Improved LC-MS Detection for High Throughput Metabolic Stability Assays
Wayne Duncan, Agilent Tech., Santa Clara, CA, Mark A. Hughes and Kenneth C. Lewis, OpAns LLC, Durham NC 27713

Abstract

Parallel and combinational synthesis techniques have dramatically increased the number of chemicals available for biological screening. Higher screening rates have translated to more lead series and the ability to rapidly, and thoroughly, investigate the SAR for each series. Companies need a way to winnow these choices down to a handful of compounds for animal studies. In-vitro high throughput screening is a proven part of this selection process.

Like many labs, we use LC-MS/MS detection for our standard micromolar stability assay. However, each batch of samples in our miniaturized high-throughput assay would require the development of hundreds of MS/MS methods with us using those methods to analyze thousands of samples. This presentation details our findings that the Agilent 1100 MSD, with the multimode source, is a better match for our high-throughput assay.

Instrumentation

We have chosen to use an Agilent 1100 LC-MSD system (fig 1) as the detection system for our high-throughput in-vitro metabolic assay. Some of the reasons we chose this over the standard ESI mode are:

• Availability of the multimode source
• Cofactor solution in buffer (usually 6 mM NADPH) Alternately a NADPH regeneration system was used (OP-23). The reaction is assembled in a stopped condition i.e. stop buffer is added prior to NADPH addition. The timed reaction is assembled (total reaction volume = 25 μL) and then stopped at the appropriate time point. Reagents and plates are peroxidized for 10 minutes in 3% H2O2 to assure complete stopping.

LC conditions

The plumbing of the binary high pressure (1100) was modified to reduce delay volume.

Solvent A: Water w/ 0.1% Formic Acid

Solvent B: Methanol w/ 0.1% Formic Acid

Gradient: Linear gradient from 5% A to 100% B in 0.70 min

Column: Zorbax Eclipse C18 2.1x30 mm

Column Temperature: 35° C

MS conditions

Capillary Voltage: 2000 V

Charging Voltage: 2000 V

Fragmentor: 150 V

SIM on Target ion: 40 msec dwell

ES-ES gradient to 100% B in 0.70 min

Capillary Voltage: 3500 V

Charging Voltage: 2000 V

Fragmentor: 150 V

SIM on Target ion: 40 msec dwell

ES-ES

Figure 1

Agilent introduced the Multimode source (fig 2) in 2005 to address the need for universal ionization of pharmaceuticals. The source has several innovative features including:

• Separate ESI and APCI regions
• Simultaneous ESI and APCI operation
• Infilled lamps for heating
• Control of the temperature in the chamber and not just the gas-phase

While universal ionization might be a stretch, it clearly has the best ionization efficiency of any source we have ever used. This was a significant advantage as we developed a genetic method that did not suffer from the ion-suppression effects that are so common with these types of high-throughput analyses.

Source Evaluation

The initial focus of the evaluation was to determine how the multimode source impacted standards.

Four different control compounds were analyzed on each 384 well HTS plate. The multimode source was operated in APCI mode for the assay. Zero time represented phosphorimetric standard.

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Instrumentation

We have chosen to use an Agilent 1100 LC-MSD system (fig 1) as the detection system for our high-throughput in-vitro metabolic assay. Some of the reasons we chose this over a triple quad or ion trap are:

• Less expensive
• Separate ESI and APCI regions
• Simultaneous ESI and APCI operation
• InfraRed lamps for heating
• Control of the temperature in the chamber and not just the gas-phase

While universal ionization might be a stretch, it clearly has the best ionization efficiency of any source we have ever used. This was a significant advantage as we developed a genetic method that did not suffer from the ion-suppression effects that are so common with these types of high-throughput analyses.

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Conclusions

The Agilent multimode source is a good choice for analyzing pharmaceutical compounds from dirty matrices. It produces higher ion counts in simultaneous ESI/APCI mode than in other mode alone when analyzing precipitated micromolar solutions.

We have observed less variance in the assay results when using the source in APCI mode instead of ESI mode. This is most probably due to ionization suppression from proteins and other matrix effects that are not separated from the analyte with fast chromatography. We have observed that operation in simultaneous ESI/APCI mode is at least equal, if not better than APCI mode. Simultaneous ESI/APCI gives higher signal intensity than APCI with variance equal to APCI.

The multimode source is very tolerant of non-volatile salts and maintains sensitivity to simultaneous ESI/APCI mode despite the buildup of salt residue.

The multimode source, independent of operation mode, is very good at ionizing compounds. When we fail to see the target compound in the incubated well, we reanalyze the sample in stand alone ESI and stand alone APCI modes.

We have observed that the multimode source is very tolerant of non-volatile salts and maintains sensitivity to simultaneous ESI/APCI mode despite the buildup of salt residue.

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For Further Information

www.OpAns.com

Contact Ken Lewis at KenLewis@OpAns.com

OpAns, LLC, 416 S. Asten Ave, Durham, NC 27713

Phone (919) 323-4180

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Contact Ken Lewis at KenLewis@OpAns.com

OpAns, LLC, 416 S. Asten Ave, Durham, NC 27713

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