The Agilent 5975 inert MSD: New Tools for the Forensic Analyst

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The Speaker

Bruce Quimby is a Senior Applications Chemist in the Chemical Analysis Solutions unit of Agilent Technologies in Wilmington, Delaware. He received a Ph.D. in analytical chemistry from the University of Massachusetts (Amherst) in 1980 and a bachelor's degree in chemistry from Mansfield State College (PA) in 1974. He has been at Agilent Technologies (formerly Hewlett-Packard) since 1979, working the first 10 years in research and development. He has authored or co-authored 18 journal articles and 10 patents in the field of gas chromatography. He is currently working in Forensic and Homeland Security applications.
Introducing the 5975 inert MSD
What’s Different?

• Does everything a 5973 does, plus…
• Synchronous SIM/Scan
• Extended mass range
• AutoCl and EI with CI source
• 6850 GC control
• QuickSwap MSD interface
• Permanent Effluent Splitters
• Deconvolution Reporting Software
Functional Front Window – Ease of Use

- Wire connections
- Filament operation
- Column insertion
- CI Source identification
Quick Access – Ease of Use

Easy analyzer access – hinged side cover
Extended Mass Range – Performance

1050 m/z for 5975 inert MSD

- Quadrupole modified
- New electronics

- 800 m/z for all 5973 MSDs
  (Software upgrade does not enable 1050 m/z)
AutoCI – Performance

- No external manual control
- Fully automated setup/tuning
- Flexible Configuration
- EI capability with CI Source
- AutoCI – as easy as EI
AutoCI Electronic Flow Control – Ease of Use

- Reagent gas automatically adjusted by tune
  - Ammonia compatibility
- Ensures easy reproduction of application

![Diagram showing gas flow](image-url)
Ion Source – Improved Response

Data from a production forensic drug lab…

Inert Ion source – Improved response

50pg LSD

Extracted Ion 253 m/z

Inert Source

~6x improvement!

LSD S/N 16

SS Source

LSD S/N 2.9
Faster Scan Speeds and 5 msec SIM Dwell

**Standard Electronics**

- $2^0 \sim 6250$ amu/sec
- $2^1$ Fall off with increasing scan speed
- $2^3 \sim 781$ amu/sec

**Performance Electronics**

- $2^0 \sim 6250$ amu/sec
- Nearly no fall off with increasing scan speed
- $2^1 \sim 781$ amu/sec

Moving from $2^3$ to $2^2$ doubles scans
Opiates, 38 ions (5 msec dwell) in 1 group, 10 SIM cycles/peak

280°C 1.75 min
200°C 0 min
20°C/min
3 min

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Synchronous SIM/Scan

What is Synchronous SIM/Scan?

Agilent’s Synchronous SIM/Scan allows for the continuous collection of both SIM and Scan data in the SAME acquisition. More data without sacrificing sensitivity or library searching capability.

Great for target analysis (SIM) and identifying unknowns (Scan):

- SIM data for quantitation of targets
- Scan data for confirmation of unknowns
Agilent Synchronous SIM/Scan

Alternating SIM (black) and scan (magenta) data collection

SIM + scan in one cycle, alternating rapidly

High sensitivity + high information throughout the whole chromatogram
Agilent Instrumentation Used

Gas Chromatograph

- 6890N with Autosampler (tray & injector) and split/splitless inlet
- Capillary NPD with extended jet
- G3180B - Microfluidic Splitter with Makeup Gas

Mass Spectrometer

- 5975 inert MSD with EI source

Software

- GC/MSD Chemstation G1701 DA version D.02.00 or higher (includes RTL and Screener)

Column

- DB-17MS 15m x 250µm x 0.25µm part # 122-4712
Method Parameters

<table>
<thead>
<tr>
<th>GC</th>
<th>Agilent 6890N</th>
</tr>
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<tbody>
<tr>
<td>Oven</td>
<td></td>
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<tr>
<td>Ramp</td>
<td>'C/min 'C Hold min</td>
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<tr>
<td>Initial</td>
<td>80 0.00</td>
</tr>
<tr>
<td>Ramp 1</td>
<td>20 320 5.00</td>
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<tr>
<td>Runtime</td>
<td>17 min</td>
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<table>
<thead>
<tr>
<th>MSD</th>
<th>Agilent 5975inert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent Delay</td>
<td>2.5 min</td>
</tr>
<tr>
<td>Scan Range</td>
<td>42 to 450</td>
</tr>
<tr>
<td>Threshold</td>
<td>0</td>
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<tr>
<td>Sampling</td>
<td>$2^2$ for scan, $2^1$ for SIMSCAN</td>
</tr>
<tr>
<td>Quad Temp</td>
<td>150 'C</td>
</tr>
<tr>
<td>Source Temp</td>
<td>280 'C</td>
</tr>
<tr>
<td>Transfer Line</td>
<td>280 'C</td>
</tr>
</tbody>
</table>

| Inlet       | Split/Splitless |
| Temp        | 280 'C          |
| Mode        | Splitless       |
| Pressure    | 23.16 psi       |
| Purge Flow  | 50 mL/min       |
| Purge time  | 2.00 min        |
| Total Flow  | 56.3 mL/min     |

| Injector    |               |
| Sample Washes | 0             |
| Sample Pumps | 6              |
| Injection volume | 2 uL          |
| Solv A washes | 4             |
| Solv B washes | 4              |
| Viscosity Delay | 3            |
| Plunger Speed | Fast          |

| Column      | DB-17MS part # 122-4712 |
| Mode        | Constant pressure      |
| Pressure    | 23.16 psi              |
| Initial Flow| 3.8 mL/min             |
| Outlet      | Splitter               |
| Outlet Pressure | 3.8 psig            |
Benzodiazepines
0.63 ng each to MSD

1. Oxazepam
2. Lorazepam
3. Diazepam
4. Flunitrazepam
5. Nitrazepam
6. Clonazepam
7. Alprazolam
Flunitrazepam - Scan Only Mode
0.63 ng

Ion 312

Ion 286

Ion 285

Ion 266

S/N_{pk-pk} 25.7
Flunitrazepam- SIM From SIM Only Run
0.63 ng

Ion 312

Ion 286

Ion 285

S/N_{pk-pk} 511

Ion 266

11.1 11.2 11.3 11.4 11.5 11.6 11.7

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Synchronous Data

Scan data file

SIM data file
Comparison of Scan Only and Scan from SIM/Scan

Extracted Ions From Scan Mode

- Ion 312
- Ion 286
- Ion 285
- Ion 266

Extracted Scan Ions From SIM/Scan Mode

- Ion 312
- Ion 286
- Ion 285
- Ion 266

S/N_{pk-pk} 26

3.51 Scans/sec

S/N_{pk-pk} 32

3.59 Scans/sec

For Research Use Only. Not for use in diagnostic procedures.
Comparison of SIM Only and SIM from SIM/Scan

**Extracted SIM Ions From Scan Mode**
- Ion 312
- Ion 286
- Ion 285
- Ion 266

25 msec dwell

**Extracted SIM Ions From SIM/Scan Mode**
- Ion 312
- Ion 286
- Ion 285
- Ion 266

25 msec dwell

- $S/N_{pk-pk}$ 511
- $S/N_{pk-pk}$ 327

6.20 Scans/sec
3.59 Scans/sec

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Flunitrazepam-Advantage for Quant with SIM/Scan

0.63 ng

Extracted Ions From Scan Mode

- Ion 312 Scan
- Ion 286 Scan
- Ion 285 Scan
- Ion 266 Scan

$S/N_{pk-pk} = 25.7$

Extracted SIM Ions From SIM/Scan Mode

- Ion 312 SIM
- Ion 286 SIM
- Ion 285 SIM
- Ion 266 SIM

$S/N_{pk-pk} = 327$
Synchronous SIM/Scan – Library Searchable Spectra

Flunitrazepam

Scan Only Spectra

NIST Forward Match 837

Spectra from SIM/Scan

NIST Forward Match 826

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## Signal to Noise Improvement with SIM/Scan

<table>
<thead>
<tr>
<th>Ret Time</th>
<th>Compound</th>
<th>Tgt Ion</th>
<th>A Scan S/N pk-pk</th>
<th>B SIM/Scan S/N pk-pk</th>
<th>C SIM only S/N pk-pk</th>
<th>D SIM/Scan S/N pk-pk</th>
<th>Improvement factor</th>
<th>D/A</th>
<th># ions in SIM</th>
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<tr>
<td>9.940</td>
<td>Oxazepam</td>
<td>267</td>
<td>43</td>
<td>49</td>
<td>687</td>
<td>676</td>
<td>16</td>
<td>4</td>
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<td>10.338</td>
<td>Lorazepam</td>
<td>239</td>
<td>87</td>
<td>60</td>
<td>787</td>
<td>1098</td>
<td>13</td>
<td>7</td>
<td></td>
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<tr>
<td>10.490</td>
<td>Diazepam</td>
<td>256</td>
<td>242</td>
<td>388</td>
<td>2002</td>
<td>2638</td>
<td>11</td>
<td>7</td>
<td></td>
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<tr>
<td>11.353</td>
<td>Flunitazepam</td>
<td>285</td>
<td>50</td>
<td>42</td>
<td>461</td>
<td>597</td>
<td>12</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>12.327</td>
<td>Nitrazepam</td>
<td>280</td>
<td>6</td>
<td>12</td>
<td>115</td>
<td>116</td>
<td>18</td>
<td>8</td>
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<tr>
<td>12.733</td>
<td>Clonazepam</td>
<td>280</td>
<td>8</td>
<td>11</td>
<td>128</td>
<td>123</td>
<td>15</td>
<td>4</td>
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<td>13.168</td>
<td>Alprazolam</td>
<td>308</td>
<td>18</td>
<td>24</td>
<td>173</td>
<td>192</td>
<td>11</td>
<td>4</td>
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<tr>
<td></td>
<td>avg</td>
<td></td>
<td>1613</td>
<td>11</td>
<td>12</td>
<td>18</td>
<td>1511</td>
<td>13.6</td>
<td></td>
</tr>
</tbody>
</table>

**SIM and Scan performance of SIM/Scan is comparable to SIM only and Scan only modes**
Permanent Splitter System GC/NPD/MSD

1:1 split NPD:MSD

- **6890N GC**
  - Column: 30 m X 0.25 mm id X 0.25 um HP-5MS

- **Auto-sampler**
  - AUX EPC: 3.8 psig

- **NPD**
  - 0.532 m X 0.18 mm id

- **Effluent Splitter with Makeup**
  - 1.44 m X 0.18 mm id

- **5975 Inert MSD**
  - 0.532 m X 0.18 mm id
uFluidic Splitter Hardware

Deactivated microfluidic splitter operates to 350 C.
Uses metal ferrules to eliminate leaks and retightening
Three Signals from 1 Injection: Scan, SIM, and NPD
1.25 ng Each Compound

Scan TIC

SIM TIC

NPD

1 Oxazepam
2 Lorazepam
3 Diazepam
4 Flunitrazepam
5 Nitrazepam
6 Clonazepam
7 Alprazolam

For Research Use Only. Not for use in diagnostic procedures.
Flunitrazepam

Extracted Ions From Scan Mode

- Ion 312 Scan
- Ion 286 Scan
- Ion 285 Scan
- Ion 266 Scan

Extracted SIM Ions From SIM/Scan Mode

- Ion 312 SIM
- Ion 286 SIM
- Ion 285 SIM
- Ion 266 SIM

NPD

- S/N_{pk-pk} 32
- S/N_{pk-pk} 327
- S/N_{pk-pk} 766

For Research Use Only. Not for use in diagnostic procedures.
Flunitrazepam
1.25 ng

- **Scan from SIM/Scan**
  - Use for confirmation

- **Extracted SIM Ions From SIM/Scan Mode**
  - **Ion 312 SIM**
  - **Ion 286 SIM**
  - **Ion 285 SIM**
  - **Ion 266 SIM**
  - \( S/N_{pk-pk} = 327 \)

- **NPD**
  - Use for confirmation and/or quant
  - \( S/N_{pk-pk} = 766 \)

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Why Does My NPD Peakshape Look So Good?
NPD Jets, Standard vs Extended Tip

Extended Tip extends ~ 9mm
Analytes are positioned closer to the bead, minimizing tailing.

G1531-80560 Standard Capillary Jet
G1534-80580 Optional Capillary Jet with extended tip
Drug Standard by NPD using Different Jets

Blue is Extended Tip, less tailing

Red is Standard Tip, more tailing
NPD Jet For Improved Nitrogen on 6890

There is a much better jet for nitrogen detection with the NPD:

- G1534-80580 NPD Jet Capillary Det
- G1534-80590 NPD Jet Universal Det
- G1530A-29 Service Note that describes best practices for optimal NPD operation
- [note: disregard suggested use of narrower collector]
QuickSwap MSD Interface – Ease of Use

Remove column w/o venting

Backflush mode
- Inlet maintenance reverse flow
- Removes heavies from column

Maintain constant flow to MSD

Requires performance turbo for flow rates > 2 cc/min

Microfluidic technology
3x Speed, 15m, Lemon Oil TIC, no spike

Longer than normal run to elute heavies, another opportunity to save time using backflush

Elution time of last pesticide = 14 min

10 min at 320C needed after last target compound to elute oil
Permanent Splitter System GC/NPD/MSD

1:1 split NPD:MSD

6890N GC

Atlas EPC 3.8 psig

NPD

Effluent Splitter with Makeup

Column

5975 Inert MSD

Auto-sampler

0.532 m X 0.18 mm id

1.44 m X 0.18 mm id

30 m X 0.25 mm id X 0.25 um HP-5MS
How Do You Backflush?

Program inlet pressure to ~1.0 psi (may have to fine tune)

Program make-up pressure to calculated value after last target peak elution time

- Use flow calculator to get pressure for make-up to splitter (aux EPC or PCM). MS restrictor flow < 10 mL/min at backflush temp

Try a 3 void-times backflush, then run a blank for an extended time to make sure system is clean

Shorten this time if blank is clean, in 0.5 min steps

Performance Turbo a must
3x Speed, 15m, Lemon Oil TIC, no spike

Blank before backflush run

Backflush for 2.5 min at 300C instead of running an extra 10 min at 320C

Blank after backflush run
Spectral Deconvolution

• Extracts cleaner mass spectra from overlap situations – easier, reliable identification

• Faster data review, a free second opinion from a mass spec expert

• When coupled with RTL and NPD data, gives higher quality identifications

• Can run searches of data not restricted by RT to find “spectral brethren” like designer drugs
AMDIS Deconvolution Pulls Out Individual Components and their Spectra

TIC & Spectrum

Deconvoluted peaks and spectra

Component 1
Component 2
Component 3

Deconvolution

matrix
interference
target

Agilent Technologies
Deconvolution Removes Spectral Interferences

3-Chloro-1,2-dibromopropane overlapped with fuel components

Raw Spectrum
70th hit in NIST search

Manual Subtraction
2nd hit in NIST search

Deconvolved Spectrum
1st hit in NIST search

NIST 05 Library
DRS: 3 Integrated Processes

**Total ion chromatogram**

Targets are identified by locked R.T.s and 3 qualifier ions, then quantified using target ion area vs ISTD cal table

AMDIS 32 deconvolutes spectra and searches target MS database using locked RT as a qualifier

Deconvoluted Target spectra confirmed by AMDIS, searched against NIST02 MS database

Quant Results

Combined quantitative and qualitative HTML Summary report

Confirmed AMDIS hits

Confirmed NIST05 hits
The NIST library was searched for the components that were found in the AMDIS target library.

<table>
<thead>
<tr>
<th>R.T.</th>
<th>Cas #</th>
<th>Compound Name</th>
<th>Agilent ChemStation Amount (ng)</th>
<th>AMDIS Match</th>
<th>R.T. Diff sec.</th>
<th>Reverse Match</th>
<th>NIST Hit Num.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.497</td>
<td>107062</td>
<td>1,2-Dichloroethane</td>
<td>2.27</td>
<td>97</td>
<td>0.6</td>
<td>94</td>
<td>1</td>
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<tr>
<td>1.540</td>
<td>563586</td>
<td>1,1-Dichloropropylene</td>
<td>7.6</td>
<td>100</td>
<td>0.5</td>
<td>96</td>
<td>1</td>
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<td>1.867</td>
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<td>4.92</td>
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<td>2.330</td>
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<td>0.9</td>
<td>95</td>
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Summary

The 5975 and associated new products bring several new tools to the forensic analyst:

- Auto-CI
- SIMScan
- Splitters
- DRS