EXACT MASS MEASUREMENT

What exact mass measurement can do for you

For the identification and characterization of compounds, exact mass measurement in MS can be used to gain high specificity for identifying nominally isobaric components. Exact mass measurement in MS/MS gives you the added power to more confidently identify compounds and characterize structural fragments, where nominal mass data may be inconclusive.

Good scientific practice also mandates the use of exact mass measurements: "...for materials with molecular weights below 1000, the measured mass should generally agree to 5ppm or better, with the calculated mass." Notice to Authors of Papers: J. Am. Chem. Soc, 1999 121(1), 7A 12A"

The high resolution capability of Waters® orthogonal time-of -flight (oa-ToF) mass spectrometers enhance the analysis of compounds from complex matrices by minimizing co-eluting interferences, resolving components of interest even at low abundance.

With Waters oa-ToF systems and LockSpray™ technology you can achieve automated, routine exact mass measurement in MS and MS/MS, enabling maximum confidence in results.

Want to significantly enhance your MS or MS/MS analyses and the confidence in your results?

- Identify unknown compounds with higher confidence using elemental composition
- Successfully identify nominally isobaric compounds and structural fragments
- Reduce false positive protein identifications and minimize manual validation of results
- Gain higher sequence coverage and confidence for characterizing novel proteins and peptide sequences

Waters® Micromass® LCT Premier™ and Q-Tof™ Mass Spectrometers enable you to achieve exact mass measurement in LC/MS and MS/MS.

Exact mass measurement in MS and MS/MS...

Waters oa-ToF mass spectrometers have enhanced resolution and high mass measurement accuracy across the full m/z range, allowing MS and MS/MS analyses to be performed rapidly with high sensitivity. These features are standard across Waters oa-ToF range, enabling you to choose the exact mass instrument that best suits your application:

- Benchtop LC/MS – Waters Micromass® LCT Premier™
- Benchtop LC/MS and MS/MS - Waters Micromass Q-Tof micro™
- High Performance LC/MS and MS/MS - Waters Micromass Q-Tof Ultima™ API, Waters Micromass Q-Tof™ API-US

...made easy...

Exact mass measurement in MS and MS/MS on Waters oa-ToF mass spectrometers is simple and reliable with the use of LockSpray technology. Using a single point lockmass correction in MS and MS/MS, exact mass measurement can be routinely and automatically achieved over long time periods. The lockmass is sampled independently from the analyte into its own data channel, resulting in exact mass measured analyte spectra free from interfering lockmass peaks.

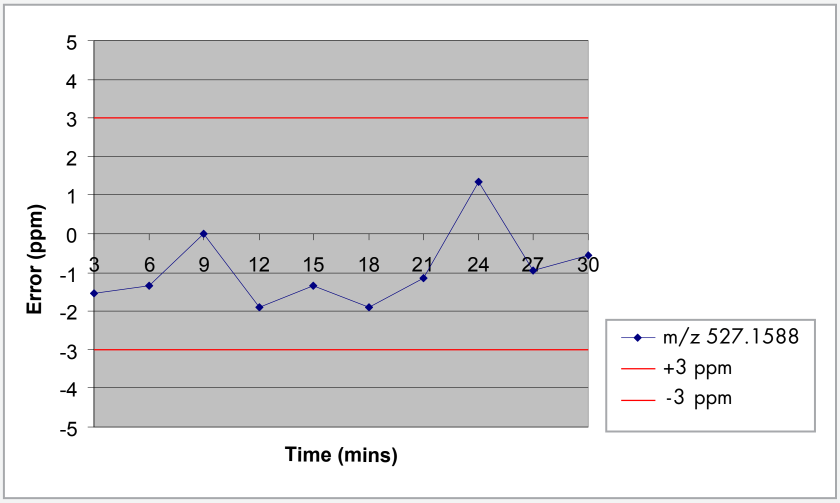
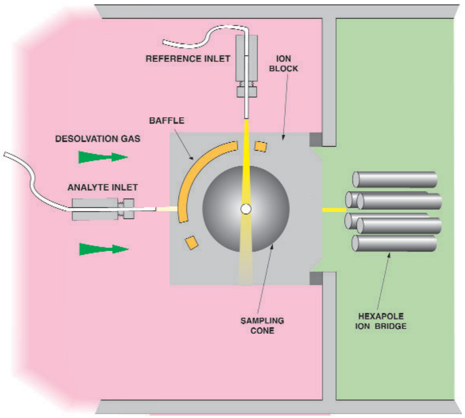


Figure 1. Routine exact mass measurement accuracy obtained from the LCT Premier equipped with an integrated LockSpray source. A small molecule, Raffinose ([M+Na]+ m/z 527.1588), was measured over a 30-minute period and the error in mass measurement was retained within +/- 3 ppm. The RMS error for all measurements is 1.32 ppm.



...and tuned for your application

- The LockSpray dual electrospray source enables automated exact mass measurement in MS and MS/MS. A lockmass solution is infused from a second sprayer, eliminating the need for post-column addition and potential ionization interference between analytes and lockmass
- The NanoLockSpray™ dual electrospray source is used for exact mass data acquisition with nanoflow LC/MS/MS and is ideal for proteomics studies or low level metabolite identification
- Exact Mass MUX-technology™ is ideal for high throughput applications where four or eight multiplexed HPLC streams can be employed to run parallel analyses into a single mass spectrometer, with an additional lockmass sprayer.

Why exact mass in MS?

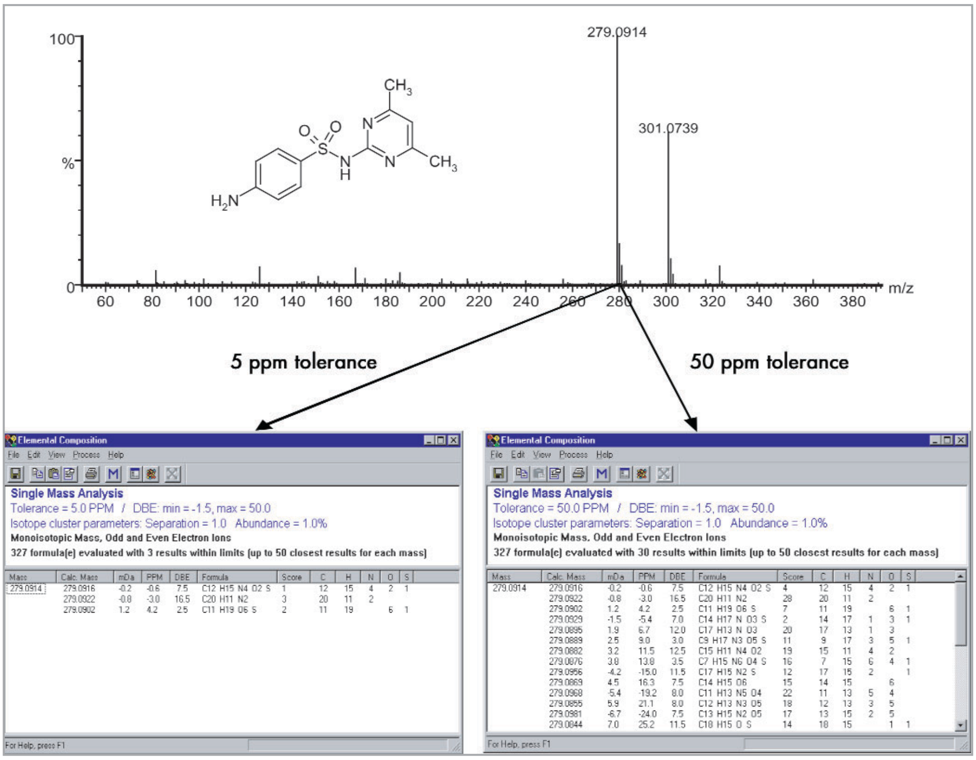
High mass measurement accuracy is a key factor to gaining more confidence in your results. When you detect a drug metabolite at 500.2 m/z in an *in vivo* sample, are you sure which biotransformation has taken place? When you detect a peptide at 976.6 m/z, could you be sure which post-translational modification is present?

With exact mass measurement you can:

- Determine the elemental composition of compounds from their exact mass measurements
- Distinguish between nominally isobaric components
- Increase specificity and data processing speed
- Confidently detect and confirm modifications on small molecules and peptides
- Increase specificity for quantitative analyses

Compond name	Actual Mass	Theoretical mass	+/- mDa	+/- ppm
Verapamil	455.2914	455.2910	0.4	1.0
M-14(1)	441.2750	441.2753	0.3	0.7
M-14(2)	441.2756	441.2753	0.3	0.7
Dextromethorphan	272.2017	272.2014	0.3	1.0
M-14	258.1859	258.1858	0.1	0.5
M-28	244.1702	244.1701	0.1	0.1
Diazepam	285.0793	285.0795	0.2	0.6
M+16	301.0745	301.0744	0.1	0.4
M-14	271.0637	271.0638	0.1	0.5
Midazolam	326.0856	326.0860	0.4	1.2
M+16(1)	342.0804	342.0809	0.5	1.7
M+16(2)	342.0807	342.0809	0.2	0.7
M+ 16(3)	342.0810	342.0809	0.1	0.2

Figure 2: This table shows exact mass measurements for 4 pharmaceuticals and their major metabolites on the Q-Tof with LockSpray and W-Optics™. Samples were protein-precipitated from 1μM microsomal incubation.



Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%
Monoisotopic Mass, Odd and Even Electron Ions
327 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)

Mass	Calc. Mass	mDa	PPM	DBE	Formula	Score	C	H	N	O	S
279.0914	279.0916	-0.2	-0.6	7.5	C12 H15 N4 O2 S	1	12	15	4	2	1
	279.0922	-0.8	-3.0	16.5	C20 H11 N2	3	20	11	2		
	279.0902	1.2	4.2	2.5	C11 H19 O6 S	2	11	19		6	1

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%
Monoisotopic Mass, Odd and Even Electron Ions
327 formula(e) evaluated with 30 results within limits (up to 50 closest results for each mass)

Mass	Calc. Mass	mDa	PPM	DBE	Formula	Score	C	H	N	O	S
279.0914	279.0916	-0.2	-0.6	7.5	C12 H15 N4 O2 S	4	12	15	4	2	1
	279.0922	-0.8	-3.0	16.5	C20 H11 N2	28	20	11	2		
	279.0902	1.2	4.2	2.5	C11 H19 O6 S	7	11	19		6	1
	279.0929	-1.5	-5.4	7.0	C14 H17 N O3 S	2	14	17	1	3	1
	279.0895	1.9	6.7	12.0	C17 H13 N O3	20	17	13	1	3	
	279.0889	2.5	9.0	3.0	C9 H17 N3 O5 S	11	9	17	3	5	1
	279.0882	3.2	11.5	12.5	C15 H11 N4 O2	19	15	11	4	2	
	279.0876	3.8	13.8	3.5	C7 H15 N6 O4 S	16	7	15	6	4	1
	279.0956	-4.2	-15.0	11.5	C17 H15 N2 S	12	17	15	2		1
	279.0869	4.5	16.3	7.5	C14 H15 O6	15	14	15		6	
	279.0968	-5.4	-19.2	8.0	C11 H13 N5 O4	22	11	13	5	4	
	279.0855	5.9	21.1	8.0	C12 H13 N3 O5	18	12	13	3	5	
	279.0981	-6.7	-24.0	7.5	C13 H15 N2 O5	17	13	15	2	5	
	279.0844	7.0	25.2	11.5	C18 H15 O S	14	18	15		1	1

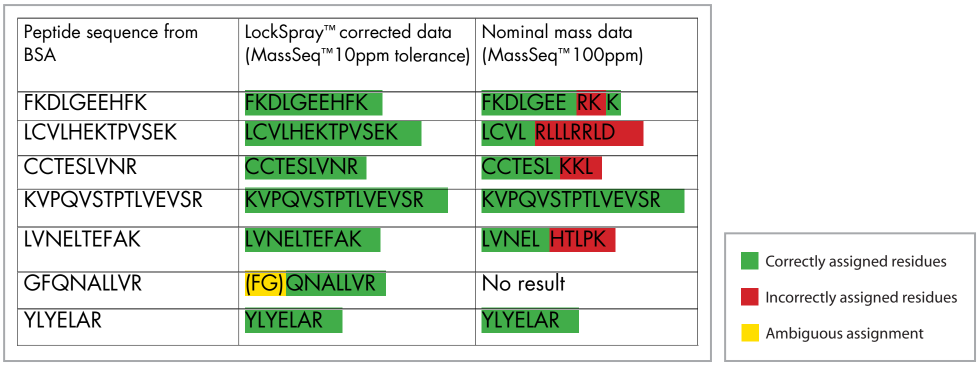
Figure 3: Enhanced compound identification: Exact mass MS measurement enables compounds to be more confidently identified. Confirming a measured m/z to within 5 ppm significantly increases confidence in assigning elemental composition.

Why exact mass in MS/MS?

The benefits of exact mass measurement in MS also apply to MS/MS analyses. At lower m/z values, exact mass is a particularly powerful tool for calculating elemental composition of structural fragments. Exact mass measurement enables the specificity of MS/MS to be extended, adding an extra dimension of confidence in the identification and characterization of compounds. For example, if you want to elucidate the structure of a metabolite, you'll be able to more clearly define the molecular sub-structures. If you want to sequence a peptide or locate a post-translational modification, you can be confident of assigning the correct amino acid residue.

With exact mass measurement in MS/MS you can:

- Confirm the nature and position of modifications on molecules with high confidence
- Distinguish between nominally isobaric product ions
- Determine elemental composition of structural fragments



Peptide sequence from BSA	LockSpray™ corrected data (MassSeq™ 10ppm tolerance)	Nominal mass data (MassSeq™ 100ppm)
FKDLGEEHFK	FKDLGEEHFK	FKDLGEE RK K
LCVLHEKTPVSEK	LCVLHEKTPVSEK	LCVL RLLLRRLD
CCTESLVNR	CCTESLVNR	CCTESL KKL
KVPQVSTPTLVEVSR	KVPQVSTPTLVEVSR	KVPQVSTPTLVEVSR
LVNELTEFAK	LVNELTEFAK	LVNEL HTLPK
GFQNALLVR	(FG) QNALLVR	No result
YLYELAR	YLYELAR	YLYELAR

🟩 Correctly assigned residues
🟥 Incorrectly assigned residues
🟨 Ambiguous assignment

Figure 4: Confident assignment of amino acids in de novo sequencing of peptides is significantly enhanced through the exact mass measurement of MS/MS fragments. Greater sequence coverage is achieved for each peptide, resulting in a higher confidence in the characterization of known or novel proteins. Results are shown for a digest of Bovine Serum Albumin analyzed with a Q-Tof and ProteinLynx™ Global SERVER 2.0 software.

For more information on the benefits of exact mass measurement specific to your application, please visit the literature section of www.waters.com. You will find specific literature using both the keywords "exact mass" and your application/product of interest; e.g. Q-Tof, LCT Premier, proteomics, pharmaceutical, environmental, etc.

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[1] Bateman et al. JASMS (2002) 13, 792-803

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