Dual Dopant Approach for Simultaneous LC-APPI-MS/MS Analysis of Low and High Proton Affinity Pesticides

Sheng-Suan (Victor) Cai, Kaveh Jorabchi, and Jack Syage
Syagen Technology, Inc., 1411 Warner Ave., Tustin, CA 92780

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Introduction

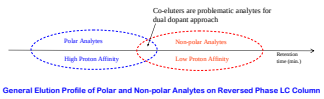
- Benefits of APPI
  - Ionizing both polar and nonpolar compounds simultaneously, allowing LC/MS users to analyze more compounds in USDA pesticide database.
  - Over 4-5 orders of dynamic linear ranges, a preferred ionizer for quantitative analysis.
  - Much reduced matrix effects than ESI, leading to simplified sample cleanup procedures, better analyte recoveries and data quality.
- Objectives
  - To explore dual dopant approach to maximizing APPI sensitivity for simultaneous analysis of low and high proton affinity compounds

Why Dual Dopants

- Low proton affinity analytes require a charge exchange dopant such as chlorobenzene, toluene and anisole
- High proton affinity analytes need a proton transfer dopant such as acetone, toluene or THF
- To maximize APPI sensitivity, dual dopants are needed for simultaneous analysis of both low and high proton affinity compounds.
- Mixing a charge exchange dopant with a proton transfer dopant does not maximize APPI sensitivity.

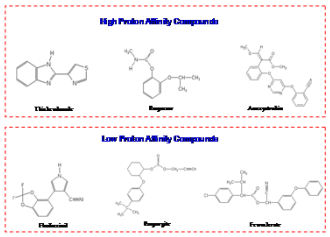
On-Column Analyte Elution Profile

- For demonstration purpose, a total of six pesticides are used. Three of them are high proton affinity compounds and the other three are low proton affinity compounds.
- In general, when a reversed phase column such C18 is used as a separation column, high proton affinity analytes (usually more polar) are less retained on column than those with low proton affinities (usually less polar or non-polar). This allows delivery of a proton transfer dopant such as toluene for sensitivity enhancement of relatively polar eluters at the same time and delivery of a charge exchange dopant such as chlorobenzene for less or nonpolar analytes.



General Elution Profile of Polar and Non-polar Analytes on Reversed Phase LC Column

Chemical Structure of Pesticides



Setup of Dopant Delivery Switch Valve

- A Rheodyne six port injection valve is used for delivery of toluene for high proton affinity analytes and chlorobenzene for low proton affinity compounds.
- Instead of using peek tubing, Restek deactivated GC fused silica guard column (0.075 mm ID) is used as a dopant transfer line. Each dopant is individually introduced into APPI source via the auxiliary gas inlet instead of by post column addition using a MicroTee assembly.
- Setup of Rheodyne Switch Valve Flow Lines
  - For toluene delivery, set Rheodyne valve in "Load" position
    - Port 2 connected to a syringe or LC pump, and Port 3 connected to APPI source via auxiliary gas inlet.
  - For chlorobenzene delivery, set Rheodyne valve in "Inject" position
    - Port 6 connected to a syringe or LC pump, and Port 5 connected to APPI source via auxiliary gas inlet.
  - Port 1 connected to a waste bottle and Port 4 connected to a purge line (capped in this work).

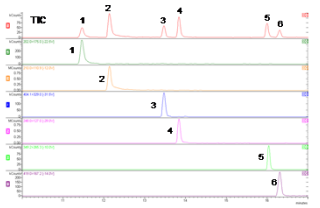
Instrument Parameters and Conditions

- Instrument: Varian 320 MS/MS with Syagen prototype APPI PhotoMate source
- Shield: 600 V
- Housing Temp: 65 °C
- Drying Gas Temp: 300 °C
- Vaporizer Gas Temp: 350 °C on column
- Drying Gas Pressure: 12 psi
- Nebulizing Gas Pressure: 30 psi
- Vaporizer Gas Pressure: 60 psi
- Collision Gas Argon Pressure: 1.5 mTorr
- APPI Repeller Voltage: 1.5 kV
- Detector: 1355 V, positive mode
- Injection volume/amount: 2.5 µL/2.5 ng each
- Column separation was performed using MeOH/water gradient elution: 10% to 100% MeOH in 10 min and hold. Mobile phase flow rate: 200 µL/min. LC Column: ACE 3 C18, 100 x 2.1 mm.
- APPI Dopant: Toluene and/or chlorobenzene, flow rate: 20 µL/min. The dopant was introduced into APPI source via auxiliary gas inlet

LC-MS/MS Acquisition Parameters

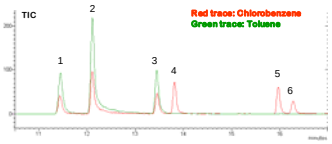
Analyte ID	Pesticide Name	Proton Affinity	Precursor Ion Type	RT (min)	MS/MS Transition	Capillary (V)	Collision Energy (V)	Dwell Time (s)
					202.0 > 130.9	88	28.5	0.025
1	Thiabendazole	High	$[M+H]^+$	11.463	202.0 > 175.0	88	22.5	0.025
					210.0 > 110.9	36	12	0.025
2	Propoxur	High	$[M+H]^+$	12.139	210.0 > 168.0	36	7	0.025
					404.1 > 329.0	30	31.5	0.025
3	Azoxystrobin	High	$[M+H]^+$	13.473	404.1 > 372.1	30	12	0.025
					248.0 > 127.0	60	29	0.025
4	Fludioxonil	Low	M^-	13.844	248.0 > 182.2	60	18.5	0.025
					350.1 > 81.1	32	17	0.025
5	Propargite	Low	M^-	15.999	350.1 > 201.0	32	9.5	0.025
					419.0 > 125.2	30	34.5	0.025
6	Fenvalerate	Low	M^-	16.307	419.0 > 167.2	30	14	0.025

On-Column Separation of Analytes



Analytes 1-3: High proton affinity, 4-6: Low proton affinity

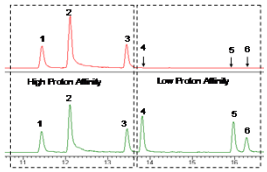
Chlorobenzene vs. Toluene



Chlorobenzene detects both high proton affinity compounds 1-3 and low proton affinity compounds 4-6. However chlorobenzene offers lower sensitivity for high proton affinity compounds relative to toluene.

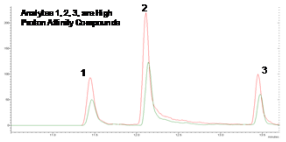
Toluene enhances intensity of high proton affinity compounds 1-3 by about two folds, but does not detect low proton affinity compounds 4-6.

Chlorobenzene vs. Toluene



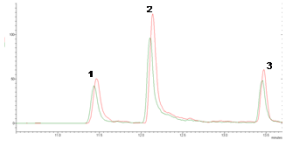
Green trace: Chlorobenzene, Red trace: Toluene. Chlorobenzene detects all six compounds 1-6. Toluene does not detect low proton affinity compounds 4-6.

No Dopant vs. Toluene



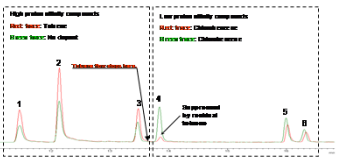
Green trace: No dopant, Red trace: Toluene. Toluene enhances intensity by about two folds for high proton affinity compounds 1-3. None of low proton affinity compounds 4-6 is detected using toluene or without a dopant (results not shown).

No Dopant vs. Chlorobenzene



Green trace: Chlorobenzene, Red trace: No dopant. Chlorobenzene does not enhance intensity of high proton affinity compounds when MeOH/H_2O is used as a mobile phase. However, chlorobenzene does promote a proton transfer when CH_3CN/H_2O is used as a mobile phase (results not shown)

Signal Suppression of Co-Eluters

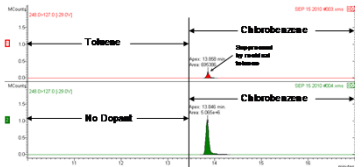


High proton affinity compounds 1-3: Toluene was used for red trace and no dopant was used for green trace.

Low proton affinity compounds 4-6: Chlorobenzene was used for both traces.

These results show that residual amount of toluene present in housing significantly suppresses the intensity of low proton affinity compounds.

Signal Suppression of Co-Eluters



For upper red trace: toluene was introduced for high proton affinity compounds between 10.0 and 13.5 min. and for lower green trace: No dopant was used within the same time frame. For both traces, chlorobenzene was used for low proton affinity analytes between 13.5 and 17.0 min. These results show that residual amount of toluene present in housing significantly suppresses the intensity of low proton affinity compound (246.0>127.0).

Limitations and Design Considerations

- When low and high proton affinity compounds co-elute or closely elute on column. This approach does not work well. In this case, chlorobenzene must be used for both compounds because chlorobenzene promotes formation of both $[M+H]^+$ and M^+ ions simultaneously although the enhancement for protonated compounds is less than toluene.
- Dual dopant switch valve must be mounted as closely to APPI source as possible, preferably being built into the source. This will minimize the delivery delay time while switching and reduce the amount of unwanted dopant leaching into the source.
- After a dopant flow stops, the residual dopant continue to be present in APPI for about 30-40 seconds. This results in suppression of analytes requiring another dopant. A purge flow can be programmed and used to rapidly flush the unwanted dopant out of APPI source, if needed.
- Purge line (port 4) can be left open if the switch valve is built into the APPI source. This will allow the unwanted dopant to be purged out of the flow line, reducing the unwanted dopant to continue to leach into APPI source.

Summary

- A programmable dual dopant delivery system allows use of optimum dopants for specific analytes based on on-column elution profile/retention times and offers several capabilities/flexibilities for achievement of maximum APPI sensitivity. The following scenarios can be performed:
  - Use of Dual Dopants (e.g., toluene vs. chlorobenzene): When high proton affinity and low proton affinity analytes are well separated on column, dual dopants can be used such as toluene for polar compounds and chlorobenzene for nonpolar compounds.
  - Use of No Dopant vs. Chlorobenzene: No dopant is used for polar compounds and chlorobenzene is used for non-polar compounds. APPI offers good sensitivity without dopant using MeOH/water as a mobile phase especially when analytes co-elute with high MeOH content. Sometimes, chlorobenzene suppresses polar compound sensitivity. In this case, chlorobenzene flow must be stopped.
  - Adjustment of Dopant Flow Rates: An optimum dopant flow rate changes from analyte to analyte and changes as mobile phase composition changes using a gradient elution. A programmable dopant delivery system offers varied flow rates for maximum sensitivity of specific compounds.

Acknowledgement

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