV (AC)

Pulsed laser beam

Analyte ion

ESI+APCI plume

Neutral analyte

TLC plate

MS inlet
Photograph of TLC-LD/ESI+APCI/MS system

- MS inlet
- TLC plate
- XY stage
- ESI+APCI source
- Laser spot
TLC-LD/ESI+APCI/MS to characterize standard mixture

**ESI only**

<table>
<thead>
<tr>
<th>Relative intensity</th>
<th>(a) EIC 284</th>
</tr>
</thead>
<tbody>
<tr>
<td>methylene blue</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relative intensity</th>
<th>(b) EIC 186</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrocene</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Relative intensity</th>
<th>(c) EIC 215</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrocenealdehyde</td>
<td></td>
</tr>
</tbody>
</table>
TLC-LD/ESI+APCI/MS to characterize standard mixture

APCI only

Relative intensity

100

0

methylene blue

Ferrocenealdehyde

Ferrocene

(a) EIC 284

(b) EIC 186

(c) EIC 215
TLC-LD/ESI+APCI/MS to characterize standard mixture

(a) EIC 284

Relative intensity

(b) EIC 186

Relative intensity

(c) EIC 215

Relative intensity
LD/ESI+APCI mass spectra of methylene blue, ferrocene, and ferrocenealdehyde

(a) Methylene blue

(b) Ferrocene

(c) Ferrocenealdehyde
ESI+APCI/MS for desorption/ionization of analytes on TLC plate
Summary

An ambient source coupling ESI with APCI was developed to simultaneously generate primary ions by ESI and APCI.

This ambient ESI+APCI source was successfully used to characterize polar and nonpolar compounds on surfaces (e.g., TLC plate) through direct desorption/ionization and two-step ionization processes. Both radical and protonated analytes ions (including singly and doubly charged ions) were detected in the ESI+APCI mode.
Acknowledgement

Dr. Sy-Chyi Cheng
Dr. Siou-Sian Jhang
Dr. Min-Zong Haung
Mr. Zuei-Hung Hung

Financial Supports:
National Sun Yat-Sen University (NSYSU)
National Science Council, Taiwan
Ministry of Education, Taiwan
National Sun-Yat Sen University
95 Unleaded Gasoline - CW LD-ESI+APCI/MS

(a) ESI on  
APCI off

(b) ESI on  
APCI (4.7 kV)

(c) ESI on 
APCI (6.2 kV)

(d) ESI on  
APCI (7.1 kV)

(e) ESI on 
APCI (8.3 kV)

(f) ESI on 
APCI (9.4 kV)