Evaluation of Accurate Mass and Dynamic Range Capabilities of Low and High-Resolution Instrumentation in Compound Identification in Drug Discovery.

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Overview

- Reliable accurate mass (AM) measurement plays an important role in compound identification.
- Accurate mass measurement typically requires use of expensive equipment and significant user expertise.
- A simpler, more robust, and user friendly system is needed for routine use in the drug discovery environment.
- Capabilities and limitations of time-of-flight (TOF) and quadrupole accurate mass measurements and compound molecular formula determination are compared.

Introduction

- In drug discovery applications, AM is typically measured using orbitrap or TOF instruments with time-to-digital (TDC) or analog-to-digital (ADC) converters.
- TDC-based TOF instruments suffer from limited dynamic range due to dead time detector limitations.
- Replacement of TDC-TOF instruments with ADC instruments or an orbitrap is not always feasible due to budgetary constraints.
- Feasibility of routine <5 ppm AM measurement and molecular formula (MF) determination was investigated using TDC-based TOF and single quadrupole MS instruments.
- For the TOF instrument, useful dynamic range was determined using Dynamic Range Enhancement (DRE).
- For the quadrupole instrument, AM and MF determination was done after spectral calibration with MassWorks™ software with isotopic pattern calibration.
- Effect of MS scan parameters on AM, formula rank, and dynamic range was investigated.
- Three model compounds with typical elemental composition in drug discovery were used.

Results

ZQ, Single Quadrupole

- MassWorks™ software calibrates spectral accuracy (SA) using a known standard. It includes mass accuracy and peak line shape calibration of the isotopic signature of the molecule.
- The calibrated spectrum is compared with the theoretical one for a molecular formula candidate. Formula rank is based on SA.
- SA (%) = 100*(1-RMSE).
- Scan speed plays important role in MassWorks™ performance.
- Mass accuracy of all measurements was <10 mDa and <5 mDa at <2500 amu/s (figure 1).
- SA improves with slower scan speeds and is not influenced by whether internal or external calibration is used (figure 2).

Table I. MassWorks molecular formula rank at 350 amu/s using external[a] spectral accuracy calibration.

Concentration[b] µg/mL	Compound 1 Rank	Compound 2 Rank	Compound 3 Rank
1000	1	1	2
100	1	1	1
10	3	2	6

[a] rank was the same with internal calibration.
[b] compounds not detected <10 µg/mL.

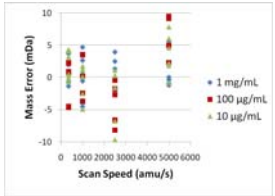

Figure 1. Influence of scan speed on mass accuracy.

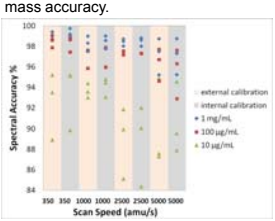

Figure 2. Influence of scan speed on spectral accuracy.

LCTp, Time-of-Flight

- DRE tool alternates between high and low intensity beam to extend the dynamic range of the instrument.
- DRE is calibrated using a lock mass in dead time saturation (attenuated lock mass) and normal lock mass (not saturated). Z-focus lens and a magnification factor are calibrated.
- Without DRE, dynamic range with AM <5 ppm is 1-2 orders of magnitude (table II).
- With DRE, dynamic range extends to 3-4 orders of magnitude.
- Isotopic fit (i-FIT™, measure of isotopic pattern accuracy) improves with decreasing concentration. Formula rank is based on i-fit and mass accuracy.

Table II. Dynamic range evaluation with and without the use of DRE.

	Conc.[a] µg/mL	Compound 1			Compound 2			Compound 3		
		AM (ppm)	i-FIT™	Rank	AM (ppm)	i-FIT™	Rank	AM (ppm)	i-FIT™	Rank
No DRE	100	*111.3*			*100.5*			*125.8*		
	10	*15.3*			3.9	13.4	3	*14.5*		
	1	2.9	0.3	1	0.0	0.9	3	-2.6	0.2	1
	0.1	-1.1	2.5	3	7.5	1.1	3	6.8	0.2	3
DRE	100	-0.3	120	1	0.7	4.9	1	-2.3	51.9	2
	10	-4.0	94.8	2	5.5	14.3	2	2.6	11.4	2
	1	2.4	1.3	1	2.6	0.3	2	3.7	3.7	5
	0.1	-2.4	2.2	2	0.9	1.6	2	-13.2		

Italics, red - in dead time saturation.
[a] Compounds not detected <0.1µg/mL.

Materials and Methods

	Single Quadrupole, ZQ	TOF, LCT Premier
Instrument	Waters ZQ + Agilent 1100	Waters LCT premier + Waters Acquity UPLC®
Column	Varian Polaris™ C18-A, 3 µm, 20 mm x 2.0 mm	Waters Acquity UPLC® HSS T3, 1.8 µm, 50 mm x 2.1 mm
Flow Rate	1.8 mL/min	1.0 mL/min
Injection Volume	1 µL	2 µL
Mobile Phase	A: 0.1% formic acid in H_2O B: 0.1% formic acid in CH_3CN	A: 0.1% formic acid in H_2O B: 0.1% formic acid in CH_3CN
Gradient	5 to 95% B in 1.75 min	5 to 95% B in 2.0 min
MS detection, ESI+	Scan speed 250, 1000, 2500, and 5000 amu/s covering the appropriate mass range	200 – 800 amu in 0.5 s
Resolution	Unit, ~0.5 amu peak width half height	~6000 FWHM
Mass Calibration	External mass calibration Internal and external Spectral Accuracy calibration using MassWorks	Fresh external mass calibration Lock mass using Lock Spray With DRE – two lock masses used
Lock Mass or calibrant	imipramine ([M+H] 281.2018), 100 µg/mL buspirone ([M+H] 386.2556), 100 µg/mL tyr-tyr-tyr ([M+H] 508.2084), 1 mg/mL reserpine ([M+H] 609.2812), 100 µg/mL	buspirone-^{13}C* ([M+H] 387.2771), 0.1 µg/mL leucine enkephalin-^{13}C* ([M+H] 557.2805), 1 µg/mL * naturally occurring ^{13}C isotopic mass peak
DRE calibration	N/A	buspirone ([M+H] 386.2556), 0.1 µg/mL leucine enkephalin ([M+H] 556.2771), 1 µg/mL

Sample	[M+H], Da	MF Search criteria	AM Criteria*
$C_{21}H_{19}N_3O_4$	378.1454	$C_{1-100}H_{1-100}N_{0-20}O_{0-20}$	5 ppm/5 mDa
$C_{25}H_{26}N_3O_6Br$	544.1083	$C_{1-100}H_{1-100}N_{0-20}O_{0-20}Br_{0-5}$	5 ppm/5 mDa
$C_{21}H_{25}N_7O_6SCl$	574.1042	$C_{1-100}H_{1-100}N_{0-20}O_{0-20}S_{0-5}Br_{0-5}$	5 ppm/5 mDa

* 5ppm TOF, 5mDa quadrupole

Concentrations: 10 ng/mL - 1 mg/mL in methanol

Quadrupole Instrument:
- Compounds mixed with internal spectral accuracy standards.
- MassWorks™ (ver. 2,0,2,0) used for spectral accuracy calibration (internal and external calibration)

TOF Instrument:
- Same day external mass calibration performed (100-1500 Da) at RMS < 5 ppm.
- DRE calibrated with ^{12}C peaks of reference compounds (in dead time).
  - Z-focus lens and magnification factor determined.
- Lock masses used were ^{13}C peaks of reference compounds.
- Compounds run with and without using DRE.

Conclusions

- TDC based TOF instrument has only 1-2 orders of magnitude dynamic range for accurate mass measurement (<5 ppm).
- It limits the usefulness in applications with wide concentration range samples.
- DRE extends the dynamic range to 3-4 orders of magnitude.
- DRE greatly simplifies instrument operation due to less stringent requirements to control ion beam intensity.
- Significant user expertise is still required to achieve the desired results.
- Accurate mass measurement and molecular formula ID is possible using a more economical single quadrupole instrument with MassWorks™ spectral accuracy calibration.
- It has three orders of magnitude dynamic range, with better performance at higher concentrations.
- Scan speed is a key parameter affecting the spectral accuracy.
- Internal calibration did not improve spectral accuracy due to high stability of quadrupole mass analyzer as compared to TOF.
- Compared to TOF, a single quadrupole instrument with MassWorks™ is easier to use, more economical and well suited in support of drug discovery chemistry operations.
- Compared to a single quadrupole instrument, a TOF instrument provides better sensitivity and is well suited to applications where sample concentration might be limited, such as in metabolite ID.

Acknowledgments

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